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Original article

Experimental infection of specific pathogen free (SPF) cats with two different strains of *Bartonella henselae* type I: A comparative study

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Abstract – Domestic cats are the reservoir of *Bartonella henselae*, the main causative agent of cat scratch disease. We compared *B. henselae* type I infection characteristics in 6 SPF cats infected with a feline strain (4.8×10^7 colony-forming units (CFU)/mL) and in 6 SPF cats infected with the reference Houston I strain (6.6×10^6 CFU/mL to 9.6×10^7 /mL). All the cats inoculated with the feline strain, but none of the cats inoculated with *B. henselae* Houston I, developed a fever within 2–12 days (mean: 5.8 days) post inoculation (PI), which lasted for 1–2 weeks. However, all 12 cats became bacteremic. The duration of bacteremia was significantly longer in the cats inoculated with the feline strain (mean: 237 days) than in the cats inoculated with Houston I strain (mean: 60 days) ($p < 0.01$). Five (83%) cats inoculated with the feline strain and none of the six cats inoculated with *B. henselae* Houston I had relapsing bacteremia ($p = 0.02$). IgG antibodies were detected by IFA within 1–2 weeks for both strains, but peaked later (week 10 versus week 3 PI) for the feline strain. By ELISA, using antigens of each *B. henselae* strain, all 12 cats developed *Bartonella* specific IgM and IgG antibodies, but the cats infected with *B. henselae* Houston I antigen yielded significantly lower optical density values ($p < 0.05$). By SDS-PAGE, PFGE and Western blotting, protein profile differences (84 to 89% homology) were observed between the two strains. If a feline vaccine is to be developed in order to prevent human infection, the choice of the vaccine strain will be critical, since major differences were identified even between strains belonging to the same sero/genotype.

Bartonella henselae **/ bacteremia / IFA / ELISA / Western blotting / cat**

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Résumé – Étude comparative de l'infection expérimentale de chats sans germe pathogène spécifique avec deux souches différentes de *Bartonella henselae* type I. Le chat domestique constitue le réservoir de *Bartonella henselae*, l'agent principal de la maladie des griffes du chat. Les caractéristiques de l'infection expérimentale de chats domestiques sans germe pathogène spécifique par une souche féline de *B. henselae* type I (6 chats recevant une dose de $4{,}8 \times 10^7$ colonies/mL) ou de la souche de référence Houston I (6 chats recevant entre $6{,}6 \times 10^6$ et $9{,}6 \times 10^7$ colonies/mL) sont présentées. Les six chats infectés avec la souche féline, mais aucun des six chats infectés avec la souche Houston I, développèrent de la fièvre 2 à 12 jours après l'inoculation (moyenne : 5,8 jours) et celle-ci dura entre 1 et 2 semaines. Cependant, les 12 chats devinrent bactériémiques, mais la bactériémie dura significativement plus longtemps chez les chats infectés par la souche féline (moyenne : 237 jours) que chez les chats infectés par la souche Houston I (moyenne : 60 jours) ($p < 0.01$). La majorité (83 %) des chats inoculés avec la souche féline présentèrent des récurrences de bactériémie, alors qu'aucun des chats inoculés avec la souche Houston I n'en présenta ($p < 0.02$). Les anticorps IgG, détectés par immunofluorescence chez tous les chats entre 1 et 2 semaines après l'inoculation, atteignirent leur acmée plus tardivement pour les chats infectés par la souche féline (10 semaines au lieu de 3 semaines après infection). En utilisant une technique ELISA, la réponse sérologique en anticorps IgM et IgG était significativement moins forte chez les chats infectés par la souche Houston I que chez les chats infectés par la souche féline ($p < 0.05$). Par électrophorèses en gel de polyacrylamide et en champ pulsé et par Western blot, les profils protéiques présentaient 84 % à 90 % d'homologie. Si un vaccin à usage félin pour prévenir les infections humaines venait à être développé, le choix de la souche vaccinale serait critique, puisque des différences majeures furent identifiées entre deux souches appartenant pourtant au même sérotype/génotype.

***Bartonella henselae* / bactériémie / chat / sérologie / Western blot**

1. INTRODUCTION

Bartonella henselae, the main causative agent of cat scratch disease, is a small gram-negative, rod-shaped, fastidious bacterium. Epidemiological and experimental studies have demonstrated that domestic cats represent the main reservoir of *B. henselae* [10, 12, 33, 49, 59] and *B. henselae* is primarily transmitted among cats by fleas (*Ctenocephalides felis*) [14, 22, 32]. Cats are persistently infected with *Bartonella* in their bloodstream for a few months up to several years [1, 35, 38]. *Bartonella henselae* have been classified in two predominant serotypes/genotypes, type I and type II, also designated as Houston I and BA-TF/Marseille based on 16S rRNA-DNA sequencing [6, 7, 20, 31, 53, 55]. Major regional variations in the prevalence of these variants have been reported in domestic cat populations. In the United States, prevalence of *B. henselae* type I reached only 18% in cats from the western United States, whereas it represented about half (52%) of the isolates from cats from the eastern United States (Chomel, unpublished data). In most of Europe, *B. henselae* type II is the dominant type [5, 7, 18, 30, 53]. On the contrary, in Asia, 70 to 80% of *B. henselae* isolates belong to type I [15, 16, 41].

