

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

Authors:

Anastasia N. Trataris^{1,2}
 Jennifer Rossouw¹
 Lorraine Arntzen¹
 Allan Karstaedt^{2,3}
 John Frean^{1,2}

Affiliations:

¹National Institute for Communicable Diseases, National Health Laboratory Service, South Africa

²University of the Witwatersrand, Parktown, South Africa

³Department of Medicine, Chris Hani Baragwanath Hospital, South Africa

Correspondence to:

Anastasia Trataris

Email:
 anastasian@nicd.ac.za

Postal address:
 Private Bag X4, Sandringham, Johannesburg 2131, South Africa

How to cite this proceeding:

Trataris, A.N., Rossouw, J., Arntzen, L., Karstaedt, A. & Frean, J., 2012, 'Bartonella spp. in human and animal populations in Gauteng, South Africa, from 2007 to 2009', *Onderstepoort Journal of Veterinary Research* 79(2), Art. #452, 8 pages. <http://dx.doi.org/10.4102/ojvr.v79i2.452>

Note:

Proceedings of the Conference of the Southern African Centre for Infectious Disease Surveillance 'One Health' held at the National Institute for Communicable Diseases, Johannesburg, July 2011.

© 2012. The Authors.
 Licensee: AOSIS
 OpenJournals. This work is licensed under the Creative Commons Attribution License.

Bartonellae are highly adaptive organisms that have the ability to evade the host immune system and cause persistent bacteraemia by occupying the host's erythrocytes. *Bartonella* spp. is under-studied and health care professionals often misdiagnose *Bartonella*-related infections. The aim of this study was to investigate the carriage of *Bartonella* spp. circulating in human and animal populations in Gauteng using culturing and polymerase chain reaction (PCR) detection. A total of 424 human, 98 cat, 179 dog, and 124 wild rodent blood samples were plated onto specialised media and 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. Deoxyribonucleic acid (DNA) was extracted from all blood samples and tested by nested PCR. *Bartonella* could only be cultured from the cat and rodent specimens. Cat isolates were > 99% similar to *Bartonella henselae* URBHLIE 9, previously isolated from an endocarditis patient, and rat isolates were > 98% similar to either RN24BJ (candidus 'Bartonella thailandensis') or RN28BJ, previously isolated from rodents in China. The PCR prevalences were 22.5% in HIV-positive patients, 9.5% in clinically healthy volunteers, 23.5% in cats, 9% in dogs and 25% in rodents. Findings of this study have important implications for HIV-positive patients.

Introduction

Background

Bartonella fall within the alpha-2 subgroup of the class Proteobacteria (Jacomo, Kelly & Raoult 2002). Recent studies have indicated that *Bartonella* species (spp.) have some degree of relatedness to other alpha-2 Proteobacteria including *Brucella* species, *Afipia* species, *Agrobacterium tumefaciens*, *Bradyrhizobium* species, and *Bosea* species (Duncan, Maggi & Breitschwerdt 2007; Greub & Raoult 2002; Houptikian & Raoult 2001; Jacomo *et al.* 2002; Pretorius, Beati & Birtles 2004; Rolain *et al.* 2004). Current knowledge suggests that there are more than 20 species and subspecies included within this genus (Márquez *et al.* 2008). Approximately 13 species have been associated with human diseases (Pérez-Martínez *et al.* 2009; Maggi *et al.* 2009; Pons *et al.* 2008) affecting both immunocompetent and immunocompromised individuals. At least six species affecting humans have been isolated from domestic cats and dogs (Chomel *et al.* 2006).

Bartonellae are pleomorphic, fastidious, oxidase-negative, Gram-negative bacilli (Jacomo *et al.* 2002; Maurin & Raoult 1996). *Bartonella* species growth is hemin-dependent (Wong *et al.* 1995), therefore the addition of hemin-rich rabbit blood or horse blood to the agar yields better growth than sheep blood (Jacomo *et al.* 2002). Growth occurs on enriched medium at 37 °C with 5% carbon dioxide (CO₂); however, growth also occurs in broth with fetal bovine serum, and in various tissue culture systems including cell lines (La Scola & Raoult 1999). On average primary isolates appear after 12 to 14 days (Jacomo *et al.* 2002), although it has been reported that primary isolation can take up to 45 days (Maurin *et al.* 1994). Subcultured colonies have been found to appear after only three to five days (Jacomo *et al.* 2002).

Objectives

In this study, human immunodeficiency virus (HIV) positive people, clinically healthy volunteers, impounded cats, impounded dogs, and commensal rodents were investigated for carriage of *Bartonella* spp. The objective was to determine the rates of infection in the study groups and to determine the species responsible for infection in the study populations.

Literature review

Various studies have been carried out globally to determine the prevalence of *Bartonella* spp. in humans and animals. There is very little data available for the culture or molecular prevalence of human *Bartonella*. The lowest prevalence reported in humans was 3% and the highest was 33.3%, in the USA (Breitschwerdt *et al.* 2007; Bonilla *et al.* 2009; Koehler *et al.* 2003). The prevalence in



cat populations was from as little as 2% in Canada (Kamrani *et al.* 2008) to as high as 53% in France (Heller *et al.* 1997), in dog populations from 0% in the United Kingdom (Birtles *et al.* 2002) to 16% in Korea (Kim *et al.* 2009), and in rodent populations from 6% in Indonesia (Winoto *et al.* 2005) to 43.5% in China (Ying *et al.* 2002).

