CHAPTER 1

Review of current knowledge

1.1 GENERAL INTRODUCTION

Bartonella is a genus of fastidious bacteria responsible for a wide range of both symptomatic and asymptomatic infections (Maurin *et al*., 1997; O'Reilly *et al*., 1999). In recent years, the number of *Bartonella* species (spp.) isolated and described has increased markedly. Often, bartonellae are considered obligate pathogens where infection is concurrent with immunological suppression of the host, although infections do present in healthy individuals (Chiu *et al.,* 2001; Kabeya *et al.,* 2002; Yamamoto *et al.,* 2003; Reine, 2004; Rampersad *et al*., 2005; Lupi *et al*., 2006).

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. A remarkable capability to mediate either an acute or chronic infection with vascular proliferation has been described for this genus (Rolain *et al.,* 2004). *Bartonella* spp. that cause infections often resolve spontaneously without treatment, although there have been reported cases where disease has led to patient death, especially if the heart has been infected (Rolain *et al.,* 2004). Before the antibiotic era, the only known and described species were *B. bacilliformis* and *R. quintana*, and treatment for related infections was limited and had poor effectiveness with a mortality rate of approximately 80% (Schultz, 1968; Sobraquès *et al*., 1999). With a mortality rate of up to 90% during the acute phase, *B. bacilliformis* is considered to cause the highest mortality rate when compared with infections caused by other *Bartonella* spp. (Zeaiter *et al*., 2002).

Some species remain relatively unknown emerging pathogens (Birtles *et al.,* 1995; Anderson and Neuman, 1997; Kabeya *et al.,* 2002; Parola *et al.,* 2002; Yamamoto *et al.,* 2003; Reine, 2004; Lupi *et al*., 2006) while other species have been known for many years. Both *B. bacilliformis* and *B. quintana* (formerly *Rochalimaea quintana*) have been described in early records (Kabeya *et al.,* 2002; Yamamoto *et al.,* 2003; Reine, 2004;

Lupi *et al*., 2006) whereas *B. clarridgeiae* has only recently been discovered and associated with disease. The majority of *Bartonella* spp. are greatly under-studied and health care professionals often misdiagnose *Bartonella*-related infections. Globally, much effort has been invested in the study of *Bartonella* biodiversity; however fewer than 30 mammal species have been studied for the prevalence of infections caused by this genus. As a result it is likely that the true extent of the differences between species within the genus is unknown. If this is the global scenario, then even less is known about the South African situation (Pretorius *et al*., 2004).

The genus *Bartonella* is largely under-represented in terms of epidemiological burden of infectious disease. To understand the bartonelloses, one should understand the characterization, morphology, history, carriage, epidemiology, diagnosis, and treatment of bartonellae and infections caused by them.

1.2 CLASSIFICATION OF *BARTONELLA* SPECIES

According to the 1984 edition of *Bergey's Manual of Systematic Bacteriology*, *B. bacilliformis* was the only species of the genus *Bartonella* (Weiss & Moulder, 1984; Johnson *et al*., 2003; Woolley *et al*., 2007). Almost a decade later, work done by Brenner *et al.* (1993) confirmed that the genus *Rochalimaea* should be incorporated into the genus *Bartonella* (Brenner *et al*., 1993; Greub and Raoult, 2002; Johnson *et al*., 2003). Two years after this reclassification, it was proposed that the genus *Grahamella* should also be included (Birtles *et al*., 1995; Rolain *et al.,* 2004). The reclassifications were based on DNA-DNA hybridization data and phylogenetic analyses performed on the 16S ribosomal ribose nucleic acid (rRNA) gene sequences. *Rochalimaea, Grahamella,* and *Bartonella* were collectively classed as *Bartonella*, family Bartonellaceae (Birtles *et al*., 1995; Clarrige *et al*., 1995; Anderson and Neuman, 1997; Greub and Raoult, 2002). The proposal to merge the genus *Grahamella* with *Bartonella* resulted in the description of 2 more species of *Bartonella*, *B. talpae* and *B. peromysci*, both non-pathogenic to humans (Maurin and Raoult, 1996; Karem *et al*., 2000).

Bartonellae fall within the alpha-2 subgroup of the class Proteobacteria (Jacomo *et al*., 2002). Recent studies have indicated that *Bartonella* spp. have some degree of

relatedness to other α-2 Proteobacteria including *Brucella* spp., *Afipia* spp., *Agrobacterium tumefaciens, Bradyrhizobium* spp.*,* and *Bosea* spp. (Houpikian and Raoult, 2001; Greub and Raoult, 2002; Jacomo *et al*., 2002; Pretorius *et al*., 2004; Rolain *et al.,* 2004; Duncan *et al*., 2007).

