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Impact of queen infection on kitten susceptibility to different strains of *Bartonella henselae*

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ABSTRACT

Domestic cats are the natural reservoir of *Bartonella henselae*, the agent of cat scratch disease in humans. In kittens, maternal IgG antibodies are detectable within two weeks postpartum, weaning in six to ten weeks postpartum and kittens as young as six to eight weeks old can become bacteremic in a natural environment.

The study's objective was to evaluate if maternal antibodies against a specific *B. henselae* strain protect kittens from infection with the same strain or a different strain from the same genotype. Three seronegative and *Bartonella*-free pregnant queens were infected with the same strain of *B. henselae* genotype II during pregnancy. Kittens from queens #1 and #2 were challenged with the same strain used to infect the queens while kittens from queen #3 were challenged with a different genotype II strain.

All queens gave birth to non-bacteremic kittens. After challenge, all kittens from queens infected with the same strain seroconverted, with six out of the seven kittens presenting no to very low levels of transitory bacteremia. Conversely, all four kittens challenged with a different strain developed high bacteremia (average 47,900 CFU/mL by blood culture and 146,893 bacteria/mL by quantitative PCR). Overall, qPCR and bacterial culture were in good agreement for all kittens (Kappa Cohen's agreement of 0.78).

This study demonstrated that young kittens can easily be infected with a different strain of *B. henselae* at a very young age, even in the presence of maternal antibodies, underlining the importance of flea control in pregnant queens and young kittens.

1. Introduction

Bartonella henselae is a fastidious gram-negative bacterium capable of infecting humans and animals. In humans, the most common syndrome caused by *B. henselae* is cat scratch disease, characterized by chronic lymphadenopathy, fever, headache, poor appetite and exhaustion. Domestic cats are the natural reservoir of *B. henselae*. *Bartonella* species have been shown to be vertically transmitted in various rodent species (Boulouis et al., 2001; Kosoy et al., 1998). However, vertical transmission has not been demonstrated in cats (Abbott et al., 1997; Guptill et al., 1998) or in gerbils (Morick et al., 2013). In cats, maternal antibodies were detected within two weeks after birth but were not detectable six weeks post-partum (Abbott et al., 1997) to 10 weeks postpartum (Guptill et al., 1998). Furthermore, all 12-week-old specific pathogens free (SPF) cats experimentally inoculated with *B. henselae* became bacteremic (Guptill et al., 1997). Experimental infection with *B. henselae* of SPF neonate kittens (between three to five days and two weeks) showed that these kittens were fully receptive to infection (Guptill et al., 1999). In that study, kittens inoculated orally or intradermally were bacteremic through 12–16 weeks post-inoculation (PI), similar to what was documented for adult cats inoculated intradermally or intravenously (Guptill et al., 1999). Only intradermally inoculated neonatal cats produced serum IgG antibodies to *B. henselae*. These studies demonstrate that in the absence of specific maternal antibodies against *B. henselae*, kittens can be readily infected with this pathogen. However, the protective effect of maternal antibodies against same genotype strains of *B. henselae* has not been investigated.

Our recent data from kittens and cats rescued at the San Francisco Society for the Prevention of Cruelty to Animals (SPCA) (Fleischman et al., 2015) indicate that kittens six to eight weeks old

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can already be *Bartonella* bacteremic in a natural environment, as prevalence was respectively 29.5% (57/193) in kittens 6–17 weeks old and more specifically 33% (8/24) in cats 6–8 weeks old. It has been shown that cats can be infected or re-infected with a wide range of *B. henselae* strains, including strains belonging to the same 16S rRNA genotype (Bouchouicha et al., 2009). Furthermore, multiple locus variable-number tandem repeat analysis (MLVA) has documented wide genetic diversity of strains present in cat populations in the USA (Bouchouicha et al., 2009). Therefore, we hypothesize that young kittens (<3 months old) are protected from infection with the same strain of *B. henselae* used to infect their mothers, but will become infected when challenged with different strains, despite maternal colostral immunity. This study focused on the experimental infection of newborn kittens born from *B. henselae* bacteremic queens and challenged with *B. henselae* strains identical or different from the strain used to infect the queens.

