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Short communication

Oral shedding of *Bartonella* in cats: Correlation with bacteremia and seropositivity

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ABSTRACT

Cats are the main reservoirs of zoonotic *Bartonella henselae*, *B. clarridgeiae* and *B. koehlerae*, transmitted among cats by cat fleas. No study has investigated the presence of *Bartonella* in the saliva of bacteremic and non-bacteremic cats to correlate it to the level of bacteremia and the presence or absence of oral lesions. Shelter cats from northern California ($n = 130$) and Michigan ($n = 50$) were tested for *Bartonella* bacteremia by blood culture, presence of *Bartonella* antibodies and *Bartonella* DNA in oral swabs. Bacteremia was detected in 45 (25%) cats, mainly from northern California ($n = 40$), which were highly flea infested and were 4 times more likely to be bacteremic than the non-flea-infested cats from Michigan. Overall, 69 (38.3%) cats had *Bartonella* PCR positive oral swabs. Bacteremic cats were almost 3 times ($P = 0.003$) more likely to have PCR positive oral swabs (59%, 26/44) than non-bacteremic cats (32.5%, 44/135). However, there was no correlation between cats being bacteremic and having oral lesions. Antibody prevalences for *B. henselae* and *B. clarridgeiae* were 30% and 42.8%. *B. henselae* and *B. clarridgeiae* seropositive cats were almost 4 times ($P = 0.0001$) and 3 times ($P = 0.003$) more likely to have oral lesions than seronegative cats. Despite a higher prevalence (odds ratio = 1.73; 95% confidence interval = 0.88–3.38) of oral lesions in cats with oral swabs testing PCR positive, no statistical association could be demonstrated in this cat population.

1. Introduction

Members of the genus *Bartonella* are Gram-negative, intra-erythrocytic bacteria and are usually vector-borne. *Bartonella henselae* is the main causal agent of cat scratch disease (CSD) in humans, which usually presents as a benign lymphadenopathy (Dolan et al., 1993). The cat flea is the main vector of *B. henselae* and domestic cats are its natural reservoir (Chomel et al., 1996). *Bartonella clarridgeiae* is also isolated from cat blood and could be another etiological agent of CSD (Chomel et al., 2006).

CSD is transmitted to humans through scratches or bites from domestic cats (Chomel et al., 2006; Jacomo et al., 2002; Lamps and Scott, 2004). However, limited scientific data are available to support the role of cat bites in the transmission of *B. henselae* between cats or to humans. Flea feces play a major role in the transmission of the infection between cats and to humans, as *B. henselae* can survive at least 3 days in flea feces (Finkelstein et al., 2002) and only intradermal injection of flea feces was able to induce bacteremia in experimentally infected cats (Foil et al., 1998). Most reports of human infections through cat or dog bites are anecdotal and identification of *Bartonella* DNA in

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the saliva of the biting animals was usually not investigated (Duncan et al., 2007; Rolain et al., 2009). In cats, *Bartonella* DNA has been detected by PCR in oral swabs with prevalence ranging from 4.8% (Demers et al., 1995) to 17.6% (Lappin and Hawley, 2009) and up to 44.1% in 102 feral cats (Kim et al., 2009). A few studies have investigated a possible correlation between stomatitis and *Bartonella* infection in cats. Dowers et al. (2010) showed no differences in the prevalence of *Bartonella* DNA detection for cats with confirmed gingivostomatitis (8/70, 11.4%) and healthy age-matched controls (5/61, 8.2%) using PCR on blood samples. Quimby et al. (2008) reported a prevalence of 11.1% in oral swabs from cats with ($n = 9$) and without stomatitis ($n = 36$), but only one cat with stomatitis was bacteremic, and cats without stomatitis were more likely to be *Bartonella* Western blot positive than cats with stomatitis ($P = 0.02$). In cats from Southern Italy, a high prevalence of *B. henselae* DNA was detected in cat saliva swabs (60%) (Pennisi et al., 2010). On the contrary, in two serological studies, stomatitis or gingivitis were more commonly observed in cats that were seropositive for *B. henselae* compared with seronegative cats (Glaus et al., 1997; Ueno et al., 1996) and in a recent study of 298 pet cats from Northern California, bacteremic cats were more likely to have oral lesions (Sykes et al., 2010). *Bartonella* DNA has also been detected in the dental pulp of cats tested hundreds of years after their death (La et al., 2004).