In humans, *B. henselae* can cause a wide variety of diseases and syndromes, such as bacillary angiomatosis, bacillary peliosis, osteomyelitis, endocarditis, neuroretinitis as well as cat scratch disease (CSD) [3, 10, 17, 34, 43, 47, 58]. In contrast to human infection with *B. henselae*, cats naturally or experimentally infected do not usually express any major clinical signs [13, 50, 59]. However, some cats experimentally infected with *B. henselae* had systemic and local clinical signs. Such signs, occurring mostly within 2 to 4 weeks after infection, included fever, erythema at the inoculation

site, lethargic neurological signs, lymphadenopathy, and reproductive disorders [25–28, 35, 44, 45]. The presence or lack of clinical signs in experimental studies could be related to the variability of pathogenicity among *Bartonella* strains, as previously suggested [45, 46].

Relapsing bacteremia, characterized by positive cultures occurring after at least two successive negative cultures, has been observed in cats infected with *B. henselae* isolates of feline origin (mainly *B. henselae* type II) [35, 44, 59]. Some authors suggested that the occurrence of such relapses could be related to antigenic variation [37, 42]. On the contrary, experimental infection of cats with *B. henselae* Houston I, a strain of human origin, which has been sub-cultured numerous times, has never led to relapsing bacteremia [50, 59]. Differences among feline *Bartonella* species are seen genotypically and phenotypically. Pulsed field gel electrophoresis (PFGE) separation of *Sma*I endonuclease digested genomic DNA fragments from *B. henselae* isolates reveals a wide diversity of profiles [5, 42, 54], and is a useful tool to differentiate *B. henselae* strains.

To date, no studies have been conducted to compare experimental infections of specific pathogen free (SPF) cats with *B. henselae* type I of feline origin and *B. henselae* Houston I, and in particular the presence of clinical signs, duration of bacteremia and presence of bacteremia relapses, and serological immune response. If the development of a feline vaccine is a feasible approach to reduce human infection, selection of the vaccine strains will be of major importance. Therefore, the objectives of this study were to: (1) determine if SPF cats, experimentally inoculated with a *B. henselae* type I strain of feline origin, could develop clinical signs after infection; (2) evaluate the bacteremia time of onset and duration, as well as the presence of bacteremia relapses in SPF cats infected with this feline strain; and (3) characterize the serological immune response to this infection. (4) Finally, to compare this infection in cats inoculated with *B. henselae* Houston I (human isolate) as well as the pheno/genotypic differences of these two *B. henselae* strains.

2. MATERIALS AND METHODS

2.1. Animals

Twelve 8–12 month old cats (4 males, 8 females) were obtained from the SPF colony of the Feline Nutrition Laboratory, School of Veterinary Medicine, University of California, Davis (courtesy of Drs. James Morris and Quinton Rogers). Before experimental infection, all 12 cats were confirmed to be *Bartonella* spp. sero-negative and culture-negative. All cats were examined clinically every day for the first two weeks and at least weekly thereafter. The cats were housed in three groups of respectively 2, 4 and 6 cats, in a controlled environment (ectoparasite-free, with restricted access, including a pre-room with a disinfectant footbath). Blood samples (3.0 mL) were collected weekly for the first month and every other week thereafter for blood culture and serological tests. Six cats were inoculated with *B. henselae* feline type I, and six cats with *B. henselae* Houston I.

2.2. Primary isolation of *B. henselae* feline type I

Blood was collected in April 1997 at the University of California Veterinary Medical Teaching Hospital from a healthy 11-month old, shorthaired, indoors domestic cat from northern California. The cat had no clinical signs, including fever. No ectoparasite infestation was observed at the time of blood collection.

Blood (1.5 mL) was transferred into a pediatric lysis-centrifugation tube (Wampole Laboratories, Cranbury, NJ, USA) and centrifuged, and the pellet was plated onto 5%

rabbit blood agar, as previously described [1]. The isolate was confirmed to be a *B. henselae* strain by the polymerase chain reaction (PCR) and determined to belong to *B. henselae* type I by 16S rRNA-DNA sequencing, as previously published [29]. The isolate was referred to as *B. henselae* feline type I (feline isolate). The level of bacteremia in this cat was 1.23×10^2 CFU/mL. IgG antibody titers, determined by the immunofluorescence antibody assay (IFA), against *B. henselae* feline type I and Houston I were both 1:256.

2.3. Experimental inoculation

Either *Bartonella henselae* feline type I or *B. henselae* Houston-I (ATCC # 49882) [47] were plated onto 5% rabbit blood agar, and incubated at 35 °C for 4–5 days. The harvested colonies were suspended into sterile saline and 0.5 mL was inoculated intradermally in 3–5 different sites, as previously described [1]. For the *B. henselae* feline type I infection, all six cats were inoculated with 4.8×10^7 colony-forming units (CFU)/mL. For the *B. henselae* Houston-I infection, the inoculum doses were respectively 6.6×10^6 CFU/mL for 4 cats, and 9.6×10^7 CFU/mL for two cats. The feline type I strain was inoculated in the SPF cats from an initial subculture of the original isolate.

2.4. Blood culture

Blood was drawn from the jugular vein of each cat weekly for the first month and every other week for the following months, up to 6 to 14 months after inoculation. Two milliliters of blood were placed into ethylenediamine tetraacetate (EDTA) tubes (Becton Dickinson, Franklin Lakes, NJ, USA) for culture and 1 mL into serum separation tubes for serological tests. The EDTA tubes were frozen at –70 °C and plated a few days later onto 5% rabbit blood agar plates, as previously described [1]. The plates were incubated at 35 °C with 5% CO_2 for four weeks. The plates were examined 2–3 times a week for any bacterial growth. The isolated strains were confirmed to be *B. henselae* by PCR/RFLP of the citrate synthase gene using *Hha* I and *Taq* I endonucleases [15]. The number of colonies was counted and calculated as CFU/mL.