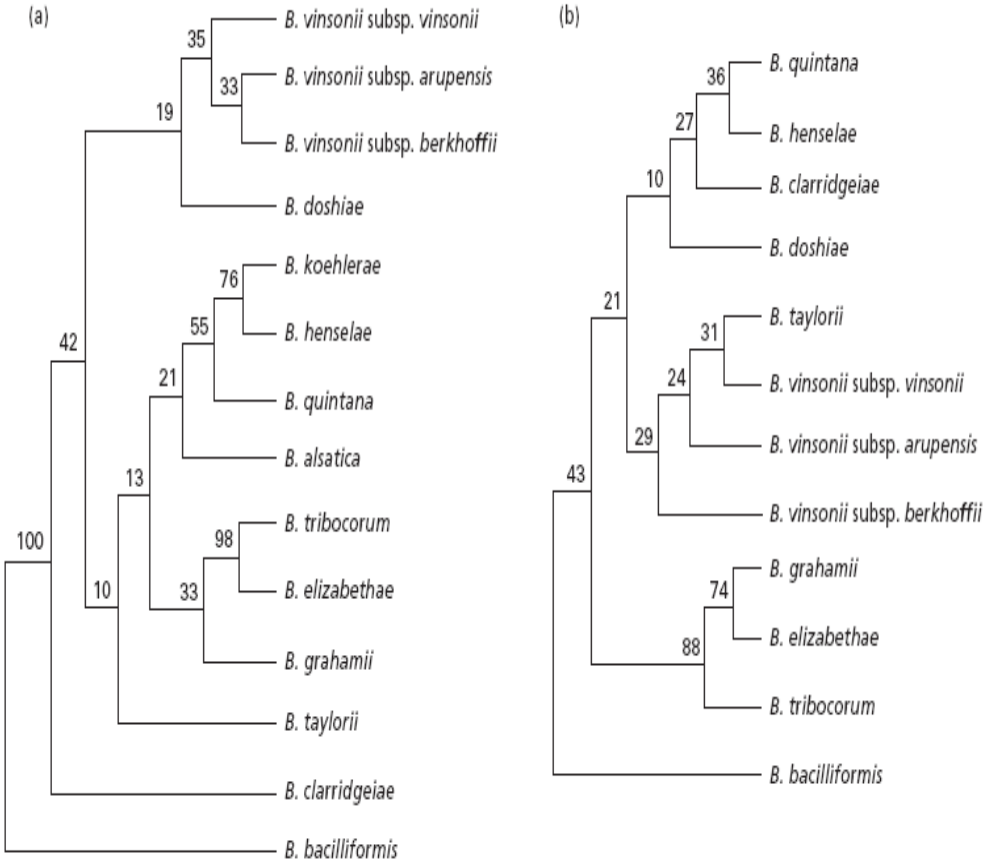

Figure 1.1 Phylogenetic trees derived from the 16S rRNA gene (a) and the citrate synthase (*gltA*) gene (b) for *Bartonella* spp., using the parsimony method. The support of each branch, as determined from 100 bootstrap samples, is indicated at the nodes. The lengths of vertical and horizontal lines are not significant. *Brucella melitensis* and *Rickettsia conorii* served as outgroups to establish the roots of the 16S rRNA tree and the *gltA* tree, respectively. (*Source*: Houpikian *et al*., 2001)

Phylogenetic studies based on 16S rRNA and citrate synthase (*gltA*) gene-sequences, mapped the genetic relatedness between 14 species/subspecies (subsp.) of *Bartonella* (Figure 1.1) (Houpikian *et al.*; 2001)

Current knowledge suggests that there are more than 20 spp. and subsp. included within this genus (Márquez *et al.,* 2008). Approximately 13 spp. have been associated with

human diseases (Pons *et al*., 2008; Pérez-Martínez *et al.,* 2009; Maggi *et al*., 2009) affecting both immunocompetent and immunocompromised individuals. Approximately 6 species affecting humans have been isolated from domestic cats and dogs (Chomel *et al*., 2006).

1.3 MORPHOLOGY

Bartonellae are generally short, pleomorphic, non-fermentative Gram-negative bacilli. They are fastidious aerobic and oxidase-negative organisms (Maurin and Raoult, 1996; Jacomo *et al*., 2002). Growth occurs on enriched medium at 37℃ with 5% carbon dioxide (CO_2), but they can also be grown in broth with fetal bovine serum and in various tissue culture systems including cell lines (La Scola and Raoult, 1999). 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). All species of *Bartonella* grow slowly on blood agar and primary isolates appear after 12 to 14 days on average (Jacomo *et al*., 2002) although it has been reported that primary isolation can take up to 45 days (Maurin *et al.,* 1994). Sub-cultured colonies have been found to appear after only 3 to 5 days (Jacomo *et al*., 2002).

Antibiotic susceptibilities of *Bartonella* spp. have been evaluated (Maurin and Raoult, 1993; Maurin *et al*., 1995; Sobraques *et al*., 1999) and they have been found to be very susceptible in vitro to beta-lactams (except oxacillin and cephalothin), aminoglycosides, macrolides (but not clindamycin), tetracyclines, and rifampicin (Jacomo *et al*., 2002). Considerable variability in the susceptibilities of isolates to the fluoroquinolones was found and only the aminoglycosides (gentamicin, tobramycin, and amikacin) were found to be bactericidal (Jacomo *et al*., 2002). Antibiotic susceptibilities are not routinely tested in clinical isolates of *Bartonella* spp. as susceptibility studies often fail to predict response to therapy (Hammoud *et al.,* 2008).

1.3.1 *Bartonella bacilliformis*

B. bacilliformis is a Gram-negative microaerophillic bacillus that despite being recognized as part of the combined genus *Bartonella*, illustrates phenotypic distinctions

between itself and organisms formerly belonging to the genus *Rochalimaea* (Anderson and Neuman, 1997). It grows best *in vitro* at 28℃, has polar peritrichous flagella, and is highly motile (Maurin and Raoult, 1996; Jacomo *et al*., 2002).

B. bacilliformis grows intra-erythrocytically, resulting in well-recognized cell lysis and anaemia. This species has also been known to adhere to and invade cultured human endothelial cells (Anderson and Neuman, 1997).