The prevalence of *Bartonella* spp. is largely unknown in South Africa. There are some case reports of bacillary angiomatosis caused by *Bartonella* spp. in HIV positive patients (Frean, Arndt & Spencer 2002). A non-random pilot survey of outpatients of three Johannesburg HIV clinics has been done on a relatively small sample group ($n = 188$ patients). This study showed a 10% prevalence of *Bartonella henselae* in blood of HIV-positive patients, determined by nested polymerase chain reaction (PCR) (Frean *et al.* 2002). A prevalence study in domestic and wild felines in southern Africa published by Kelly *et al.* (1996) reported 23% ($n = 171$) prevalence. A study of rodents in South Africa showed a high rate of infection (44%) with a wide range of subtypes of *bartonellae* (Pretorius *et al.* 2004).

Materials and methods

Sample collection

Ethical considerations

Ethical approval for collection of samples from humans and animals was granted by the Human Research Ethics Committee (Medical) of the University of the Witwatersrand (Ethics Approval Number: M070637), the National Health Laboratory Service Animal Ethics Committee (Ethics Approval Number: 2007/112), and the University of the Witwatersrand Animal Ethics Screening Committee (AESC) (Ethics Approval Number: 2008/56/03).

Recruitment of participants and informed consent

A convenience sample of 382 HIV-positive patients was recruited from the Chris Hani Baragwanth Hospital (Soweto, Gauteng), HIV-clinic. HIV-status was known as the clinic only treats HIV-positive patients. Specimen collection started in September 2007 and was completed by the end of January 2008. Survey objectives and consent forms were explained to prospective participants by an available HIV-counselor employed at the clinic, a laboratory aid or by the phlebotomists drawing the blood samples. Patients were assured that the study was voluntary and results would not directly benefit nor disadvantage them in any way. Informed consent forms were completed by the participating patients before blood collection. The recruited volunteers were provided with an information sheet containing the contact details of the Special Bacterial Pathogens Reference Laboratory (SBPRL) at the National Institute for Communicable Diseases (NICD) to which queries were addressed.

Clinically healthy volunteers were recruited from the National Institute for Communicable Diseases and a local animal shelter for blood collection. Due to ethical considerations, the volunteers were not required to disclose their HIV status, and health was assumed on the basis of no observable signs of illness at the time of blood collection. These volunteers were also required to complete an informed consent form and

participation was completely voluntary. Two 4 mL tubes of blood were collected from each volunteer, 1 tube containing the anticoagulant ethylenediaminetetra-acetic acid (EDTA) and the other a plain red-top tube in which the blood was allowed to clot. Blood specimens were stored at minus 20 °C until use.

Animal samples

Blood was collected from dogs and cats that were undergoing euthanasia, surgery or sterilisation procedures at a local animal shelter. Two 2-mL tubes of blood (as described above) were collected from each cat and dog. Samples were stored at 4 °C until use.

Rodents were prospectively sourced from a pest control company operating in the Ekurhuleni Metropolitan area. Rodents were trapped alive, anaesthetised using CO₂, and exsanguinated by cardiac puncture. Blood was collected using a syringe and needle from each rodent and was divided into two tubes, one red top and one purple top (as above). Specimens were stored at 4 °C until use.

Procedures

Culturing of *Bartonella* spp.

All blood samples were plated onto 5% rabbit blood-supplemented Columbia agar (hereafter referred to as *Bartonella* medium). Culturing was carried out in a class two biohazard safety cabinet to reduce the risk of contamination. Ethylenediaminetetra-acetic acid blood was predominantly used for culturing, unless the EDTA specimen was insufficient (especially for cat and dog samples), in which case some of the clotted blood (packed cells excluding serum) was used. Approximately 100 µL – 150 µL of each whole blood sample was inoculated onto fresh *Bartonella* media and spread over the surface using sterile glass spreaders. Inoculated media were incubated in 5% CO₂ at 37 °C for seven ± two days before being checked for growth.

If small dry colonies, pitting into the media were present, they were Gram stained, and plated out onto fresh media. Media with faint or no growth were re-incubated as above to a maximum of two months with periodic examinations every seven days. All blood culture isolates were confirmed by PCR and stored in tryptic soy broth (TSB) with 10% (v/v) glycerol at minus 80 °C.

Genus specific polymerase chain reaction amplification

All culture isolates and blood samples were tested by genus specific PCR amplification of the intergenic spacer (ITS) region between the 16S and 23S rRNA genes for evidence of *Bartonella* Deoxyribonucleic acid (DNA). Deoxyribonucleic acid extractions were carried out using the QIAamp DNA mini kit (Qiagen, Germany) as per kit protocol.

Published genus-specific primers were selected as shown in Table 1 (Roux & Raoult 1995; Seki *et al.* 2006). The reverse primer QHVE-14 (Seki *et al.* 2006) was modified by elongating the 5' end by three nucleotides to decrease non-specific amplification.

Polymerase chain reaction confirmation of cultured isolates was carried out using primers QHVE-1 and QHVE-3, whereas the blood-extracted DNA required a nested PCR using the QHVE-12 and QHVE-14b inner primers. Table 2 shows the primer binding localities and product sizes of the six most commonly isolated human *Bartonella* spp., although other species within the genus are also detectable using these primers.

