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Human seroreactivity against *Bartonella* species in the Democratic Republic of Congo

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ABSTRACT

Objective: To assess the presence and identity of *Bartonella* species in a pool of human blood samples from DRC Congo. **Methods:** Blood (±120 μL) was collected anonymously from Congolese patients and placed on calibrated filter papers. *Bartonella* serology determination was performed using an indirect immunofluorescence assay (IFA) against six specific *Bartonella* antigens and *Coxiella burnetii (C. burnetii)* antigen. The end cut-off value for *Bartonella* sp. was a titre greater than 1:200. **Results:** None of the patients was positive for *Bartonella elizabethae, Bartonella vinsonii* subsp. *vinsonii* or *Bartonella vinsonii* subsp. *arupensis* nor for *C. burnetti*, but 4.5% of the 155 samples were positive for either *Bartonella henselae*, *Bartonella quintana*, or *Bartonella clarridgeiae*. **Conclusions:** This preliminary study presents the first report of *Bartonella* species in the DR Congo and the first report of antibodies to *Bartonella clarridgeiae* in an African human population. Although few experimental trials have established the link between fleas and *Bartonella* transmission, the repeated detection of similar *Bartonella* species in fleas and humans in several countries suggests that Bartonellosis could be another flea-borne disease which specific reservoirs are still unknown.

1. Introduction

Bartonella are Gram-negative bacteria which mostly infect erythrocytes and endothelial cells, often leading to persistent blood-borne infections. Fleas and other ectoparasites are reportedly capable of carrying and transmitting several *Bartonella* strains. Transmission rate depends on the *Bartonella* strain, host and specific vector[1,2]. While infection of domestic animals and arthropods may increase the risk of human infection, few African countries have yet to report cases of human bartonellosis. The reason for this may coincide with recent recognition of bartonellosis emergence worldwide, and the fact that infection can either be asymptomatic or closely resemble symptoms associated with other infections. Thus, *Bartonella* may go undiagnosed or be markedly underreported, especially in resource-poor or politically unstable areas. Indeed, the region surveyed was restricted to a 30 km radius circle around Rethy village (02°05′N; 30°54′E), Ituri district of the DR Congo due to threats of rebel attacks and interethnic tensions. However, previous observations in this area identified domestic fleas [3] and rodents (Gundi et al, unpub. data) to be infected with *Bartonella* spp. which prompted this investigation of human patients' sera.

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2. Material and methods

Patients enrolled in this study resided in 12 villages located within the Rethy Health Zone. The average age of female and male patients was (30.4±15.1) and (33.7±15.1) years old, respectively. The respondent's answers revealed that 78.0% of residents slept on the floor of the house and that only 52.7% of the houses had at least one bed. None of the households visited (n=160) contained cows or sheep, while 15.6% kept goats. Blood collection was performed between 19th March and 4th September 2007 on filter papers (LDA 22, Ploufragan, France) distributed among local health clinics. Participants were patients presenting with febrile signs who were negative for malaria on a thick-smear blood test. This prompted clinicians to perform venipuncture for additional diagnostic tests. All samples were collected anonymously and six drops of blood were placed on calibrated filter papers, with each impregnated dot (6 per sample) corresponding to 20 μL of whole blood. Papers were air dried, stored at room temperature, and sent to the CDC, Fort Collins (CO, USA) for *Bartonella* serology determination using an indirect immunofluorescence assay (IFA). To accomplish this blood from filter paper was eluted in PBS overnight at 4 ℃. The eluate was added to slides fixed with various purified Bartonella antigens, made by infecting Vero E6 cells with the selected *Bartonella* strains [*Bartonella henselae (Bartonella henselae)*, *Bartonella quintana (B. quintana)*, *Bartonella elizabethae(B.elizabethae)*, *Bartonella vinsonii* subsp. *vinsonii(B. vinsonii* subsp. *vinsonii)*, *Bartonella vinsonii* subsp. *arupensis* (*B. vinsonii* subsp. *arupensis*), and *Bartonella clarridgeiae(B. clarridgeiae)*]. Slides were then incubated in a moist chamber at 35 ℃ for 30 min, washed with PBS for 10 min, rinsed with distilled water, and air dried. Anti-human fluorescein isothiocyanate-labeled IgG conjugate was added to the slides which were processed as before. Slides were then mounted and read on a fluorescent microscope. All positive samples were then serially diluted in PBS, and an IFA-endpoint titer was determined using the same procedure; the end cut-off value for *Bartonella* sp. was a titre greater than 1:200. Since cross reactivity with *Coxiella burnetti (C. burnetti)* has been noted[4], and the presence of antibodies to *C. burnetii* in humans had been previously reported from DR Congo[5], we further tested both Bartonella positive and negative samples for antibodies to *C. burnetii* by IFA. Known positive and negative sera served as controls for this assay.