1.3.2. *Bartonella henselae*

B. henselae bacilli are small, Gram-negative bacteria that appear as small, dry colonies on specialized media. They often appear brownish in colour with a metallic sheen and tend to pit into the media, but are non-hemolytic. Organisms of this genus have a tendency to adhere to each other in tissues and in culture. This characteristic may contribute to variable tissue pathogenesis observed in cats, dogs, and humans (Abbott *et al*., 1997). Little is known about the metabolic requirements of *B. henselae* (Chenoweth *et al.,* 2004).

The three human-pathogenic serotypes are referred to as *B. henselae* Houston-16S type I (Regnery *et al.,* 1992); *B. henselae* Marseilles-16S type II, (Drancourt *et al*., 1996; La Scola *et al.,* 2002), and Berlin (Arvand *et al.,* 2001), and serotypic distinctions are based on 16S rRNA sequences and chemotaxonomic examinations of cellular proteins by sodium-dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and multilocus sequence typing (MLST) (Bergmans *et al*., 1996; Drancourt *et al*., 1996; Arvand *et al.,* 2001).

1.3.3. *Bartonella quintana*

B. quintana bacteria have been described as short, often curved, Gram-negative rods similar to rickettsiae. *B. quintana* are both catalase and oxidase negative, approximately 0.3 – 0.5 µm wide and 1.0 – 1.7 µm long. Growth is best supported on rabbit blood-supplemented media and growth appears as slightly mucoid, grey, translucent colonies, deeply pitted into the agar. For primary isolation from a clinical sample, at least 12 – 14 days of incubation is required at 35 ± 2°C (Maurin and Raoult, 1996). *B. quintana* has been found to utilize succinate, pyruvate and glutamine/glutamate during growth, but not

glucose. An increase in concentration of CO_2 in the growth environment enhances growth; however the organism is also capable of growing independently of CO_2. It is a hemin-dependent organism and requires sodium bicarbonate ($NaHC0_3$) for growth.

This organism has traditionally been described as epicellular; however growth in human endothelial cell lines has been described since 1997 (Anderson and Neuman, 1997). The genome size has been estimated at 1700 Kb (Maurin and Raoult, 1996).

1.3.4. *Bartonella elizabethae*

B. elizabethae resembles *B. henselae* in morphology. They are small, Gram-negative bacilli that appear as small, white, rough, and dry colonies. The colonies have a raised appearance and tend to pit into the media. *B. elizabethae* is differentiated from *B. henselae* by its ability to cause incomplete haemolysis on the rabbit blood-supplemented agar (Maurin and Raoult, 1996). Colonies are self-adherent and are difficult to separate and transfer (Chomel and Kasten, 2004).

1.3.5. *Bartonella clarridgeiae*

B. clarridgeiae organisms are faintly Gram-negative rods that grow in microaerophillic conditions as described for the other species (Jacomo *et al*., 2002). It has been reported that *B. clarridgeiae* grows weakly or inconsistently on solid media described for other species and growth is best supported by chocolate agar, Fildes enrichment medium, and hemin-supplemented *Brucella* broth (Arvand *et al.,* 2001). Visualization is easier when cultivated in Vero cells and stained by the Gimenez technique. Motility has not been detected in motility agar following 1 week of incubation, although twitching motion is observed in a hanging drop preparation. Negative staining with 2% phosphotungstic acid illustrated the presence of lophotrichous flagella when examined by transmission electron microscopy (Figure 1.2) (Kordick *et al*., 1997).

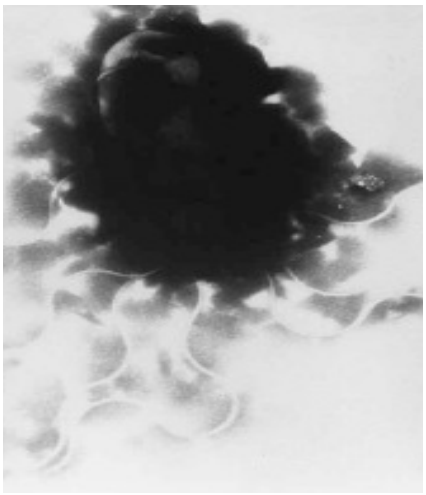

Figure 1.2 *B. clarridgeiae* stained with 2% phosphotungstic acid (pH 7.2), illustrating the presence of lophotrichous flagella using transmission electron microscopy. Magnification: 331 000x. (source: Kordick *et al*., 1997)

1.3.6. *Bartonella vinsonii*

B. vinsonii is recognized as a fastidious, non-motile, small, Gram-negative rod very similar in morphological characteristics to *B. quintana*. Rods are approximately 0.3 – 0.5 µm wide and 1.0 – 1.7 µm long. Growth is visible on chocolate agar after 7 days incubation and it has been shown to grow independently of CO_2. Colonies of *B. vinsonii* are non-hemolytic, pinpoint, white, opaque, dry, and smooth with a metallic sheen. *B. vinsonii* tests negative for both oxidase and catalase and has been shown to not require $NaHCO_3$, unlike *B. quintana* (Maurin and Raoult, 1996; Welch *et al*., 1999; Houpikian *et al*., 2001). *B. vinsonii* genome size has been estimated to be about 2100 Kb (Maurin and Raoult, 1996).

1.3.7 *Bartonella grahamii*

B. grahamii are also Gram-negative, faintly-staining short bacilli, first isolated from the blood of a mouse (*Clethrionomys glareolus*) in the United Kingdom (Birtles *et al.,* 1995). *B. grahamii* is said to grow best on tryptic soy agar supplemented with 5% defibrinated sheep blood, although cultures grow just as well on 5% defibrinated rabbit blood-supplemented Columbia agar. Colonies are flat, pinpoint, and translucent.

