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***Bartonella henselae* transmission by blood transfusion in mice**

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Abstract

BACKGROUND—*Bartonella* spp. are neglected fastidious Gram-negative bacilli. We isolated *Bartonella henselae* from 1.2% of 500 studied blood donors and demonstrated that the bacteria remain viable in red blood cell units after 35 days of experimental infection. Now, we aim to evaluate the possibility of *B. henselae* transmission by blood transfusion in a mouse model.

STUDY DESIGN AND METHODS—Eight BALB/c mice were intraperitoneal inoculated with a 30µL of suspension with 10⁴ CFU/mL of *B. henselae* and a second group of eight mice were inoculated with saline solution and used as control. After 96 hours of inoculation, the animals were euthanized. We collected blood and tissue samples from skin, liver, and spleen. Thirty microliters of blood from four *Bartonella*-inoculated animals were transfused into a new group (n=4). Another group received blood from the control animals. *B. henselae* infection was investigated by conventional and nested polymerase chain reaction (PCR).

RESULTS—Blood samples from all 24 mice were negative by molecular tests though half of the tissue samples were positive by nested PCR in the intraperitoneal *Bartonella*-investigated animals. Tissues from two of the four mice that received blood transfusions from *Bartonella*-inoculated animals were also nested PCR positives.

CONCLUSIONS—Transmission of *B. henselae* by transfusion is possible in mice even when donor animals have undetectable bloodstream infection. The impact of human *Bartonella* sp. transmission through blood transfusion recipients must be evaluated.

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CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest

Bartonella spp. are fastidious Gram-negative bacilli with worldwide distribution. They are reemerging and neglected zoonotic agents. These bacteria can cause several human diseases including Peruvian bartonellosis, trench fever, cat scratch disease, bacillary angiomatosis, and endocarditis. *Bartonella* infection can be fatal, especially in immunocompromised patients. Its prevalence among blood donors is unknown, and screening of blood supplies for this pathogen is not routinely performed because the fastidious characteristics of this organism pose a major challenge in reaching a diagnosis.¹ Moreover, automated detection is based on CO₂ production and *Bartonella* spp. do not generate sufficient CO₂ to give a positive signal.²

The human-specific species *Bartonella quintana* and *Bartonella bacilliformis* and the zoonotic cat-related species *Bartonella henselae* are typically associated with human manifestations. Infection of immunocompetent patients with *B. henselae* results in cat scratch disease, a typically self-limiting swelling of lymph nodes often associated with fever. In immunocompromised patients infection with *B. quintana* or *B. henselae* can develop serious complications with vasoproliferative lesions known as bacillary angiomatosis. Long-term infection with *Bartonella* spp. can involve skin, liver, spleen, lung, bone, or brain.^{1, 3}

The transmission of *Bartonella* spp. to humans usually occurs by traumatic contact with infected animals or by blood-sucking arthropods. *B. henselae* can multiply in the cat flea and persist in flea feces in the environment for at least 9 days.³ Blood transfusion also represents a risk: cats have been experimentally infected with *B. henselae* and *Bartonella clarridgeiae* by intravenous or intramuscular inoculation with infected cat blood.⁴ *B. bacilliformis* human blood transmission has already been documented⁵ and solid organ transplant recipients can also be infected by *Bartonella* spp. that may result in fatal outcomes.^{1, 6}

The fastidious growth characteristics of *Bartonella* spp. and its capacity to partially evade the host immune system and typically to cause a chronic infection still pose a problem for diagnosis and treatment. The intracellular location, frequent genetic rearrangements, and alteration of outer membrane proteins are considered important for the maintenance of persistent bacteremia.⁷

After intradermal inoculation the *Bartonella* infection spreads to a still enigmatic primary infection niche, although it likely includes the vascular endothelium. Inside the mammalian reservoir host, the infection spreads to the blood where bacteria invade red blood cells (RBCs) and may cause a long-lasting intraerythrocytic bacteremia, an hallmark of *Bartonella* infection.⁸

Bartonella spp. are able to infect and survive inside RBCs.⁹ In vitro *Bartonella* spp. will invade RBCs, multiply within, and persist for the lifetime of the infected host cell.⁸ Previous studies from our group using transmission electron microscopy and culture isolation have documented the ability of *B. henselae* to survive in stored blood for 35 days of storage at 4°C.¹⁰ This study was considered important to include *Bartonella* spp. as a pathogen with risk of transmission by transfusion.¹¹ Ruiz and colleagues¹² also demonstrated the ability of *B. bacilliformis* to survive long periods of time in blood samples stored at 4°C.

Allogenic blood transfusion can be a source of bloodstream infection.¹³ Approximately 13,785,000 units of RBCs and whole blood were transfused in 2011 only in the United States.¹⁴ In 2012, a total of 3,127,957 transfusions were performed in Brazil.¹⁵ The screening of bacterial contamination in donated blood components is a challenge since the initial bacteremia can be extremely low and difficult to detect. Efficient and rapid laboratory tests are not available.¹⁶

Bartonella transmission was reported in two accidental percutaneous punctures in veterinarians with contaminated animal blood.^{17, 18} Recently, we isolated *Bartonella* spp. from 1.2% of 500 Brazilian blood donors and demonstrated *B. henselae* DNA in blood samples of 3.2% of these donors.¹ We hypothesized that *B. henselae* could cause infection in a mouse model taking blood from a *Bartonella*-bacteremic donor to a healthy recipient.