2.5. Serology

2.5.1. Immunofluorescent antibody (IFA) test

IFA antibody titers were determined against whole organisms of three feline *Bartonella* species, as previously described [12, 13]. The feline serum samples were diluted to 1:64 with phosphate-buffered saline containing 5% skim milk (PBS, pH 7.4) and placed on a 12-well polytetrafluoroethylene slide sensitized with either *B. henselae* feline type I or Houston I whole cells. The slides were incubated for 30 min at 37 °C and were washed with PBS for 10 min. Fluorescein conjugated goat affinity purified antibody against cat IgG (whole molecule) (Cappel, Organon Teknika Corp., Durham, NC, USA) was diluted 1:800 with PBS containing 5% skim milk and 0.001% Evan blue, and placed onto the slides. The slides were incubated for 30 min at 37 °C and washed with PBS for 10 min. Two readers independently and blindly scored the levels of fluorescence and determined the end-point dilution (the last dilution with a 50% fluorescence identified by the 2 readers). A titer of $\geq$ 1:64 was considered to be sero-positive [12].

2.5.2 Enzyme immunosorbent assay (ELISA)

The outer membrane proteins (OMPs) of two *B. henselae* strains, feline type I and Houston I, were prepared as previously described [57]. Then ELISA testing was

performed, as previously published [12]. Serum samples were diluted to 1:50 for IgM and to 1:100 for IgG, and anti-cat-IgM (heavy and light chain peroxidase-labeled conjugate) (Kirkegaard and Perry Laboratories [KPL], Gaithersburg, MD, USA) or anti-cat IgG phosphatase-labeled conjugate (KPL) were diluted at 1: 100 dilution for IgM and at 1: 2000 for IgG conjugate, respectively. The optical density (O.D.) of each well was quantified for IgM at 450 nm with a second wave length at 570 nm as a reference, and for IgG at 410 nm, using a Spectra Max 340 (Molecular Devices, Sunnyvale, CA, USA).

2.6. SDS-PAGE and Western blotting

Each *Bartonella* type (*B. henselae* feline type I and Houston I) was cultured, harvested and suspended in 0.5 mL of sterile PBS [57]. SDS-PAGE sample buffer (0.5 mL), composed of 125 mM Tris-HCl (USB™, Cleveland, OH, USA) pH 6.8, 20% (v/v) of glycerol, 4% (w/v) of SDS (BIO-RAD Laboratories, Hercules, CA, USA), 10% (v/v) of 2-β-mercaptoethanol, 0.004% (w/v) of bromophenol blue with sterile distilled water**,** was added to the suspension. Similarly, OMPs of each *Bartonella* species were diluted with sample buffer and adjusted to 1.25 µg protein/µL. Both preparations, whole organisms and OMPs of *Bartonella*, were then solubilized for 5 min at 100 °C. Whole organisms and OMPs were electrophoresed through discontinuous SDS-polyacrylamide gel (4% stacking gel and 12% resolving gel) in a SE-600 (Hoefer Scientific Instruments, San Francisco, CA, USA) apparatus at 8.5 mA for 16 hours. Proteins, stained with SYPRO® orange (BIO-RAD), were visualized under ultra violet (UV) light by a gel documentation system, Gel Doc 1000® (BIO-RAD). Comparative analysis of these protein profiles was performed to generate similarity values by the unweighted-pair group method with an arithmetic mean (UPGMA), using Molecular Analyst® software, Fingerprinting Plus (BIO-RAD).

The Western blotting strips were prepared using SDS-PAGE as above. The electrophoretically separated proteins were transferred onto a sheet of nitrocellulose (0.2 µm pore size, Hoefer Scientific Instruments), using a TE 50X (Hoefer Scientific Instruments) unit at about 900 mA for 2 hours at 10 °C. The nitrocellulose sheet was dried and cut into 4.0 × 100 mm strips, then soaked in a blocking solution (20 mM Tris-HCl, 500 mM NaCl, 3% gelatin, pH 7.5). Each serum sample was tested against its homologous antigen, i.e., cats inoculated with *B. henselae* feline type I were tested against the same antigen. Serum samples from cats inoculated with *Bartonella*, diluted at 1:100 in a buffer solution (20 mM Tris-HCl, 500 mM NaCl, 5% of skim milk, pH 7.5), were added to the strips and incubated for 1 hour at room temperature. The strips were washed 3 times for 5 min and peroxidase labeled goat anti-cat IgG (H + L) conjugate diluted at 1:3000 (KPL) was added and incubated for 1 hour at room temperature. The strips were rinsed 3 times for 5 min and then, TMB (3,3',5,5'-tetramethylbenzidine) membrane peroxidase substrate (KPL) was added.

2.7. Pulsed-field gel electrophoresis (PFGE)

The PFGE procedure was performed as previously described [11, 42, 52] with some very minor modifications. The two *B. henselae* strains were cultured on 5% rabbit blood agar, scraped, and suspended in 1.0 mL of sterile PBS. Digestion of the proteins was performed using proteinase K solution (10 mM Tris, 150 mM NaCl, 2 mM EDTA, 1% SDS [BIO-RAD], 0.25% Triton X-100, 1 mg/mL proteinase K [GIBCO, BRL, Gaithersburg, MD, USA]) and DNA from each *Bartonella* species was digested with *Sma*I endonuclease. Cluster analysis using the Molecular Analyst Software

(BIO-RAD) was performed and dendrograms based on the results of the matrix of similarity values were created with UPGMA clustering.

