2014

Host-specialization of Bartonella in flea vectors collected from black-tailed prairie dogs

Mark D. Massaro
University of Richmond

Host-specialization of Bartonella in flea vectors collected from black-tailed prairie dogs

By

Mark D. Massaro

Honors Thesis

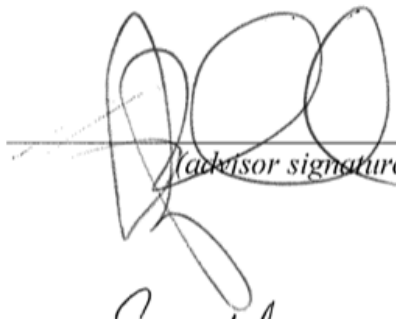
In

Department of Biology
University of Richmond
Richmond, VA

April 25, 2014

Advisor: Dr. R. Jory Brinkerhoff

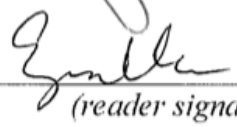
This thesis has been accepted as part of the honors requirements in the Department of Biology.



(advisor signature)

4/25/14

(date)



(reader signature)

4/25/14

(date)

Host-specialization of *Bartonella* in flea vectors collected from black-tailed prairie dogs

Mark D Massaro, R Jory Brinkerhoff

Department of Biology, University of Richmond, Richmond, VA

Abstract

At least 22 species of *Bartonella* bacteria have been named and many are associated with one of a list of potential mammalian reservoirs and arthropod vectors. One example of such a system is the parasitism of black-tailed prairie dogs (*Cynomys ludovicianus*) of the Western U.S. by *Oropyslla hirsuta* and *Pulex simulans* fleas. These vectors are thought to maintain *B. washoensis* infection in these mammals, but little is known about their role in the specialization of this strain to this reservoir. We found that the more specialist *O. hirsuta* fleas were much more likely to be infected with *B. washoensis* than the generalist *P. simulans* vector. Also, as expected, fleas collected from infected hosts were more likely to carry that infection than those collected from blood culture negative hosts. However, some generalist and host culture negative fleas were found to carry *B. washoensis*. Therefore, we believe that *O. hirsuta* fleas are the primary vector of *B. washoensis* infection in black-tailed prairie dogs in the Western U.S., and that these vectors can jump from host to host, feeding on multiple organisms in a lifetime.

Introduction

Bacteria of the genus *Bartonella* are gram-negative, fastidious organisms that are known to infect the erythrocytes and endothelial cells of a wide range of hosts from loggerhead sea turtles to bats, but are most commonly studied in mammalian reservoirs

(Breitschwerdt and Kordick 2000). *Bartonella* are thought to specialize to such reservoirs, which include small mammals such as field mice and rats, as well as cats, dogs, and domestic cattle. Although not usually found to carry *Bartonella* capable of infecting humans, small rodent communities are some of the most commonly discussed *Bartonella* reservoirs, with prevalence measured at 42 and 62% in the southeastern U.S. and the UK respectively (Breitschwerdt and Kordick 2000). The most commonly implicated vectors of *Bartonella* are fleas, sandflies, and body lice, but studies showing infection in ticks have identified them as another potential vector (Chang et al. 2001). At least 22 species of *Bartonella* have been named, and several others described, 13 of which are known to cause human disease (Vayssier-Taussat et al. 2009). The most common human diseases caused by *Bartonella* infection include cat scratch disease, trench fever, and Carrion's disease, which can cause symptoms ranging from fever and endocarditis to bacillary angiomatosis. In addition, *Bartonella* bacteria have been found to show high levels of host-specificity, possibly due to the narrow host ranges of some of their vectors (Vayssier-Taussat et al. 2009).

One example of such host-specificity may be found in the spread of the species *B. washoensis* among black-tailed prairie dogs (*Cynomys ludovicianus)* of the Western U.S. (Brinkerhoff 2010). *B. washoensis* is maintained in ground squirrels but is known to have caused cardiac disease in humans (Kosoy et al. 2003). One such ground squirrel is the black-tailed prairie dog (BTPD). In fact, *B. washoensis* is thought to be specialized to BTPDs and has been found at prevalence levels as high as 46.5% in some colonies during the summer months (Bai 2008). BTPDs are known to be parasitized by several flea species, but are thought to fed on primarily by *Oropsylla hirsuta*. Studies have shown that *Oropsylla hirsuta* make up approximatley 84.1% of fleas collected from BTPD burrows, and even up to 98% of those collected from trapped BTPDs, and are rarely found on other species of small mammals (Salkeld and Stapp 2008, Brinkerhoff 2008). A moderate proportion of the rest of the fleas

