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Review article

Ecological fitness and strategies of adaptation of *Bartonella* species to their hosts and vectors☆

Bruno B. CHOMEL[1]*, Henri-Jean BOULOUIS[2], Edward B. BREITSCHWERDT[3], Rickie W. KASTEN[1], Muriel VAYSSIER-TAUSSAT[2], Richard J. BIRTLES[4], Jane E. KOEHLER[5], Christoph DEHIO[6]

[1] Department of Population Health and Reproduction, School of Veterinary Medicine, University of California, Davis, CA 95616, USA
[2] UMR BIPAR ENVA/AFSSA/INRA/UPVM, École Nationale Vétérinaire d'Alfort, 7 avenue du Général de Gaulle, 94704 Maisons-Alfort, France
[3] Center for Comparative Medicine and Transitional Research, College of Veterinary Medicine, North Carolina State University, 4700 Hillsborough St., Raleigh, NC 27606, USA
[4] Department of Veterinary Pathology, Faculty of Veterinary Science, University of Liverpool, Leahurst, CH64 7TE, United Kingdom
[5] Division of Infectious Diseases, University of California, San Francisco, 513 Parnassus Avenue, Room S-380, San Francisco, CA, 94143-0654, USA
[6] Focal Area of Infection Biology Biozentrum, University of Basel, Klingelbergstrasse 50/70, CH-4056 Basel, Switzerland

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Abstract – *Bartonella* spp. are facultative intracellular bacteria that cause characteristic host-restricted hemotropic infections in mammals and are typically transmitted by blood-sucking arthropods. In the mammalian reservoir, these bacteria initially infect a yet unrecognized primary niche, which seeds organisms into the blood stream leading to the establishment of a long-lasting intra-erythrocytic bacteremia as the hall-mark of infection. Bacterial type IV secretion systems, which are supra-molecular transporters ancestrally related to bacterial conjugation systems, represent crucial pathogenicity factors that have contributed to a radial expansion of the *Bartonella* lineage in nature by facilitating adaptation to unique mammalian hosts. On the molecular level, the type IV secretion system VirB/VirD4 is known to translocate a cocktail of different effector proteins into host cells, which subvert multiple cellular functions to the benefit of the infecting pathogen. Furthermore, bacterial adhesins mediate a critical, early step in the pathogenesis of the bartonellae by binding to extracellular matrix components of host cells, which leads to firm bacterial adhesion to the cell surface as a prerequisite for the efficient translocation of type IV secretion effector proteins. The best-studied adhesins in bartonellae are the orthologous trimeric autotransporter adhesins, BadA in *Bartonella henselae* and the Vomp family in *Bartonella quintana*. Genetic diversity and strain variability also appear to enhance the ability of bartonellae to invade not only specific reservoir hosts, but also accidental hosts, as shown for *B. henselae*. Bartonellae have been identified in many different blood-sucking arthropods, in which they are typically found to cause extracellular infections of the mid-gut epithelium. Adaptation to specific vectors and reservoirs seems to be a common strategy of bartonellae for transmission and host diversity. However, knowledge regarding arthropod specificity/

☆ This manuscript is dedicated to the memory of Professor Yves Piemont (1951–2009) from Strasbourg University (France) whose significant contribution to the field of Bartonella knowlegde expended the number of known species and its epidemiology.

* Corresponding author: bbchomel@ucdavis.edu

restriction, the mode of transmission, and the bacterial factors involved in arthropod infection and transmission is still limited.

Bartonella **/ vector / ecological fitness / host adaptation / pathogenesis**

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1. INTRODUCTION

Bartonella spp. are fastidious, hemotropic Gram-negative bacteria that cause long-lasting intra-erythrocytic infections in their mammalian reservoirs and are mainly transmitted by arthropod vectors. With the exception of *Bartonella bacilliformis* and *Bartonella quintana*, most species or subspecies of *Bartonella* have been isolated or described on the basis of DNA sequences within the last fifteen years [19]. At least one mammalian reservoir host is known for each of the described *Bartonella* species. Infection studies employing cell cultures or animals, in combination with bacterial genetic analysis, allowed delineation of common parameters of the infection cycle in the mammalian reservoir host, as well as the identification of key bacterial factors involved in the infection process [48, 51]. In contrast, the predominant arthropod vector(s) and the mode of transmission for many novel *Bartonella* species remain elusive or essentially unstudied, as only three *Bartonella* species are known to infect and replicate in the digestive tract of their respective vectors: *B. quintana* in body lice [58, 107], *Bartonella henselae* in cat fleas [67] and, more recently, *Bartonella schoenbuchensis* in the deer ked [49]. Research, during the past two decades, has focused primarily on the natural reservoirs of *B. henselae*, the etiological agent of cat scratch disease (CSD), whereas many novel *Bartonella* spp. that are animal and human pathogens remain unstudied [19, 38, 39]. This review will consider the ecological fitness and different strategies of adaptation among various *Bartonella* species, their hosts and their vectors. Recent advances in the knowledge of such relationships between the pathogens, their hosts and their vectors will be

emphasized, and the substantial gaps that still exist in our knowledge of the adaptation of these bacteria to their hosts and vectors will be considered.

2. ADAPTATION OF *BARTONELLA* SPECIES TO THE ARTHROPOD VECTOR

In addition to sandflies for *B. bacilliformis* and body lice for *B. quintana*, fleas appear to play an important role in the maintenance and transmission of many *Bartonella* species among cat and rodent populations [95, 113, 128]. Data concerning the role of ticks and biting flies as potential vectors of *Bartonella* spp. are beginning to support tick transmission of these bacteria in nature [14, 42, 45, 49, 62, 96, 109].

2.1. Sandflies and *B. bacilliformis*

Lutzomyia verrucarum (Townsend, 1913) (Diptera: Psychodidae) is the natural vector of *B. bacilliformis,* agent of human bartonellosis (verruga peruana or Carrion's disease) [130]. Townsend was the first to propose *Phlebotomus* (now *Lutzomyia*) *verrucarum* as a potential vector of the Oroya fever agent based on epidemiological data [130]. Noguchi et al. [101] identified three *Lutzomyia* species as potential vectors and were able to reproduce *B. bacilliformis* bacteremia in monkeys by using crushed *L. verrucarum* and *L. noguchii* sandflies from Peru [101]. Battistini [10] established that sandfly bites can induce verruga peruana in monkeys under experimental conditions, and that disease manifestations could be reproduced in two Rhesus macaques (*Macaca mulatta*) inoculated with crushed sandflies collected in an area where Oroya fever was endemic. In 1942, Hertig [65, 66] definitively established the role of *L. verrucarum* as vector of Oroya fever by experimental transmission of *B. bacilliformis* to monkeys by wild *L. verrucarum* females. He also demonstrated the presence of *B. bacilliformis* in the midgut and feces of the sand flies and isolated the bacteria from the tip of the proboscis of *L. verrucarum* [66]. *L. verrucarum* is a native sandfly species of Peru [10]. Its geographic distribution occurs between latitudes 5° and 13° 25′ South in the occidental and inter-Andean valleys of the Andes. The altitudinal distribution of *L. verrucarum* in the different valleys is as follows: Occidental between 1 100 and 2 980 m above sea level and Inter-Andean from 1 200 to 3 200 m above sea level. Some discrepancies between the distribution of Carrion's disease and *L. verrucarum* suggest the existence of secondary vectors that cause transmission of *B. bacilliformis* in areas where *L. verrucarum* is not present [27, 28]. For instance, *L. peruensis* was the vector implicated in the outbreak that occurred in Cusco in the late 1990s [133]. Human bartonellosis has a seasonal transmission, with most cases reported in the months of January to June when rainfall is greatest [28]. Unfortunately, the adaptation of *B. bacilliformis* to *Lutzomyia* spp. has been poorly studied. Nevertheless, it was recently shown that a temperature shift from 37 °C (mammalian blood temperature) to 20 °C (expected temperature of vector) had a significant influence on the expression of the invasion-associated locus B (*ialB*) gene of *B. bacilliformis* [44]. Quantification of *ialB* protein using SDS-PAGE and immunoblotting showed the greatest amounts of IalB under acidic conditions or at 20 °C and lowest protein concentrations under basic conditions or at 37 °C.