2.8. Statistical tests

The non-parametric Mann-Whitney rank sum test was performed with MINITAB™ statistical software Release 13.1 (MINITAB Inc., State College, PA, USA) for the duration of the bacteremia, and antibody detection by IFA and ELISA for comparison of the two groups of cats for each antigen. For the peak level of the bacteremia, the time to reach the maximum level of bacteremia, and IFA titers/ELISA OD values for comparison of the two groups of cats with homologous antigens, an equal variance t-test was performed using MINITAB™ software. For univariate analysis, the Fisher exact test was used for the association between *B. henselae* strains and bacteremia relapses (Epi-info version 6.04b, CDC Atlanta, GA, USA).

3. RESULTS

3.1. Clinical observation and bacteremia characteristics

The only obvious clinical sign observed in cats inoculated with *B. henselae* feline type I was fever. All six cats, inoculated with *B. henselae* feline type I, developed fever (≥ 39.2 °C) within 2–12 days (mean: 5.8 days) after inoculation. The duration of the fever in cats inoculated with *B. henselae* feline type I lasted from 1 to 2 weeks (mean: 7.8 days). On the contrary, none of the six cats inoculated with *B. henselae* Houston I showed any obvious clinical signs, including fever. All twelve cats became bacteremic within a week after the experimental inoculation (Fig. 1). Bacteremia peaked between 21 and 28 days (mean: 23.3 days) in cats inoculated with *B. henselae* feline type I and between 21 and 44 days (mean: 28.3 days) in cats inoculated with *B. henselae* Houston I. No significant differences in time to reach the bacteremia peak were observed between the cats infected with *B. henselae* feline type I and *B. henselae* Houston I ($p = 0.31$). The maximum level of bacteremia reached $5.20 \times 10^4 – 1.58 \times 10^5$ (mean: 1.01×10^5) in cats inoculated with *B. henselae* feline type I and $1.90 \times 10^3 – 4.48 \times 10^5$ CFU/mL (mean: 2.41×10^5 CFU/mL) in cats inoculated with *B. henselae* Houston I, a difference not statistically significant ($p = 0.94$). The duration of bacteremia in cats inoculated with *B. henselae* feline type I lasted from 98 to 357 days (mean: 237 days). It was much shorter in cats inoculated with *B. henselae* Houston I, since it lasted 37 to 77 days (mean: 60 days) ($p < 0.01$). Bacteremia relapses, as defined above, occurred in 83% ($n = 5/6$) of the cats inoculated with *B. henselae* feline type I, whereas none of the 6 cats inoculated with *B. henselae* Houston I had a relapse, a difference which was statistically significant ($p = 0.02$).

3.2. Serology

3.2.1. IFA

IgG antibodies were detected by IFA in all 12 cats within 1–2 weeks post-infection (PI). Using homologous antigens, IgG antibody titers peaked between 3 to 4 weeks PI in the cats inoculated with *B. henselae* Houston I, whereas it peaked at week 10 PI in the cats inoculated with *B. henselae* feline type I (Tab. I). Furthermore, the IFA titers in the cats inoculated with *B. henselae* feline type I were significantly higher after week 8 until week 24 PI than those in cats inoculated with *B. henselae* Houston I ($p < 0.05$). The IgG antibody response towards *B. henselae* Houston I antigen was stronger in the *B. henselae* feline type I inoculated cats than in the *B. henselae* Houston I inoculated cats. That difference,

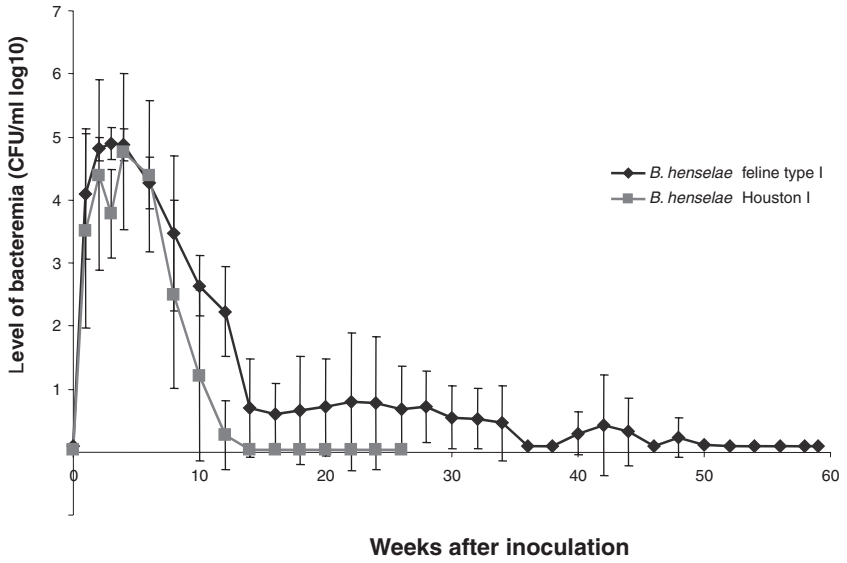

Figure 1. Mean bacteremia level in specific-pathogen-free (SPF) cats experimentally inoculated with *B. henselae* feline type I ($n = 6$) and Houston I ($n = 6$). The results are expressed as mean and standard deviations (± SDs).