The single or first round PCR amplifications were carried out in a reaction mixture (50 µL) containing 20 pmol of each primer (QHVE-1 and QHVE-3), 1 x Buffer II, 2 mM MgCl₂, 1.5 U AmpliTaq DNA polymerase (Applied Biosystems, USA), 200 µM of each deoxyribonucleotide triphosphate (dNTP) (Thermo Scientific, United Kingdom), and 1% (v/v) Triton-X 100 surfactant additive. The amount of DNA was used depended on the source of the DNA. For the culture DNA, 20 ng of template was included into the reaction, whereas 5 µL of the total blood extracted DNA was included into the reaction. Polymerase chain reactions were performed on a VERITI Thermocycler (Applied Biosystems) under the following conditions: 2 min initial denaturation step at 94 °C, followed by 35 cycles of the following steps: denaturation at 94 °C for 30 s, primer annealing at 52 °C for 30 s, and elongation at 72 °C for 60 s. A final elongation step concluded the amplification at 72 °C for 6 min. Polymerase chain reaction products were maintained at 4 °C until being added to the reaction mixtures of the nested round. For the nested PCR reactions, a final volume of 50 µL consisted of 2 µL of first-round product, 1 x Buffer II, 1.5 mM MgCl₂, 30 pmol of each inner primer (QHVE-12 and QHVE-14b), 200 µM of each dNTP, and 1.5 U AmpliTaq DNA polymerase. The reactions were amplified as previously described, with a variation in the annealing temperature (55 °C).

Amplicon analysis was performed on a 2% (w/v) Tris-acetic acid-EDTA (TAE) buffer agarose (WhiteSci, USA) gel, supplemented with 0.5 µg/mL ethidium bromide. Electrophoresis was carried out at 100 V in 1 x TAE for 40 min. Gels were visualised by ultraviolet illumination.

TABLE 1: Published genus-specific primers.

Name	Sequence	Bp	Reference
QHVE-1	5' – TTC AGA TGA TGA TCC CAA GC – 3'	20	Roux and Raoult 1995; La Scola and Raoult 1999
QHVE-3	5' – AAC ATG TCT GAA TAT ATC TTC – 3'	21	Roux and Raoult 1995; La Scola and Raoult 1999
QHVE-12	5' – CCG GAG GGC TTG TAG CTC AG – 3'	20	Seki <i>et al.</i> 2006
QHVE-14a	5' – CAC AAT TTC AAT AGA AC – 3'	17	Seki <i>et al.</i> 2006
QHVE-14b	5' – CCT CAC AAT TTC AAT AGA AC – 3'	20	Unpublished

Note: Please see the full reference list of the article, Trataris, A.N., Rossouw, J., Arntzen, L., Karstaedt, A. & Frean, J., 2012, 'Bartonella spp. in human and animal populations in Gauteng, South Africa, from 2007 to 2009', *Onderstepoort Journal of Veterinary Research* 79(2), Art. #452, 8 pages. <http://dx.doi.org/10.4102/ojvr.v79i2.452>, for more information.

TABLE 2: Primer binding localities and product sizes of the six most commonly isolated human *Bartonella* spp.

Species	Outer primers			Inner primers		
	Bp	QHVE-1	QHVE-3	Bp	QHVE-12	QHVE-14
<i>Bartonella henselae</i>	723	318–337	1021–1041	568	448–467	1000–1016
<i>Bartonella quintana</i>	640	353–372	973–993	500	468–487	952–968
<i>Bartonella vinsonii</i>	661	336–355	977–997	481	491–510	956–972
<i>Bartonella elizabethae</i>	788	359–378	1135–1147	572	558–577	1114–1130
<i>Bartonella claridgeiae</i>	711	313–332	1004–1024	573	425–445	982–998
<i>Bartonella grahamii</i>	736	311–330	1026–1046	487	538–557	1005–1024

amphotericin B was therefore discontinued. Due to the low circulating bacterial load often observed in natural infection, the risk of suppression, although slight, was not one worth taking. If a culture became contaminated with fungal growth, the culture was repeated. To ensure that *Bartonella* was not missed, any cultured organisms were Gram-stained, and any Gram-negative pleomorphic bacilli were sub-cultured and *Bartonella* was excluded by PCR.

A total of 42 clinically healthy volunteers were recruited to determine normal infection rates. Seven animal shelter staff and 35 National Institute for Communicable Diseases' staff members participated in this study. All specimens were freshly cultured onto the *Bartonella* media. Only one of the 42 blood specimens illustrated growth morphologically consistent with *Bartonella* spp. Unfortunately, the culture failed to grow on sub-culture.

The seven animal shelter volunteers had daily exposure to many different animals. *Bartonella* spp. infections have been associated with cats and dogs (Barnes *et al.* 2000; Ketring *et al.* 2004) and it was therefore expected that this group would be most likely to demonstrate *Bartonella* bacteraemia. This was not the case in this study, but the sample size was inadequate.

Dog ($n = 179$) blood specimens yielded no *Bartonella* culture isolates, but cat ($n = 98$) blood specimens yielded five (approximately 5%) *Bartonella* culture isolates that were confirmed by PCR. It was concerning that there were so few cultured isolates and that three of the five isolates obtained came from a single batch of seven feline blood samples received from the animal shelter, whereas preceding batches had not yielded culture isolates. To exclude the possibility of cross-contamination within this batch, the blood samples were re-cultured and subjected to PCR confirmation.

A total of 124 rodent samples taken from *Rattus norvegicus* and *Rattus rattus* were cultured on *Bartonella* medium. *Bartonella* was isolated from 16 (13%) rodents. All isolates were Gram-negative, pleomorphic bacilli with morphology consistent with *Bartonella*. Primary isolation occurred between five and nine days, with sub-cultures growing within four to five days. At least two different colony morphologies (Table 3) were observed for the rodent isolates, and these were later confirmed and identified by sequencing.

Molecular detection and confirmation of *Bartonella*

A total of 21 culture isolates were confirmed as *Bartonella* by PCR. Amplicon sizes varied between 728 and 809 bp.