1.4 HISTORY OF BARTONELLAE AND BARTONELLOSES

Bartonellae have been responsible for causing various diseases for centuries, although the genus as we now have it has only been recognised since 1992 (Eremeeva *et al.,* 2007). Excavations in the northern suburbs of Vilnius (Verkiu Street, Siaures Miestelis Territory) in 2001 saw the discovery of mass graves on the site of a former Soviet Army barracks. According to local record archives, the graves contained French troops, buried around December 1812 during the retreat of Napoleon's Grand Army from Moscow. Various louse-borne diseases, including *B. quintana,* were tested for by amplification of DNA of ancient lice and dental pulp of buried soldiers. The presence of *B. quintana* was confirmed (Raoult *et al.*, 2006).

La *et al.* (2004) tested the dental pulp of 19 domestic cats, buried in France between the 13th and the 16th centuries, by molecular techniques for presence of *Bartonella* spp. DNA. The finding of *Bartonella* bacteraemia in cats provides a unique model for the study of the antiquity of the relationship between cats, *Bartonella* spp. and the co-evolution between the bartonellae and felid hosts. *B. henselae groEL* protein complex and *pap31* hemin-binding protein gene fragments were detected in the dental pulp of 3 cats dating from the 13th, 14th, and 16th centuries, suggesting persistence of bartonellae among French cats for at least 800 years (La *et al.,* 2004).

Table 1.1 Time-line of the progression of research in *Bartonella* spp.

1895	Peru	Common bacterial origin of verruga peruana and Oroya fever (bartonellosis) established by Daniel Carrión.
1905	United Kingdom	***B. talpae*** was discovered (Birtles *et al.,* 1995).
1909	Peru	***B. bacilliformis*** was isolated by Alberto Barton (Maurin *et al.*, 1997).
1914	Globally	***B. quintana*** was discovered (Kostrzewski, 1949).
1915		***B. quintana*** was acknowledged as the causative agent of trench fever during the First World War (Roux and Raoult, 1995).

1919		***B. bacilliformis*** was cultured (Jacomo *et al*., 2002).
1942	USA	***B. peromysci*** was discovered (Birtles *et al*., 1995).
1946	Canada	***B. vinsonii*** was isolated from voles in Grosse Isle, Quebec, Canada (Baker, 1946; Roux and Raoult, 1995).
1950		Cat scratch disease was described (Chomel *et al*., 1995; Dehio, 2001; Seubert *et al*., 2002).
1961		***B. quintana*** was isolated (Maurin and Raoult, 1996).
1966		***B. quintana*** was successfully cultured (Maurin *et al*., 1997).
1980s		Bartonellae bacteria were identified in patient samples by Warthin-Starry staining (Margolis *et al*., 2003).
1982		***B. vinsonii*** was characterized further by Weiss & Dasch (1982), who proposed the name *Rochalimaea vinsonii* (Weiss & Dasch, 1982; Houpikian *et al*., 2001).
1983		***B. quintana*** associated with endocarditis and bacteraemia in homeless people; and with bacillary angiomatosis (BA) (Maurin and Raoult, 1996; Jacomo *et al*., 2002).
1984		*Bergey's Manual of Systematic Bacteriology* divided the order *Rickettsiales* into three families: 1) Rickettsiaceae, 2) Bartonellaceae, and 3) Anaplasmataceae (Maurin and Raoult, 1996).
1986		***B. elizabethae*** was discovered (Daly *et al.,* 1993).
1990		***B. henselae*** was cultured (Slater *et al.,* 1990) and identified by PCR amplification of the gene for bacterial 16S rRNA (Maurin and Raoult, 1996; Jacomo *et al*., 2002).
1992		***B. henselae*** and ***B. elizabethae*** were renamed; formerly known as *Rochalimaea henselae* and *Rochalimaea elizabethae,* respectively (Maurin and Raoult, 1996). ***B. henselae*** was shown to be an agent of various other diseases in human patients (Maurin *et al*., 1997; Bernit *et al.,* 2003; Ridder *et al.,* 2005).
1993		***B. elizabethae*** was cultured (Daly *et al.,* 1993).

1994		Domestic cats (*Felis domesticus*) were found to be a reservoir for ***B. henselae*** (O'Reilly *et al*., 1999; Breitschwerdt and Kordick, 2000; Pretorius *et al.,* 2004).
1995	Houston	Clarridge *et al.,* (1995) published a description of laboratory diagnostic approach for cat scratch disease (CSD) and gave case presentations of two patients found to have the disease. (Clarridge *et al*., 1995). ***B. clarridgeiae*** was discovered (Clarridge *et al*., 1995) and first cultured (Kordick *et al.,* 1997) from a human case of endocarditis.
	N. Carolina	***B. vinsonii*** **subsp.** ***berkhoffii*** was discovered and cultured from a case of canine endocarditis (Breitschwerdt *et al.,* 1995).
	United Kingdom	***B. grahamii, B. taylori,*** and ***B. doshiae*** were discovered and cultured (Birtles *et al.,* 1995).
1996		***B. vinsonii*** **subsp.** ***berkhoffii*** was described and named as an agent of canine endocarditis (Kordick *et al*., 1996; Houpikian *et al*., 2001). Canadian vole agent was first cultured (Gurfield *et al.,* 1997) and renamed ***B. vinsonii*** **subsp.** ***vinsonii*** (Kordick *et al*., 1996; Houpikian *et al*., 2001). It was discovered that there are 2 ***B. henselae*** genotypes associated with cat scratch disease (CSD) and endocarditis. Proposal to divide ***B. henselae*** into 2 serogroups (Drancourt *et al.,* 1996; Drancourt *et al.,* 2000).
1998		***B. tribocorum*** was discovered and cultured (Heller *et al.,* 1998) from the blood of a wild rat (*Rattus norvegicus*) near the Rhine River in France. Has not been implicated in human disease.
1999	USA	Species ***B. vinsonii*** was implicated in human disease when a new strain was recovered from the blood of a cattle rancher with acute febrile illness (Welch *et al*., 1999; Houpikian *et al*., 2001).
	California	***B. koehlerae*** was discovered and cultured from the blood of 2 cats during a prevalence study of *B. henselae* in

