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EPIDEMIOLOGY OF *BARTONELLA* INFECTION IN DOMESTIC FRENCH CATS

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Quatre cent trente six chats de la region parisienne furent testes pour la presence d'une bacteriemie causee par diverses especes de Bartonelles et pour la presence d'anticorps anti-bartonelliques. 71 chats (16.3%) etaient bacteriemiques: 37 avec Bartonella henselae BA-TF (8.4%), 15 avec B. clarridgeiae (3.4%), 11 avec B. henselae Houston-1 (1.8%). Six chats (1.4%) etaient co-infectes par B. henselae et B. clarridgeiae et deux chats (0.5%) par les types Houston-1 et BA-TF. Parmi les71 chats bacteriemiques, 21 (29.5%) etaient infectes par B. clarridgeiae. 165 chats (38%) avaient des anticorps anti-B.henselae. Les 89 etudiants veterinaires testes etaient tous seronegatifs.

INTRODUCTION

Cat scratch disease (CSD) was first described in France by Debré *et al.* [6]. Various etiologic agents were proposed over the ensuing 42 years, but it was not until 1992 that the causative agent of CSD, *Bartonella* (formerly *Rochalimaea*) *henselae* was identified by serological and microbiological studies [14]. Subsequently, two 16S rRNA variants of *B. henselae* were described, Houston-1 and BATF variants, as well as several other RFLP types [2,16]. Additionally, a novel *Bartonella, B. clarridgeiae* was isolated from the blood of a cat [5,11]. *B. henselae* has been linked to several syndromes that differ depending on the person immune status. In immunocompetent individuals, persistent necrotizing inflammation of the lymph nodes draining the inoculation site occurs during CSD. The disease is usually self limiting after several weeks and is not antibiotic responsive. In immunodeficient people, *B. henselae* induces a vasculoproliferative disease, called bacillary angiomatosis (BA) [14,16]. In contrast to CSD, BA is responsive to antibiotic treatment. Improper treatment may be fatal. The distinction between immunocompetent and immunocompromised hosts is not absolute, with some immunocompetent individuals contracting BA. Other rare manifestations of *B. henselae* infection occur, such as encephalitis and retinitis. In 1992, it was determined that 24,000 cases of CSD occurred in the United States, 2,000 of whom required hospitalization [8]. Cats have been identified as the reservoir for B. *henselae* and the cat flea, *Ctenocephalides felis*, as the vector [3,4,9,10,15]. Epidemiological studies in the United States have shown cat populations containing 4-55% seropositive cats with as many as 41% bacteremic [3,9]. Young cats with high flea exposure are most at risk for being infected and high infection prevalences are associated with high flea population densities [3]. Infected cats may remain bacteremic for several months to up to 2 years with elevated antibody levels [1,9,10]. Infection is inapparent and cats remain asymptomatic throughout their bacteremia [1].

Although several epidemiological studies of CSD had been performed in the U.S., none had been performed in France in the Paris area. The purposes of this study were to 1) describe the prevalence of infection and exposure in domestic cats of the Parisian area, 2) identify the bacterial species involved, 3) determine risk factors for infection and 4) assess the degree of human exposure to the CSD agent(s).

MATERIAL AND METHODS

Cats presented to the Maisons-Alfort veterinary school clinic and some private clinics in the Paris area from February through June 1996 were recruited for the study. Owners completed a questionnaire which described their cat's signalment, history, lifestyle, and degree of flea infestation. A 3 ml blood sample was drawn from the cats and cultured on defibrinized rabbit blood agar at 35.5°C in 5% CO_2 for 4 weeks. Plates were checked regularly for growth. Positive cultures were enumerated and then identified via PCR/RFLP, PCR-sequencing of a segment of the 16s rRNA gene analysis [2,9,12,14]. Cat serum was tested by IFA for anti-*B. henselae* antibodies using a cat fetal cell line infected with *B. henselae*., as previously described [3].