2.2. Human body lice and *B. quintana*

The role of body lice (*Pediculus humanus corporis*) in the transmission of *B. quintana* was reported as early as 1920 [26]. Several experiments demonstrated that trench fever could be induced in human volunteers [134] and rhesus monkeys [99] by injection of cultured *B. quintana*. Experiments also revealed that the trench fever agent was not transmitted transovarially to the offspring of infected lice [25]. When feces from offspring of infected lice were scarified into the skin of non-infected patients, no clinical symptoms developed; whereas, infectious louse feces induced a febrile illness following scarification and application to human skin. The bacteria multiply in the gut lumen of naturally [135] or intra-rectally [69]

infected lice, without interfering with the viability of the lice [136]. *B. quintana* replicates extracellularly within the louse intestinal tract and attaches to the luminal surface of epithelial cells. Following intra-rectal inoculation of laboratory-reared lice, Vinson et al. [134] demonstrated viable *B. quintana* in the louse gut lumen. Furthermore, *B. quintana* was also visualized in feces collected from lice feeding on an infected patient for xenodiagnostic purposes [134]. Lice fed on human blood need to feed every day and generally feed at least 5 times a day [107]. An adult louse usually lives 20 to 30 days and prefers a temperature of 29 to 32 °C. Lice die at and above 50 °C [107]. Louse multiplication potential is impressive (up to 10% of females produce offspring daily) and louse eggs usually can survive for about two weeks after being laid. Lice infected with *B. quintana* remain so, and the bacterium multiplies throughout the louse intestinal tract, until they die [107]. The presence of chronically-infected, asymptomatic bacteremic patients is important to facilitate the continuous spread of *B. quintana* infection by the louse. Infectious organisms are excreted in louse feces [26, 58, 59, 69, 107]. *B. quintana* survives very well in louse feces and can remain infectious for up to one year [82]. Infection is thought to be transmitted by inoculation of *B. quintana* infected feces from lice to humans [82, 107]. Louse feces are inoculated when the person scratches himself or herself: lice usually defecate when feeding, providing a source of local irritation that results in itching, scratching and inoculation of *B. quintana*-containing feces [107].

A few years ago, a laboratory colony of human body lice was experimentally infected by feeding on rabbits made artificially bacteremic with a green fluorescent protein-expressing *B. quintana* [58]. *B. quintana* was detected in the louse gut where dense clusters of bacteria were identified in the intestinal lumen with masses of bacteria occupying an extracellular location. Between 7 and 20 (mean ± standard deviation = 16.3 ± 2.9) colony forming units (CFU) of *B. quintana* were obtained from each louse and that number did not decrease over time. *B. quintana* was also grown and PCR amplified from fecal samples harvested up to the death of the lice (about 35 days). As previously reported the life span of the infected louse was not modified by infection with *B. quintana*. In agreement with historical studies, no growth or PCR amplification of *B. quintana* was obtained from the 300 eggs and 100 larvae tested [58]. Furthermore, growth kinetics of *B. quintana* in body lice was recently characterized by a quantitative analysis of bacterial multiplication rate [124]. *B. quintana* started to proliferate in body lice by day 4 after ingestion and was constantly excreted in the feces for at least 3 weeks. The number of bacteria in feces reached a maximum of 10^7/louse per day on day 15 with an estimated *B. quintana* doubling time of 21.3 h. Scanning electron microscopy showed the presence of bacterial masses contained within a mesh-like structure in lice feces, which were confirmed to be *B. quintana* by immunofluorescence using a specific monoclonal antibody. Scanning electron microscopy identified *B. quintana* attached to the exopolysaccharide (EPS)-like matrix and fecal materials. It is generally considered that biofilms are characterized by an EPS matrix created by various bacteria, and this EPS matrix plays a vital role in the survival of bacteria [30], providing ecological advantages to the organisms. Therefore, the biofilm-like structure could contribute to the long survival of *B. quintana* in a fecal environment [82, 124]. *B. quintana* has been isolated or detected by PCR not only from humans and body lice, but also from a few naturally infected mammals (a cynomolgus monkey, two cats and two dogs with endocarditis) [22, 75, 85, 103] and from cat fleas [113] and questing ticks [33]. These recent data suggest that the epidemiological cycle of *B. quintana* could be more complex than previously thought, as several vectors and mammalian reservoirs could be involved in the transmission of *B. quintana*.

2.3. Fleas

B. henselae was first isolated from cat fleas (*Ctenocephalides felis*) collected from bacteremic cats in the early 1990s in northern California [77]. It was demonstrated experimentally that transmission of infection among cats could

be accomplished only when *B. henselae*-infected fleas were present [36]. Vector-mediated transmission of *B. henselae* isolates was evaluated by removing fleas from naturally bacteremic, flea-infested rescued cats and transferring these fleas to specific-pathogen-free (SPF) kittens housed in a controlled, arthropod-free animal facility. *B. henselae* bacteremia was detected in 89% of the 47 naturally infected cats. From these naturally infected cats, a total of 132 fleas were removed and were tested individually for the presence of *B. henselae* DNA by PCR. *B. henselae* DNA was detected in 34% of 132 fleas but without an association between the presence or the level of bacteremia in the corresponding cat. Fleas removed from the bacteremic cats transmitted *B. henselae* to five SPF kittens in two separate experiments. However, control SPF kittens housed with highly bacteremic kittens in the absence of fleas did not become infected.

It has been shown that *B. henselae* can multiply in the digestive system of the cat flea and survive several days in the flea feces. Fleas artificially fed a concentration of 1×10^5 CFU/mL of *B. henselae* in blood were examined over time using an immunofluorescent antibody assay and PCR [67]. Bacteria were present in the flea gut at 3 h post-feeding, and persisted up to 9 days after infection. Qualitatively, the density of *B. henselae* was greater in the flea gut at day 9, indicating that replication was occurring within the flea gut. *B. henselae* was also detected in the feces of infected fleas 9 days after infection and produced viable colonies upon inoculation onto 5% rabbit blood agar plates. In another experiment, the quantity of *B. henselae* in flea feces was below the level of detection when the fleas were transferred from the infected cats to a recipient cat; but after the fleas had fed on the recipient cat for 6 days, a bacteria level of 4×10^3 CFU/mg was detected in the flea feces [56]. Subsequently, the bacteria level increased for 4 days and then declined. In another experiment, the bacterial level in the flea feces was 1.8×10^3 CFU/mg at 2 h after collection and 3.33×10^2 CFU/mg at 72 h after collection [54]. These data confirm that *B. henselae* can multiply in the cat flea and indicate that *B. henselae* can persist in flea feces in the environment for at least 3 days.