		domestic cats in the greater San Francisco Bay area of northern California (Droz *et al.*, 1999).
	Wyoming	***B. vinsonii* subsp. *arupensis*** was discovered and cultured from blood cultures of a cattle rancher and genotypically related to characterized murine strains from naturally infected mice (Welch *et al.,* 1999).
	France	***B. alsatica*** was discovered and cultured from the blood of wild rabbits (Heller *et al.,* 1999).
2000	Utah & Illinois	***B. birtlesii*** was discovered and cultured isolated from wild *Apodemus* spp., although this animal genus should not be regarded as a specific host since it was successfully inoculated into laboratory mice and bacteraemia was long-term (Bermond *et al.,* 2000).
	France& USA	***B. weissii*** was discovered and cultured from ruminant livestock, i.e. cattle (Breitschwerdt**,** *et al.,* 2001). First human infection of ***Bartonella vinsonii* subsp. *berkoffii*** was detected in a patient with endocarditis (Bermond *et al.*, 2002).
2001	N. Carolina	***B. bovis***, a species almost identical to ***B. weissii,*** was isolated from beef (Breitschwerdt *et al.*, 2001; Bermond *et al.,* 2002).
2002	South Africa	Blood samples taken from 65 African lions in Free State Province confirmed that lions are susceptible to infection by ***B. henselae***. The study illustrated a 1.5% prevalence of infection and a 29% exposure rate (Pretorius *et al.*, 2004).
	South Africa	Pilot survey of HIV clinic outpatients showed 10% prevalence by PCR of *B. henselae* in blood of HIV-positive patients (Frean *et al.*, 2002).
2007	USA	***B. rochalimaea*** was isolated by blood culture from a woman traveling in Peru. The woman experienced numerous insect bites on her travel and illustrated symptoms 16 days after having arrived back in the USA (Eremeeva *et al.*, 2007).

2008	Thailand	**B. *tamiae*** was isolated from three human patients who said that they had recent contact with rats in their homes (Kosoy *et al.,* 2008).
2009	N. Carolina	Candidatus ***B. melophagi*** was isolated from the blood cultures of 2 women proposed to have contracted the infection from sheep (Maggi *et al*., 2009).
	Thailand	Candidatus ***B. thailandensis*** has been proposed for isolates from rodents in Thailand. The species was proposed based on comparisons of concatenated sequences of four genes (Saisongkorh *et al.,* 2009).

1.5 NATURAL HISTORY, CARRIAGE, AND TRANSMISSION OF BARTONELLAE

Prevalence of *Bartonella* infections differs among geographic areas. It has been suggested that seroprevalence is higher in more humid regions (Glaus *et al*., 1997; Skerget *et al.,* 2003). Infection with bartonellae is widespread in certain animal populations and although there have been human infections caused by various *Bartonella* spp., there are some species that have not been associated with human disease at all and have only been isolated from various mammal reservoirs (Pretorius *et al*., 2004). *B. vinsonii* subsp. *vinsonii* has only been isolated from Canadian voles (Karem *et al*., 2000).

Since *Bartonella* spp. reside within the red blood cells of the host (Figure 1.3), the transmissibility of the bacteria is greatly facilitated. Residing intra-erythrocytically allows the bacteria to evade the host's immune system and thus chronically infect the host (Greub and Raoult, 2002). Prolonged bacteraemia allows for more time for transmission to occur between hosts. Transmission is further aided by blood-sucking vectors, such as ticks, fleas, and lice, internalizing the infected blood meal and consequently transferring the infection to the next mammal host.

The life cycle of *Bartonella* varies slightly depending on species. Bartonellae affecting humans are incidentally transmitted from animal or arthropod carriers.

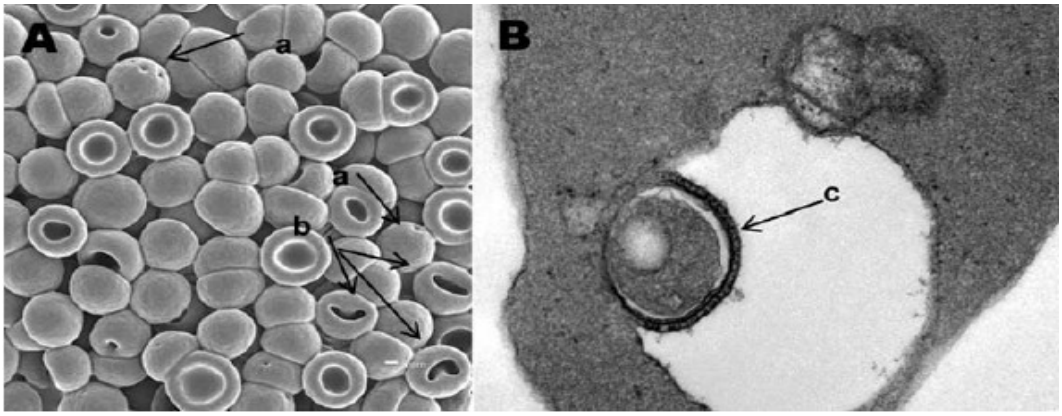

Figure 1.3 A) Scanning electron micrograph of peripheral blood. B) Transmission electron micrograph of erythrocytes. a, membrane invaginations; b, stomatocytes and spherostomatocytes; c, erythrocyte with vacuole-enclosed suspect organism. (Source: O'Rourke *et al.,* 2005)