Polymerase chain reaction of the HIV-positive population yielded a prevalence of 22.5% (86/382) (95% confidence;

TABLE 3: Two different colony morphologies observed for the rodent isolates.

Morphology type 1	Morphology type 2
Tiny, pin-point, smooth, moist, and slightly metallic sheen	Tiny, pin-point, drier, rougher, and metallic sheen
Grew on surface of medium	Variable degrees of pitting into the medium
Highly self-adhesive, but easily scraped off the surface of the medium	More difficult to scrape off the medium due to pitting

18.5–27.1), whereas the clinically healthy group had a prevalence of 9.5% (4/42) (95% confidence; 3.1–23.5). This is a significant difference (p -value: 0.05; chi-square statistic: 3.818 with one degree of freedom) in the proportion of current infection for the two populations. This difference is unlikely to have occurred through mere chance, although the limited healthy volunteer sample size is a major limitation.

The feline blood tested by PCR indicated a 23.5% (23/98) (95% confidence; 15.8–33.3) *Bartonella* prevalence. This was significantly different (p -value: 0.0002; chi-square statistic: 13.500 with one degree of freedom) to the culture prevalence (5%). Both test techniques test for current infection; however, due to the fastidious nature of the bacteria, PCR is the far more efficient method for detection of *Bartonella* spp. as it does not rely on the viability of the bacteria.

The dog PCR prevalence was 9% (16/179) (95% confidence; 5.4–14.4), which is significantly lower than the prevalences in felines (p -value: 0.0009; chi-square statistic: 11.053 with one degree of freedom) and rodents (p -value: 0.0001; chi-square statistic: 14.419 with one degree of freedom).

Rat bloods tested by PCR indicated 25% prevalence (31/124) (95% confidence; 17.9–33.7). There was a significant difference (p -value: 0.0151; chi-square statistic: 5.907 with one degree of freedom) between PCR prevalence and culture prevalence (13%). When the prevalence for rats was compared with that of the felines, there was no significant difference (p -value: 0.7918; chi-square statistic: 0.070 with one degree of freedom).

Deoxyribonucleic acid sequencing and analysis of *Bartonella* isolates

Comparison with GenBank (NCBI website) sequences showed that the rodent isolates ranged in percentage similarity from 97% – 99% to either the recently named novel species *candidatus 'Bartonella thailandensis'* (RN24BJ;

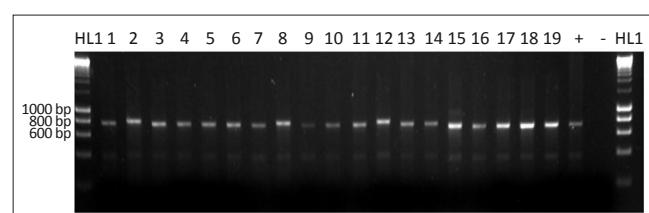


FIGURE 1: Molecular detection and confirmation of *Bartonella*.

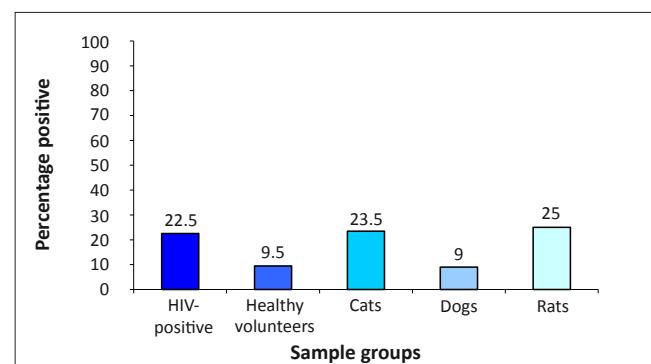


FIGURE 2: The positive percentage for the sample group.

accession number: EF190333.1) or RN28BJ (accession number: EF213776.1). Both isolates were described in a recent publication by Saisongkhor *et al.* (2009) where the rodents in Beijing, China were tested for bartonellae.

The rodent isolates were slightly more variable and a tree of relatedness was constructed (Figure 3). Contingent from the ITS data using parsimony and distance methods illustrated two well-supported (more than 90% bootstrap values) clusters within the isolates. The first cluster placed RN24BJ with 12 of the isolates (BART0272, BART0323, BART0268, BART0354, BART0379, BART0381, BART0312, BART0324, BART0359, BART0361, BART0357 and BART0358) and the second group clustered RN28BJ with the remaining three isolates (BART0271, BART0355 and BART0377). *Bartonella elizabethae* (GenBank accession number: L35103) and *Bartonella grahamii* (GenBank accession number: AJ269785) were used for comparison. *Bartonella elizabethae* was found to be most similar to the rodent isolates from this study.

The five cat isolates were 99% – 100% similar to *B. henselae* URBHLIE 9 (accession number: AF312496.1). Primers (QHVE1 & QHVE3) amplified a region consisting of 687 bp (excluding primers) for all the feline isolates. BART0480 and BART0483 were 100% identical to the *B. henselae* URBHLIE-9 strain, and had only one nucleotide difference from *B. henselae* Houston-1 (accession number: L35101) strain at position 98. BART0519 was 99% similar to URBHLIE-9 with a heterogenous nucleotide at position 285, where adenosine (A) or guanine (G) is equally expressed. BART0519 and BART0484 were identical to each other and 99% similar to URBHLIE-9. One nucleotide difference was observed at position 660.

Discussion

Bartonella remains one of the most difficult organisms to detect via blood or tissue culture. Frean *et al.* (2002) looked at the prevalence of *Bartonella* in HIV-positive out-patients at several hospitals in Johannesburg and found a 10% PCR prevalence of *Bartonella*. A conservative expectation for the present study was to find at least 10% culture-positive specimens for *Bartonella* spp.