The main objectives of this study were to determine the presence of *Bartonella* in the saliva of both bacteremic and non-bacteremic cats and to correlate it to the level of bacteremia, whether or not the cats had oral lesions. Our hypotheses were that bacteremic cats were more likely to yield *Bartonella* positive oral swabs and that cats with high levels of bacteremia were more likely to yield positive oral swabs than cats with low levels of bacteremia. We also wanted to investigate a possible association between *Bartonella* bacteremia or infection (presence of *Bartonella* antibodies) and presence of oral lesions in cats.

2. Materials and methods

2.1. Study population

Cats were selected from two shelters, one in Michigan (Muskegon NOAH project, 50 cats) and one from northern California (Sacramento SPCA, 130 cats). For the cats from Michigan, a blood sample (2 mL collected in a plastic EDTA tube) and one oral swab were obtained during physical examination of the cats in June 2008. The blood was stored at 4 °C and shipped overnight to the Veterinary Public Health Laboratory (VPHL) at the University of California, Davis. Blood samples (2 mL in a plastic EDTA tube) and two oral swabs were collected from 32 cats from Sacramento shelter during a spay and neuter operation in June 2008, from 43 cats in August 2008 and from 55 cats in September 2008 and transported to the VPHL on the day of collection. Information about estimated age, sex, clinical status (well or specific disease or pathology) was recorded. A mouth examination was also performed by a veterinarian on each cat at both facilities at the time of the saliva swab collection and presence or absence of any oral lesions (gingivitis, palatoglossitis, stomatitis, bleeding or oral ulceration) was reported.

2.2. Laboratory procedures

2.2.1. Whole blood samples

The EDTA tubes were frozen at −70 °C upon reception and plated a few days to a few weeks later onto 5% rabbit blood agar plates, as previously described (Chomel et al., 1995). The plates were incubated at 35 °C with 5% CO_2 for 4 weeks and examined 2–3 times a week for any bacterial growth. The number of colonies was counted and calculated as colony forming units (CFU) per milliliter of blood.

2.2.2. PCR, PCR/RFLP and sequencing

The isolated strains were confirmed to be *B. henselae* or *B. clarridgeiae* by PCR/RLFP of the citrate synthase gene using HhaI, TaqI and MseI endonucleases, as previously described (Chomel et al., 1999; Molia et al., 2004). The 16S rRNA gene type was determined by PCR, using previously designed primers (Maruyama et al., 2000). To confirm that our PCR products were *Bartonella* specific, a random subset of the oral swabs were submitted to Davis Sequencing, Davis, CA and sequencing was run using a fluorescence-based automated sequencing system.

2.2.3. Serology

Specific antibodies against *B. henselae* and *B. clarridgeiae* were detected using an immunofluorescent antibody (IFA) test, as previously described (Henn et al., 2007). The intensity of bacillus-specific fluorescence at dilutions of 1:32 and 1:64 was scored subjectively for each serum sample and a fluorescence score of ≥ 2 at 1:64 was defined as a positive result (Henn et al., 2007).

2.2.4. Oral swabs

2.2.4.1. DNA extraction. The saliva swabs were vortexed and incubated for 15 min in 400 μL of PBS and DNA was extracted, using the DNeasy Tissue Kit (Qiagen, Valencia, CA).

2.2.4.2. Bartonella-specific PCR. Oral swab DNA extracts were tested in a nested-PCR with *Bartonella* primers specific for the 16S–23S rDNA intergenic region (Ramper-sad et al., 2005). Primary reactions (primers P-bhenfa and P-henr1) were performed in a 25 μL volume as follows: 1 μL extracted DNA, 0.2 mM each dNTP, 0.5 μM each primer, 3 mM MgCl2, 1× Taq Gold reaction buffer, 0.5 U Taq Gold polymerase. PCR cycle conditions were 94 °C for 10 min, 40 cycles of: 94 °C for 15 s, 48.2 °C for 30 s, 72 °C for 30 s; followed by 72 °C for 5 min. Nested reactions (primers N-bhenf1a and N-henr) were performed in a 25 μL volume as follows: 1 μL primary amplicon, 0.2 mM each dNTP, 0.5 μM each primer, 1.5 mM MgCl2, 1× Tag Gold reaction buffer, 0.5 U Taq Gold polymerase. PCR cycle conditions were 94 °C for 10 min, 40 cycles of: 94 °C for 15 s, 56 °C for 30 s, 72 °C for 30 s; followed by 72 °C for 5 min. A positive control, consisting of purified *B. henselae* and *B. clarridgeiae* DNA, and a negative control (water blank) were included with each run. Extraction controls were not included, as