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Molecular study on selected vector-borne infections in urban stray colony cats in northern Italy

Eva Spada[1], Daniela Proverbio[1], Paola Galluzzo[2], Alessandra Della Pepa[1], Roberta Perego[1], Giada Bagnagatti De Giorgi[1] and Elisabetta Ferro[1]

Abstract

Feline vector-borne diseases can be caused by a range of pathogens transmitted by arthropods. Many of these infections have zoonotic implications, and stray cats are potential sentinels for human and pet health. This study investigated the prevalence of selected vector-borne infections in stray colony cats in Milan. Blood samples from 260 stray cats were evaluated, using conventional polymerase chain reaction tests (cPCRs), for the presence of DNA associated with *Rickettsia* species, *Anaplasma phagocytophilum* and *Ehrlichia* species. Positive cPCR results occurred in 127/260 subjects (48.9%; 95% confidence interval [CI] = 40.7–58.1), with a prevalence of 31.9% (83/260, 95% CI = 25.4–39.6) for *Rickettsia* species, 17.7% (46/260, 95% CI= 13.0–23.6) for *A phagocytophilum*, and 5.4% (14/260, 95% CI = 2.9–9.0) for *Ehrlichia* species. There was no statistical association between a positive PCR test for vector-borne infections surveyed and colony location, age, gender, body condition score or complete blood count abnormalities, nor feline immunodeficiency virus, feline leukaemia virus or *Toxoplasma gondii* status. The only variable linked to positive PCR results was detection of signs of ocular infection and PCR positivity for *Rickettsia* species (P = 0.04 odds ratio [OR] = 2.2, 95% CI = 1.1–4.4, P = 0.02). There is a significant prevalence of vector-borne infections with zoonotic potential in urban stray cats in Milan. Thus, dogs and pet cats with outdoor access should be monitored and treated for ectoparasites on a regular basis to minimise risks of disease and the potential transmission of zoonotic agents to people.

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Feline vector-borne diseases (FVBDs) are an emerging problem worldwide owing to their frequency and morbidity. Furthermore, most FVBDs are zoonotic, and stray cats can be potential sentinels for human infection. FVBDs, such as rickettsiosis, anaplasmosis and ehrlichiosis, are caused by diverse pathogens transmitted by arthropod vectors, especially fleas and ticks.[1–3]

Stray cats are predisposed to FVBDs because they live outdoors, have constant exposure to ticks and fleas, and prey on urban wildlife that may harbor pathogens. Additionally, stray cats are often neither monitored nor treated for vector-borne pathogens. Although several studies worldwide have reported on the epidemiology and emergence of FVBDs in owned healthy or sick pet cats,[4–14] only a few studies have focused on stray colony cats[15–18] and, in particular, there have been only studies looking for *Bartonella* species infections in stray cats in northern Italy.[19,20]

Free-roaming stray cats are a public health concern in urban and rural areas of Italy.[21] However, the environmental and medical impact of free-roaming cats is not well understood owing to a lack of scientific data on these populations. Identification and epidemiological surveillance of the various zoonotic diseases in cats may advance the development, and implementation, of protective measures by national and regional agencies. Therefore, an understanding of the prevalence of FVBDs could help to limit the

[1]Veterinary Transfusion Unit (REV), Department of Health, Animal Science and Food Safety (VESPA), University of Milan, Milan, Italy
[2]National Centre of Reference for Anaplasma, Babesia, Theileria and Rickettsia (CRABaRT), Experimental Zooprophylaxis Institute of Sicily, Palermo, Italy

Corresponding author:
Eva Spada DVM, PhD, Veterinary Transfusion Unit (REV), Department of Health, Animal Science and Food Safety (VESPA), University of Milan, via G. Celoria 10, 20133 Milan, Italy
Email: eva.spada@unimi.it

spread of zoonotic diseases in feline, canine and human populations.

This study evaluated the prevalence of *Rickettsia* species, *Anaplasma phagocytophilum*, and *Ehrlichia* species in stray colony cats in Milan, and identified possible risk factors for these infections.

During a 2 year collection period (January 2008–January 2010), blood samples were taken from 260 stray cats from urban colonies in Milan (northern Italy), under a trap–neuter–release programme approved by the city council. The programme was conducted as previously described.[22] Briefly, stray cats, trapped by volunteers and presented to the University of Milan, were anaesthetised (tiletamine and zolazepam plus tramadol intramuscularly) while confined in the trap. General anaesthesia was maintained with isoflurane administered by mask. Before surgery clinical examinations were performed and blood samples were taken from each cat. After surgery cats were hospitalised for at least 5 days (depending on health status) before being released into the location from where they had been trapped.

