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.

No conflicts of interest declared.

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 \geq 1:128, \geq 1:64 and \geq 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 \leq 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 \leq 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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| Facial destification tumber of fasa Species detected by PCR in fleas Species detected by PCR in fleas <i>Because</i> by PCR in fleas <i>Because</i> by PCR in fleas <i>A phagocytophilum</i> 1* ND ND Bartonilla spp. 256 250 320 2* ND ND Bartonilla spp. - >512 - 4* ND ND Bartonilla spp. - - - 5* ND ND Bartonilla spp. - - - 6* ND ND Bartonilla spp. - - - - 9* ND ND ND Bartonilla spp. - - - - 12* ND ND ND - - - - 12* ND ND Bartonilla spp. - - - - 12* ND ND Bartonilla spp. 128 64 - - 12* C, fela (D) B. darridgciae, R, felis < | Cat No. | Flea identification (number of fleas) | Species detected by PCR in fleas | Species detected by PCR in blood | IgG titre determined by IFA | | |
|--|-----------------------|--|-------------------------------------|-------------------------------------|-----------------------------|------------|--------------------|
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| 16*C, felis (1)Batronella spp.12864-17*C, felis (3)B. clarridgeiae, R. felisND | 15 ^a | C. felis (1) | B. clarridgeiae, R. felis | ND | ND | ND | ND |
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| 20° 21° <br< td=""><td>19^a</td><td>ND</td><td>ND</td><td>Bartonella spp.</td><td>_</td><td>32</td><td>_</td></br<> | 19 ^a | ND | ND | Bartonella spp. | _ | 32 | _ |
| 21bC, feits (1)-NDNDNDND22bC, feits (2)-NDNDNDND24cC, feits (2)-NDNDNDND24cC, feits (1)B. clarridgeiae, R. felisNDNDNDND25bC. caris (1)B. clarridgeiae, R. felisNDNDNDND26cC. felis (1)B. clarridgeiae, R. felisNDNDNDND27cC. felis (1)G. felis (1)NDNDNDND27cC. felis (1)R. felisNDNDNDND27cC. felis (1)R. felisNDNDNDND27cNDNDNDNDNDNDND27cNDNDNDNDNDNDND27cC, felis (1)R. felisNDNDNDND27cNDNDNDNDNDNDND27cNDNDNDNDNDNDND27cNDNDNDNDNDNDND27cNDNDNDNDNDNDND27cNDNDNDNDNDNDND27cNDNDNDNDNDNDND27cNDNDNDNDNDNDND27cNDNDNDNDNDNDND <td>20^a</td> <td>C. felis (2)</td> <td>_</td> <td>Bartonella spp.</td> <td>64</td> <td>_</td> <td>-</td> | 20 ^a | C. felis (2) | _ | Bartonella spp. | 64 | _ | - |
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| 2pbC fails (2)-NDNDNDNDND24°C fails (1)B. clarridgeiaeNDNDNDND25°C canis (1)-NDNDNDND26°C fails (4)B. clarridgeiae, R. felisNDNDNDND26°C fails (1)-NDNDNDND27°C fails (1)-NDNDNDND28°C fails (1)-NDNDNDND29°Ctenocephalides spp. (1)R. felisNDNDNDND29°NDNDNDBartonella spp8031°NDNDND-64648034°NDND-2566435°NDNDNDBartonella spp.6435°NDNDNDBartonella spp.6436°NDNDBartonella spp.6436°NDNDNDNDNDNDNDND36°NDNDNDNDNDND36°NDNDNDNDNDNDND36°NDNDNDNDNDNDND36°NDNDNDNDNDND </td <td>22^b</td> <td>C. felis (2)</td> <td>_</td> <td>ND</td> <td>ND</td> <td>ND</td> <td>ND</td> | 22 ^b | C. felis (2) | _ | ND | ND | ND | ND |