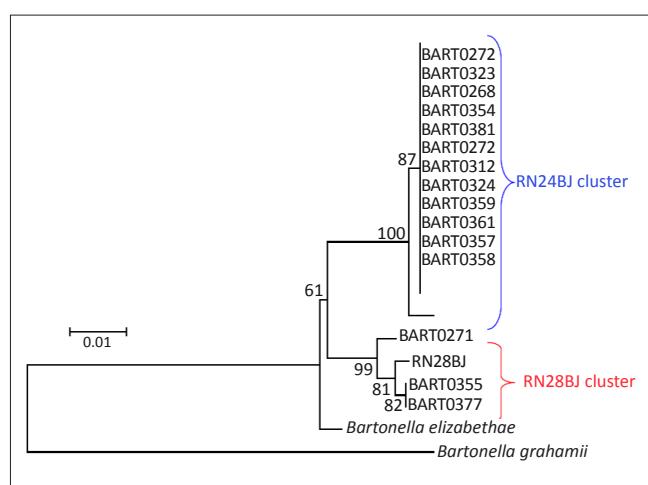


FIGURE 3: Tree of relatedness.

Bartonella henselae was first isolated from the bloodstream of an AIDS patient (Regnery *et al.* 1992a). Severely immunocompromised people are most at risk of contracting a *Bartonella* infection (Boulouis *et al.* 2005) and those with bacillary angiomatosis remain bacteremic for a number of weeks (Koehler & Tappero 1993). HIV-infected patients with CD4+ cell counts of less than 50/mm³ are more likely to develop bacillary angiomatosis lesions (Boulouis *et al.* 2005; Koehler & Tappero 1993), and as a precaution, broad-spectrum antibiotic treatments are periodically prescribed for these patients. The effect of broad-spectrum antibiotics on *Bartonella* infections lacks clarity. Research has been conducted on *in vivo* and *in vitro* antimicrobial sensitivities of *Bartonella* to various drug classes. *In vitro* testing has shown bactericidal efficacy, but *in vivo* tests have demonstrated mostly bacteriostatic activity, with the exception of aminoglycosides which are bactericidal (Florin *et al.* 2008). The bacteriostatic activity of the broad-spectrum drugs may be suppressing circulating bacteremia to undetectable levels for culture. It is probable that the HIV-positive patients we tested were on a range of broad-spectrum antibiotics as prophylaxis against opportunistic infections. Information regarding whether or not these patients were on the antibiotic treatment, the type of antibiotic treatment taken, the prescribed doses, and duration of treatment administered was not collected at the time of specimen collection and is thus unavailable. It was speculated that this would influence *Bartonella* isolation from the specimens.

Published methodologies were followed for culture, including freeze-thawing of the specimens (La Scola & Raoult 1999). The methodologies were later refined as it was discovered that inoculating fresh packed red-blood cell samples onto the *Bartonella* media yielded better results. Although culture is regarded as the gold standard for detection, it is limited and problematic. The lack of cultured *Bartonella* in human blood does not indicate that the prevalence is zero, since *Bartonella* spp. was detected by PCR.

This study has shown an even higher *Bartonella* prevalence (22.5%) in HIV-positive out-patients than the 10% previously reported (Frean *et al.* 2002). In immunocompromised individuals *B. henselae* infections are usually associated with exposure to cats and cat fleas (Boulouis *et al.* 2005; Koehler & Tappero 1993). The highest prevalences found in this study were for cats (23.5%) and rats (25%). These prevalences were not as high as some of the other reports published on the prevalence of bartonellae in animals. Dog and cat samples were stored in the fridge at 4 °C for up to three weeks before being processed. It is hypothesised that *Bartonella* bacilli (if present in the blood) became non-viable due to prolonged storage time. Supporting this hypothesis is the fact that isolates were obtained from blood specimens that were processed within one week of collection. This is further supported by the fact that PCR detected *Bartonella* DNA in the blood at a much higher rate than culture (23.5% vs 5%).

An interesting finding for the present study was the isolation of *B. henselae* URBHLIE9 from all five 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 & Raoult 2001).

Polymerase chain reaction results indicate that there is a high prevalence of *bartonellae* in human and animal populations in Gauteng Province, South Africa. More work is required to fully understand the extent of disease resulting from these *Bartonella* infections.

Limitations

Bartonella prevalence for HIV-positive patients in Gauteng was found to be much higher than previously reported. Based on the 22.5% (95% confidence) PCR prevalence, it is speculated that approximately 1 in 4 HIV-positive patients is infected with *Bartonella*. Although most patients are consistently subjected to broad-spectrum antibiotic treatment, the efficacy of treatment is not known since treatment efficacy varies from patient-to-patient. The fact that 22.5% of the tested samples were PCR positive for *Bartonella* indicates that the bacteria are present in the host. *In vitro* efficacy of antibiotic use against *Bartonella* has been limited (Florin *et al.* 2008). In most cases antibiotics have had an inhibitory effect rather than a bactericidal one. The lack of cultured isolates from HIV-positive human samples could be attributed to this inhibitory effect.

Treatment is onerous as it has to be administered for prolonged periods to suppress infections, such as *Bartonella* infections. There is limited information on the economic burden of *Bartonella* infections, since diseases caused by *Bartonella* are not notifiable. Furthermore, due to the difficulty in diagnosis of *Bartonella*, infections often go undetected and untreated.

