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Ctenocephalides felis an *in vitro* potential vector for five *Bartonella* species

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ABSTRACT

The blood-sucking arthropod *Ctenocephalides felis* has been confirmed as a vector for *Bartonella henselae* and is a suspected vector for *Bartonella clarridgeiae*, *Bartonella quintana* and *Bartonella koehlerae* in *Bartonella* transmission to mammals. To understand the absence of other *Bartonella* species in the cat flea, we have developed an artificial flea-feeding method with blood infected successively with five different *Bartonella* species. The results demonstrated the ability of these five *Bartonella* species to persist in *C. felis* suggesting an ability of fleas to be a potential vector for several *Bartonella* species. In addition, we demonstrated a regurgitation of *Bartonella* DNA in uninfected blood used to feed *C. felis* thus suggesting a potential horizontal transmission of *Bartonella* through *C. felis* saliva. On the contrary, no vertical transmission was detected in these artificial conditions.

1. Introduction

Bartonellae are Gram-negative bacteria belonging to the class of alpha-proteobacteria and have vertebrates as reservoir/host. Animal hosts of *Bartonellae* include cats, dogs, rabbits, bovines, and multiple rodent species [1,2]. Among the 26 species and subspecies of *Bartonella* described, about half are known to infect healthy or immunocompromised humans [3–5]. These bacteria can infect and invade erythrocytes and endothelial cells leading to persistent infections and some *Bartonella* species were associated with endocarditis, bacillary angiomatosis and myocarditis [6–9]. Among these species, three are associated with well characterized diseases: Carrion disease for *Bartonella bacilliformis* (*B. bacilliformis*), trench fever for *Bartonella quintana* (*B. quintana*) and cat scratch disease for *Bartonella henselae* (*B. henselae*) [10]. Various arthropods showed the ability to be potential vectors for transmission of *Bartonella* to a mammalian host. The list of arthropods species found naturally infected with *Bartonella* is still growing [11,12]. To date, five arthropods were confirmed as competent vectors for *Bartonella*: the sandfly *Lutzomyia verrucarum* for *B. bacilliformis* [13], the louse *Pediculus humanus humanus* for *B. quintana* [14], *Ctenophtalmus nobilis* for *Bartonella grahamii* and *Bartonella taylorii* [15] and *Ctenocephalides felis* (*C. felis*) for *B. henselae* [16] and *Ixodes ricinus* for *Bartonella birtlesii* [17]. *C. felis* commonly called the "cat's flea" is the most common external parasite found worldwide on cats and dogs [18–20]. There are four recognized subspecies of *C. felis* all of them mainly parasites of carnivores: *C. felis strongylus* (Jordan, 1925) and *C. felis damarensis* [21] mainly found in Africa, *C. felis orientis* in southeast Asia and in the East Indies [22] and *C. felis felis* (Bouché, 1835) in North