| 24°C. felis (1)B. clarridgeiaeNDNDNDNDND25°C. cauis (1)-NDNDNDND25°C. felis (4)B. clarridgeiae, R. felisNDNDNDND27°C. felis (2)B. clarridgeiae, R. felisNDNDNDNDND28°C. felis (1)-NDNDNDNDND26°Ctenoce/phildies spp. (1)R. felisNDNDNDND30°NDNDNDBartonella spp.128-8031°NDNDNDNDNDND35°NDNDND-6464935°NDNDND-25664-35°NDNDNDNDNDNDND36°NDNDNDNDNDNDND36°NDNDNDNDNDNDND36°NDNDNDNDNDNDND36°NDNDNDNDNDNDND36°NDNDNDNDNDNDND36°NDNDNDNDNDNDND36°NDNDNDNDNDNDND36°NDNDNDNDNDNDND36°NDNDNDNDND <td< td=""><td>23^b</td><td>C. felis (2)</td><td>-</td><td>ND</td><td>ND</td><td>ND</td><td>ND</td></td<> | 23 ^b | C. felis (2) | - | ND | ND | ND | ND |
| 2^5 C. cauis (1)-NDNDNDNDND 26^c C. felis (4)B. clarridgeiae, R. felisNDNDNDNDND 27^c C. felis (2)B. clarridgeiaeNDNDNDNDND 28^c C. felis (1)-NDNDNDNDND 29^c Ctenocephalides spp. (1)R. felisNDNDNDNDND 29^c Ctenocephalides spp. (1)R. felisNDNDNDNDND 30^c NDNDNDNDNDNDNDND 30^c NDNDNDBartonella spp 32^c NDNDND-646480- 34^c NDNDNDPartonella spp.6432 35^c NDNDND-12864 35^c NDNDNDNDNDNDNDND 36^c NDNDNDNDNDNDND 36^c NDNDNDNDNDNDND 36^c NDNDNDNDNDNDND 36^c NDNDNDNDNDNDND 36^c NDNDNDNDNDNDND 46^c NDNDBartonella spp128 </td <td>24^c</td> <td>C. felis (1)</td> <td>B. clarridgeiae</td> <td>ND</td> <td>ND</td> <td>ND</td> <td>ND</td> | 24 ^c | C. felis (1) | B. clarridgeiae | ND | ND | ND | ND |
| 26° C. felis (4)B. clarridgeiae, R. felisNDNDNDND 27° C. felis (2)B. clarridgeiaeNDNDNDND 28° C. felis (1)-NDNDNDND 29° Ctenocephalides spp. (1)R. felisNDNDNDND 30° NDNDNDNDNDND 30° NDNDNDBartonella spp 32° NDNDNDBartonella spp 32° NDNDND-6464- 33° NDNDND-25664- 35° NDNDND-12864- 35° NDNDNDNDNDNDND 36° NDNDNDNDNDNDND 36° NDNDNDNDNDND 36° NDNDNDNDNDND 36° NDNDNDNDNDND 36° NDNDNDNDNDND 36° NDNDNDNDNDND 36° C. felis (2)R. felisBartonella spp128- 40° C. felis (3)R. felisBartonella spp.256 41° NDNDBartonella spp.128- <td>25^b</td> <td>C can is (1)</td> <td>-</td> <td>ND</td> <td>ND</td> <td>ND</td> <td>ND</td> | 25 ^b | C can is (1) | - | ND | ND | ND | ND |
| 2° C. felis (2)B. clarridgeineNDNDNDND 28° C. felis (1)-NDNDNDND 29° Ctenocephalides spp. (1)R. felisNDNDNDND 30° NDNDNDBartonella spp.128-80 31° NDNDND-6464- 32° NDNDND-6464- 34° NDND-25664 35° NDNDND-128 35° NDNDND-25664 35° NDNDNDNDNDNDND 35° NDNDNDNDNDNDND 35° NDNDNDNDNDND 35° NDNDNDNDNDND 35° NDNDNDNDNDND 40° C. felis (2)R. felisBartonella spp128- 41° C. felis (3)R. felisBartonella spp.25664- 42° NDNDNDNDNDND 45° NDNDNDNDNDND 45° NDNDNDNDNDND 45° NDNDNDNDND | 26° | C felis (4) | B clarridoeiae R felis | ND | ND | ND | ND |