Although there are various methodologies available for detection of *Bartonella*, each method has its own limitations. Most methods are only used for research purposes and highly-skilled laboratory staff. The first test dealt with in this study is culture. Most microbiology laboratories are proficient in this method and culture is generally regarded as the gold standard for most bacterial diagnoses. Culture of *Bartonella* differs from most conventional bacteria culture as it is more labor-intensive. Fresh EDTA blood must be centrifuged and the pellet is plated onto specialised media supplemented with rabbit blood. Isolation requires a prolonged incubation period from 7–21 days at 37 °C under microaerophilic conditions. Most laboratories do not have the capacity to maintain these cultures for prolonged periods or prevent contamination. Assuming the above conditions are met, there still remains the task of confirmation of the culture as *Bartonella*. *Bartonellae* do not visibly metabolise compounds provided in rapid biochemical test panels. *Bartonella* spp. is oxidase and catalase negative and stain faintly as Gram negative, slightly curved, bacilli. Small diagnostic laboratories often do not have the means of confirming the culture isolate as *Bartonella*.

Polymerase chain reaction has become a relatively affordable option when testing for bacterial diseases, although affordability is relative. A skilled and competent operator

performing a PCR may not have problems carrying out the test, particularly if it is a routine test, but if the operator does not routinely perform the test (especially for *Bartonella*) the method is relatively labour-intensive and prone to contamination. Much research has gone into developing nested PCR, high-tech real-time PCR and amplicon sequencing. These tests remain largely for research purposes only. Staff would have to be trained; primers and other reagents would have to be readily available and expensive equipment such as thermocyclers, electrophoresis power packs and tanks, UV light-boxes, software and computers would have to be purchased and maintained.

Recommendations

It is recommended that operator-friendly tests such as the IFA become more affordable for use in laboratories. Perhaps an ELISA as previously described (Bergmans *et al.* 1997; Vermeulen *et al.* 2007) would be a more cost effective method. Control culture is relatively easy to grow and the antigens of the culture can be sonicated from the growth and consequently be used to coat the microtitre wells. This method can be optimised and made 'in-house' thus mitigating the need for expensive commercially available kits. *Bartonella* testing is especially required for immunocompromised patients presenting with clinical symptoms and histories.

Training and out-reach programs directed towards doctors and the generally public should be carried out to ensure the indicative symptoms are recognised, and reported when presenting for medical assistance. Pest control and fleas control methods should be advised and implementable solutions should also be discussed.

Conclusion

In this study, results were within global trends for *Bartonella* prevalence in both human and animal populations. The objectives of the study were met and a better understanding of *Bartonella* prevalence in human and animal populations in Gauteng, South Africa is now available. This study has confirmed that the primary concern is human health, particularly for immunocompromised people who are at higher risk of contracting various opportunistic infections including *Bartonella*.

Bartonella is prevalent in human and animal populations in Gauteng, South Africa, and could be responsible for a number of unresolved or misdiagnosed diseases in immunocompromised patients. Although the broad-spectrum antibiotics offered to HIV-positive patients in particular maybe suppressing the infection, the treatment would have to be constant and there are possible side-effects that could cause the patient more discomfort. It is important to emphasise that immunocompromised patients be very careful when handling domestic animals.

Furthermore, attempts should be made to decrease the risk of rat exposure. By keeping food in sealed containers off



the floor, and ensuring proper sanitation in and around the home is followed, the household rodent population would be largely reduced.

Too little is known about *Bartonella* and further research is required to fully understand the extent of disease related to this emergent pathogen.

Acknowledgements

It is with gratitude that we would like to acknowledge the following; firstly the staff of SBPRL – CEZD for their support and assistance with the project, Mr Justice Matebula (Laboratory aid) who assisted with the translations and explanations of the informed consent in the recruitment of volunteer participants, the HIV-clinic councilors and phlebotomists for their assistance with translations, explanations and recruitment of HIV-positive volunteers as well as specimen collection, and to the staff of NICD and the animal shelter (wished to remain anonymous) for 'bleeding' for the cause. The animal shelter is furthermore thanked for assisting us with obtaining the cat and dog specimens. A special thank you is owed to Mr Lee Ashford and his enthusiastic staff at 'Scientific Supakill' pest-control company for providing us with the live rodents. And finally thank you to the NICD/NHLS for their support and use of facilities in this study.

Competing interests

The authors declare that they have no financial or personal relationship(s) which may have inappropriately influenced them in writing this paper.

References

Birtles, R.J., Laycock, G., Kenny, M.J., Shaw, S.E. & Day, M.J., 2002, 'Prevalence of *Bartonella* species causing bacteraemia in domesticated and companion animals in the United Kingdom', *Veterinary Record* 151, 225–229. <http://dx.doi.org/10.1136/vr.151.8.225>, PMID:12219899

Bonilla, D.L., Kabeya, H., Henn, J., Kramer, V.L. & Kosoy, M.Y., 2009, 'Bartonella quintana in body lice and head lice from homeless persons, San Francisco, California, USA', *Emerging Infectious Diseases* 15, 912–915. <http://dx.doi.org/10.3201/eid1506.090054>, PMID:19523290

Boulouis, H.J., Chang, C.C., Henn, J.B., Kastenc, R.W. & Chomel, B.B., 2005, 'Factors associated with the rapid emergence of zoonotic *Bartonella* infections', *Veterinary Research* 36, 383–410. <http://dx.doi.org/10.1051/vetres:2005009>, PMID:1584231

Branley, J., Wolfson, C., Waters, P., Gottlieb, T. & Bradbury, R., 1996, 'Prevalence of *Bartonella henselae* bacteremia, the causative agent of cat scratch disease, in an Australian cat population', *Pathology* 28, 262–265. <http://dx.doi.org/10.1080/00313029600169124>, PMID:8912359