Table I. Mean IgG antibody response to homologous and heterologous antigens by indirect fluorescent antibody (IFA) in specific-pathogen free experimentally inoculated with *B. henselae* feline type I ($n = 6$) or Houston I ($n = 6$).

Inoculation Antigen tested Weeks PI	*B. henselae* feline type I		*B. henselae* Houston I	
	B. h. feline I mode (range)	*B. h.* Houston I mode (range)	*B. h.* feline I mode (range)	*B. h.* Houston I mode (range)
0	0	0	0	0
2	256 (128–1024)	1024 (512–2048)	1024 (512–1024)	512 (512–4096)
4	512 (128–1024)	1024 (512–2048)	1024 (512–1024)	1024 (512–1024)
8	1024 (256–2048)	512 (256–2048)	512 (256–512)	512 (128–1024)
10	1024 (1024–2048)	512 (512–1024)	256 (128–512)	256 (256–512)
12	1024 (512–1024)	512 (256–1024)	256 (128–256)	256 (128–512)
16	512 (512–1024)	256 (256–512)	256 (128–256)	256 (128–256)
20	512 (256–1024)	256 (128–512)	128 (128–256)	256 (128–256)
24	512 (256–2048)	256 (128–512)	256 (128–256)	128 (128–256)
26	256 (128–512)	256 (128–512)	128 (128–256)	128 (128–256)

however, was not statistically significant (Tab. I).

3.2.2. ELISA

Bartonella specific IgM and IgG antibodies against OMP antigens from either *B. henselae* feline type I or *B. henselae* Houston I were detected in all 12 cats. IgM antibodies were detected and peaked by week 1 PI in the cats inoculated with either *B. henselae* feline type I or Houston I. In the two cat groups, the IgM antibody response was significantly ($p < 0.05$) stronger towards

the feline strain antigen than towards the Houston I antigen (Tab. II); even though the baseline values were quite high for the feline I inoculated cats with the homologous antigen. The IgM OD values returned to the baseline value for most of the cats.

IgG antibody response to *B. henselae* OMP antigens (both *B. henselae* feline type I and *B. henselae* Houston I) started to rise within 1 to 2 weeks PI in both groups and peaked by week 8 PI, as shown in Table III, for both groups. As with the IgM antibody response, IgG antibody OD values against *B. henselae* feline type I antigen were significantly higher than those against *B. henselae* Houston I in both groups ($p < 0.05$).

3.3. SDS-PAGE analysis

The SDS-PAGE analysis of whole organisms and OMPs for the two *Bartonella* strains feline type I and Houston I showed almost identical protein profiles (Figs. 2 and 3). At least 25 prominent protein bands were identified between 14 kDa and 200 kDa for both *B. henselae* feline type I and *B. henselae* Houston I whole organism lysates, with estimated molecular weights of 14, 22, 26, 30, 33, 37, 39, 46, 49, 56, 65, 78, and 93 kDa. For OMPs, at least 12 protein bands were identified between 20 kDa and 200 kDa, with prominent bands at approximately, 30, 33, 37, 65, 73, and 85 kDa. However, the intensity of the protein bands, especially at 33 kDa, was much stronger for *B. henselae* feline type I than for *B. henselae* Houston I.

A pairwise comparison of the protein profiles between *B. henselae* feline type I and *B. henselae* Houston I showed that for whole organisms, *B. henselae* feline type I had an 89.1% similarity with *B. henselae* Houston I. Similarly, protein profile comparisons for OMPs showed that *B. henselae* feline type I had an 84.2% similarity with *B. henselae* Houston I.

3.4. Western blotting

Serum samples from the twelve cats inoculated with either *B. henselae* feline

Table II. Mean IgM antibody response to homologous and heterologous antigens by ELISA in specific-pathogen free experimentally inoculated with *B. henselae* feline type I ($n = 6$) or Houston I ($n = 6$).

Inoculation Antigen tested Weeks PI	*B. henselae* feline type I		*B. Henselae* Houston I	
	B. h. feline I Mean (±SD)	*B. h.* Houston I (Mean (±SD)	*B. h.* feline I Mean (±SD)	*B. h.* Houston I (Mean (±SD)
0	0.246 (0.087)	0.036 (0.016)	0.127 (0.039)	0.042 (0.011)
1	0.400 (0.021)	0.201 (0.004)	0.486 (0.021)	0.147 (0.031)
2	0.333 (0.029)	0.154 (0.014)	0.450 (0.029)	0.107 (0.041)
4	0.316 (0.044)	0.119 (0.034)	0.330 (0.010)	0.068 (0.021)
6	0.277 (0.079)	0.070 (0.031)	0.281 (0.030)	0.051 (0.017)
8	0.260 (0.073)	0.053 (0.032)	0.219 (0.023)	0.044 (0.010)
10	0.220 (0.061)	0.058 (0.028)	0.210 (0.037)	0.052 (0.014)
12	0.259 (0.041)	0.080 (0.041)	0.198 (0.040)	0.038 (0.009)
14	0.273 (0.065)	0.074 (0.041)	0.192 (0.030)	0.050 (0.025)
16	0.260 (0.066)	0.077 (0.031)	0.196 (0.023)	0.043 (0.011)
18	0.242 (0.073)	0.076 (0.035)	0.179 (0.016)	0.036 (0.015)
20	0.239 (0.068)	0.060 (0.018)	0.178 (0.016)	0.038 (0.013)
22	0.244 (0.068)	0.061 (0.024)	0.214 (0.066)	0.045 (0.014)
24	0.253 (0.048)	0.073 (0.025)	0.184 (0.027)	0.041 (0.014)
26	0.259 (0.086)	0.069 (0.025)	0.172 (0.042)	0.047 (0.014)