Cats (n = 260) were collected from eight of the nine decentralisation zones of Milan, including three from zone 1 (1.2%), 11 from zone 2 (4.2%), 108 from zone 4 (41.5%), 12 from zone 5 (4.6%), 27 from zone 6 (10.4%), 55 from zone 7 (21.2%), 22 from zone 8 (8.5%) and 22 from zone 9 (8.5%). All cats were domestic shorthairs. Age distribution, estimated by dentition, included 118 (45.4%) kittens and 142 (54.6%) adult cats. The group consisted of 90 males (34.6%) and 170 females (65.4%). Body condition score (BCS) was recorded for 243 cats, with a median score of 4.3 ± 0.73 (range 2–8/9). Moreover, 225 cats (92.6%) had a normal weight with a BCS score range of 4–6. Only 18 (7.4%) cats were underweight (BCS = 1–3).

Health evaluation (n = 260) revealed 72 (27.7%) cats to be healthy and 188 (72.3%) cats to be unhealthy. Clinical abnormalities detected included enlarged lymph nodes (n = 133, 51.2%), pale mucous membranes (n = 14, 5.4%), stomatitis (n = 101, 38.8%), signs of respiratory tract infection (n = 22, 8.5%) and signs of ocular infection (n = 40, 15.4%). The cats were not systematically examined for the presence of ticks or fleas and so the rates of ectoparasitism were not recorded.

Blood samples were collected aseptically, from the jugular or cephalic vein, into EDTA-treated and serum separator tubes. Complete blood count (CBC, n = 150) was performed on EDTA-anticoagulated blood within 24 h of sample collection, using the ADVIA 120 System (Siemens Healthcare Diagnostics). Surplus blood was stored at −20°C and used for polymerase chain reaction (PCR) assay. Sera separated from whole blood were tested for antibodies to feline immunodeficiency virus (FIV) (gp40 and p24 FIV antigens; n = 166) and feline leukaemia virus (FeLV) p27 antigen (n = 166) using a commercial ELISA kit (SNAP FeLV/FIV Combo Plus Test; IDEXX Laboratories). The presence of immunoglobulin G (IgG) antibodies against *Toxoplasma gondii* (n = 113) was evaluated using a commercial indirect fluorescent antibody test (IFAT) kit (Fuller Laboratories). An antibody titre ⩾1:64 was considered indicative of *T gondii* exposure.

Conventional PCR (cPCR) was performed on blood samples to amplify pathogen-associated DNA associated with *Rickettsia* species, *A phagocytophilum* and *Ehrlichia* species. Primer names, sequences and amplicon size (base pairs) are summarised in Table 1, and PCR conditions have been previously reported.[23–25] Control reactions were performed in the absence of template DNA to rule out contaminations during PCR.

The prevalence of each pathogen was calculated and univariate analysis of categorical data was performed using the χ^2 test (cell frequencies of >5) or Fisher's exact test (cell frequencies of ⩽5). Any parameters statistically linked to positive PCR results were used in a logistic regression model to test for independent risk factors associated with infection. Associations were considered statistically significant when P <0.05. Data were analysed using MedCalc Software (version 12.3.0).

Overall, 127 cats (48.9%, 95% confidence interval [CI] = 40.7–58.1) tested cPCR positive to at least one of the agents. Prevalence of each infection and co-infections are reported in Table 2. Median haematocrit (Ht) was 25.2% (range 13–38%), median white blood cell (WBC)

Table 1 Names, sequences and amplicon size (base pairs [bp]) of polymerase chain reaction primers used in this study

Organism detected	Primer name	Nucleotide sequence (5'-3')	Amplicon size (bp)	Reference
Rickettsia species	Tz-15-19	TTCTCAATTCGGTAAGGGC	246	Tzianabos et al[23]
	Tz-16-20	ATATTGACCAGTGCTATTTC		
Ehrlichia species	PER1	TTTATCGCTATTAGATGAGCCTATG	451	Munderloh et al[24]
	PER2	CTCTACACTAGGAATTCCGCTAT		
Anaplasma phagocytophilum	MSP4AP5	ATGAATTACAGAGAATTGCTTGTAGG	849	de la Fuente et al[25]
	MSP4AP3	TTAATTGAAAGCAAATCTTGCTCCTATC		

Table 2 Prevalence of vector-borne infections in 260 stray colony cats based on polymerase chain reaction detection of bacterial DNA in blood

