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Short communication

Serological and molecular evidence of exposure to arthropod-borne organisms in cats from northeastern Spain

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Abstract

One hundred sixty-eight cat sera from Spain were tested for IgG antibodies to *Rickettsia conorii* (*Rc*), *Ehrlichia canis* (*Ec*), *Anaplasma phagocytophilum* (*Ap*) and *Bartonella henselae* (*Bh*) antigens using IFA and for FeLV antigen and FIV antibody by ELISA. For 47 whole blood samples, PCR testing was performed for *Rickettsia*, *Ehrlichia* and *Bartonella*. Seroprevalences were: *Bh* (71.4%), *Rc* (44%), *Ec* (11.3%), FeLV (8.5%), FIV (7.4%) and *Ap* (1.8%). *Bh* antibodies were associated with seroreactivity to both *Ec* and *Rc* antigens. FIV antibodies were associated with illness and cats older than 2 years. *Bartonella henselae* and *B. clarridgeiae* (*Bcl*) DNA was amplified from seven and one sample, respectively.

Keywords: Spain; Cat; Vector-borne pathogens; Retrovirus

1. Introduction

Bartonella henselae (*Bh*) is currently the most commonly encountered *Bartonella* zoonosis (Guptill-Yoran, 2006). Asymptomatic infection with *Bh* or *Bcl* is frequently reported in cats, which are therefore considered to be a major reservoir for human infection. Several *Bartonella* species have been identified in wild and domestic cats: *Bh*, *B. koehlerae*, *Bcl* and *B. bovis* (Guptill-Yoran, 2006). A large body of literature has been generated regarding bartonellosis in humans and cats. However, in Spain, there is limited information related to *Bh* infection in humans (Blanco et al., 1999) and cats (Pons et al., 2005).

There is serological and molecular evidence that cats can be infected with intracellular bacteria of the genus *Ehrlichia* and *Anaplasma*. *Ehrlichia*-like bodies

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or morulae have been detected in monocytes, eosinophils and neutrophils of cats with febrile illness and thrombocytopenia (Lappin et al., 2006; Lappin and Breitschwerdt, 2006).

The role of the cat as a sentinel or reservoir for SFG rickettsiae such as *Rickettsia rickettsii* or *Rickettsia conorii* has only been investigated serologically in cats from South Africa and Zimbabwe (Matthewman et al., 1997). No studies related to *Rc* infection in cats have been reported from Europe. To further characterize patterns of exposure to vector-borne pathogens, we investigated the *Rc*, *Ec*, *Ap* and *Bh* seroprevalence in cats from Spain. Due to immunosuppressive effects induced by both feline retroviruses, FeLV antigen and FIV antibody testing was also performed.

2. Material and methods

One hundred sixty eight cats from Spain (Tarragona ($n = 12$), Barcelona ($n = 113$) and Mallorca ($n = 43$)) were studied. Eighty-eight cats were male and sixty cats were female. Seventy cats were clinically healthy and sixty were sick with various illnesses at the time of sampling. The age was known for 118 cats with a mean $\pm$ S.D. of 4.6 ± 4.4 years old. Age ranged from 3.4 months to 17 years. The breed was known from 146 cats: 118 domestic short hair, nine Siamese, three mixed, 14 Persian, one Korat and one *chinchilla*.

All cat sera were tested for IgG antibodies to *Bh*, *Rc*, *Ec* and *Ap* antigens by an immunoflorescence assay (IFA) with minor modifications, as previously described (Solano-Gallego et al., 2006). Fluorescein conjugated goat anti-cat immunoglobulin (whole molecule immunoglobulin G; Cappel, Organon Teknika Corp., Durham, N.C.) was used. Twenty sera from specific pathogen free cats did not contain antibodies to any test antigen, therefore an arbitrary cutoff titer of $\geq$64 was used to define a reactive sera in this study. Sera from cats experimentally infected with *Bh* (titer 2048) and naturally infected with *Rc* (titer 2048), *Ec* (titer 128) and *Ap* (titer 256) and non-reactive serum from a specific pathogen free cat were used as positive and negative controls for all IFA testing. Ninety-four serum samples were assessed for FeLV antigen and FIV antibody with SNAP® FIV/FeLV Combo Test (IDEXX Laboratories, Portland, Maine).

To minimize the potential risks for PCR contamination, DNA extractions, PCR amplification and agarose gel electrophoresis were performed in separate rooms. DNA was isolated by double phenol–chloroform extraction from 47 cat samples (40 from Mallorca and seven from Tarragona). Of this sample subset, 27 cats were healthy and 20 cats were sick. The glyceraldehyde-3-phosphate dehydrogenase (GAPDH)) gene was used as a housekeeping target to verify amplifiable DNA as previously described (Birkenheuer et al., 2003). *Bartonella* species PCR amplifications were performed as previously described (Jensen et al., 2000) with primers Bart-ITS1.2 (5′-(C/T)CTTCGTTTCTCTTTCTTCA-3′) and Bart-ITS3 (5′-AACCAACTGAGCTACAGCC-3′). Due to the similar number of base pairs (bp) between *B. clarridgiae* (154 bp) and *B. quintana* (157 bp), a *Mse*I restriction enzyme was used to discriminate between these organisms following instructions (New England BioLabs, Beverly, MA). The recognition site for the *Mse*I enzyme is only found in the *B. quintana* PCR product. *Ehrlichia/Anaplasma* genus PCR was performed as described previously (Breitschwerdt et al., 2002) with primers designated ECC-mod (5′-AGAACGAACGCTGGCGGCAAG-3′) and HE3-Rmod (5′-CTTCTATAGGTACCGTCATTATCTTCCCTATTG-3′). A one-tube nested PCR was used as previously described for detection of both spotted fever and typhus group *Rickettsia* species (Breitschwerdt et al., 1999).

For univariate analysis, non-parametric tests (Chi-square, Fisher's exact test) were used to test for associations between proportions and putative explanatory factors. Differences were considered significant if P-value was <0.05.

3. Results

Total seroprevalences were: *B. henselae* 71.4%, *R. conorii* 44%, *E. canis* 11.3%, *A. phagocytophilum* 1.8%, FeLV antigen 8.5% and FIV 7.4%. *Bartonella henselae* titers ranged from 64 to 8192 with a geometric median titer of 253. *Rickettsia conorii* titers ranged from 64 to 8192 with a geometric median titer of 283. *Ehrlichia canis* titers ranged from 64 to 512 with a geometric median titer of 118. *Anaplasma phagocytophilum* titers ranged from 128 to 512 with a geometric median titer of 128. Two cats were