found on BTPDs belong to the genus *Pulex*, particularly *Pulex simulans*, which are also known to parasitize carnivores and larger mammals. However, despite this apparent host-specialization, recent studies have shown high rates of coinfection with mutiple *Bartonella* strains in fleas collected from BTPDs and other small mammals atypical of their respective host species (Brinkerhoff et al. 2010, Abbot et al. 2007). In addition, fleas collected from blood culture-negative BTPDs have been shown to test positive for *Bartonella* infection by PCR, further suggesting the ability of these vectors to feed on multiple hosts, including those outside of their normal range (Brinkerhoff et al. 2010). In this study we have explored this issue of flea host-specialization in BTPDs by screening *O. hirsuta* and *P. simulans* collected from both blood culture positive and negative BTPDs for *Bartonella* infection. In addition, the fleas were tested for host blood DNA to approximate the amount of host-switching that might have occurred before landing on their final destination. We hypothesized that most if not all of *Bartonella* found in these fleas should match with *B. washoensis*, and that positive hosts should yield more positive fleas, but did not rule out the possibility of finding infected fleas on culture negative hosts. The possibility of host-switching by these vectors was also thought to be minimal, but was not discounted.

Materials and Methods

Flea specimens were collected from live-caught black-tailed prairie dogs in Boulder County, Colorado in 2006 and were since frozen at -20°C. Both *O. hirsuta* and *P. simulans* were selected from culture positive and negative hosts. DNA extractions were performed on each sample using *MACHERY-NAGEL Nucleospin® Tissue* kits. Each sample was screened by PCR for the presence of *Bartonella* and host bloodmeal DNA.

Bartonella presence was indicated by amplification of the gene *rpoB*, which codes for the RNA polymerase beta-subunit (Renesto et al. 2001). The primers 1400F (5'-CGCATTGGCTTACTTCGTATG-3') and 2300R (5'-GTAGACTGATTAGAACGCTG-3') were used at conditions of a 2 min. denaturation at 94°C and a cycle of 94°C for 30 sec., 53°C for 30 sec., and 72°C for 1 min. repeated 35 times. Positive samples were purified using *MACHERY-NAGEL Nucleospin® Gel and PCR Cleanup* kits and cloned using competent JM-109 *E. coli* cells and *Promega pGEM-T* ligation vector. Multiple colonies were selected from pure culture plates for sequencing in order to detect a coinfection with multiple strains of *Bartonella* if they were present.

Host bloodmeal DNA was detected through the amplification of the vertebrate 16S rDNA region using the primers VerU-1 (5′-AAGACGAGAAGACCCYATGGA-3′) and VerU-2 (5′-CCTGATCCAACATMGAGGTCGTA-3′) (Kim et al. 2009). After denaturation at 94°C for 10 min. a cycle of 95°C for 30 sec., 55 °C for 30 sec. and 72°C for 90 sec. was repeated 35 times. Positive samples were purified using *MACHERY-NAGEL Nucleospin® Gel and PCR Cleanup* kits and sequenced.

Results

DNA was successfully extracted from 50 *O. hirsuta* each from culture positive and negative BTPD hosts and 30 *P. simulans* from culture positive and 50 *P. simulans* from culture negative hosts. Of the *O. hirsuta* collected, 16 (32%) tested positive for *Bartonella* from culture positive hosts and 8 (16%) tested positive from culture negative hosts. However, only 1 (3.3%) of *P. simulans* collected from culture positive hosts and 0 from culture negative hosts showed the presence of *Bartonella* DNA (Table 1). All positive samples sequenced to *B. washoensis*. In addition the majority of the samples tested positive for the

presence of vertebrate host bloodmeal DNA. Without the genetic sequence of BTPD (*C. ludovicianus*) uploaded to GenBank®, the closest match for all positive samples was white-tailed prairie dogs (*C. leucurus*).

Table 1: *Bartonella* prevalence (%) detected in fleas collected from both culture positive (H+) and negative (H-) black-tailed prairie dog hosts

Flea species	*Bartonella* prevalence	
	H+	H-
O. hirsuta	16/50 (32%)	8/50 (16%)
P. simulans	1/30 (3.3%)	0/50 (0%)

Bartonella presence was indicated by PCR amplification of the gene *rpoB*, which codes for the RNA polymerase beta-subunit

Discussion

Bartonella bacteria are associated with a number of natural mammalian reservoirs and different strains are thought to be relatively specific to each host species (Breitschwerdt and Kordick 2000). One proposed explanation for this host-specificity is that the spread of each strain may be limited by the narrow host range of the vector(s) that transmit it (Vayssier-Taussat et al. 2009). For example, some species of *Borrelia*, the Lyme disease agent, have been found to specialize to certain tick vectors that contain salivary proteins with immunosuppressive properties that assist the bacteria in infecting a host (de Taeye *et al.* 2013). We recognized a model system to study another potential example of such

specialization in the cycle of *B. washoensis* infection in black-tailed prairie dogs of the western U.S., parasitized by the highly specific *O. hirsuta* fleas. We found that these specialized vectors were indeed more likely than the more generalist *P. simulans* fleas also found on BTPDs to carry *B. washoensis*. In addition, fleas collected from *Bartonella*-infected hosts were the most likely to also be infected, but some individuals collected from uninfected hosts were also found to harbor *B. washoensis*.