MATERIALS AND METHODS

Animals

The experiments were conducted using 24 isogenic, female, 8-week-old, BALB/c mice (20–22 g of weight). All the experimental protocols were approved by Institutional Animal Care and Use Committee of the University of Campinas.

Bacterial suspension

A bacterial suspension was prepared using *B. henselae* (Houston 1, American Type Culture Collection, Rockville, MD; ATCC 49882T) with 10⁴ colony forming bacterium units (CFU) concentration in 0.9% NaCl.

Experimental design and transfusion

An overview of the experimental performed procedures is presented in Fig. 1. Eight mice were infected by intraperitoneal inoculation with 30µL of a suspension with 10⁴ CFU/mL *B. henselae*. Eight control mice were inoculated with the same volume of saline. After 96 hours, four *Bartonella*-inoculated and four control mice were euthanized with overdose of thiopental. We collected blood and tissue fragments of skin, liver and spleen. We collected blood in sodium heparin tubes from the other four *Bartonella*-inoculated animals and immediately transfused 30µL, by injection in the ocular venous plexus, into a new group of mice (n=4) that were anesthetized with ketamine and xylazine (150 mg/Kg/10 mg/Kg). The same blood collection and transfusion procedures were performed from saline-inoculated mice to a second set of uninfected animals used as control group (n=4). On the fourth day post transfusion, the animals were euthanized with pentobarbital and we collected blood samples and skin, liver, and spleen fragments. We did not observe any clinical signs in infected animals.

Polymerase chain reaction screening

DNA from whole blood and from skin, liver, and spleen fragments were extracted using Bacteria DNA mini kit (RTP, Stratec Molecular) in accordance with the manufacturer's instructions. DNA samples were tested by genus specific conventional polymerase chain reaction (PCR) targeting 16S to 23S ribosomal RNA intergenic spacer.¹⁹

We also performed *B. henselae* species-specific nested PCR targeting *ftsZ* region using primers previously described.²⁰ To avoid contamination, these techniques were performed as described by Pitassi and colleagues.

RESULTS

Our sensitivity in conventional PCR was 50 copies/mL and in nested PCR it was 10 copies/mL. In two of the four animals inoculated intraperitoneally with a *B. henselae* suspension, one liver and two spleen fragments were *B. henselae* positive by nested PCR. Two of the four animals that received blood transfusion from *Bartonella*-intra-peritoneal inoculated mice also had positive spleen nested PCR, even though blood molecular tests were negative to *B. henselae* from the 24 animals (Table 1).

DISCUSSION

It is estimated that *Bartonella* spp. bacteremia in asymptomatic subjects is approximately 10 CFU/ μ L blood, which may be below the detection limit of most conventional or real-time PCR assays.²¹ We were able to detect tissue infection only using nested PCR, which suggest slow bacteremia once nested PCR is admittedly more sensitive than conventional PCR.²⁰

B. henselae typically causes self-limiting, asymptomatic infection in humans, but, for some individuals, the bacteremia can become chronic and sometimes the infection can be fatal.¹ Stramer and colleagues^{11, 22} describe the potential threat of emerging infectious disease agents to transfusion safety and our data indicate that *Bartonella* spp. could fall into that category. Other *Bartonella*-related organisms such as *Borrelia burgdorferi*, ehrlichiae, and *Rickettsia rickettsii* had biologic plausibility of blood transfusion transmission. Despite this, these agents have been conclusively documented only for *Babesia* sp. (>20 cases) and *R. rickettsia* (one case).²³

Bartonella spp. have the ability to course with subclinical chronic infection. Because of the slow doubling time of *Bartonella* spp. manifestation of bartonellosis in a patient that has been transfused with *Bartonella*-infected blood could occur much later and therefore not be linked with the transfusion procedure. These data reinforce that most transfusion transmissions with this bacterium are underrecognized.

B. henselae transmission through transfusion blood had already been demonstrated in cats.²⁴ This study shows that transmission of *B. henselae* by transfusion is possible in mice even when the blood of the donor animals had no detectable bacteremia. We could not detect, by conventional or nested PCR, *B. henselae* in the blood of animals at day 4 post intra-peritoneal inoculation with *B. henselae*. These data reinforce the possibility of *B. henselae* infection by blood transfusion and that blood-negative PCR does not exclude the possibility of infection by this bacterium.

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Fig. 1.
Animal infection and samples collection scheme

Table 1

Diagnosis results of the molecular techniques used to detected bartonella infection

	Control-inoculated (IP) ^{*,#}	Bartonella-inoculated (IP) ^{*,#}	Bartonella-tranfused (IV) ^{*,+}	Control-transfused (IV) ^{*,+}
Convencional PCR	Blood	0/8	0/4	0/4
	Skin	0/4	0/4	0/4
	Liver	0/4	0/4	0/4
	Spleen	0/4	0/4	0/4
Nested PCR	Blood	0/8	0/4	0/4
	Skin	0/4	0/4	0/4
	Liver	0/4	1/4	0/4
	Spleen	0/4	2/4	0/4

* No. of positive samples/total of samples tested

IP: Intraperitoneal

+ IV: Intravenous