Isolation of *Bartonella henselae* from a serologically negative cat in Bloemfontein, South Africa

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ABSTRACT

Sera collected from apparently healthy 6–12-month-old cats (n = 31) presented to the Society for the Prevention of Cruelty to Animals Veterinary Clinic in Bloemfontein for neutering were tested for antibodies reactive to *Bartonella henselae* (Houston-1 strain) by indirect fluorescent antibody testing. Whole blood collected from the cats was used in isolation experiments and subsequent identification of *Bartonella* species was based on comparison of the nucleotide base sequence of polymerase chain reaction-amplified citrate synthase gene fragments. While none of the cats had antibodies reactive with *B. henselae* at titres ≥1/64, an organism with a partial citrate synthase gene sequence identical to that of *B. henselae* (Houston-1) was isolated from 1 cat.

Key words: *Bartonella henselae,* cats, isolation, serological survey, South Africa.

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INTRODUCTION

Bartonella henselae is a recently described Gram-negative, oxidase-negative, fastidious, aerobic, rod-shaped, slow-growing bacterium[21,24]. Clinical manifestations in humans infected with this emerging pathogen include cat-scratch disease[3], bacillary angiomatosis[22,24], peliosis hepatitis[24], septicaemia[21,24], endocarditis[6,9,20] and neurological disorders[7,17,26]. A report in the popular press[8] claimed that there are at least 3000 cases per year of cat-scratch disease in people in South Africa and patients with bacillary angiomatosis have been reported from South Africa[2,15] and Zimbabwe[4].

The domestic cat is recognised to be the major reservoir of *B. henselae,* and contact with cats and their fleas is a major risk factor for acquiring *B. henselae* infections[23,27]. Up to 40 % of cats in the USA have been shown to be infected with *B. henselae* and these cats may have asymptomatic bacteraemias for up to 17 months[5,13]. In cats experimentally infected with *B. henselae,* subtle clinical abnormalities have been observed, including intermittent fever, transient anaemia, lymphadenopathy and self-limiting non-localising CNS abnormalities[14].

In Gauteng, South Africa, and Harare, Zimbabwe, cats have been shown to have high prevalences (20 %) of antibodies reactive to *B. henselae* in indirect fluorescent antibody assays (IFA)[12]. More recently *B. henselae* has been isolated from the blood of domestic cats and a cheetah in Zimbabwe[11]. To provide further information on the epidemiology of *B. henselae* infections in cats in South Africa, we tested cats from Bloemfontein, Free State, for antibodies reactive to the organism by immunofluorescence (IFA), and attempted to isolate the organism from their blood. The results of these experiments are described in this report.

MATERIALS AND METHODS

Serology

Sera were obtained from apparently healthy domestic cats 6–12 months of age that were presented by their owners to be neutered at the Society for the Prevention of Cruelty to Animals Veterinary Clinic in Bloemfontein between April and June 1998. Positive and negative control sera were obtained from the Unité des Rickettsies, Faculté de Médecine, Université Aix-Marseille, France. As antigen, *B. henselae* Houston-1 (ATCC 49882) was grown in Vero cells. When 90 % of the cells were infected they were pelleted, washed in phosphate-buffered saline (PBS), resuspended in 0.5 % bovine serum albumin in PBS, applied (5 $\mu\ell$ aliquots) to the wells of 32-well Teflon slides and air-dried. Reactive antibodies in cat sera were detected using previously reported IFA procedures and fluorescein isothiocyanate-labelled protein G conjugate (Biogenesis Inc, Sandown, USA). Based on the results of previous studies[10,12,13], sera with IFA titres of ≥1:64 were regarded as positive for previous exposure to *B. henselae.*

Isolation

Whole blood was collected aseptically from all the cats described above and stored at –70 °C. After thawing in a waterbath at 37 °C, aliquots (300 μl) of the blood were plated onto 10 % blood-enriched agar and incubated at 35 °C with 5 % CO_2. When no growth could be detected on the plates after 45 days the blood was regarded as being culture negative for *Bartonella* species. When colonies were observed they were harvested in 5 % brain heart infusion (BHI) and stored at –70 °C until identified as described below.

Amplification and sequence analysis of citrate synthase gene (gltA) fragments

A crude DNA extract was prepared by boiling a heavy suspension of the isolated organisms mixed (4:1) with a 20 % (w/v) solution of Chelex 100 (Bio-Rad Laboratories Ltd, UK) in distilled water. Polymerase chain reaction (PCR)-based amplification followed by nucleotide base sequence determination of an approximately 700 base pair 3′ *glt*A fragment was carried out as described previously[1].

RESULTS AND DISCUSSION

We were unable to detect significant antibody titres against *B. henselae* (Houston-1) in any of the cats we studied, while the positive control serum had the expected titre of 1:128 and the negative control serum had a titre of <1:64. From 1 cat, however, an isolate was obtained that had a partial *glt*A sequence identical to that of *B. henselae* (Houston-1)[1].

The isolation of *B. henselae* from appar-

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ently serologically negative cats has been reported previously[5,13] and the organism may also be isolated from serologically negative people[16,19]. The absence of antibody responses in these cases may be the result of immunosuppression resulting from FIV or HIV infections or because blood for isolation of the organism was collected early in the course of infection, before antibodies against *B. henselae* could be produced. In experimentally-infected cats it has been shown that delays of up to 181 days may occur before reactive antibodies can be detected by IFA[14].

The absence of serologically positive cats in our study contrasts with results in a previous report where 20 % of cats from the SPCA in Gauteng, and 21 % of cats from the SPCA in Harare, were found to have antibodies against *B. henselae*[12]. In a study on cats from 33 sites in the USA, marked differences in seroprevalences (0–71 %) against *B. henselae* in different geographical locations were also found[10]. The reasons for the geographical differences in seroprevalences in this study were attributed to climatic factors, in particular average daily temperatures and annual rainfall, which would influence the numbers of potential arthropod vectors, mainly cat fleas (*Ctenocephalides felis*). Further studies are needed to determine whether similar climatic factors influence the prevalence of *B. henselae* infections in cats in southern Africa.

An alternative explanation of our failure to detect antibodies may be serological variation among *B. henselae* strains. Recognition of this phenomenon led to the description of a distinct serogroup of *B. henselae* named the 'Marseille' serogroup[6] and the isolate obtained in our study has been assigned to this group on the basis of its reactivity to a 'Marseille'-specific monoclonal antibody (D Raoult, unpublished observation). Antisera obtained from humans infected with these serovariant strains were found to yield markedly lower IFA titres when tested against antigen prepared from the type strain, *B. henselae* Houston-1, rather than from a 'Marseille' serogroup representative[6].

Unfortunately, we were unable to test any of the sera we obtained against antigen other than that prepared from the Houston-1 strain, but our findings should at least alert workers planning future serological surveys in the area to the shortfalls in using only a single antigen of *B. henselae* for antibody detection.

B. henselae has been encountered throughout the world, including North America, Europe, Israel, Japan, Sri Lanka and Australia[12,18]. It is only recently, however, that the organism been shown to occur in Africa, with *B. henselae* isolates being made from 2 domestic cats and a cheetah from Zimbabwe[11]. Our finding that *B. henselae* is also present in South Africa provides evidence that the organism could be widely distributed in the region. It is important, then, that medical health workers should be aware of the possibility of *B. henselae* infections in their patients and that health laboratories in the region develop appropriate diagnostic tests to detect infections. Similarly, veterinarians should be prepared to answer questions from concerned cat owners relating to *B. henselae* infections in their animals[12].

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