Figure 1.4 illustrates the natural history of infection of *B. henselae*. To understand the life cycle of *Bartonella* spp., one must remember that humans are mostly secondary hosts that become infected due to contact with the reservoir hosts. Infected animals are contagious through their blood and the infection is therefore transmitted through ectoparasites such as fleas and ticks, or sometimes through contaminated saliva in the event of bleeding gums (Skerget *et al.,* 2003).

The primary mode of transmission of *B. henselae* to humans is through a cutaneous scratch by a cat and transmission is less likely to occur by cat bite as shedding of *B. henselae* in cat saliva has not been clearly documented. Direct transmission of *B. henselae* to humans by the cat flea (*Ctenocephalides felis*) is not yet experimentally proven and is largely speculative. The presence of cat fleas does, however, remain essential for the maintenance of the infection within the cat population (Chomel *et al.*, 2006). Bacteraemia prevalence in cats is highest when flea infestation is high and hosts may remain infected for months to years, (Kordick *et al.,* 1999; Muñana *et al.,* 2001) although this varies globally. Various prevalence surveys have illustrated that a significant number of cats globally are asymptomatically infected with *Bartonella* and consequently have the potential to be reservoirs for human infection (Baneth *et al.,* 1996; Kordick *et al.,* 1999).

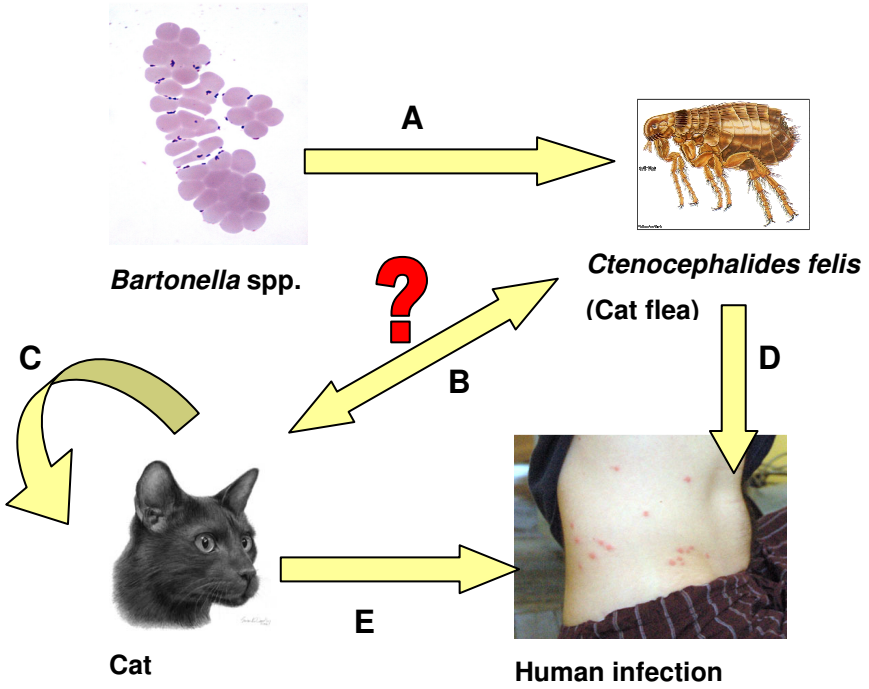

Figure 1.4 Life cycle of *Bartonella henselae* typically transmitted by cat flea (*Ctenocephalides felis*) to animal and human hosts. A, the bacillus infects fleas via ingestion of infected cat blood; B, fleas bite and infect other cats with *Bartonella*; C, cats become reservoirs and re-infect other cats; D, fleas often bite humans that come into contact with them; E, cats also transmit the infection to humans by scratch or bite.

Studies have shown that *B. henselae* can multiply in the digestive system of the cat flea and survive several days in flea faeces (Chomel *et al.,* 2006). In an experiment, only cats inoculated with flea faeces, compared to those on which fleas were deposited in retention boxes or flea fed, became bacteraemic. The main source of infection thus appears to be flea faeces that contaminate a cat's claws when cats attempt to scratch away the flea irritation.

B. quintana is carried by human body lice (*Pediculus humanus*) and human hosts form the reservoir (Roux and Raoult, 1995; Chang *et al.,* 2001). *B. quintana* are capable of intracellular growth in human epithelial cells (Batterman *et al.,* 1995).