It has been suggested that flea feces, analogous to louse feces and trench fever transmission, could be the principal infectious material inoculated by a cat scratch ultimately resulting in CSD [57]. In a series of experiments, only cats inoculated intradermally with infected flea feces (1 mL of saline containing 45 mg of feces from *B. henselae*-exposed fleas) became bacteremic, in contrast to those cats on which infected fleas were deposited in retention boxes, or cats that were fed infected fleas [57]. The cats that were injected with feces from infected fleas were positive by blood culture for *B. henselae* at 1 or 2 weeks after exposure and were the only cats to become bacteremic or seropositive by week 20. Therefore, the main source of infection for cats, and potentially humans, appears to be the flea feces that are inoculated by contaminated cat claws. The transmission strategy for *B. henselae* thus appears to involve replication in the gut of the arthropod vector (the cat flea), excretion in the flea feces, with subsequent survival in the environment for several days within the flea feces.

A role for cat saliva and for cat bites in the transmission of *B. henselae* or *B. quintana* has been suggested but never been fully investigated [22, 52, 70]. Finally, no work has been conducted to determine the sequential dispersion of *B. henselae* within cat fleas except to document the presence of *B. henselae* in the lumen of the flea gut. It will be of interest to determine whether viable *B. henselae* or other *Bartonella* species may also reside within the salivary glands of cat fleas and could therefore be a source of infection during a flea bite and blood meal.

Different genera of rodent fleas have been shown to be infected by some of the *Bartonella* species specifically adapted to rodents (e.g., *Xenopsylla* spp., *Ctenophthalmus* spp.) [95, 126]. However, documenting presence of *Bartonella* DNA in fleas by PCR testing does not establish vector competency. Nevertheless, *Ctenophthalmus nobilis nobilis* was demonstrated to be a competent vector for *Bartonella taylorii* and *B. grahamii* [20]. Because two different *Bartonella* species were transmitted by a

single species of flea, it suggests that vector-bacteria specificity among rodent *Bartonella* spp. may be less restricted. As stated by Bown et al. [20]: "Investigating the efficiency of different flea species in transmitting a variety of *Bartonella* species would be valuable as would determining whether fleas infected with a number of *Bartonella* species transmit one species more efficiently than the others. Studies such as these would help expand the current knowledge on vector-*Bartonella* specificity and determine its importance in influencing the diversity of *Bartonella* species." Although infection with rodent-adapted *Bartonella* spp. has been reported in both dogs and human beings, the route(s) of infection remains unknown.

2.4. Ticks

The first demonstration of the role of ticks in *Bartonella* infection was made by Noguchi who showed that *B. bacilliformis* could be recovered from tick viscera after feeding on bacteremic monkeys [100]. *B. bacilliformis* was transmitted from infected to normal rhesus monkeys by the bite of the tick, *Dermacentor andersoni*, only after a long period of feeding. The infection transmitted by ticks was mild, but definite, as shown by the recovery of *B. bacilliformis* from the lymph nodes and blood [100].

For *B. henselae*, the first report of a statistically significant association between CSD and exposure to ticks was published from Connecticut in a case control study showing that CSD cases were 29 times more likely to have been exposed to a kitten with fleas and 5.5 times more likely to have removed at least one tick than controls [140]. Lucey et al. published the first case report suggestive of human *B. henselae* infection associated with tick bite transmission [91]. A few years later, Eskow et al. [55] reported *B. henselae* as a potential human tick-borne pathogen. *B. henselae*-specific DNA was detected in four patients and in live deer ticks obtained from the households of 2 of the 4 patients. Some publications from Europe and Russia also reported the detection of *Bartonella* DNA in patients exposed to tick bites (reviewed in [14]).

Since the first reports of Kruzweska and Tylewska-Wierzbanowska [83], Schouls et al. [119] and Chang et al. [33, 34], hard ticks, including *Ixodes* spp., *Dermacentor* spp., *Rhipicephalus* spp., and *Haemaphysalis* have been shown to harbor *Bartonella* DNA when tested from many parts of the world [14]. Several epidemiological studies have associated the presence of *Bartonella* antibodies or DNA in dogs and infections by several tick borne pathogens, suggesting possible co-infections acquired from ticks [14, 39]. However, no proof of natural vector capacity has been published so far for *Bartonella* transmission by ticks.

Nevertheless, a recent publication reported successful experimental transmission of *B. henselae* from *I. ricinus* salivary glands to cats [45]. Using a membrane-feeding technique to infect *I. ricinus* with *B. henselae*, transmission of *B. henselae* within *I. ricinus* was demonstrated across developmental stages, as was migration or multiplication of *B. henselae* in salivary glands after a second meal, and transmission of viable and infective *B. henselae* from ticks to blood. Nine nymphs (100%) and 67% of the six adults fed on *B. henselae* infected blood at their preceding stages carried *B. henselae* in their gut, but bacterial DNA was not detected in the salivary glands. When molted nymphs ($n = 7$) and female ticks ($n = 3$) potentially infected with *B. henselae* at their previous developmental stage were fed again on uninfected blood, viable *B. henselae* were detected in 70% of the salivary glands after 84 h of engorgement, suggesting that during the initial period the bacteria were restricted to the gut, but after 84 h, *Bartonella* had disseminated to the salivary glands in the majority of ticks.

In order to determine whether the *B. henselae* present in salivary glands of nymph and adult ticks was infectious, two *Bartonella*-free cats were inoculated respectively with a pair of the salivary glands from 1 of the 7 nymphs and one pair of the salivary glands of 1 of the 3 female adult ticks. Viable and infective *B. henselae* was detected in the blood of the cats 1 to 2 weeks after experimental inoculation. To confirm that cats can be infected with *B. henselae* by tick bites, cat models of *B. henselae* transmission using infected ticks are needed. Further investigations and experiments are also needed to

evaluate the natural capacity of *I. ricinus* to transmit *B. henselae* to cats and humans and to evaluate the epidemiological importance of such transmission. Even if infrequent, tick transmission of *B. henselae* to individual patients would be of clinical importance. At present, limited attempts have been made to explore the molecular basis of *Bartonella* adaptation to arthropods, especially ticks, which is in contrast to the increasing body of knowledge relating to the molecular basis of arthropod exploitation by other tick-borne bacteria, such as *Borrelia burgdorferi* [131]. However, thanks to in vivo and in vitro infection models, effective molecular tools and the availability of the complete genome sequence data for an increasing number of *Bartonella* species, we can achieve a better understanding of the overall mechanisms of *Bartonella* adaptation to their vectors, and more specifically to ticks, in the coming years.