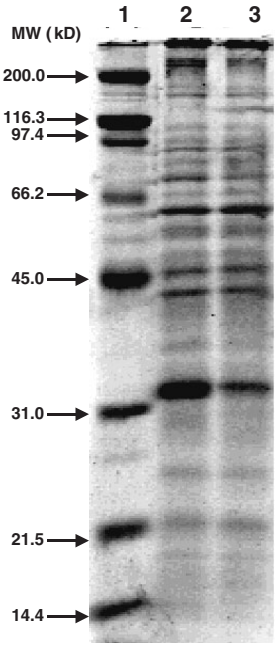

Figure 2. Comparison of protein profiles from whole organism lysates of two *Bartonella* strains separated in a 12% gel by SDS-PAGE: Lane 1, molecular weight standard; lane 2, *B. henselae* (feline type I strain); lane 3, *B. henselae* (Houston I strain, ATCC 49882).

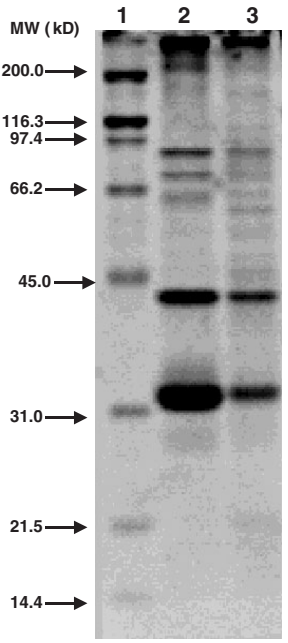

Figure 3. Comparison of outer membrane protein (OMP) profiles from *Bartonella henselae* strains separated in a 12% gel by SDS-PAGE: Lane 1, molecular weight standard; lane 2, *B. henselae* (feline type I strain); lane 3, *B. henselae* (Houston I strain, ATCC 49882).

type I ($n = 6$) or Houston I ($n = 6$), were examined by Western blotting to identify specific IgG seroreactivity patterns against OMP antigens. Similar Western blotting profiles for each group were identified, but they differed from group to group. At least 20 seroreactive bands were identified between 14 and 180 kDa (Figs. 4 and 5). Specific IgG seroreactivity was first detected between week 1 to week 2 PI in both groups. Prominent specific bands against the homologous antigen were observed within 1 to 5 weeks PI for the cats inoculated with *B. henselae* feline type I at approximately 24, 28, 33, 35, 37, 38, 44, 65, 73, 78, 83 and 85 kDa and for *B. henselae* Houston I at approximately 33, 35, 44, 65, 73, 78, and 85 kDa, and persisted until the end of the experiment. The following bands at 37, 38, 65, 73, 78 kDa for *B. henselae* feline type I and at 44, 48, 65, 78 kDa for *B. henselae* Houston I were all detected by week 2 PI.

Protein bands against *B. henselae* Houston I OMP antigens in cats inoculated with *B. henselae* feline type I were detected approximately at 35, 37, 38, 65, 73 and 78 kDa by week 2 PI (data not shown). Whereas protein bands in cats inoculated with *B. henselae* Houston I against *B. henselae* feline type I appeared approximately at 33,

Table III. Mean IgG antibody response to homologous and heterologous antigens by ELISA in specific-pathogen free experimentally inoculated with *B. henselae* feline type I ($n = 6$) or Houston I ($n = 6$).

Inoculation Antign tested Weeks PI	*B. henselae* feline type I		*B. henselae* Houston I	
	B. h. feline I Mean (±SD)	*B. h.* Houston I (Mean (±SD)	*B. h.* feline I Mean (±SD)	*B. h.* Houston I (Mean (±SD)
0	0.097 (0.032)	0.032 (0.042)	0.143 (0.071)	0.054 (0.050)
1	0.458 (0.081)	0.052 (0.050)	0.198 (0.058)	0.057 (0.047)
2	0.895 (0.104)	0.133 (0.059)	0.525 (0.112)	0.152 (0.048)
4	1.245 (0.219)	0.214 (0.084)	0.822 (0.049)	0.284 (0.078)
6	1.347 (0.211)	0.275 (0.087)	0.912 (0.082)	0.322 (0.115)
8	1.410 (0.203)	0.305 (0.074)	1.000 (0.110)	0.372 (0.143)
10	1.286 (0.275)	0.346 (0.137)	1.014 (0.073)	0.386 (0.162)
12	1.380 (0.290)	0.278 (0.037)	0.971 (0.091)	0.362 (0.153)
14	1.404 (0.309)	0.307 (0.067)	0.986 (0.055)	0.364 (0.157)
16	1.309 (0.233)	0.418 (0.114)	0.966 (0.117)	0.354 (0.153)
18	1.323 (0.306)	0.385 (0.236)	0.973 (0.184)	0.364 (0.222)
20	1.333 (0.299)	0.355 (0.114)	0.963 (0.075)	0.372 (0.193)
22	1.386 (0.287)	0.312 (0.078)	0.746 (0.142)	0.321 (0.160)
24	1.451 (0.295)	0.359 (0.088)	0.965 (0.073)	0.339 (0.165)
26	1.444 (0.314)	0.317 (0.058)	1.056 (0.120)	0.380 (0.144)

