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Evidence of *Bartonella* spp., *Rickettsia* spp. and *Anaplasma phagocytophilum* in domestic, shelter and stray cat blood and fleas, Portugal

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INTRODUCTION

Cats are reservoirs of several infectious agents and potential sources of infection to humans. Examples of these are *B. henselae* and *B. clarridgeiae*, agents of cat scratch disease (CSD). The transmission occurs mainly by the scratch of contaminated cat claws. However, the possibility of direct transmission by cat fleas should not be excluded. Moreover, it is known that the presence of cat fleas (*Ctenocephalides felis*) is essential for the maintenance of the infection within cat populations. Cats may also be involved in the maintenance cycle of other flea-borne agents such as *Rickettsia felis* that cause human disease.

To our knowledge no previous studies have been performed to detect the presence of *Bartonella* spp., *R. felis* and *A. phagocytophilum* in Portuguese cat fleas. This study also evaluated the prevalence of antibodies against *Bartonella* spp., *Rickettsia* spp. and *A. phagocytophilum* and the detection of *Bartonella* bacteraemia by PCR in cat blood.

METHODS

Fifty-one cats (domestic, shelter and stray) from Lisbon and Évora were enrolled in the study between August 2007 and April 2008.

DNA was extracted from each flea and tested by PCR using Rp877F/Rp1258R and 120-M59/120-807 primers, which amplify *Rickettsia* spp.; *Bartonella* DNA was amplified with BhCS.781p/BhCS.1137n primers and *A. phagocytophilum* with a nested PCR using HS1/HS6 and HS43/HS45 primers.

Cat blood samples were used to perform serological and molecular assays. Nested PCR with P-bhenfa/P-bhenr1 and N-bhenf1a/N-bhenr primers was used to detect *Bartonella* DNA.

The amplicons were sequenced and compared with the available corresponding sequences in the GenBank/EMBL database, using the BLAST software.

Serologic testing was performed by in-house IFA using *R. conorii* Malish, *A. phagocytophilum* and *B. henselae*. Sera tested for *Bartonella* and *Rickettsia* were diluted 1:32, 1:64 and 1:128; sera were diluted 1:40 and 1:80 for *A. phagocytophilum*. Serial two-fold dilutions were made of positives to obtain an endpoint titre. IgG titres ≥1:128, ≥1:64 and ≥1:40 were considered positive for *R. conorii*, *B. henselae*, and *A. phagocytophilum*, respectively.

RESULTS

Out of 51 cats, 27 (52.9%) were female and 37 (72.5%) were less than ≤1 year old. Twenty-five (49.0%) lived indoors at the time of the survey, but more than 80% of them lived outdoors before adoption.

Thirty-two fleas were collected from 18 Lisbon cats, 29 of which (90.6%) were *C. felis*, one (3.1%) was *C. canis* and two (6.3%) were unidentifiable. Only *C. felis* fleas were infected, six (40.0%) with *B. clarridgeiae* and six (40.0%) with *R. felis*; three (20.0%) were co-infected. No positive result was found for *A. phagocytophilum*. The infection prevalence of *B. clarridgeiae* was higher in domestic (43.8%) than in shelter cat fleas (28.6%). However, the infection rate of *R. felis* was higher in shelter (42.9%) than in domestic cat fleas (25.0%). Stray cat fleas were only infected with *R. felis* (11.1%).

Twenty-five cats (67.7%) were bacteraemic (Table 1). Twenty-one of them (84.0%) were less than ≤1 year old, 15 (60.0%) were female and 10 (40.0%) had no *Bartonella* spp. antibodies, one of which (10.0%) was more than 1 year old. The prevalence of *Bartonella* bacteraemia is higher in shelter (76.9%) than in domestic cats (68.2%) and all stray cats tested (n = 2) were positive.

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Table 1. PCR and antibody testing for rickettsia and Bartonella spp. in cat blood and fleas