Vector-borne pathogen	Number of infected cats	Observed prevalence (%)	95% CI
Rickettsia species	83	31.9	25.4–39.6
Anaplasma phagocytophilum	46	17.7	13.0–23.6
Ehrlichia species	14	5.4	2.9–9.0
A phagocytophilum + *Rickettsia* species	14	5.4	2.9–9.0
A phagocytophilum + *Ehrlichia* species	0	0	0
Rickettsia species + *Ehrlichia* species	2	0.8	0.09–2.80
A phagocytophilum + *Rickettsia* species + *Ehrlichia* species	0	0	0

CI = confidence interval

count was 10,770/µl (range 1516–23,240/µl) and the median thrombocyte count was 374 × 10^9/l (range 90–800). Anaemia (Ht <24%) was found in 69/150 (46.0%) cats, leukopenia (WBC <10,570/µl) in 14/150 (9.3%) cats, leukocytosis (WBC >14,390/µl) in 5/150 (3.3%) cats, thrombocytopenia (PLT <200,670/µl) in 10/150 (6.7%) cats. Thirteen (7.8%) cats tested positive for FIV and six (3.6%) were positive for FeLV. An antibody titre for *T gondii* ⩾1:64 was detected in 31/113 (27.4%) cases.

There were no correlations between colony location, age, gender, BCS, CBC abnormalities, FIV, FeLV, *T gondii* status and infections. The only variable linked to PCR-positive results was the presence of sign of ocular infections in association with *Rickettsia* species infection (Table 3).

This is the first study to investigate the prevalence of selected FVBDs in urban stray cats in Milan. It is difficult to compare these results to the worldwide prevalence of feline infections owing to the limited data available, difficulties in comparing information from studies using different diagnostic tools (ie, molecular and serological) and confounding factors associated with distinct study populations (ie, stray vs owned cats, healthy vs sick cats).

Rickettsia species have been detected in up to 15.7% of ticks collected from public parks in northern Italy.[26] Despite their presence in arthropod vectors, rates of rickettsial infection in cats are generally low worldwide. Our prevalence was significantly higher than in previous studies on stray cats from Ireland in which no cats tested positive for *Ehrlichia* species DNA in blood.[18] Also in a study of stray cats in Ontario, Canada, no *Rickettsia felis* DNA was found in blood, despite 18% of fleas being found to test positive for *R felis* DNA.[17] There was an association between cats that tested PCR positive for *Rickettsia* species and the presence of signs of ocular infection in these cats (OR = 2.2, P = 0.02). In dogs, rickettsial infection can cause ocular abnormalities[1] and, although not previously documented, we suggest that this may also be true for cats.

In this study, 17.7% of cats were infected with *A phagocytophilum*, which is higher than other studies performed on feral cats in northern Florida,[15] Arizona[16] and Ireland[18] where no cats tested positive for *A phagocytophilum* DNA on PCR of blood. Studies have shown that, in some cases, *A phagocytophilum* DNA was not detected by PCR even if cats were seropositive (mainly at IFAT test) in the USA (4.3% seropositivity),[10] and northern (1.8%)[7] and central Spain (4.9%).[5]

The *Ehrlichia* species that infect cats have been not fully determined.[3] The majority of epidemiological data on *Ehrlichia* species infections in cats are based on serological surveys. Only two studies have looked at prevalence of *Ehrlichia* species in central Italy. One study was able to detect serological evidence of *Ehrlichia* species in 10.2% of stray cats from shelters in Tuscany.[4] Another study from the Abruzzo region, detected two seropositive cats out of 203 (1%), although both cats were PCR-negative for *Ehrlichia* species DNA in blood samples.[6] No *Ehrlichia* species-associated DNA was amplified by PCR from blood of stray cats in Ireland[18] nor from feral cats in Arizona (USA)[16] or Florida.[15]

Our study had certain limitations as some pathogens were investigated only at the genus level (eg, *Rickettsia* species and *Ehrlichia* species), and information about the species infecting the study population was lacking. Ectoparasitism was not investigated and, consequently, potential vectors associated with the pathogens in this area were not identified. Risk factors analysed were not available for all 260 cats. Regardless of these limitations, we believe that this study provides new and useful information on FVBDs in Italy.

Conclusions

We found that FVBDs were present with significant prevalence in the stray urban feline population of Milan. Pet cats with outdoor access in this region should be regularly monitored and treated for ectoparasites to minimise health risks to humans and pets.