There have been various attempts to induce illness in animals through inoculation of *B. quintana* and *B. bacilliformis,* in order to detect possible animal hosts for these 2 species; however, this only succeeded when primates were injected with the bacteria (Maurin and Raoult, 1996). *B. bacilliformis* has humans as the only reservoir host, and

the sand fly, genus *Lutzomyia,* is the transmitting vector. *B. henselae, B. clarridgeiae, B. koehlerae,* and *B. weissii* are cat-specific species, although they may infect unusual hosts. *B. henselae* and *B. clarridgeiae* are etiologic agents of cat scratch disease (CSD) in humans, usually transmitted by direct contact with the infected reservoir (Dehio, 2001; Seubert *et al*., 2002; Parola *et al*., 2002; Bernit *et al.,* 2003; Dyachenko *et al.,* 2005; Ridder *et al.,* 2005; Loa *et al*., 2006) or with the fleas feeding on the infected reservoir host. There are other zoonotic species of *Bartonella* (*B. elizabethae, B. vinsonii,* and *B. grahamii*) that are also transmitted by fleas. The reservoirs of these species are mostly dogs and rodents (Dehio, 2001). The pathways for transmission are similar, although there are species that have been newly described and further investigations are required. Other species within the genus have been found to infect small mammals such as mice, voles, rabbits, roe deer, and rats. *B. grahamii, B. taylorii,* and *B. doshiae* have been isolated from voles and mice by Birtles *et al.* (1995), but have not been implicated in human disease (Maurin and Raoult, 1996).

1.6 CLINICAL IMPORTANCE

Bartonellae affect both immunocompromised and immunocompetent patients, although depending on the species, the infections present in different ways (Slater *et al*., 1992; Wong *et al*., 1997). Bartonellae are obligate pathogens. The probability of infection is heightened in immunosuppressed transplant patients, chemotherapy patients, and patients who suffer from immunosuppressive disorders such as HIV/AIDS (Tappero *et at.,* 1993).

Human pathogenicity is related to incidental infection contracted from carrier species. A wide spectrum of diseases has been described following *Bartonella* infection (Dehio, 2001; Seubert *et al*., 2002; Bernit *et al.,* 2003; Ridder *et al.,* 2005). Active investigation into pathogenesis of *Bartonella* infections has recently been sparked due to the increase in both the number of newly characterized *Bartonella* spp. and the re-emergence of older diseases characteristic of *Bartonella* (Greub and Raoult, 2002).

Clinical disease ranges from painless lymphadenopathy to large cervical abscesses (Kabeya *et al.,* 2002; Seubert *et al*., 2002; Ridder *et al.,* 2005; Rie *et al.,* 2003). Lymph

nodes most commonly involved are in the axilla, neck, and groin (Marakaki *et al.,* 2003; Dyachenko *et al.,* 2005). Although rare, serious diseases such as encephalopathy, pulmonary disease, pneumonia, neuroretinitis, endocarditis, aseptic meningitis, and osteomyelitis may complicate *Bartonella* infections, even in immunocompetent individuals (Ciervo *et al.,* 2005; Ridder *et al.,* 2005; Loa *et al.,* 2006). In immunocompromised patients, disseminated and systemic infections such as fever, bacillary angiomatosis (BA) and peliosis hepatis (PH) may occur (Johnson *et al*., 2003). In severe cases, HIV-positive patients develop cerebral lesions (Spach *et al.,* 1992; Muñana *et al*., 2001) and dementia associated with relapsing *B. henselae* bacteraemia (Lucey *et al.,* 1992; Schwartzmann *et al.,* 1995; Muñana *et al*., 2001). The pathogenesis and dysfunction of central nervous system (CNS) is not fully understood (Muñana *et al*., 2001). Other diseases include meningo-encephalitis, stellar retinitis, Parinaud's oculoglandular syndrome, erythema nodosum, arthritis, pulmonary nodules (Giladi *et al.,* 2001), myelitis, granulomatous hepatitis, and lesions of almost every organ system including the heart, liver, spleen, bone and bone marrow, lymphatics, muscle and soft tissue, and central nervous system (Anderson and Neuman, 1997; Greub and Raoult, 2002).

Perhaps the most interesting observation of the interaction of *Bartonella* spp. with its host is the proliferation of vascular endothelial cells. This neovascularization occurs during infection with *B. henselae*, *B. bacilliformis*, or *B. quintana* and begins with the production of the endothelial cells lining small blood vessels. *Bartonella*-induced angiogenesis results in the lesions observed in patients with BA and the verruga peruana of Carrión's disease.

1.6.1 Carrión's disease and verruga peruana

B. bacilliformis infects the erythrocytes of patients diagnosed with bartonellosis or Carrión's disease, a biphasic disease once seen as a medical enigma (Maurin *et al*., 1997; Houpikian and Raoult, 2001). The acute phase of the disease, Oroya fever, is characterised by severe hemolytic anaemia (Chang *et al.,* 2001) and life-threatening septicemia, as a result of which 40 to 85% of untreated patients die. It is a disease that predominantly affects immunologically naïve persons travelling to the Andes of Peru, where the disease is endemic. Prior to the antibiotic era, a blood transfusion was the only available treatment for Oroya fever. This treatment lacked efficacy and the mortality

rate remained high (Rolain *et al.,* 2004). Chronic Carrión's disease, termed verruga peruana, presents as distinctive, benign, cutaneous vascular lesions typically consisting of round papules on the skin (Anderson and Neuman, 1997). These papules are often inflamed and bleed, and osteoarticular pain usually occurs in association with these vascular lesions. A very small percentage (~5%) of patients diagnosed with verruga peruana recall acute febrile illness prior to the chronic bacteraemia. Verruga peruana generally affects the native population of the Andes of Peru (Rolain et al., 2004).