RESULTS AND DISCUSSION

Four hundred and thirty-six cats from the greater Paris area were sampled. They ranged from 4 months to 9 years old and were divided approximately equally between males and females. Eighty- four percent were intact and 89% were European breed. Fifty-one percent of the cats lived predominantly outdoors (>90% of their lives outdoor), 23% divided their time equally between living outdoors and indoors and 23% lived predominantly indoors (>90% of their lives indoor). From this population of cats, we found that 71 (16.3%) were bacteremic with *Bartonella*: 37 with *B. henselae* BA-TF (8.4%), 15 with *B. clarridgeiae* (3.4%), 11 with *B. henselae* Houston-1 (1.8%). Six cats (1.4%) were co-infected with *Bartonella henselae* and *Bartonella clarridgeiae* and two cats (0.5%) were co-infected with the 16S rRNA gene variants Houston-1 and BA-TF. Of the 71 bacteremic cats, 21 (29.5%) were infected with *B. clarridgeiae*. One hundred sixty five cats (38%) were seropositive to *B.henselae* .

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Age, sex, lifestyle, flea presence and concurrent illness were analyzed as risk factors for infection and seropositivity for all 436 cats. Cats were grouped by age categories of <6 months, 6-12 months and >12 months old. Bacteremic cats ranged from 14% to 20% and seropositive cats ranged from 38.5% to 50% in these groups.
Among male cats, 13% were infected and 39% seropositive, while 19% and 40% of the females cats were infected and seropositive, respectively. Twenty-one percent of cats living outdoor were infected and 43% were seropositive. Infection in indoor/ outdoor cats was 19% and seropositivity was 37%. Of the indoor cats, 27% were infected and 39% were seropositive. No statistically significant correlations were observed between bacteremia nor seropositivity and age, sex, lifestyle nor disease status . However, cats with a history of flea infestation within the preceding six months were twice as likely to be bacteremic as those without flea infestation (25% versus 13.3%, p=0.007). Seropositivity between the flea and non-flea infested groups was similar at 45% and 35%, respectively. Possible explanations for the lack of correlation of infection and serological status with the age and lifestyle of the cats include that the cats may have been infected at a young age and then remained positive for many months to years. Unlike FIV, which is more prevalent in male than female cats due to transmission during fighting, *Bartonella* infection prevalence was not different between sexes. This supports an inter-cat mode of transmission other than via scratches and bites, (unlike cat-to-man transmission). Cat infection of *Bartonella* via fleas was supported by this study, as well as other previous studies [3,4,7,9] It is unknown if fleas may infect man with *Bartonella*. Natural co-infection with multiple *Bartonella* variants and species was previously unrecognized, and all permutations of coinfection were seen. All co-infected cats had been exposed to fleas and had lived primarily outdoors. Bacteremia levels ranged from 100 to almost 2,667 CFU per milliliter of blood. Two different colony sizes were observed in the mixed species co-infected cultures: 2-4 mm diameter colonies and 1 mm or less diameter colonies. Using the methods indicated previously, the larger colonies were identified as *B. henselae* and the smaller colonies as *B. clarridgeiae*. Gram stain of *B. henselae* and *B. clarridgeiae* colonies both revealed small gram negative rods.
To assess the degree of human exposure to *B. henselae*, serum samples were collected from 89 veterinary students mainly in their first year of school. All were seronegative to B. *henselae*, however, 75% were seropositive to *Toxoplasma gondii* using a latex agglutination test assay. The students will be tested each year to assess the change.
It is important to determine if one species or subspecies is more predominant within the same infected cat and, if possible, if human coinfection by accidental inoculation of two *Bartonella* species can occur. No human CSD case has yet been reported to be caused by *B. clarridgeiae*. It is possible that cats carry a variety of *Bartonella* species or variants and that only a limited number of these types can cause disease in immunocompetent human hosts. The lack of isolation of *B. clarridgeiae* from immunocompromised individuals until present would support such a hypothesis. Different prevalences of strain types existing at different geographic regions may also explain why different studies have found different strain prevalences in CSD patients. The diversity of *Bartonella* isolates raises important concerns regarding the sensitivity of diagnostic procedures. Studies have shown that up to 60% of CSD patients may test negative to conventional *B. henselae* seroassays [13]. Accurate diagnosis of CSD, therefore, requires development of assays that detect the various serogroups of *Bartonella*. Epidemiological studies are necessary to elucidate these groups. The development of a polyvalent vaccine targeted against the most pathogenic or invasive strains may be a means of protecting cats and man from infection.

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