| L_2 C_1 (b) D_1 D_1 D_1 D_1 D_1 D_1 28^c C (clinocephalides spp. (1) R , felisNDNDNDND 30^c NDNDNDNDNDND 30^c NDNDBartonella spp. $ 32^c$ NDNDND $ 64$ 64 $ 33^c$ NDNDND $ 64$ 64 $ 35^c$ NDND $ 256$ 64 $ 35^c$ NDNDND $ 128$ 64 $ 35^c$ NDNDNDNDNDND 35^c NDNDNDNDNDND 35^c C, felis (2) R , felisNDNDND 35^c C, felis (3) R , felisNDNDND 35^c NDNDNDNDNDND 35^c C, felis (3) R , felisNDNDND 40^c C , felis (2) R , felisBartonella spp. $ 128$ $ 45^c$ NDNDNDNDNDNDND 45^c NDNDNDNDNDND 45^c NDNDNDNDNDND 45^c NDNDNDNDNDND 45^c NDNDNDNDNDND 45^c <td< td=""><td>20 27^c</td><td>C felis (2)</td><td>B clarridgeige</td><td>ND</td><td>ND</td><td>ND</td><td>ND</td></td<> | 20 27 ^c | C felis (2) | B clarridgeige | ND | ND | ND | ND |
| 29° Chemocephalides spp. (1)R. felisNDNDNDNDND 30° NDNDNDBartonella spp.128-80 31° NDNDBartonella spp 32° NDNDND-6464- 33° NDNDND-646480 34° NDND-6464- 35° NDNDNDBartonella spp.6432- 35° NDNDNDNDNDND 40° C_felis (2)R_felisNDNDND 40° C_felis (3)R_felisBartonella spp128- 42° NDNDNDBartonella spp.25664- 42° NDNDNDNDNDND 45° NDNDNDNDNDND 45° NDNDNDNDNDND 45° | 28 ^c | C felis (1) | | ND | ND | ND | ND |
| 25Chrone particular spip. (1)N pointN point <t< td=""><td>20°</td><td>Ctenocenhalides spp (1)</td><td>R falic</td><td>ND</td><td>ND</td><td>ND</td><td>ND</td></t<> | 20° | Ctenocenhalides spp (1) | R falic | ND | ND | ND | ND |
| 31°NDBartonella spp.1.00 $ -$ 32°NDND $ -$ 33°NDND $ -$ 34°NDND $ 256$ 64 $-$ 35°NDNDBartonella spp. 64 32 $ 36°$ NDNDBartonella spp. 64 32 $ 36°$ NDNDBartonella spp. 64 32 $ 36°$ NDNDBartonella spp. 64 $ 37°$ $C_{felis}(2)$ R_{felis} NDNDNDND $38°$ NDNDBartonella spp. 64 $ 39^{b}$ $C_{felis}(3)$ R_{felis} Bartonella spp. $ 128$ $ 41°$ $C_{felis}(1)$ $ -$ Bartonella spp. 256 64 $ 42°$ NDNDBartonella spp. 256 64 $ 45°$ NDNDBartonella spp. 128 $ 45°$ NDNDNDNDNDNDND $45°$ NDNDBartonella spp. 128 $ 45°$ NDNDBartonella spp. 128 $ 45°$ NDNDBartonella spp. 128 $ 45°$ NDNDBartonella spp. $32.$ < | 30 ^c | ND | ND | Bartonella enn | 128 | - | 80 |
| 32° NDND $ -$ | 21 ^c | ND | ND | Bartonella con | 120 | - | 80 |
| 32° NDND- 64° 64° $ 33^{\circ}$ NDND- 64° 64° 80° 34° NDND- 256° 64° $ 35^{\circ}$ NDND $ 128^{\circ}$ 64° $ 36^{\circ}$ NDND- 128° 64° $ 37^{\circ}$ $C_{felis}(2)$ R_{felis} NDNDND 38° NDNDNDNDND 38° $C_{felis}(3)$ R_{felis} NDNDND 40° $C_{felis}(3)$ R_{felis} Bartonella spp. $ 128^{\circ}$ $ 41^{\circ}$ $C_{felis}(1)$ $-$ Bartonella spp. $ 128^{\circ}$ $ 42^{\circ}$ NDNDNDBartonella spp. 256° 64° $ 43^{\circ}$ NDNDBartonella spp. 128° $ 45^{\circ}$ NDNDBartonella spp. 128° $ 45^{\circ}$ NDNDBartonella spp. 128° $ 45^{\circ}$ NDNDBartonella spp. 64° $ 45^{\circ}$ NDNDBartonella spp. 128° $ 45^{\circ}$ NDNDBartonella spp. 64° $ 45^{\circ}$ NDNDBartonella spp. 128° $ 49^{\circ}$ ND <t< td=""><td>21^c</td><td>ND</td><td>ND</td><td>bartonena spp.</td><td>61</td><td>- 64</td><td>_</td></t<> | 21 ^c | ND | ND | bartonena spp. | 61 | - 64 | _ |
