Bartonella spp. in human and animal populations in Gauteng, South Africa, 2007 - 2009

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Special Bacterial Pathogens Reference Unit

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Bartonellae are:

- Gram-negative fastidious bacilli
- genus of opportunistic
  - affecting mostly immunocompromised individuals
- fall within the alpha-2 subgroup of the class Proteobacteria
- generally transmitted from animals to human hosts
  - Different spp. affect different hosts
- newly emerging pathogens
  - > 20 spp. and subsp.
  - ~ 13 spp. associated with human diseases
- highly adaptive organisms
  - ability to evade the host immune system
  - cause persistent bacteraemia
- under-studied and often missed or misdiagnosed.
• Prevalence differs among geographic areas
  – higher prevalence in more humid regions
• Widespread infection in human and animal populations
• Reside within host red blood cells
  – facilitated transmissibility
    • evade the host’s immune system → chronically infection
    • Prolonged bacteraemia → more time for transmission between hosts
    • blood-sucking vectors (e.g. ticks, fleas, sandflies, and lice )
• Life cycle varies slightly depending on species
Life cycle of *Bartonella henselae* typically transmitted by cat flea (*Ctenocephalides felis*) to animal and human hosts. A, the bacillus infects fleas via ingestion of infected cat blood; B, fleas bite and infect other cats with *Bartonella*; C, cats become reservoirs and re-infect other cats; D, fleas often bite humans that come into contact with them; E, cats also transmit the infection to humans by scratch or bite.
Clinical Importance

- Affect both immunocompromised and immunocompetent patients
- Infections present in different ways (depending on the species)
- Probability of infection heightened in immunosuppressed patients
  - (e.g. transplant patients, chemotherapy patients, and HIV/AIDS patients)
- Human pathogenicity related to incidental infection
- A wide spectrum of new diseases and re-emergence of older ones
Clinical disease

- **Carrion’s disease & verruga peruana**
  - *B. bacilliformis* infection of the erythrocytes
  - Transmitted by sandflies

- **Bacillary angiomatosis (BA)**
  - *B. quintana* → transmitted by human body lice
  - *B. henselae* → transmitted by fleas and direct inoculation by cat scratch

- **Trench fever**
  - aka 5-day fever, Wolhynia fever, or quintan fever
  - *B. quintana* infection of erythrocytes
  - transmitted by human body lice

- **Cat scratch disease (CSD)**
  - *B. henselae* is the most common causative pathogen
  - Transmitted by fleas and direct inoculation by cat scratch

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AIMS/OBJECTIVES

Main objective:
To investigate the carriage of *Bartonella* spp. circulating in human and animal populations in Gauteng using culture and PCR detection.

Specific objectives:
- To determine the prevalence of *Bartonella* infections in HIV-positive patients presenting for treatment.
- To determine to what extent bartonellae affect the general healthy population.
- To investigate cats and dogs in Johannesburg for carriage of bartonellae.
- To investigate commensal and wild rodents for carriage of bartonellae.
METHODS

Blood samples were collected from:
- 424 humans
  - 382 HIV-positive patients
  - 42 clinically healthy volunteers
- 98 cats
- 179 dogs
- 124 wild rodents
- plated onto specialized media
  - 5% rabbit blood supplemented Columbia agar
- incubated for 7-21 days at 37°C in CO₂.

Culture isolates morphologically similar to *Bartonella* control strains were confirmed by PCR and sequenced to determine species.

DNA was extracted from all blood samples and tested by nested PCR.

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HIV-positive patients

- A total of 382 HIV-positive patients volunteered to take part in this study.
- EDTA blood samples were plated out after having been freeze-thawed from -20 °C.
- No HIV-positive patient blood cultures yielded *Bartonella* spp isolates.
- ~130/382 of the blood cultures were contaminated due to the prolonged incubation periods.
  - Amphotericin B reduces fungal contamination and suppresses bacterial growth.
  - Contaminated cultures were retested.
- Any cultured Gram-negative pleomorphic bacilli were sub-cultured and *Bartonella* was excluded by PCR.
Healthy volunteers

- Determine the normal infection rates in healthy individuals.
- A total of 42 healthy volunteers were recruited;
  - 7 specimens → staff at an animal shelter
  - 35 specimens → staff at the NICD.
- All specimens were freshly cultured onto the *Bartonella* media.
- 1/42 illustrated growth indicative of *Bartonella* spp.
  - morphology and Gram-stain were consistent with *Bartonella*.
- Culture failed to grow on sub-culture
- Final confirmation done by PCR of DNA extracted from the blood of the volunteer.
  - Found to be PCR negative
No dog (n=179) blood specimens yielded *Bartonella* isolates.