3. Results

A total of 155 human samples were tested for seroreactivity against the six *Bartonella* antigens selected. Seven samples had antibodies (titre ⩾ 1:200) reactive against one of the six *Bartonella* antigens tested, yielding an overall seroprevalence rate of 4.5% for *B. henselae, B. quintana* and *B. clarridgeiae*. The specific antigen(s) to which each sample was positive and the highest antibody titre found for each antigen can be found in Table 1. None of the patients sampled was positive for *B. elizabethae, B. vinsonii* subsp. vinsonii, *B. vinsonii* subsp. *arupensis*, or other *Bartonella* species as well as *C. burnetti*.

Table 1
Positive results for the serological screening of the Congolese samples from Ituri with IFA titers.

Village	Patient sex	Patient age (Years)	Species			
			Bh	*Bq*	*Bc*	*Cb*
Jalussene	F	19	256	<32	16 384	<32
Jalussene	F	19	<32	<32	4 096	<32
Kokpa	F	UN	<64	<64	2 048	<32
Kpandruma	M	17	2 048	<32	<32	<32
Rethy	F	UN	<32	2 048	<32	<32
Wanyale	F	47	<32	<32	16 384	1:32
Wanyale	UN	18	<32	<32	2 048	<32

F: female, M: male, *Bh: B. henselae ; Bq: B. quintana; Bc: B. clarridgeiae, Cb: C. burnetti.* UN: Unrecorded.

4. Discussion

This preliminary study presents the first report of *Bartonella* species in the DR Congo and the first report of antibodies to *B. clarridgeiae* in an African human population. The robustness of the test is supported by a relatively high cut-off value (1:200) compared to the commonly used cut-off (1:64) for *Bartonella* spp. serology[6]. The test also proved specific enough to detect titres reactive against different *Bartonella*-specific antigens, allowing us to discriminate between *Bartonella* species. While not all samples were tested against *C. burnetti* antigens, we demonstrated that none of the *Bartonella* seropositive patients reacted positively to *C. burnetti*. In an attempt to understand risk factors of exposure to potential *Bartonella* carriers and vectors we investigated the animal husbandry practices and sleeping habits within the villages investigated. We found that the only ruminants kept peridomestically

are goats (15.6%) which are known carriers of *C. burnetti*, but have not been shown to be infected with *Bartonella*. Questionnaires revealed that the majority of the respondents (78%) sleep on the floor of huts exposing them to domestic arthropod bites and rodent ectoparasites. Moreover, previous studies revealed that domestic fleas and rodents from the same villages were PCR-positive for *Bartonella* spp. which closely aligned with pathogenic *B. vinsonii* arupensis, *B. rochalimae* and *B. clarridgeiae* for fleas[3], and remained genetically close to *B. elizabethae*, or *B. tribocorum* for rodents (Gundi *et al*, unpub. data). Although there is still limited experimental evidence to firmly establish the link between fleas and *Bartonella* transmission[7,8], the repeated detection of *Bartonella* species in fleas, rodents, and humans in several countries[9-11] suggests that Bartonellosis could be another flea borne disease where the specific reservoirs are unknown. Either way, this study raises a potential public health concern in the DR Congo which should prompt the joint efforts between local veterinarians and medical officers to add this to their list of differentials and address this issue. Finally, to our knowledge this is the first report of *Bartonella* seropositive patients in sub-saharan Africa.

Conflict of interest statement

We declare that we have no conflict of interest.

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