A total of 24 of 100 (24%) of the specialized *O. hirsuta* fleas tested positive for *B. washoensis*, while only 1 of the 80 (1.25%) generalist *P. simulans* fleas were found to contain *B. washoensis* DNA. These results indeed suggest that *O. hirsuta*, the primary parasite found on BTPDs, is also the primary vector of *B. washoensis* infection, but that *P. simulans* may also be capable of transmitting the infection at a lower efficiency. These results also have implications for human health as *B. washoensis* has been shown to cause cardiac disease in people (Kosoy et al. 2003). Future work may include investigating the exact mechanism of potential *Bartonella* transmission to humans, and which areas and BTPD colonies pose the greatest risk to human populations based on which has the greatest proportion of *O. hirsuta* to other fleas found on these animals. However, the presence of more generalist fleas such as *P. simulans* must also be taken into account as these are the individuals that may carry the infection outside of the natural reservoir.

As expected, fleas collected from culture positive hosts were more likely to harbor *B. washoensis* infection than those collected from negative hosts, but some culture negative fleas were found to be infected. The fact that some individuals collected from uninfected hosts were found to carry *B. washoensis* is evidence that these vectors can feed on multiple hosts in a lifetime. In addition, the finding that not all fleas collected from positive hosts actually tested positive for *Bartonella* DNA reminds us that infection prevalence data for vectors does not always directly correlate to the infection prevalence in the host species. For

instance, due to the small size of the bloodmeal taken by flea vectors, it is possible that an infected flea may just not transmit a large enough dose of bacteria for infection to develop in the host. Therefore, it is crucial that researchers consider these trends as we continue to investigate *Bartonella* infection in these mammals as well as other natural reservoirs.

Acknowledgements

We would like to thank the Howard Hughes Medical Institute, the University of Richmond School of Arts and Sciences, and the University of Richmond Office of International Education for funding this research, as well as the members of the Nihon University College of Bioresource Sciences Laboratory of Veterinary Public Health in Fujisawa, Japan for their collaboration and guidance.

References

Abbot P, Aviles AE, Eller L, Durden LA. 2007. Mixed infections, cryptic diversity, and vector-borne pathogens: evidence from *Polygenis* fleas and *Bartonella* species. *Appl Environ Microbiol* 73: 6045–6052.

Bai, Y, Kosoy, MY, Ray, C, Brinkerhoff, RJ, Collinge, SK. 2008. Temporal and spatial patters of *Bartonella* infection in black-tailed prairie dogs (*Cynomys ludovicianus*). *Microb Ecol* 56: 373-82.

Breitschwerdt, EB and DL Kordick. 2000. *Bartonella* infection in animals: carriership, reservoir potential, pathogenicity, and zoonotic potential for human infection. *Clinical Microbio Reviews* 13(3): 428-438.

Brinkerhoff, RJ, Kabeya, H, Bai, Y, and S. Maruyama. 2010. Detection of multiple *Bartonella* species in digestive and reproductive tissues of fleas collected from sympatric mammals. *ISME* 4: 955-58.

Chang, CC, Chomel, BB, Kasten, RW, Romano, V, and N Tietze. 2001. Molecular evidence of *Bartonella* spp in questing adult *Ixodes pacificus* ticks in California. *J. Clin. Microbiol.* 39(4): 1221-26.

de Taeye, SW, Kreuk, L, van Dam, AP, Hovius, JW, and TJ Schuijt. 2013. Complement evasion by *Borrelia burgdorferi*: it takes three to tango. *Trends in Parasitology* 29(3): 119-28.

Kim, KS, Tsuda, Y, and A Yamada. 2009. Bloodmeal identification and detection of avian malaria parasite from mosquitoes (*Diptera: Culicidae*) inhabiting coastal areas of Tokyo Bay, Japan. *J. of Medical Ent.* 46(5): 1230-34.

Kosoy, M, Murray, M, Gilmore Jr., RD, Bai, Y, and KL Gage. 2003. *Bartonella* strains from ground squirrels are identical to *Bartonella washoensis* isolated from a human patient.

Renesto, P, Gouvernet, J, Drancourt, M, Veronique Roux, and D. Raoult. 2001. Use of *rpoB* gene analysis for detection and identification of *Bartonella* species. *J. Clin. Microbiol*. 29(2): 430-37.

Salkeld, DJ and P Stapp. 2008. Prevalence and abundance of fleas in black-tailed prairie dog burrows: implications for the transmission of plague (*Yersinia pestis)*. *J. Parasitol.* 94(3): 616-21.

Vayssier-Taussat, M, Le Rhun, D, Bonnet, S, and V Cotte. 2009. Insights in *Bartonella* host specificity. *Rickettsiology and Rickettsial Diseases-Fifth International Conference: Ann. N.Y. Acad. Sci.* 1166: 127–132.