Cat blood (n=98)
- cultures yielded 5 (~5% culture prevalence) PCR-confirmed *Bartonella* spp. isolates
  - confirmed by PCR
- Samples were stored at 4 °C for up to 3 weeks before being processed.
  - **hypothesis**: if present *Bartonella* bacilli die if outside the host for prolonged periods.
  - Isolates obtained when blood specimens were plated out within 1 week of collection

Rodent blood (n=124)
- *Rattus norvegicus* and *R. rattus*
- Cultures yielded 16 (13% culture prevalence) *Bartonella* isolates
  - confirmed by PCR.
  - Primary isolation occurred between 5 and 9 days, with sub-cultures growing within 4-5 days.
  - from blood of adult *Rattus rattus* and *Rattus norvegicus*, with no apparent bias for gender (8 female & 8 male).
- At least 2 different morphologies (later identified by sequencing)
  - **Morphology type 1**: colonies were tiny, pin-point, smooth, moist, and slightly metallic. This species grew on the *Bartonella* media surface rather than pitting into it and were highly adhesive, and quite difficult to emulsify in sterile water.
  - **Morphology type 2**: colonies were drier and rougher, with variable degrees of pitting into the media. They too had a metallic sheen and were tiny pin-point colonies, but were far more difficult to scrape off the media, although emulsification of the bacteria in sterile water was easier.
PCR RESULTS

- HIV-positive population → 22.5% (86/382) prevalence
- Clinically healthy group → 9.5% (4/42) prevalence
  - significant difference (p-value: 0.05) in the proportion of current infection for the two populations.

Cat PCR prevalence → 23.5% (23/98)
  - significantly higher (p-value: 0.0002) than culture prevalence (5%).
  - PCR is the far more efficient method for detection of Bartonella spp. as it does not rely on the bacteria being alive in the blood to be detected.

Rat PCR prevalence → 25% prevalence (31/124)
  - significant difference (p-value: 0.0151) between PCR prevalence and culture prevalence (13%).

- Dog PCR prevalence → 9% (16/179)
  - significantly lower than the prevalences of the felines (p-value: 0.0009) and rodents (p-value: 0.0001).

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• Cat isolates were >99% similar to *B. henselae* URBHLIE 9
  – previously isolated from an endocarditis patient

• Rat isolates were >98% similar to either RN24BJ (*B. thailandensis*) or RN28BJ
  – previously isolated from rodents in China.
• *B. henselae* was first isolated from the bloodstream of an AIDS patient (Regnery *et al.*, 1992a).

• Severely immunocompromised people with bacillary angiomatosis remain bacteremic for a number of weeks (Koehler & Tappero, 1993) and it is this group that is most at risk of contracting a *Bartonella* infection (Boulouis *et al.*, 2005).

• Findings of this study have important implications for HIV-positive patients since 22.5% of the HIV-positive blood tested by PCR was positive for bartonellae.

• The isolation of *B. henselae* URBHLIE9 from all 5 culture-positive cat isolates. This strain was previously isolated from the blood of a patient presenting with endocarditis and implies a strong link between humans and cats as reservoirs for bartonellae (Houpikian and Raoult; 2001).

• Although our study yielded no dog isolates, the 9% PCR prevalence indicates that Dogs are carrying the organism, and may be a potential reservoir for human infection.
Bartonella is prevalent in Gauteng human and animal populations.

Cats and rodents are the principle transmission concerns, however dogs are also a possible concern.

The true extent of the burden of this disease is not yet known in this country.

More work is required to fully understand the extent of disease resulting from these Bartonella infections.