Breitschwerdt, E.B., Maggi, R.G., Duncan, A.W., Nicholson, W.L., Hegarty, B.C. & Woods, C.W., 2007, 'Bartonella species in blood of immunocompetent persons with animal and arthropod contact', *Emerging Infectious Diseases* 13, 938–941. <http://dx.doi.org/10.3201/eid1306.061337>, PMID:17553243

Chomel, B.B., Boulouis, H.J., Maruyama, S. & Breitschwerdt, E.B., 2006, 'Bartonella spp. in pets and effect on human health', *Emerging Infectious Diseases* 12, 389–394. <http://dx.doi.org/10.3201/eid1203.050931>, PMID:16704774

Duncan, A.W., Maggi, R.G. & Breitschwerdt, E.B., 2007, 'A combined approach for the enhanced detection and isolation of *Bartonella* species in dog blood samples: Pre-enrichment liquid culture followed by PCR and subculture onto agar plates', *Journal of Microbiological Methods* 69, 273–281. <http://dx.doi.org/10.1016/j.mimet.2007.01.010>, PMID:17346836

Florin, T.A., Zaoutis, T.E. & Zaoutis, L.B., 2008, 'Beyond cat scratch disease: Widening spectrum of *Bartonella henselae* infection', *Pediatrics* 121, 1413–1425. <http://dx.doi.org/10.1542/peds.2007-1897>, PMID:18443019

Frean, J., Arndt, S. & Spencer, D., 2002, 'High rate of *Bartonella henselae* infection in HIV-positive outpatients in Johannesburg, South Africa', *Transactions of the Royal Society of Tropical Medicine and Hygiene* 96, 549–550. [http://dx.doi.org/10.1016/S0035-9203\(02\)90437-2](http://dx.doi.org/10.1016/S0035-9203(02)90437-2)

Greub, G. & Raoult, D., 2002, 'Bartonella: New explanations for old diseases', *Journal of Medical Microbiology* 51, 915–923. PMID:12448674

Heller, R., Artois, M., Xemar, V., De Briel, D., Gehin, H., Jaulhac, B. et al., 1997, 'Prevalence of *Bartonella henselae* and *Bartonella clarridgeiae* in stray cats', *Journal of Clinical Microbiology* 35, 1327–1331. PMID:9163438

Houpikian, P. & Raoult, D., 2001, '16S/23S rRNA intergenic spacer regions for phylogenetic analysis, identification, and subtyping of *Bartonella* species', *Journal of Clinical Microbiology* 39, 2768–2778.

Jacomo, V., Kelly, P.J. & Raoult, D., 2002, 'Natural history of *Bartonella* infections (an exception to Koch's postulate)', *Clinical and Diagnostic Laboratory Immunology* 9, 8–18. PMID:11777823

Kamrani, A., Parreira, V.R., Greenwood, J. & Prescott, J.F., 2008, 'The prevalence of *Bartonella*, hemoplasma, and *Rickettsia felis* infections in domestic cats and in cat fleas in Ontario', *The Canadian Journal of Veterinary Research* 72, 411–419. PMID:19086373

Kelly, P.J., Matthewman, L.A., Hayter, D., Downey, S., Wray, K., Bryson, N.R. & Raoult, D., 1996, 'Bartonella (Rochalimae) henselae in southern Africa – evidence for infections in domestic cats and implications for veterinarians', *Journal of the South African Veterinary Association* 67, 182–187. PMID:9284029

Kim, C.M., Kim, J.Y., Yi, Y.H., Lee, M.J., Cho, M.R., Shah, D.H. et al., 2005, 'Detection of *Bartonella* species from ticks, mites and small mammals in Korea', *Journal of Veterinary Science* 6(4), 327–334. PMID:16293997

Kim, Y.S., Seo, K.W., Lee, J.H., Choi, E.W., Lee, H.W., Hwang, C.Y. et al., 2009, 'Prevalence of *Bartonella henselae* and *Bartonella clarridgeiae* in cats and dogs in Korea', *Journal of Veterinary Science* 10(1), 85–87. <http://dx.doi.org/10.4142/jvs.2009.10.1.85>, PMID:19255530

Koehler, J.E. & Tappero, J.W., 1993, 'Bacillary angiomatosis and bacillary peliosis in patients infected with human immunodeficiency virus', *Clinical Infectious Diseases* 17, 612–624. <http://dx.doi.org/10.1093/clinids/17.4.612>, PMID:8268340

Koehler, J.E., Sanchez, M.A., Tye, S., Garrido-Rowland, C.S., Chen, F.M., Maurer, T. et al., 2003, 'Prevalence of *Bartonella* infection among human immunodeficiency virus infected patients with fever', *Clinical Infectious Diseases* 37, 559–566. <http://dx.doi.org/10.1086/375586>, PMID:12905141

La Scola, B. & Raoult, D., 1999, 'Culture of *Bartonella quintana* and *Bartonella henselae* from Human Samples: A 5-Year Experience (1993 to 1998)', *Journal of Clinical Microbiology* 37, 1899–1905.