2. Materials and methods

2.1. Animal infection protocol

Five seronegative and *Bartonella*-free pregnant queens (age range 3–8 years old) were infected with the same strain of *B. henselae* (genotype II, strain UCD-U4) at mid to late pregnancy (Table 1). Kittens born from these queens were tested every 10–15 days for presence of antibodies by indirect immunofluorescence assay (IFA) and bacteremia by blood culture and real time PCR and then were challenged between 5 and 9 weeks of age with the same strain (UCD-U4) of *B. henselae* type II used to infect the queens or a different strain (*B. henselae* genotype II strain 269,608) of feline origin. This study was approved by the University of California Davis Institutional Animal Care and Use Committee (IACUC) under protocol #17683.

2.2. Blood sample collection

2.2.1. Queens

A blood sample (1.5 mL) was collected in plastic EDTA tubes for culture and serology (on supernatant) prior to mating, at time of inoculation (mid-term or later), 2 weeks after inoculation and at time of delivery. After the birth of the kittens, a blood sample was collected from the queens every other week until weaning.

2.3. Kittens

A blood sample (300–400 μL dispersed in equal aliquots in two different sterile EDTA microvials) was collected by jugular venipuncture from the kittens at time of birth (within the first 24 h) and every other week after birth until weaning (eight weeks). After being challenged with strains of *B. henselae*, whole blood samples were collected once a week for the first four weeks and every other week during the following month. For the kittens, serology, blood culture, conventional PCR following DNA extraction were performed for the samples collected for each kitten. Real-time PCR was also performed during the first 40 days of life.

2.4. Blood culture

Whole blood was plated onto fresh 5% rabbit blood agar plates (<8 days old), as previously described (Fleischman et al., 2015; Chomel et al., 2014), incubated at 35 °C in a 5% CO_2 atmosphere and observed for 4 weeks to detect bacterial growth.

2.5. DNA extraction

Genomic DNA (gDNA) was extracted from whole blood samples using DNeasy Blood and Tissue kit (QIAGEN Inc., Valencia, CA). gDNA was extracted according to the kit's protocol with one modification: each column was incubated with heated elution buffer (70 °C) for 2 min prior to centrifugation in order to increase the gDNA yield. The final eluted volume was 100 μL per sample. An aliquot with PBS was used as control for each batch of blood samples processed (herein called "extraction control"). gDNA quantity and purity was assessed by spectrophotometry, with average gDNA yield of 23.3 ng/dL (range 2.3–67.5 ng/dL) and the average 260:280 ratio of 1.84 (range 1.62–2.29). The absence of PCR inhibitors was confirmed by the amplification of a fragment of the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) protein gene (Diniz et al., 2007).

2.6. Conventional PCR

The extracted gDNA was used as a template for amplification of fragments of the citrate synthase gene (*glt*A). Approximately 380 base pairs (bp) of the *glt*A gene were amplified using previously described primers and methods (Norman et al., 1995).

2.7. Real-time PCR (qPCR)

An aliquot of the blood samples collected in EDTA tubes was shipped to the College of Veterinary Medicine Research Laboratory at Western University of Health Sciences for DNA extraction and real-time PCR diagnostics. A qPCR assay designed to amplify a fragment of the 16S–23S ribosomal RNA (rRNA) intergenic

Table 1
List of queens and kittens enrolled in this study, with respective age, pregnancy duration at the time of inoculation, inoculum concentration and age at the time of challenge.

Queens					Kittens						
Number	Age	Pregnancy duration at inoculation	*B. henselae* inoculum concentration	Bacteremia at delivery date	Litter number	Number and gender	Age at challenge	*B. henselae* type II inoculum strain & concentration	IFA titer range	CFU/ml range	GE/ml range
#1	7 years	55 days	1.48×10^8 CFU/ml	TMTC	#1	3 males	9 weeks	Strain UCD-U4 3.5×10^8 CFU/ml	0–256	0–16	0–16
#2	7 years	28 days	8.21×10^8 CFU/ml	91 CFU/mL	#2	3 males 1 female	5 weeks	Strain UCD-U4 3.5×10^8 CFU/ml	0–1024	0–7360	0–7360
#3	7 years	28 days	3.02×10^8 CFU/ml	4640 CFU/mL	N/A	4 dead fetuses		N/A			
#4	3 years	35 days	4.5×10^8 CFU/ml	77,200 CFU/mL	#3	2 males 2 females	9 weeks	Strain 269608 1.57×10^7 CFU/ml	0–1024	400–406,400	909,212–6,230,133
#5	8 years	28 days	8.7×10^8 CFU/ml	40,000 CFU/mL	N/A	3 kittens[a]		N/A			

IFA: immunofluorescence assay. CFU: colony forming units. GE: genome equivalents. TMTC: too many to count.
[a] Emergency caesarian was required, and kittens died shortly after.