Table 3 Prevalence of *Rickettsia* species, *Anaplasma phagocytophilum* and *Ehrlichia* species in stray colony cats based on polymerase chain reaction (PCR) detection of bacterial DNA and statistical analysis of risk factors

Factor	Category	*Ehrlichia* species-positive, n (%)	*P*	*A phagocytophilum*-positive, n (%)	*P*	*Rickettsia* species-positive, n (%)	*P*
Origin of the cats	Zone 1	1/3 (33.3)	0.38	0/46 (0.0)	0.96	1/83 (1.2)	0.57
	Zone 2	2/11 (18.1)	0.22	3/46 (6.5)	0.66	3/83 (3.6)	0.99
	Zone 4	5/109 (4.9)	0.83	24/46 (52.2)	0.15	41/83 (49.4)	0.10
	Zone 5	1/12 (8.3)	0.84	0/46 (0.0)	0.21	2/83 (2.4)	0.40
	Zone 6	1/27 (3.7)	0.97	0/46 (0.0)	0.18	4/83 (4.8)	0.07
	Zone 7	1/54 (1.9)	0.34	15/46 (32.6)	0.06	19/83 (22.9)	0.76
	Zone 8	2/22 (9.1)	0.76	0/46 (0.0)	0.05	9/83 (10.8)	0.48
	Zone 9	1/22 (4.5)	0.76	4/46 (8.7)	0.82	4/83 (4.8)	0.23
Age	Juvenile (⩽6 months)	8/118 (6.8)	0.53	21/46 (45.7)	0.90	42/83 (50.6)	0.31
	Adult (>6 months)	6/142 (4.2)		25/46 (54.3)		41/83 (49.4)	
Gender	Male	4/90 (4.4)	0.84	14/46 (30.4)	0.63	22/83 (26.5)	0.08
	Female	10/170 (5.9)		32/46 (69.6)		61/83 (79.5)	
BCS	Poor (1–3/9)	1/18 (5.5)	0.66	1/44 (2.3)	0.26	5/83 (6.0)	0.74
	Good (4–6/9)	11/225 (4.9)	0.66	43/44 (97.7)		78/83 (94.0)	
Health status	Healthy	4/72 (5.5)	0.82	18/46 (39.1)	0.08	24/83 (28.9)	0.88
	Unhealthy	10/188 (5.3)		28/46 (60.9)		59/83 (71.1)	
Clinical abnormalities in unhealthy cats	Lymph node enlargement	6/133 (4.5)	0.72	21/46 (45.7)	0.51	45/83 (54.2)	0.59
	Pale mucous membranes	1/14 (7.1)	0.76	4/46 (8.7)	0.46	1/83 (1.2)	0.08
	Stomatitis	5/101 (5.0)	0.97	14/46 (30.4)	0.26	25/83 (30.1)	0.07
	Signs of respiratory tract infection	1/22 (4.5)	0.76	3/46 (6.5)	0.82	9/83 (10.8)	0.48
	Signs of ocular infection	1/40 (2.5)	0.62	7/46 (15.2)	0.85	19/83 (22.9)	**0.04 OR = 2.2 CI = 1.11–4.38 *P* = 0.02***
CBC abnormalities	Anaemia	0/70 (0.0)	0.54	17/37 (45.9)	0.89	35/71 (49.3)	0.59
	Leukopenia	0/14 (0.0)	0.44	2/37 (5.4)	0.53	5/71 (7.0)	0.53
	Leukocytosis	0/5 (0.0)	0.08	3/37 (8.1)	0.18	2/71 (2.8)	0.90
	Thrombocytopenia	0/10 (0.0)	0.30	3/37 (8.1)	0.99	6/71 (8.5)	0.63
A phagocytophilum PCR results	Positive	0/46 (0.0)	0.15	–	–	14/83 (16.9)	
	Negative	14/14 (100)		–	–	69/83 (83.1)	
Rickettsia species PCR results	Positive	2/14 (14.3)	0.83	14/46 (30.4)	0.95	–	–
	Negative	12/14 (85.7)		32/46 (69.6)		–	
Ehrlichia species PCR results	Positive	–		0/46 (0.0)	0.15	2/83 (2.4)	0.25
	Negative	–		46/46 (100)		81/83 (97.5)	
FIV test results	Positive	0/13 (0.0)	0.74	5/39 (12.8)	0.35	5/78 (6.4)	0.68
	Negative	13/13 (100)		34/39 (87.2)		73/78 (93.6)	
FeLV test results	Positive	0/6 (0)	0.34	0/39 (0.0)	0.36	2/78 (2.6)	0.75
	Negative	6/6 (100)		39/39 (100)		76/78 (97.4)	
T gondii test results	Positive	0/31 (0.0)	0.93	6/23 (26.1)	0.94	18/65 (27.7)	0.83
	Negative	2/81 (2.5)		17/23 (73.9)		47/65 (72.3)	

BCS = body condition score; CBC = complete blood count; FIV = feline immunodeficiency virus; FeLV = feline leukemia virus; OR = odds ratio; CI = 95% confidence interval

P values in bold are statistically significant (P <0.05). **P* from logistic regression analysis

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