2.5. Biting flies

In recent years, biting flies have also been identified to carry *Bartonella* DNA [42, 49, 62, 96, 109]. Because of the very high prevalence of bacteremia in ruminants [31, 94] and the fact that fleas are generally not considered to be important ectoparasites of livestock [43], it was suspected that biting flies might be candidate vectors for the transmission of ruminant *Bartonella* spp. As blood-sucking ectoparasites of ruminants, flies of the family Hippoboscidae should be considered as candidates for the transmission of *Bartonella*. Among Hippoboscidae, *Lipoptena*, *Hippobosca*, and *Melophagus* are the three main fly genera that parasitize mammals [62]. The deer ked *Lipoptena cervi*, the predominant *Lipoptena* species in Europe, parasitizes cervids, whereas the louse fly (*Hippobosca equina*) parasitizes cows and horses, and the sheep ked (*Melophagus ovinus*) is a permanent ectoparasite of sheep (*Ovis aries*). In North America, the deer ked *Lipoptena mazamae* infests white-tailed deer (*Odocoileus virginianus*) [109].

The putative role of biting flies in *Bartonella* transmission among ruminants was investigated in France [62]. By amplification of the *Bartonella* citrate synthase gene, *Bartonella* DNA was detected in 85.5% of 83 Hippoboscidae, including 94% of 48 adult *L. cervi* flies, 71% of 17 adult *H. equina* flies, 100% of 20 adult *M. ovinus* flies, and 100% of 10 *M. ovinus* pupae. The amplified sequences were identical or closely related to *B. schoenbuchensis* and *B. chomelii*. Similarly, presence of *Bartonella* DNA was investigated in 104 horn flies (*Haematobia spp.*), 60 stable flies (*Stomoxys* spp.), 11 deer flies (*Chrysops* spp.), and 11 horse flies (*Tabanus* spp.) collected on cattle in California [42]. Partial sequencing identified *B. bovis* DNA in the horn fly pool and *B. henselae* genotype I DNA in one stable fly. However, these two studies did not investigate the mode of transmission of *Bartonella* spp. by these biting flies (mechanical versus biological). In another study, *B. schoenbuchensis* was isolated from deer keds (*L. cervi*) and shown to localize to the midgut of these blood-sucking arthropods, suggesting again potential stercorarian (fecal) transmission [49]. Interestingly, French foals were observed to seroconvert to *B. bovis* antigens during the spring-summer season[1], suggesting the role of *H. equina* in the transmission of *B. bovis* from its bovine reservoir to horses.

In the USA, 40 deer keds, *L. mazamae*, collected from white-tailed deer and humans in Georgia and South Carolina, were screened for the presence of DNA from *Bartonella* spp. by PCR using primers specific to the *Bartonella* riboflavin synthase gene [109]. *Bartonella* species closely related to *B. schoenbuchensis* and to *B. henselae* were detected in 10 keds and 1 ked, respectively. European deer keds (*L. cervi*) are thought to have been introduced into New England from Europe during the 1 800s [96]. A study was recently conducted to determine whether *L. cervi* from Massachusetts deer contained evidence of infection by *B. schoenbuchensis*, which appears to be maintained by *L. cervi* in Europe. Five of six keds were found to contain *B. schoenbuchensis* DNA [96].

[1] Boulouis H.J., unpublished data.

3. ADAPTATION OF *BARTONELLA* SPECIES TO THE MAMMALIAN HOST

3.1. Life cycle in the mammalian reservoir host

The common theme in *Bartonella* infection in mammalian hosts is a prolonged period of intra-erythrocytic bacteremia, which represents a specific adaptation to the mode of transmission by blood-sucking arthropods. The establishment of a chronic intra-erythrocytic bacteremia takes place exclusively in the mammalian reservoir host(s). Most *Bartonella* species appear to be restricted to cause intra-erythrocytic infection in a single mammalian species, e.g., *B. bacilliformis* in humans or *B. henselae* in domestic cats, while other species have several, but typically closely related mammalian reservoir hosts, e.g., *B. bovis* or *B. schoenbuchensis* infecting various ruminant hosts, such as roe deer, red deer [47], mule deer, elk and cattle [31, 114]. Depending on the level of host adaptation, these infections in the reservoir host range from an asymptomatic or sub-clinical (most animal-specific species) to clinical manifestations with low morbidity and limited mortality (such as human-specific *B. quintana* infections), or even to life-threatening disease, such as severe hemolytic anemia associated with the human-specific infection by *B. bacilliformis*. Besides erythrocytes, endothelial cells represent another major target cell type for bartonellae within their mammalian hosts. The phenomenon of host-restriction might be predominately determined by the specific capacity of a *Bartonella* species to preferentially infect the erythrocytes of a given mammalian host, while endothelial cells may also become infected during incidental infection of non-reservoir hosts (e.g., human infection by cat-specific *B. henselae*, which may lead to bacillary angiomatosis (BA) lesions where bacteria are found in close association with proliferating endothelial cells) [50]. However, further studies in multiple species need to be conducted to verify this hypothesis.

The establishment and course of the intra-erythrocytic bacteremia as an infection strategy of the bartonellae has been studied by experimental animal infection using different models [1, 18, 78, 121, 141]. The characteristics and course of intra-erythrocytic bacteremia are similar for all these models, with the most detailed information being available for the rat model of *B. tribocorum* infection [121] (Fig. 1).

Following intravenous inoculation with in vitro-grown *B. tribocorum*, bacteria appeared unable of entering erythrocytes and instead seemed to be rapidly cleared from circulating blood and remain below detectable levels for about four days. The niche that supports bacterial replication during this time has not been identified experimentally. However, the marked tropism of *Bartonella* spp. for endothelial cells and their proximity to the bloodstream suggest that endothelial cells are an important constituent of this primary niche [50]. Although not yet supported by experimental data, it is tempting to speculate that the primary niche may also comprise other cell types of the reticulo-endothelial system, i.e., migratory cells, such as dendritic cells, which might assist the passage of bacteria from the site of inoculation (e.g., bacteria in arthropod feces that are superficially scratched into the skin) to the primary site of infection. Typically, on day 5 post-infection with *B. tribocorum*, large numbers of bacteria are released from the primary niche into the bloodstream [47]. Further episodes of synchronous release of bacteria follow at intervals of approximately five days, probably as a result of the five-day infection cycle that is triggered by re-infection of the primary niche by bacteria released at the end of each cycle. The approximately five-day periodicity of human trench fever caused by *B. quintana* could reflect these bursts of synchronous bacterial release into the bloodstream [47]. When *B. tribocorum* is released into the bloodstream of a rat, these bacteria efficiently adhere to mature erythrocytes, indicating that they become competent for erythrocyte interaction during colonization of the primary niche. Following adhesion to erythrocytes, bacteria invade and replicate intracellularly within a membrane-bound compartment until a critical density is reached. Thereafter, the number of intracellular bacteria remains static for the remaining lifespan of the infected erythrocytes, which is indistinguishable from that of uninfected erythrocytes

