

Bartonella henselae infection induces a persistent mechanical hypersensitivity in mice

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ABSTRACT

Bartonella spp. are re-emerging and neglected bacterial pathogens. The natural reservoirs for several species of this genus are domestic animals such as cats and dogs, the most common pets in the USA and Brazil. Some cat studies suggest that the infection is more prevalent in tropical and poverty-stricken areas. These bacteria were associated with a wide spectrum of clinical manifestations: fever of unknown origin, endocarditis, angiomatosis, chronic lymphadenopathy, hepatitis, fatigue, paresthesia and pain. Our group has already demonstrated that *B. henselae*-infected sickle cell disease mice present with hyperalgesia. We hypothesized that even immunocompetent mice infected by *B. henselae* would show an increased and persistent mechanical sensitivity. Five ten-week old male BALB/c mice were intraperitoneally inoculated with a 30 μ L of suspension containing 10^4 CFU/mL of *B. henselae*, while five others were inoculated with an equal volume of saline solution. Four days after bacterial inoculation, the mechanical paw withdrawal threshold was measured using von Frey filaments in all animals, for five consecutive days. The infected animals showed hypersensitivity to mechanical stimuli for five consecutive days. The present study has demonstrated that *B. henselae* infection induces persistent mechanical hypersensitivity, a signal consistent with pain.

KEYWORDS: Bartonella. Hypersensitivity. Mice. Hyperalgesia.

INTRODUCTION

Bartonella spp. are Gram-negative bacilli with worldwide distribution that can cause a wide range of clinical manifestations and can be fatal, especially in immunodeficient patients¹. Among all Bartonella species, *Bartonella bacilliformis*, *Bartonella quintana* and *Bartonella henselae* are the most common cause of human infections². *B. henselae* is a zoonotic pathogen transmitted to humans by fleas and ticks. Cats are main carriers of this infection³. The *B. henselae* can infect erythrocytes, macrophages, endothelial cells, and pericytes^{4,5}. *B. henselae* has been associated with asymptomatic infection in humans including blood donors⁶. This species is the agent of Cat Scratch Disease (CSD) and may induce fever of unknown origin, endocarditis, angiomatosis, chronic lymphadenopathy, hepatitis, fatigue, paresthesia, and pain in infected hosts⁷.

Our group has already demonstrated that *B. henselae*-infected sickle cell disease mice present with hyperalgesia⁸. Therefore, the aim of this study was to evaluate if *B. henselae*-infected immunocompetent mice would show persistent mechanical hypersensitivity.

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MATERIALS AND METHODS

Animals

All procedures were performed in accordance with the policies in the Guide for the Care and Use of Laboratory Animals, after approval from the Committee of the Institution of Animal Care and Use (protocol N° 2848-1). All possible measures were taken by the involved scientists to minimize animal suffering during the experiments. Ten-week-old male BALB/c mice, weighing 22-23 g, were maintained in a 12-hour light/dark cycle room in a climate-controlled environment, with access to food and water *ad libitum*. Animals were provided by the UNICAMP Multidisciplinary Center for Biological Research.

Bacterial suspension and infection

The bacterial suspension was aseptically prepared with NaCl 0.9% and 10⁴ Colony Forming Units (CFU) of *B. henselae* / mL (Houston 1 strain, American Type Culture Collection, Rockville, MD, USA). Five mice were intraperitoneally inoculated with a 30 µL of this suspension and five control mice received an equal volume of NaCl 0.9%.

Mechanical nociceptive threshold

To assess sensitivity to a mechanical stimulus, paw withdrawal threshold was determined using von Frey filaments (27) (1601C, Life Science Instruments®), with precision ranging between 0.1 to 80 g of weight. Each animal was placed into an individual transparent acrylic box (9 cm × 9 cm × 13 cm) with a wire mesh platform floor made with non-malleable 1 mm thick wire. Mirrors placed at 30 cm below the experimentation boxes allowed visualization of the paw sole. Mice were placed in these individual boxes 30 minutes before the experiment for acclimatization. The von Frey filaments were applied with progressive pressure into the mid plantar surface of a left hind paw through the mesh floor until the animal lifted up its paw.

We tested the animals prior to and after infection with *B. henselae*. Animals were tested for five days, from day four after inoculation until day eight. The stimulus was repeated six times, with five-minute intervals, and similar measures were observed. Two different researchers individually the behavior of each animal. (GVD, MNS).