Cat No.	Flea identification (number of fleas)	Species detected by PCR in fleas	Species detected by PCR in blood	IgG titre determined by IFA		
				B. henselae	*R. conorii*	*A. phagocytophilum*
1[a]	ND	ND	*B. henselae*	256	256	320
2[a]	ND	ND	*Bartonella* spp.	128	64	80
3[a]	ND	ND	*Bartonella* spp.	–	>512	–
4[c]	ND	ND	*Bartonella* spp.	–	>512	–
5[c]	ND	ND	*Bartonella* spp.	–	–	–
6[c]	ND	ND	*Bartonella* spp.	–	–	–
7[c]	ND	ND	–	64	32	–
8[c]	ND	ND	–	32	–	–
9[a]	ND	ND	–	512	64	–
10[a]	ND	ND	*Bartonella* spp.	–	–	–
11[a]	ND	ND	*Bartonella* spp.	64	–	–
12[a]	ND	ND	–	64	–	–
13[a]	ND	ND	*B. henselae*	64	–	–
14[a]	ND	ND	*B. henselae*	32	–	–
15[a]	*C. felis* (1)	*B. clarridgeiae*, *R. felis*	ND	ND	ND	ND
16[a]	*C. felis* (1)	–	*Bartonella* spp.	128	64	–
17[a]	*C. felis* (3)	*B. clarridgeiae*, *R. felis*	ND	ND	ND	ND
18[a]	ND	ND	–	32	128	80
19[a]	ND	ND	*Bartonella* spp.	–	32	–
20[a]	*C. felis* (2)	–	*Bartonella* spp.	64	–	–
21[b]	*C. felis* (1)	–	ND	ND	ND	ND
22[b]	*C. felis* (2)	–	ND	ND	ND	ND
23[b]	*C. felis* (2)	–	ND	ND	ND	ND
24[c]	*C. felis* (1)	*B. clarridgeiae*	ND	ND	ND	ND
25[b]	*C. canis* (1)	–	ND	ND	ND	ND
26[c]	*C. felis* (4)	*B. clarridgeiae*, *R. felis*	ND	ND	ND	ND
27[c]	*C. felis* (2)	*B. clarridgeiae*	ND	ND	ND	ND
28[c]	*C. felis* (1)	–	ND	ND	ND	ND
29[c]	*Ctenocephalides* spp. (1)	*R. felis*	ND	ND	ND	ND
30[c]	ND	ND	Bartonella spp.	128	–	80
31[c]	ND	ND	Bartonella spp.	–	–	–
32[c]	ND	ND	–	64	64	–
33[c]	ND	ND	–	64	64	80
34[c]	ND	ND	–	256	64	–
35[c]	ND	ND	*Bartonella* spp.	64	32	–
36[c]	ND	ND	–	128	64	–
37[c]	*C.felis* (2)	*R. felis*	ND	ND	ND	ND
38[c]	ND	ND	*Bartonella* spp.	64	–	–
39[b]	*C. felis* (3)	*R. felis*	ND	ND	ND	ND
40[c]	*C .felis* (2)	*R. felis*	*Bartonella* spp.	–	128	–
41[c]	*C .felis* (1)	–	*Bartonella* spp.	64	128	–
42[c]	ND	ND	*Bartonella* spp.	>256	256	–
43[c]	ND	ND	*Bartonella* spp.	256	64	–
44[c]	ND	ND	*Bartonella* spp.	128	–	–
45[c]	*C. felis*(2)	–	ND	ND	ND	ND
46[c]	ND	ND	*Bartonella* spp.	128	–	–
47[b]	ND	ND	*Bartonella* spp.	64	64	–
48[b]	ND	ND	*Bartonella* spp.	32	64	–
49[c]	ND	ND	–	128	–	–
50[c]	ND	ND	–	>128	–	–
51[c]	ND	ND	–	–	–	–

[a]Shelter cats; [b]stray cats; [c]domestic cats; ND, not determinate; –, negative.

The IFA test detected reactive antibodies in 24 (64.9%), seven (18.9%) and five (13.5%) cats with *B. henselae*, *R. conorii* and *A. phagocytophilum*, respectively. Six (16.2%) were seropositive for more than one agent. Nine (37.5%) of the seropositive cats for *B. henselae* were not bacteraemic.

CONCLUSIONS

To our knowledge this is the first report of molecular detection of *B. clarridgeiae* and *R. felis* in *Ctenocephalides felis* fleas from Portugal. *R. felis* had only been previously reported in other flea species collected in Portuguese rodents [1]. In our study, the prevalence of 40% found for *R. felis* and *B. clarridgeiae* in fleas was higher compared with other reports of 8.1% and 28.4% for *R. felis* and 17.8% and 6.8% for *B. clarridgeiae* in France and Spain, respectively. However, in the USA 93% of the fleas were infected with *R. felis*. We did not detect *A. phagocytophilum* and *B. henselae* in fleas in this study, but we did detect the latter in 67.7% of cat blood samples. No correlation was observed between *Bartonella* found in fleas and blood, as the positive fleas did not belong to the same cats.

In Europe the highest prevalence of bacteraemia was reported in 22% of domestic cats in the Netherlands and in 53% of stray cats in France [2]. In our study the high prevalence of bacteraemia (67.7%) could be explained by the fact that the cats were mostly outdoors and ≤1 year old.

B. henselae antibody prevalence (64.9%) was much higher when compared with a previous Portuguese study (6.7%) [3]. Nevertheless, it is similar to other levels found in cats from the Netherlands (56%), Denmark (46.9%) and Italy (38%) [2]. The seropositivity of 18.9% found for *R. conorii* may suggest that feline rickettsioses are more relevant than expected and future studies should be considered.

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