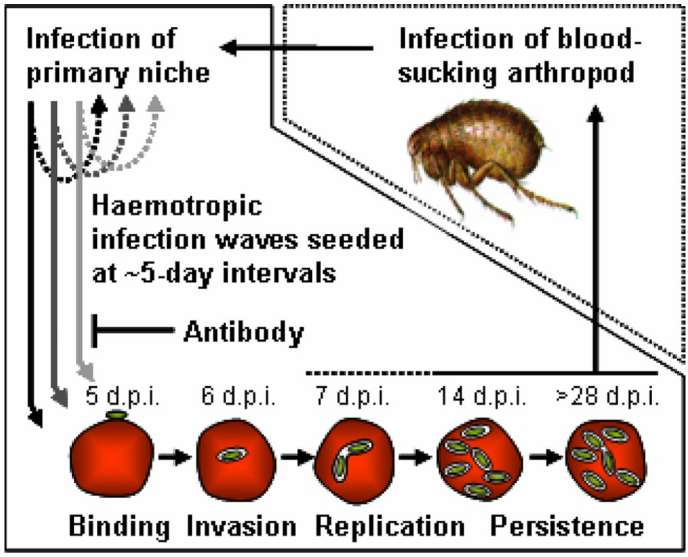


Figure 1. Model of the common infection cycle of *Bartonella* spp. in their revervoir host(s). The different infection stages in the mammalian reservoir have been resolved by tracking bacteria in the blood of experimentally infected animals (lined box), while little is known on the mode of infection of the blood-sucking arthropod vector (dashed-lined box); d.p.i. (days post-infection). Adapted from Schulein et al. [121].

[121]. *B. tribocorum* can therefore persist for several weeks in circulating blood in an immunologically privileged intracellular niche without causing apparent harm to the infected rat. This hemotropic infection strategy is probably a specific adaptation to the transmission by blood-sucking arthropod vectors and is presumably shared by most *Bartonella* species. The exception to this rule is *B. bacilliformis*, which triggers massive hemolysis of colonized human erythrocytes, giving rise to an often fatal hemolytic anemia.

The intra-erythrocytic bacteremia caused by *B. tribocorum* in rats drops below detectable levels after approximately 10 weeks, and a similar duration of detectable bacteremia has been observed in some other experimental models of *Bartonella* infection [105, 137, 138]. However, long-lasting bacteremia has been demonstrated in cats infected with feline strains of *B. henselae* or *B. clarridgeiae* [6, 137, 138]. The *B. grahamii* model of mouse infection has shown an important role for antibodies in controlling intra-erythrocytic infection [78]. However, antibodies are unlikely to function against infected erythrocytes, as the lifespan of these cells is similar to that of uninfected erythrocytes. Instead antibodies might neutralize bacteria that are released form the primary niche and thereby abrogate the infection of additional erythrocytes as well as prevent re-infection of the primary niche.

3.2. *Bartonella* pathogenicity factors involved in adaptation to the mammalian host

In recent years, studies of *Bartonella* pathogenesis and the involved bacterial pathogenicity factors have taken a big step forward. The combination of bacterial genomics, genetics, as well as in vitro cell culture and in vivo animal models of *Bartonella* infection provided initial insight into the molecular mechanisms that govern the evolution, ecology and host interactions of these elusive pathogens [48, 51, 54].

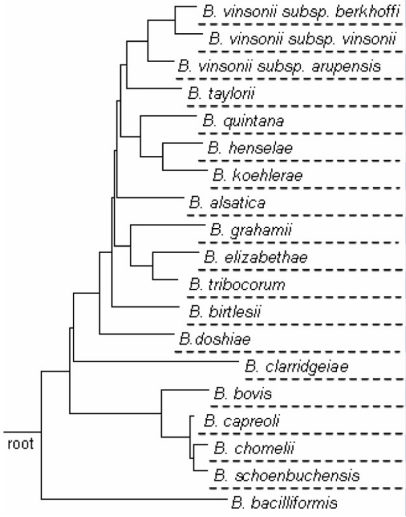
With the release in 2004 of complete genomic sequences of *B. henselae* and *B. quintana*, the genus *Bartonella* entered the post-genomic

area [3]. Since then, the complete genome sequences of *B. bacilliformis* (GenBank accession number CP000525) and *B. tribocorum* were also released [116] and genome sequencing of several other *Bartonella* species is in an advanced state [54]. The circular chromosomes of the four sequenced bartonellae range in size from 1.44 Mb (encoding 1 283 genes) to 2.62 Mb (encoding 2 136 genes). Comparative genome analysis revealed that only 959 genes are conserved in all these genomes. This relatively small core genome reflects specific adaptations to the genus-specific lifestyle. For instance, a striking example of host-integrated metabolism is represented by hemin. This important source for iron and porphyrin is particularly abundant in the host niches colonized by bartonellae, i.e., the intracellular space of erythrocytes and the mid-gut lumen of blood-sucking arthropods. The strict hemin requirement for growth of *B. quintana* (and probably other bartonellae) in vitro correlates with the presence of multiple genes encoding hemin binding and hemin uptake protein, because no hemin biosynthesis enzyme is encoded by this organism [3]. A large-scale signature tagged mutagenesis screen (STM) [115] in the *B. tribocorum*-rat model identified several of the hemin-uptake genes as essential for establishing intra-erythrocytic infection [116]. Moreover, this screen revealed that the majority of pathogenicity factors required for establishing intra-erythrocytic bacteremia is encoded by the core genome of the four available genome sequences (66 of 97 pathogenicity genes), indicating that the genus-specific infection strategy is primarily dependent on a conserved set of core genome-encoded pathogenicity factors.

All genes that are not strictly conserved in all four sequenced *Bartonella* species are excluded from the core genome and thus constitute the accessory genome of the bartonellae. To a large extent, the genes of the accessory genomes are organized in genomic islands, which were acquired by horizontal gene transfer and could have been lost again by deletion in a lineage-specific manner. The large-scale STM screen performed by Saenz et al. [116] in the *B. tribocorum*-rat model indicated that some of the genomic islands encoded by the accessory genome play an important role in *Bartonella*-host cell interaction and, moreover, represent factors that contribute to the capacity of the bartonellae to adapt to their hosts. The so-called type IV secretion systems represent the most prominent example of such a host adaptability factor.

3.2.1. *Role of* Bartonella *type IV secretion systems in host adaptation*

Type IV secretion systems are transporters of Gram-negative bacteria that are ancestrally related to bacterial conjugation systems mediating interbacterial DNA transfer. A variety of bacteria that intimately interact with eukaryotic hosts – both pathogens and symbionts – have adopted type IV secretion systems to translocate bacterial effector molecules from the bacterial cytoplasm directly into the host cell cytoplasm, where they subvert cellular functions in favor of the interacting bacterium [41]. The accessory genome of *Bartonella* encodes at least three distinct type IV secretion systems, which all are absent from the genome of *B. bacilliformis*. This deadly human pathogen holds an isolated position in the *Bartonella* phylogeny as the sole representative of an ancestral lineage. All other species evolved in a separate "modern" lineage by radial speciation and represent highly host-adapted pathogens of limited virulence potential. This high degree of evolutionary adaptation influences disease expression in persistently infected individuals, particularly in those instances in which concurrent or sequential infection with other pathogens occurs or when there is viral, age-related or drug induced immunosuppression. Unlike *B. bacilliformis*, the species of the modern lineage encode at least one of the type IV secretion systems, VirB/VirD4, Vbh or Trw [116] (Fig. 2). As exemplified by the ancestral *B. bacilliformis* lineage, the common infection strategy of the bartonellae (i.e., colonization of endothelial cells and erythrocytes leading to a long-lasting intra-erythrocytic infection) evolved initially without the contribution of type IV secretion systems. However, within the radially expanding modern lineage, the horizontally-acquired type IV secretion systems adopted diverse