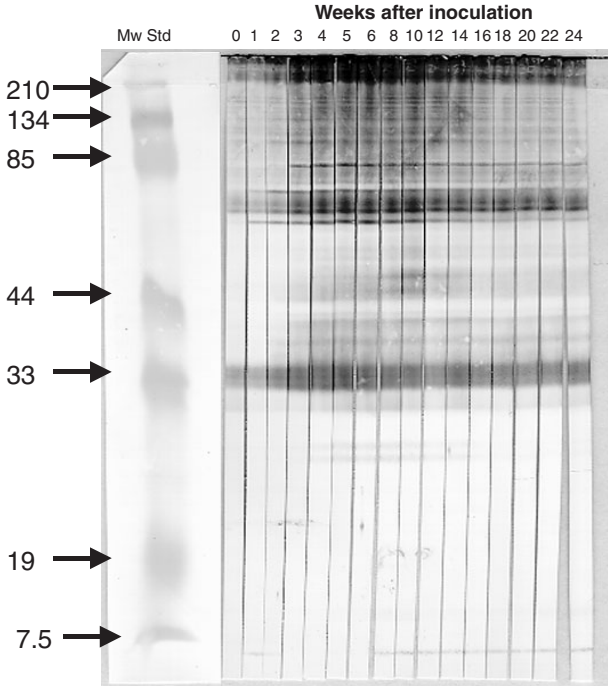

Figure 4. Western blotting results from the analysis of OMP antigen specific seroreactivity against *B. henselae* feline type I in a cat inoculated with *B. henselae* feline type I.

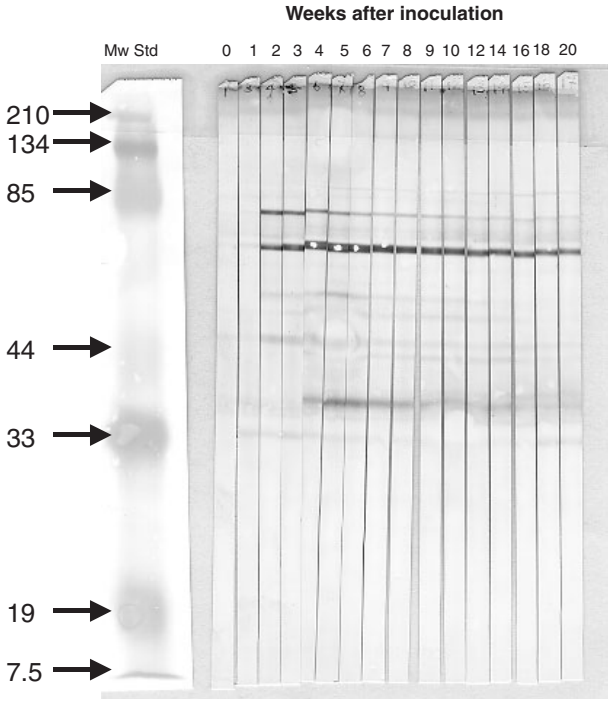

Figure 5. Western blotting results from the analysis of OMP antigen specific seroreactivity against *B. henselae* Houston I strain in a cat inoculated with *B. henselae* Houston I strain.

35, 44, 46, 65, 73, 78, and 85 (data not shown).

3.5. PFGE

The PFGE profiles of *B. henselae* feline type I and Houston I showed a range of fragment sizes between 19 Kb to 230 Kb (Fig. 6). The genome size of the two *B. henselae* strains was estimated to be approximately 1.4 Mbp. The *Bartonella henselae* feline type I and *B. henselae* Houston I showed a wide range of bands (at least 14 bands) with quite similar, but not identical profiles. Using UPGMA, we found a similarity of 84.2% between the profiles of restriction fragments from these two *B. henselae* strains.

4. DISCUSSION

We report the first study comparing the infection of SPF cats with a feline *B. henselae* type I and the reference strain, *B. henselae* Houston I. This reference strain, which is also a type I strain based on its 16S rRNA gene sequence [6, 20], was isolated from the blood of an HIV positive patient [48]. The feline strain of *B. henselae* type I caused a different type of infection in cats than the reference *B. henselae* Houston I strain, by inducing fever, longer bacteremia and bacteremia relapses.

Clinical signs, such as fever, lethargy anorexia and lymphadenopathy have been observed in cats experimentally inoculated mainly with *B. henselae* type II strains [26,

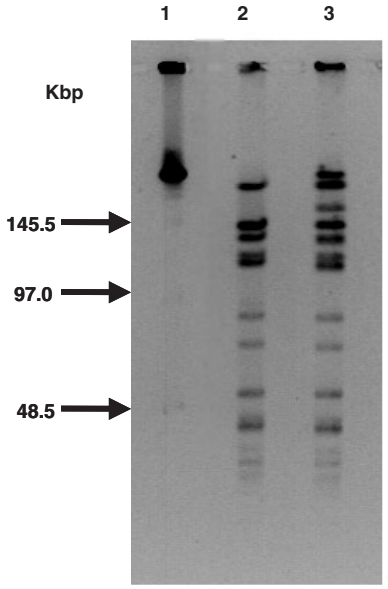

Figure 6. Pulsed field gel electrophoresis (PFGE) analysis of *Sma*I-digested genomic DNA from *Bartonella henselae* feline type I and Houston I: Lane 1, molecular size marker (48.5 to 970 kbp); Lane 2, *B. henselae* genomic DNA (Houston I strain, ATCC 49882); Lane 3, *B. henselae* genomic DNA (feline type I strain).