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America, North Africa and Europe. *C. felis* is a sedentary parasite spending almost its entire life on their host [23] and rarely move from on host to the other [24]. Once adult flies had acquired a host, the blood meal starts almost immediately. Female *C. felis* can gain until 15 times their weight in blood; they can consume 13.6 μL of blood daily [23]. Real Time PCR assays performed on *C. felis* fed on dogs showed that after 15 min spent on their host almost all fleas were similarly engorged and the amount of blood uptake was 1.1 nL for both males and females. Saturation uptake was observed at 30 min and 60 min after the introduction of fleas on dogs and was 118 and 100 nL of blood respectively [25]. Feces (or dejection) are small dark pellets of dried blood and their emission occurs quickly after the beginning of the blood meal: on membrane feeding system they were excreted within the 8–9 min after the contact with blood [23]. Consumption of blood is necessary before *C. felis* could initiate reproduction. Mating occurs after fleas have fed. Females lay between 20 and 30 eggs per day. Laid eggs fall onto the ground and after three larval stages give rise to nymphs followed by adults. After hatching, the young adults or imago, jump onto the host animal [23,26]. *C. felis* has been experimentally shown as a competent vector for *B. henselae* transmission. Fleas collected from bacteriemic cats were placed on five specific-pathogen-free cats. After 2 weeks, four cats were found bacteriemic for *B. henselae* [16]. *C. felis* acquire *B. henselae* after feeding on infected cat and the bacteria persists in fleas and feces [27,28]. The potential mechanisms of transmission of *B. henselae* among cats could include the inoculation of contaminated flea feces through skin lesions, ingestion of infected fleas or infected feces. A successful transmission was demonstrated after intradermal injection of flea feces to cats [29]. More recently Bradbury and Lappin confirmed that *C. felis* can transmit *B. henselae* among cats and that cats treated with a topical combination of imidacloprid and moxidectin were efficiently protected from fleas infestations and thus from *B. henselae* transmission [30]. For human *B. henselae* infection could occur when flea fecal material is introduced via cat scratch or bite [31]. The contamination of a woman with B. quintana, following a bite by a feral cat, was demonstrated. Although the contamination route of cats in this study remained unknown authors suggested a flea transmission to the cats [32]. *B. quintana* infections were reported for patients with close contact with cats and flea-infested kitten [33,34]. DNA of *B. henselae*, *B. clarridgeiae*, *B. quintana* and *B. koehlerae* was detected in fleas collected in France. These findings led the authors to propose that *C. felis* was a potential vector for *B. clarridgeiae*, *B. quintana* and *B. koehlerae* [35]. The detection of these species in fleas was also described in many other countries [36]. Besides *Bartonella* transmission, fleas can be vectors of several other pathogens such as *Mycoplasma* spp., *Rickettsia felis* [35–37] and are intermediate host for the tapeworm *Dipylidium caninum* present in both cats and dogs [38]. The absence of others *Bartonella* species than *B. henselae*, *B. clarridgeiae*, *B. quintana* and *B. koehlerae* in cat fleas may be related to the specificity of fleas toward their hosts which are animal reservoirs for these four *Bartonella* species. Another explanation may be that only *B. henselae*, *B. clarridgeiae*, *B. quintana* and *B. koehlerae* are able to survive and develop inside *C. felis*. To investigate this question, we undertook the feeding of *C. felis* with blood artificially infected with five *Bartonella* species whose genomes were sequenced. The vertical transmission of these five *Bartonella* species in *C. felis* was checked. Finally, we investigated for the contamination of free-fresh-blood by *C. felis* already fed on infected blood.

2. Materials and methods

2.1. Bacterial strains

Five reference strains of Bartonella were used: *B. henselae* Houston-1 ATCC 49882 [39], *B. tribocorum* CIP 105476T [40], *B. quintana* str. Toulouse [41], *B. birtlesii* LL-WM9 [42] and *B. clarridgeiae* 73 [43].

2.2. Media and growth conditions

All *Bartonella* strains were grown on sheep blood agar medium (CBA) (BioMerieux, Craponne, France) in humidified atmosphere at 35 °C in 5% CO_2 atmosphere. For flea infection assays, *Bartonella* strains were collected after 5 days growth on CBA plates and suspended in PBS sterile buffer. The bacterial suspension was diluted with PBS to obtain a cell density of 2×10^8 bacteria per ml. The survival of bacteria in PBS buffer was not significantly decreased after 24 h storage at room temperature (data not shown). To obtain dead *Bartonella*, Kanamycin was added to bacterial suspension at 50 μg/ml final concentration and incubates one night at room temperature.

2.3. Fleas maintenance and supply

Strain *C. felis* (Siphonaptera: Pulicidae) originating from a wild strain harvested from a cat has been maintained on cat under laboratory conditions since 1990. A new generation is obtained every 2–4 weeks. Fleas were controlled to be PCR negative for *Bartonella* spp. using primers CS.140 5′-TTACTTATGATCCTGGTTTTAC-3′ and BhCS 5′-AATGCAAAAAGAACAGTAAACA-3′ [44].

2.4. Feeding of C. felis with Bartonella spp.-infected blood

Dog blood used in all experiments was obtained from 3 beagles (15 ml obtained from each dog) from the Ectoparasite Laboratory of the National Veterinary School in Toulouse, France. The absence of *Bartonella* spp. in the blood of these dogs was confirmed by PCR using primers CS.140 5′-TTACTTATGATCCTGGTTTTAC-3′ and BhCS 5′-AATGCAAAAAGAACAGTAAACA-3′ [44]. Lithium heparin-coated vacutainer tubes (Venosafe, Terumo Europe) were used to draw blood by venipuncture. Blood functional complement was deactivated by maintaining blood samples at room temperature for 1 h after blood test and before storing them at 4 °C. Blood samples were stored less than 48 h at 4 °C. When required, Kanamycin, that kills *B. henselae*, was added to blood at 50 μg/ml final concentration. Kanamycin was previously determined to have no impact on *C. felis* feeding, viability and eggs production (data not shown). 500 unfed (males and females aged between 8–10