Arthropod host(s)	Mammalian host(s)		T4SS locus		
	Reservoir	Incidental	*vbh*	*virB*	*trw*
Tick	Dog	Human	–	+	+
Vole ear mite	Vole		–	+	+
Tick	Mouse	Human	–	+	+
?	Mouse, vole		–	+	+
Body louse	Human		–	+	+
Cat flea, tick	Cat	Human	–	+	+
?	Cat	Human	–	+	+
?	Rabbit	Human	–	+	+
Fleas	Mouse, vole	Human	+	+	+
?	Rat	Human	+	+	+
?	Rat		+	+	+
?	Mouse		+	+	+
?	Vole		+	+	+
?	Cat		–	+	–
?	Cattle		+	–	–
?	Roe deer		+	–	–
?	Cattle		+	–	–
Deer ked	Roe deer		+	–	–
Sandfly	Human		–	–	–

Figure 2. Phylogeny, arthropod vectors and mammalian hosts of the genus *Bartonella* and the distribution of type IV secretion systems. Left: Phylogenetic tree of the genus *Bartonella* based on multilocus sequence analysis (MLSA, [116]). Right: summary table of the host specificity and the presence and absence of loci encoding type IV secretion systems in the different *Bartonella* species. Adapted from Dehio [51].

essential functions in this common infection process [120, 122, 125]. These include the subversion of vascular endothelial cell functions by bacterial effector proteins translocated by the closely related VirB-like type IV secretion systems VirB/VirD4 and Vbh [116–118, 123], and the interaction with erythrocytes by pilus-associated variant surface proteins expressed by the Trw type IV secretion system [125], which is not known to translocate any effector. Importantly, these type IV secretion systems have probably also facilitated the remarkable evolutionary success of the modern lineage by conferring host adaptability, which resulted in reduced virulence properties in a given host (providing a long-term fitness advantage for bacteria due to reduced direct structural or immunological damage to the infected host), and the adaptation of the generally host-restricted bartonellae to novel hosts [51]. The molecular mechanisms facilitating type IV secretion system–dependent host adaptability remain elusive. However, gene duplication and diversification by combinatorial sequence shuffling and point mutations seem to have contributed to the fast evolution of the translocated protein effectors of the VirB-like type IV secretion systems [116] and the surface-expressed pilus components of the Trw type IV secretion system [102]. Future research should focus on the elucidation of the molecular interactions of type IV secretion systems and their effectors with mammalian host cells and the mechanisms of molecular evolution that govern host adaptability as a novel role for type IV secretion systems in bacterial pathogenesis [51].

3.2.2. *Role of trimeric autotransporter adhesins (TAA) in host adaptation and pathogenicity of* Bartonella *species*

Bacterial adhesins mediate a critical, early step in the pathogenesis of most bacterial infections. Adhesins bind to extracellular matrix components of host cells [89], and can facilitate

approximation of the bacterium and the host cell so that Type III and Type IV secretion of bacterial effectors into the host cell can occur. The best-studied adhesins in bartonellae are the orthologous TAA, BadA in *B. henselae* [110], and the Vomp family in *B. quintana* [141]. The *B. henselae* BadA is a very large, 340 kDa protein; in contrast, there are four, 100 kDa *B. quintana* Vomp family members that are encoded by four tandemly arranged, closely related *vomp* genes. BrpA, a *B. vinsonii* subsp. *arupensis* ortholog, has also been described, and is a 382 kDa protein [60].

These *Bartonella* TAA are non-pilus-associated adhesins on the bacterial cell surface, which have a demonstrated role in fibronectin and endothelial cell binding by *B. henselae* [110], and in autoaggregation and collagen binding by both *B. quintana* [141] and *B. henselae* [110]. Recent functional characterization of BadA revealed that the head domain confers the ability of *B. henselae* to bind host endothelial cells and collagen, but not fibronectin [74]. Finally, it has been shown that the *B. quintana* Vomp constitutes critical virulence determinants for in vivo infection [92], and it is likely that the orthologous TAA in other *Bartonella* species are similarly necessary for infection in vivo.

Because the *Bartonella* TAA are adhesins, they represent critical, surface-exposed virulence factors that are rapidly targeted by the host immune system. Indeed, several groups have demonstrated that the *Bartonella* TAA represent immunodominant antigens [16, 60, 110]. When the host produces antibodies targeted against the invading microorganism, the infecting pathogen is usually killed. However, if the pathogen alters the protein expressed (antigenic variation), or no longer expresses the protein on its surface (phase variation), the microorganism can survive and multiply in the host [111]. For vector-borne pathogens, this strategy enables the pathogen to persist and achieve the high density in the bloodstream necessary to be passed from the mammalian host to the hematophagous arthropod vector in order to be transmitted to a new host [8].

It has been hypothesized that the modular, repetitive DNA sequences in the stalk domains of BadA and the Vomp increase the frequency of recombination to escape host immune targeting [88]. In addition to the potential for intragenic *badA* or *vomp* recombination, the *vomp* locus consists of four highly conserved genes, each of which has the internally repetitive stalk domains. In addition, they are tandemly arranged, both of which can facilitate recombination and deletion of *vomp* genes, with potential generation of altered Vomp on the surface, or loss of surface expression of one or more Vomp (phase variation). Evidence for in vivo phase variation of the *vomp* was demonstrated in an animal model of *B. quintana* infection, with deletion occurring within the *vomp* locus during persistent bloodstream infection [141]. These phase and antigenic variation strategies could be utilized by *Bartonella* species to accomplish binding to different host cell types as well as to avoid immune targeting by the host.

BadA and the Vomp are multifunctional proteins: in addition to host cell binding, they are involved in eliciting a proangiogenic host cell response that is unique to *B. henselae* and *B. quintana* infections in immunocompromised humans. Immunocompromised patients infected with *B. quintana* or *B. henselae* can develop vascular skin lesions known as BA [76, 86]. Histopathological examination of these BA lesions reveals micro colonies of *Bartonella* bacilli adjacent to proliferating endothelial cells [86]. Although the precise signaling cascade involved in BA angiogenesis is not yet identified, expression of BadA or the Vomp is important for activation of HIF-1 and secretion of proangiogenic cytokines such as vascular endothelial growth factor. This angiogenesis could represent a further adaptation of hemin-requiring *Bartonella*: when a compromised immune system permits exit of *Bartonella* from the bloodstream and multiplication in the tissues where access to hemin is limited, new vessels are formed that could provide this essential nutrient. Further study of the many virulence functions of the *Bartonella* TAA will provide insight into the mechanisms involved in the dynamic interaction between *Bartonella* species and their cognate mammalian host.