27, 35, 36, 45]. Cats inoculated by the intradermal route with a pathogenic strain of *B. henselae* type II (LSU16) developed fever after 6 to 16 days PI, which lasted for 1 to 8 days [45]. Our present data were in contrast to all our previous results, using a *B. henselae* type II strain, that did not cause any fever or clinical signs [1, 59]. These clinical observations may support strain dependent differences in the pathogenicity of *B. henselae*, as previously suggested [45, 51]. Although fever genesis is not clear in cats as well as in humans, fever could be caused by the release of endogenous pyrogens, including cytokines, such as interleukin (IL)-1β, IL-6, IL-8 and tumor necrosis factor α, as previously reported [2, 19]. Similarly, lipopolysaccharides (LPS), which can induce these pyrogenous cytokines, are known components of gram-negative bacteria, including *Bartonella* [9, 21].

All 12 cats became bacteremic within one week PI and bacteremia peaked within 3 to 4 weeks PI at levels (10^3 to 10^6 CFU/mL) similar to previous observations in cats inoculated with either *B. henselae* or *B. clarridgeiae* [1, 22, 25-27, 38, 46, 50, 59]. However, the duration of bacteremia in cats inoculated with *B. henselae* feline type I was significantly longer than in cats inoculated with *B. henselae* Houston I, but in the same range as in cats infected with either feline *B. henselae* type II strains or *B. clarridgeiae* [35, 59]. None of the six cats infected with *B. henselae* Houston I showed relapsing bacteremia, as previously reported [50]. On the contrary, most of the cats infected with *B. henselae* feline type I did relapse, as did these cats that were naturally and experimentally infected with *B. clarridgeiae* or various strains of *B. henselae* type II [35, 44, 59].

The IgG antibody response against *B. henselae* feline type I in cats inoculated with that strain was higher than for cats inoculated with *B. henselae* Houston I and the IgG antibody response towards *B. henselae* Houston I antigen was stronger in the *B. henselae* feline type I inoculated cats. The IgG titers were similar for both antigens in the cats inoculated with *B. henselae* Houston I. A specific IgM antibody response was detected by ELISA in all cats within 1 to 2 weeks PI and declined rapidly, as previously reported [26, 44, 45]. The *B. henselae* feline type I strain appeared to induce a stronger antibody response in cats infected to *B. henselae* feline type I than in cats infected with *B. henselae* Houston I.

The feline *B. henselae* type I inoculum used in this experimentation was obtained from the first subculture of the original isolate. For *B. henselae* Houston I, it is not known exactly how many passages this strain has been submitted to, but according to an ATCC source, it was passed at least five times to make the initial culture stock

(Jane Tang, personal communication). Serial passages of *Bartonella* strains on blood agar, even in limited numbers, could lead to the attenuation of the strains and induce a lower antigenic response when compared to freshly isolated strains from cat blood [1, 26, 27, 44, 45]. Isolates with very limited passages on blood agar could harbor antigenic components that are not present or present in smaller amounts in strains that have been submitted to more passages [4, 8]. Multiple passages of *B. henselae* leads to a loss of pilus expression by the bacteria, which is associated with a reduced adhesion to and entry into epithelial cells [8]. It will be useful to verify such a hypothesis by experimentally infecting cats with the same strain directly from blood isolation and after a few serial passages on blood agar. Finally, the selection of *B. henselae* type I strains used as diagnostic antigens in humans and in domestic animals needs to be carefully assessed, as previously reported for the detection of *B. henselae* IFA antibodies [56].

Differences of protein profiles among *Bartonella* species, especially for proteins smaller than 54 kDa, has been shown by SDS-PAGE analysis [38–40, 53]. In this study, the pairwise comparison of protein profiles revealed 84 to 89% similarity between the two *B. henselae* strains. Similarly, Western blotting results also showed differences in reactive OMP protein profiles, especially at low (< 30 kDa) and high (> 80 kDa) molecular weights. All reactive bands appeared within 1 to 5 weeks after infection, as previously reported [23]. As recently reported for *B. henselae* Houston I, *B. henselae* OMPs could play a pathogenic role by inducing NF-κB activation and adhesion molecule expression [24]. Therefore, differences in OMPs between these two strains could explain the differences in pathogenicity. Genotypic differences between the two strains were observed by PFGE (only 84% similarity). However, no study has been performed as yet to compare the various *B. henselae* strains in relation to the OMPs composition and their pathogenicity.

Little is known about the pathogenic determinants of *Bartonella* infection in humans and in cats. Two sero/genotypes of *B. henselae* were described and have been used for epidemiological and microbiological investigations [6, 20, 30, 31, 42, 53-55, 59]. Recently, Arvand et al. [4] demonstrated virulence differences between the *B. henselae* Berlin I strain and *B. henselae* Houston I, which both belong to the same genotype, despite the fact that they were indistinguishable by PFGE and 16S rRNA typing. We were also able to document clinical differences within the same genotype I, also suggesting strain variations. If a feline vaccine were to be developed in order to prevent human infection, the choice of the vaccine strain will be critical, since major differences were identified even between the strains belonging to the same sero/genotype.

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