Statistical analysis

Data are expressed as mean ± standard error. Von Frey tests were analyzed using the Statistical Analysis System

for Windows (SAS 9.4, SAS Institute Inc., Cary, NC, USA). The means were compared with using ANOVA Repeated Measures and data were transformed into ranks. Differences were considered significant at $p < 0.05$.

RESULTS

Von Frey test

No differences were noted in sensitivity in the control group ($p=0.5014$). Paw withdrawal thresholds were lower in infected mice compared to control group ($p < 0.05$) from the 4th day after infection and after. **Table 1** shows the mean, median and standard deviation (SD) of all measures. **Figure 1** shows the intensity of mechanical sensitivity, before and after *B. henselae* infection.

Table 1 - Von Frey test values of *Bartonella henselae*-infected mice and controls. Time was expressed in days.

	Mean	Median	SD	pvalue
Before				
Control	9.47	9.47	1.64	
Infected	9.80	9.37	1.32	0.6873
4th day				
Control	8.94	8.93	0.52	
Infected	7.77	8.10	0.82	0.0158
5th Day				
Control	8.79	8.63	1.08	
Infected	7.08	7.13	0.21	0.0093
6th day				
Control	9.79	9.70	0.70	
Infected	6.95	6.67	0.54	0.0001
7th day				
Control	9.43	9.53	0.44	
Infected	8.06	7.97	0.49	0.0010
8th day				
Control	9.26	8.83	0.92	
Infected	7.71	7.70	0.20	0.0015

DISCUSSION

In humans, *B. henselae* infection has been associated with persistent back, chest, arms, shoulder, joint and peripheral neuropathic pain⁹. Intermittent headache and bone pain have also been reported⁹. Many patients have no known immunodeficiency¹⁰. As a translational way to analyze if *B. henselae* can be related to pain in infected immunocompetent organisms, we observed if

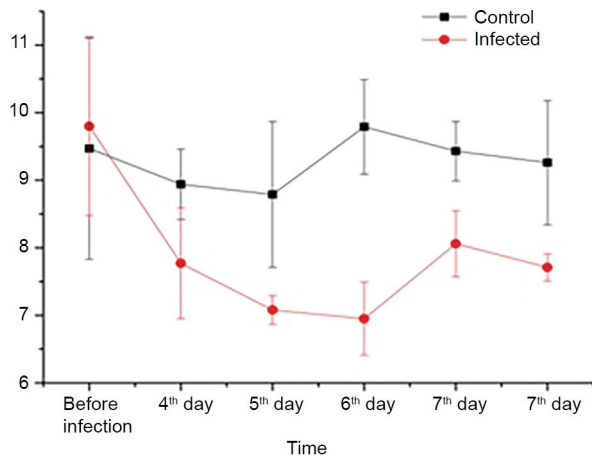


Figure 1 - Y-axis: Paw pressure threshold (g). Infected animals had a decreased intensity of nociception. Mechanical nociceptive threshold was evaluated before experimentation and for five consecutive days after *B. henselae* infection. All data are expressed as mean \pm SD for the five mice.

immunocompetent mice have increased sensitivity after experimental *B. henselae* infection.

Bacterial infection can produce pain in mice¹¹. We have already demonstrated that *B. henselae* induced hyperalgesia in sickle cell disease mice after intraperitoneal inoculation of a bacterium suspension⁸. As pain can be related to behaviors,^{8,12} we used the von Frey test to analyze the sensitivity in infected animals infected by *B. henselae*, and we observed that infected immunocompetent mice show more sensitivity than uninfected ones, for five consecutive days beginning on day four after the infection.

The pain mechanism that can be triggered by *B. henselae* remains unclear. Understanding the molecular and cellular mechanisms of infection and the host immune response is crucial to clarify *B. henselae* pathophysiology. Immune cells stimulated by different infectious pathogens increase the production of endothelin-1¹³, which plays an important role in the pathogenesis of infectious diseases¹⁴. Because *B. henselae* infects endothelial cells, these cells may produce and secrete endothelin-1. This could be a mechanism involved in *B. henselae* infection related pain.

In summary, the present study demonstrated that *B. henselae* infection induces a persistent mechanical hypersensitivity, a signal consistent with pain in immunocompetent mice.

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AUTHORS' CONTRIBUTIONS

GVD and AAR: carry out the experiments, conceived and planned the experiments, wrote the article; MNS, BGL, TCBS, MRD, KAL: helped to carry out the experiments; PENFMEE, KG: contributed to the design and implementation of the research; PENF: supervise the Project, contributed to the design and implementation of the research, to the analysis of the results. All authors contributed to the final version of the manuscript.

CONFLICT OF INTERESTS

None to declare.

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