Maggi, R.G., Kosoy, M., Mintzer, M. & Breitschwerdt, E.B., 2009, 'Isolation of *Candidatus Bartonella melophagi* from Human Blood', *Emerging Infectious Diseases* 15(1), 66–68. <http://dx.doi.org/10.3201/eid1501.081080>, PMID:19116054

Márquez, F.J., Rodríguez-Liébana, J.J., Pachón-Ibáñez, M.E., Docobo-Pérez, F., Hidalgo-Fonteros, A., Bernabeu-Wittel, M. et al., 2008, 'Molecular Screening of *Bartonella* Species in Rodents from South Western Spain', *Vector-Borne and Zoonotic Diseases* 8(5), 695–700. <http://dx.doi.org/10.1089/vbz.2007.0257>, PMID:18620508

Maurin, M., Roux, V., Stein, A., Ferrier, F., Viraben, R. & Raoult, D., 1994, 'Isolation and characterisation by immunofluorescence, sodium dodecyl sulfate-polyacrylamide gel electrophoresis, western blot, restriction fragment length polymorphism-PCR, 16S rRNA gene sequencing, and pulsed-field gel electrophoresis of *Rochalimaea quintana* from a patient with bacillary angiomatosis', *Journal of Clinical Microbiology* 32, 1166–1171. PMID:7519628

Maurin, M. & Raoult, D., 1996, 'Bartonella (Rochalimae) quintana infections', *Clinical Microbiology Reviews* 9, 273–292. PMID:8809460

Pérez-Martínez, L., Venza, J.M., González-Acuña, D., Portillo, A., Blanco, J.R. & Oteo, J.A., 2009, 'Bartonella rochalimae and other *Bartonella* spp. in Fleas, Chile', *Emerging Infectious Diseases* 15(7), 1150–1152. <http://dx.doi.org/10.3201/eid1507.081570>, PMID:19624952

Pons, I., Sanfelix, I., Nogueras, M.M., Sala, M., Cervantes, M., Amengual, M.J. et al., 2008, 'Seroprevalence for *Bartonella* spp. infection in HIV patients in Catalonia, Spain', *BioMed Central Infectious Diseases* 8, 58. <http://dx.doi.org/10.1186/1471-2334-8-58>, PMID:18452613

Pretorius, A., Beati, L. & Birtles, R.J., 2004, 'Diversity of bartonellae associated with small mammals inhabiting Free State province, South Africa', *International Journal of Systematic and Evolutionary Microbiology* 54, 1959–1967. <http://dx.doi.org/10.1099/ijss.0.03033-0>, PMID:15545418

Rampersad, J.N., Watkins, J.D., Samal, M.S., Deonanan, R., Ramsubeik, S. & Ammons, D.R., 2005, 'A nested-PCR with an Internal Amplification Control for the detection and differentiation of *Bartonella henselae* and *B. clarridgeiae*: An examination of cats in Trinidad', *BioMed Central Infectious Diseases* 5, 63–68. <http://dx.doi.org/10.1186/1471-2334-5-63>, PMID:16098227

Regnery, R.L., Anderson, B.E., Clarridge, J.E., Rodriguez-Barradas, M.C., Jones, D.C. & Carr, J.H., 1992, 'Characterization of a novel *Rochalimaea* species, *R. henselae* sp. Nov., isolated from blood of a febrile, human immunodeficiency virus-positive patient', *Journal of Clinical Microbiology* 30, 265–274. PMID:1371515

Rolain, J.M., Locatelli, C., Chabanne, L., Davoust, B. & Raoult, D., 2004, 'Prevalence of *Bartonella clarridgeiae* and *Bartonella henselae* in domestic cats from France and detection of the organisms in erythrocytes by Immunofluorescence', *Clinical and Diagnostic Laboratory Immunology* 11(2), 423–425. PMID:15013998



Roux, V. & Raoult, D., 1995, 'Inter- and intraspecies identification of *Bartonella (Rochalimaea)* species', *Journal of Clinical Microbiology* 33, 1573–1579. PMid:7650189

Sander, A., Buhler, C., Pelz, K., Von Cramm, E. & Bredt, W., 1997, 'Detection and identification of two *Bartonella henselae* variants in domestic cats in Germany', *Journal of Clinical Microbiology* 35(3), 584–587. PMid:9041393

Seki, N., Sasaki, T., Sawabe, K., Sasaki, T., Matsuoka, M., Arakawa, Y. et al., 2006, 'Epidemiological Studies on *Bartonella quintana* Infections among Homeless People in Tokyo, Japan', *Japanese Journal of Infectious Diseases* 59(1), 31–35. PMid:16495631

Winoto, I.L., Goethert, H., Ibrahim, I.N., Yunihherlina, I., Stoops, C., Susanti, I. et al., 2005, '*Bartonella* species in rodents and shrews in the Greater Jakarta Area', *Southeast Asia Journal of Tropical Medicine and Public Health* 36(6), 1523–1529. PMid:16610656

Wong, M.T., Thornton, D.C., Kennedy, R.C. & Dolan, M.J., 1995, 'A chemically defined liquid medium that supports primary isolation of *Rochalimae (Bartonella) henselae* from blood and tissue specimens', *Journal of Clinical Microbiology* 33, 742–744. PMid:7538511

Yabsley, M.J., McKibben, J., Macpherson, C.N., Cattan, P.F., Cherry, N.A., Hegarty, B.C. et al., 2008, 'Prevalence of *Ehrlichia canis*, *Anaplasma platys*, *Babesia canis vogeli*, *Hepatozoon canis*, *Bartonella vinsonii berkhoffii*, and *Rickettsia* spp. in dogs from Grenada', *Veterinary Parasitology* 151, 279–285. <http://dx.doi.org/10.1016/j.vetpar.2007.11.008>, PMid:18160223

Ying, B., Kosoy, M.Y., Maupin, G.O., Tsuchiya, K.R. & Gage, K.L., 2002, 'Genetic and ecologic characteristics of *Bartonella* communities in rodents in Southern China', *American Journal of Tropical Medicine and Hygiene* 66(5), 622–627. PMid:12201602