| 35NDND-6464-34°NDND-25664-35°NDNDBartonella spp.6432-36°NDND-12864-37°C,felis (2)R, felisNDNDNDND38°NDNDBartonella spp.6439°C. felis (3)R, felisNDNDNDND40°C, felis (2)R, felisBartonella spp128-41°C, felis (1)-Bartonella spp.64128-42°NDNDBartonella spp.25664-43°NDNDBartonella spp.25664-44°NDNDBartonella spp.25664-44°NDNDBartonella spp.12845°C, felis(2)-NDNDNDND45°NDNDBartonella spp.12845°NDNDBartonella spp.12845°NDNDBartonella spp.3264-45°NDNDBartonella spp.12845°NDNDBartonella spp.12845°NDND-12845°NDND-128-< | 32 22 ^c | ND | ND | _ | 64 | 64 | - |
| 35° NDND 2 artonella sp. 200 64 2 36° NDNDBartonella sp. 64 32 $ 36^{\circ}$ NDND $ 128$ 64 $ 37^{\circ}$ $C_{felis}(2)$ R_{felis} NDNDNDND 38° NDND R_{felis} NDNDNDND 40° $C_{felis}(3)$ R_{felis} R_{felis} NDNDNDND 40° $C_{felis}(1)$ $ Bartonella sp.$ $ 128$ $ 42^{\circ}$ NDNDNDBartonella sp. 64 128 $ 42^{\circ}$ NDNDNDBartonella sp. 256 64 $ 44^{\circ}$ NDNDBartonella sp. 256 64 $ 45^{\circ}$ NDNDBartonella sp. 128 $ 45^{\circ}$ NDNDBartonella sp. 128 $ 45^{\circ}$ NDNDBartonella sp. 64 64 $ 47^{\circ}$ NDNDBartonella sp. 64 $ 45^{\circ}$ NDNDBartonella sp. 64 $ 45^{\circ}$ NDNDBartonella sp. 64 $ 47^{\circ}$ NDND $ 49^{\circ}$ NDND $ -$ < | 33 24 ^c | ND | ND | _ | 256 | 64 | 80 |
| 35NDNDDarionella spp.6452 $-$ 36'< | 25° | ND | ND | - Bartonalla con | 230 | 22 | _ |
| 30NDNDPP | 35 26° | ND | ND | burtonettu spp. | 129 | 52 | _ |
| 37° $C, fells (2)$ $N, fells$ ND ND ND ND ND ND 38° NDNDND ND NDNDND 39^b $C. felis (3)$ $R. felis$ NDNDNDND 40° $C. felis (2)$ $R. felis$ $Bartonella spp.$ $ 128$ $ 41^\circ$ $C. felis (1)$ $ Bartonella spp.$ 256 256 $ 42^\circ$ NDND $Bartonella spp.$ 256 64 $ 43^\circ$ NDND $Bartonella spp.$ 256 64 $ 44^\circ$ NDND $Bartonella spp.$ 256 64 $ 45^\circ$ C. felis (2) $-$ NDNDNDND 45° NDND $Bartonella spp.$ 128 $ 45^\circ$ NDND $Bartonella spp.$ 128 $ 45^\circ$ NDND $Bartonella spp.$ 64 64 $ 47^b$ NDND $Bartonella spp.$ 32 64 $ 49^\circ$ NDND $ 128$ $ 49^\circ$ NDND $ 51^\circ$ NDND $ 51^\circ$ NDND $ 51^\circ$ NDND $ 51^\circ$ NDND $ -$ <t< td=""><td>20 27^c</td><td>ND C felia (2)</td><td>ND R falia</td><td>- ND</td><td>120 ND</td><td>04 NID</td><td>- NID</td></t<> | 20 27 ^c | ND C felia (2) | ND R falia | - ND | 120 ND | 04 NID | - NID |
| 35 ND ND bartonella spp. 64 - - 40° C, felis (3) R, felis ND ND ND ND 40° C, felis (2) R, felis Bartonella spp. - 128 - 41° C, felis (1) - Bartonella spp. 64 128 - 42° ND ND Bartonella spp. 526 256 - - 45° ND ND Bartonella spp. 256 64 - 45° ND ND Bartonella spp. 256 64 - 45° ND ND Bartonella spp. 128 - - 45° ND ND Bartonella spp. 128 - - 45° ND ND Bartonella spp. 64 64 - 47° ND ND Bartonella spp. 32 64 - 49° ND< | 37 20° | C.jells (2) | K. Jelis | IND Rantou alla ann | ND 64 | ND | ND |