3.3. Strategies utilized by *Bartonella* to escape the host immune response: current evidence

Bartonellae are probably able to escape the host immune response by surface antigen variation in clonal populations (see section above) and through the wide diversity of strains and species that can co-infect the host (as shown for *B. henselae* strains in cats). Co-infection of cats with *B. henselae* was first described by Bergmans et al. [13] and Gurfield et al. [61] in the 1990s, as was re-infection of cats with different strains [6]. Interestingly, most of the strains involved in human cases of CSD reported at that time belonged to genotype I (also known as Houston I type), whereas most strains identified in cats were within the genotype II group [12, 53]. Examination of the 16-23S intergenic spacer region revealed different point mutations in different strains [53]. These authors concluded that human isolates come from a limited subset of *B. henselae* strains. More recent analysis using multi-locus sequence typing (MLST) [5, 68, 87, 132] or multiple-locus variable-number tandem-repeat analysis (MLVA) also support the wide diversity of feline strains and the clustering of human cases within the broad range of feline isolates [17, 98]. A small percentage of *B. henselae* isolates may harbor two different 16S rRNA gene copies and these isolates may have emerged by horizontal gene transfer [132].

The emergence of genetically distinct organisms at various peaks of bacteremia could also contribute to the establishment of persistent infection in the naturally infected cats, as reported by Kabeya et al. [71]. Although antibodies appear to play an important role in the immune response that follows the initial infection with a *Bartonella* species, humoral immunity may contribute to ongoing suppression of the persistent or relapsing bacteremia [72, 73]. Based on experimental infection studies involving *B. vinsonii* subsp. *berkhoffii* in dogs, it is also possible that some *Bartonella* species directly suppress several components of the host immune response [104, 105]. In addition, lipopolysaccharide (LPS) from *B. quintana* has been shown to be a Toll-like receptor 4 (TLR-4) antagonist, which results in inhibition of both mRNA transcription and the release of tumor necrosis factor alpha, interleukin 1beta (IL-1beta), and IL-6 from human monocytes when stimulated by *Escherichia coli* LPS [106]. Administration of *B. quintana* LPS to mice resulted in TLR-4 antagonism, suppression of the severity of experimental arthritis and interruption of the inflammatory loop in a murine model of autoimmune destructive arthritis [2]. The structurally resolved LPS of *B. henselae* was also reported to lack any TLR-4 activating activity, which probably reflects penta-acetylation of the lipid A moiety and the incorporation of a long-chain fatty acid [139]. Infection with a *Bartonella* species, particularly in an incidental host, could result in systemic and local suppression of immunity which would facilitate persistent *Bartonella* infection and could result in concurrent opportunistic infections, autoimmunity or immune-mediated target organ destruction.

Generation of genetic variants is a possible strategy to circumvent the host specific immune responses. In the study by Berghoff et al., up to three genetic variants were detected within 20 (58.8%) isolates, indicating that most primary isolates display a mosaic-like structure [11]. The close relatedness of the genetic variants within an isolate was confirmed by MLST. In contrast to the primary isolates, no genetic variants were detected within the progeny of 20 experimental clones generated in vitro from 20 primary isolates, suggesting that the variants were not induced in vitro during the procedure as defined by PFGE analysis. Hence, the genetic variants within a primary isolate most likely originally emerged in vivo and potentially due to immune pressure. In the study by Iredell et al. [68], the distribution of sequence types among *B. henselae* isolates recovered from human infections was not random, and such isolates were significantly more often associated with one particular sequence type, lending further support to the suggestion that specific genotypes contribute disproportionately to the disease burden in humans. All but one isolate belonged to lineages that bore the representative strain of either the Houston or Marseille subtype. The inheritances of several of the genes studied could not be reconciled

with one another, providing further indication for horizontal gene transfer among *B. henselae* strains and suggesting that recombination has a role in shaping the genetic character of bartonellae.

Phase variation has also been reported [84]. Outer-membrane protein variation is seen in association with phase variation, but LPS expression is preserved in piliated as well as extensively passaged non-piliated isolates [84]. The EagI/HhaI infrequent restriction site-PCR fingerprint, which has been previously used to discriminate between serotypes Marseille and Houston, is altered with phase variation in vitro, and there is evidence that genetic change accompanies these events. The extent of genetic and phenotypic variability of phase-variant *B. henselae* must be considered when studying persistent infection or re-infection.

4. FITNESS OF *BARTONELLA* SPECIES TO THEIR NATURAL HOSTS: EPIDEMIOLOGICAL AND CLINICAL ASPECTS

Commensalism or even symbiotic interactions are optimal strategies for bacteria to adapt to their host [46]. However, in some instances or under certain poorly defined circumstances, the commensal relationship of reservoir-adapted *Bartonella* species to the host is imperfect, resulting in a stealth pathogen strategy [97]. Studies on the heritable symbionts of insects have yielded valuable information about how bacteria infect host cells, avoid immune responses, and manipulate host physiology. Furthermore, some symbionts use many of the same mechanisms as pathogens to infect hosts and evade immune responses. In order to allow successful transmission for blood-borne vector-borne pathogens, it is necessary to have sufficient susceptible individuals, but also enough infected animals to sustain vector infection and subsequent transmission cycles. Furthermore, it is also important for transmission that blood feeding insects have access to reservoir animals that are bacteremic for a long enough period of time and at levels such that the vectors can become fully infected. Finally, subclinical infections or healthy carriage could favor long-term infection and provide a source of infected animals from which vectors can themselves become infected.

4.1. High prevalence and long-term *Bartonella* bacteremia in reservoir mammals

High prevalence and long-term bacteremia in reservoir mammals represent common ecological and clinical strategies of the bartonellae. Most mammals that are reservoirs of *Bartonella* species have at any time about one fourth to one third of their population that is bacteremic. For example, bacteremia levels have been shown to be between 53% and 84% in stray cats from France and California [35, 63]. Similarly, 28% of 109 coyotes were found to be bacteremic with *B. vinsonii* subsp. *berkhoffii* in central coastal California [32] and prevalence of bacteremia in rodents also ranges between 42.2% (118 of 279 samples) in the southeastern USA [81] and 64% (70 of 109 samples) in woodland rodents in the United Kingdom [15]. Much higher prevalence of bacteremia has been detected in domestic and wild ruminants, with active infection in up to 90% of some deer and cattle herds [31, 94]. In cats and dogs, bacteremia has been detected in some animals for several months and for periods up to 2 years [1, 6, 79, 80]. However, it cannot be excluded that some of these cats were re-infected over time. In humans experimentally infected with *B. quintana*, bacteremia lasted up to 300–443 days after the onset of trench fever [82]. Recently, evidence of persistent blood infection with *B. henselae* and *B. vinsonii* subsp. *berkhoffii* has been reported in immunocompetent humans in the USA [21, 23].