| 39C. fells (3)R. fellsRDNDNDNDND 40° C. fells (2)R. fellsBartonella spp128- 41° C. fells (1)-Bartonella spp.64128- 42° NDNDBartonella spp.>25664- 43° NDNDBartonella spp.25664- 44° NDNDBartonella spp.128 45° C. felis(2)-NDNDNDND 46° NDNDBartonella spp.128 45° NDNDBartonella spp.128 45° NDNDBartonella spp.6464- 45° NDNDBartonella spp.6464- 45° NDNDBartonella spp.3264- 45° NDND-128 45° NDND-128 45° NDND-128 45° NDND128 45° NDND 45° NDND-128 45° NDND 45° NDND 50° NDND <td>38 .</td> <td>ND</td> <td>ND B. (clic</td> <td>Bartonella spp.</td> <td>04 NID</td> <td>-</td> <td>-</td> | 38 . | ND | ND B. (clic | Bartonella spp. | 04 NID | - | - |
| 40C. felis (2)K. fenisBartonella spp128- 41° C. felis (1)-Bartonella spp.64128- 42° NDNDBartonella spp.>256256- 43° NDNDBartonella spp.25664- 43° NDNDBartonella spp.25664- 45° C. felis(2)-NDNDND 45° NDNDBartonella spp.128 45° NDNDBartonella spp.128 45° NDNDBartonella spp.128 45° NDNDBartonella spp.6464- 45° NDNDBartonella spp.3264- 49° NDND-128 49° NDND-128 51° NDND 51° NDND | 39 | $C_{ij} = (1) + (2)$ | K. felis | ND Bastan dia ang | ND | ND 129 | ND |
| 41C. fells (1)-bartonelia spp.64128- 42° NDNDBartonelia spp.>256256- 43° NDNDBartonelia spp.25664- 44° NDNDBartonelia spp.128 45° C. felis(2)-NDNDNDND 46° NDNDBartonelia spp.128 47° NDNDBartonelia spp.128 47° NDNDBartonelia spp.6464- 49° NDND-128 50° NDND-128 51° NDND128 51° NDND | 40 | $C_{ijelis}(2)$ | K. felis | Bartonella spp. | - | 128 | - |
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| 44° ND ND Bartonella spp. 128 - - - 45° C. felis(2) - ND ND ND ND 45° ND ND Bartonella spp. 128 - - 45° ND ND Bartonella spp. 128 - - 47^b ND ND Bartonella spp. 64 64 - 48^b ND ND Bartonella spp. 32 64 - 49° ND - 128 - - - 50° ND ND - 128 - - 50° ND ND - - - - 51° ND ND - - - - - | 43 | ND | ND | Bartonella spp. | 256 | 64 | - |
| 45° C. felis(2) - ND ND ND ND 46° ND ND Bartonella spp. 128 - - 47^b ND ND Bartonella spp. 64 64 - 48^b ND ND Bartonella spp. 64 64 - 49° ND ND - 128 - - 50° ND ND - 128 - - 51° ND ND - >128 - - | 44 ^c | ND | ND | Bartonella spp. | 128 | - | _ |
| 46° 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 - >128 - - | 45° | C. felis(2) | _ | ND | ND | ND | ND |
| 47° 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 - - - - | 46° | ND | ND | Bartonella spp. | 128 | - | - |
| 48° ND Bartonella spp. 32 64 - 49° ND ND - 128 - - 50° ND ND - >128 - - 51° ND ND - >128 - - | 475 | ND | ND | Bartonella spp. | 64 | 64 | _ |
| 49° ND ND - 128 - - 50° ND ND - >128 - - 51° ND ND - >128 - - 51° ND ND - - - - | 480 | ND | ND | Bartonella spp. | 32 | 64 | - |
| 50° ND - >128 - - 51° ND ND - - - - | 49 ^c | ND | ND | - | 128 | - | - |
| 51° ND ND – – – – – | 50 ^c | ND | ND | - | >128 | - | - |
| | 510 | ND | ND | - | - | - | - |

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

^aShelter cats; ^bstray cats; ^cdomestic 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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