4.2. The mammalian reservoir hosts as healthy carriers of *Bartonella* species

Another adaptive strategy of bartonellae is to achieve infection that does not result in massive organ damage but instead produces chronic infection with asymptomatic, prolonged bacteremia. For example, native populations of the Andes can develop verruga peruana, a benign cutaneous infection caused by *B. bacilliformis*

that can lead to a carriage of the bacterium in up to 10% of the local population. In contrast, the non-native population, when infected, often develops a severe and potentially fatal disease known as Oroya fever [9, 37]. Similarly, asymptomatic carriers of *B. quintana* have been reported since the 1940s, and persistent bacteremia in homeless people with few or no symptoms was identified in urban outbreaks of *B. quintana* infection [82]. In France, 14% of 71 homeless people were bacteremic with *B. quintana*, and of those bacteremic patients, 80% were afebrile [24]. Such healthy carriage has been reported for most animal reservoir species of bartonellae, including cats [19], coyotes [32], or cattle [31, 94].

Infectivity can also be enhanced by immunocompromising human behaviors, as shown for the increased level of *B. quintana* erythrocyte infection in alcoholics [112]. When comparing the growth and the number of bacteria per erythrocyte in vitro in laboratory-infected red blood cells from alcoholic patients versus normal blood donor erythrocytes, the distribution of bacteria per erythrocyte in the two groups was different, although the number of bacteria after 4 days of incubation was similar in the two groups of erythrocytes. Erythrocytes from alcoholics contain significantly more bacteria per cell than erythrocytes from blood donors. These results suggest that alcoholics may be more prone to *B. quintana* infections, potentially due to more efficient invasion or replication within macrocytes as compared to mature erythrocytes.

4.3. Reservoir specialization: a co-evolution strategy

Bartonellae have also evolved towards adaptation to a specific environmental and ecological niche and have specialized to optimize fit with a given vector and reservoir host. A large number of *Bartonella* species have adapted to fleas, especially flea species infecting rodents or felids [20, 36]. However, sand flies, biting flies, including deer and sheep keds, and ticks have also been shown to harbor *Bartonella* species, even though not all these insects have been shown yet to be competent vectors. Host adaptation is also evident, as some *Bartonella* species are mainly found in very specific mammalian species, which are their natural reservoirs. For example, *B. vinsonii* subspecies *berkhoffii* has mainly been isolated in canids (domestic dogs, coyotes, and gray foxes) and exceptionally in other species, such as felids or humans, but never in the thousands of rodents tested for *Bartonella* infection. Experimental inoculation of cats with a canid strain of *B. vinsonii* subsp. *berkhoffii* did not produce any detectable bacteremia in these cats[2]. *B. bacilliformis* has been isolated only from humans and *B. alsatica* only from rabbits, except in two human cases for whom rabbit exposure was demonstrated [4, 64, 108]. In rodents, adaptation to a specific host seems to occur in many parts of the world [81, 129], whereas it is less evident in some other rodent populations, such as those described in the United Kingdom [15]. However, recent data support the hypothesis of rodent adaptation [128, 129]. Whereas previous studies had failed to uncover species differences, *B. doshiae* and *B. taylorii* were more prevalent in wood mice, and *B. birtlesii* was more prevalent in bank voles [129]. *B. birtlesii*, *B. grahamii* and *B. taylorii* peaked in prevalence in the fall, whereas *B. doshiae* peaked in spring. For *B. birtlesii* in bank voles, density dependence was direct, but for *B. taylorii* in wood mice density dependence was delayed. *B. birtlesii* prevalence in wood mice was related to bank vole density. Similar trends were revealed in a study of temporal and spatial patterns of *Bartonella* infection in black-tailed prairie dogs (*Cynomys ludovicianus*) [7]. Infection rates exhibited a sigmoidal response to body mass, such that 700 g may prove to be a useful threshold value to evaluate the likelihood of *Bartonella* infection in black-tailed prairie dogs. *Bartonella* prevalence increased throughout the testing season for each year, as newly emerged juveniles developed bacteremia. Data from recaptured animals suggest that *Bartonella* infections did not persist in individual animals, which may explain the relatively low prevalence of *Bartonella* in black-tailed

[2] Chomel B.B. et al., unpublished data.

prairie dogs compared to other rodent species. No association was found between *Bartonella* prevalence and host population density.

Interestingly, bartonellae appear to have evolved within their own reservoirs in disparate geographic locations, because several genotypes of *B. henselae* [11, 12, 18, 38] or *B. vinsonii* subsp. *berkhoffii* have now been identified [29, 39, 93] and specific genotypes appear to be found more often in specific geographical areas. Similarly, bartonellae isolated from wild felids (pumas, bobcats or cheetahs) are genetically distinct from *B. henselae* isolated from domestic cats [40].

4.4. Biological competition and disruption of host-*Bartonella* systems by introduction of non-native host species

The potential of biological invasions to threaten native ecosystems is well recognized and introduction of an alternative host can modify native host-parasite dynamics. The influence of the bank vole (*Clethrionomys glareolus*) accidentally introduced in Ireland on the epidemiology of infections caused by flea-transmitted bartonellae in native wood mice (*Apodemus sylvaticus*) has been quantified [127]. *Bartonella* infections were detected on either side of the introduction zone (front), but occurred exclusively in wood mice, despite being highly prevalent in both rodent species elsewhere in Europe. Bank vole introduction has, however, affected the wood mouse-*Bartonella* interaction, with the infection prevalence of both *B. birtlesii* and *B. taylorii* declining significantly with increasing bank vole density. Whilst flea prevalence among wood mice increases with increasing wood mouse density in areas without bank voles, no such relationship is detected in vole-invaded areas. The results are consistent with the dilution effect hypothesis [90]. This predicts that for vector-transmitted parasites, the presence of less competent host species may reduce infection prevalence within the principal host. In addition a negative relationship between *B. birtlesii* and *B. taylorii* prevalence was found, indicating that these two intravascular microparasites may compete within the host.

5. CONCLUSIONS

Bartonellae have adapted to a very specific environment allowing them to escape the host immune response by invading mammalian red blood cells. Furthermore, their vector strategy, demonstrated for at least a few species of *Bartonella*, is based on stercorarian transmission with replication in the vector midgut and survival in the environment in the vector feces. Host specificity of bartonellae is at the convergence of an ecosystem made from three main components, including the host specificity of the arthropod vectors, the mammalian host specificity of *Bartonella* species and the environment in which the hosts, the bacteria and vectors evolved. This ecosystem depends on:

- Host specificity of the arthropod vectors, because there is a preferential host for each *Bartonella* vector, such as *Lutzomyia* for humans, body lice for humans, Hippoboscidae for ruminants, and fleas for cats or rodents. However, this vector-host specificity is not exclusive, explaining the transmission to accidental hosts.
- Mammalian host specificity of *Bartonella* species, as there is an adaptation of *Bartonella* species to a specific mammalian host, such as *B. henselae* to the cat, *B. bacilliformis* to humans, or candidatus "*B. washoensis*" to ground squirrels. However, that specificity is again not exclusive, because some *Bartonella* species can be isolated from mammals different from their preferred host.
- And environment, in which the hosts, the bacteria and the vectors evolved, which has limited the conditions in which the bacteria, the vectors and the preferred hosts can interact. Massive natural or man-made changes to historically stable ecosystems that result in alterations in vector biology and reservoir host density, increased international movement of a wide range of reservoir hosts across continents, recent human behavioral and societal changes that bring animals into increasingly close human contact, as well as medical interventions, HIV infection and an aging immunosenescent human population contribute to ongoing and dynamic interactions among *Bartonella* species and their hosts and vectors.

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