1.6.2 Cat scratch disease (CSD)

CSD is common in both children and adults scratched by, or exposed to domestic cats and their fleas (Karem *et al*., 2000; Dyachenko *et al.,* 2005; Loa *et al*., 2006). The majority (approximately 60%) of the cases reported are individuals below the age of 20 years (O'Connor *et al*., 1991; Herremans *et al*., 2007). CSD has been recognized for over half a century (Margolis *et al*., 2003), but it was not until 1993 that *B. henselae* was acknowledged as the most common causative pathogen (Regnery *et al.,* 1992; Herremans *et al*., 2007). CSD is often clinically misdiagnosed as tuberculosis. This is especially true for HIV-infected patients, who are already at a higher risk of both *Mycobacterium tuberculosis* reactivation and primary infection (Bernit *et al.,* 2003). In an immunocompetent host, atypical manifestations of CSD (with or without lymphadenopathy) occur in 5% to 20% of all cases diagnosed and affect various organs (Ridder *et al.,* 2005). Typical CSD of immunocompetent patients presents as gradual lymphadenopathy at the nodes draining the site of the cat scratch preceded by localized lesions with redness, swelling, and pustules also appearing at the site of initial inoculation. These are however, inconsistent symptoms that are not seen in all CSD patients. In some cases the patient presents with low-grade fever and weight loss (O'Reilly *et al.,* 1999; Skerget *et al.,* 2003; Rolain *et al*., 2004).

CSD often presents with ophthalmic manifestations that are commonly benign and self-limiting with excellent vision recovery (Gray *et al.,* 2004). Parinaud's oculoglandular syndrome consists of conjunctival granuloma in one eye accompanied by swelling of the preauricular lymph nodes and is often associated with tularemia, and tuberculosis. Some manifestations such as neuroretinitis (Monahan, 2000), optic nerve masses, vascular-occlusive events including both central retinal artery and vein occlusion, choroidal masses, retinitis, choroiditis, and intermediate uveitis as well as some cases of

neovascular glaucoma, may result in severe ocular damage and even the total loss of vision (Cunningham and Koehler, 2000; Skerget *et al.,* 2003; Gray *et al.,* 2004; Ridder *et al.,* 2005; Curi *et al.,* 2006).

1.6.3 Trench fever

Trench fever, also known as 5-day fever, Wolhynia fever, or quintan fever, is caused by *B. quintana* and is characterized by headaches, skin rashes, inflamed eyes, leg pains, and unexplained fever for up to 8 days, concurrent with the onset of erythrocyte infection (Maurin *et al.*, 1997; Rolain *et al.*, 2004). Although the majority of trench fever cases were reported before the antibiotic era, no fatalities have been attributed to acute infection, and clinical illness resolves in 4 to 6 weeks (Maurin *et al.*, 1997; Rolain *et al.*, 2004). Trench fever refers to the acute stage of *B. quintana* infection and is marked with the onset of nonspecific symptoms (Maurin *et al.*, 1997; Guibal *et al.,* 2001). It is a disease commonly associated with homeless people and alcohol abusers. It is transmitted by human body lice (Chang *et al.,* 2001) and disease incubation period ranges from 15 to 25 days. Anaemia often occurs in chronically ill patients (Maurin *et al.*, 1997).

1.6.4 Bacillary angiomatosis (BA)

BA is a disease that is characterized by unusual neoplasia of the microvascular tissue of the skin, and other organs of the body (Maurin *et al.*, 1997; Koehler *et al.,* 1997; Rolain *et al.*, 2004). Initially described in patients with HIV/AIDS, BA also affects healthy people (Tappero *et at.,* 1993; Maurin *et al.*, 1997; Rolain *et al.*, 2004). BA has an appearance similar to that of Kaposi's sarcoma and both *B. quintana* and *B. henselae* are the species responsible for this manifestation (Stoler *et al.,* 1983; Chang *et al.,* 2001; Houpikian and Raoult, 2001; Johnson *et al.*, 2003). Nodules form from gradually enlarging papules which are the primary skin lesions. Lesions tend to bleed when punctured and are either solitary or multiple, superficial, dermal or subcutaneous (Maurin *et al.*, 1997). Often, the liver, bone marrow, lymph nodes, and spleen are involved (Rolain *et al.*, 2004) and the angiogenesis is said to be triggered by an unknown protein that stimulates endothelial cell proliferation up to 3 times more than if the protein was absent (Garcia *et* al., 1990; Greub and Raoult, 2002).

1.6.5 Peliosis hepatis (PH)

PH is caused by *B. henselae* and is particularly a problem for HIV-positive patients (Maurin *et al.,* 1994; Rolain *et al*., 2004). It is defined as a vascular proliferation of sinusoidal hepatic capillaries resulting in spaces filled with blood in the liver (Maurin *et al*., 1997; Rolain *et al*., 2004). Typically, patients with tuberculosis, advanced cancers, or those utilizing anabolic steroids, are considered most prone to develop PH. *B. henselae* has been described as the main infectious cause of PH in HIV-infected patients and often occurs simultaneously with peliosis of the spleen and BA of the skin (Maurin *et al*., 1997; Rolain *et al*., 2004). PH is considered the visceral manifestation of BA and the term bacillary peliosis has been proposed to define it (Maurin *et al*., 1997).

1.6.6 Infective endocarditis (IE)

Bartonella spp. are increasingly recognised as a cause of endocarditis although isolation from blood by routine methods is not always reliable. Diagnosis is usually made serologically (Breathnach *et al.,* 1997) or by surgical biopsy. Evidence of *Bartonella* infection has been found in 3% of all patients diagnosed with endocarditis (Maurin *et al*., 1997; Rolain *et al*., 2004). Seven of the 20 species of *Bartonella* are known to cause infective endocarditis (IE) in humans. These include *B. quintana*, *B. henselae*, *B. elizabethae*, *B. vinsonii s*ubsp. *berkhoffii*, *B. vinsonii* subsp. *arupensis*, *B. koehlerae,* and *B. alsatica* (Johnson *et al*., 2003; Lepidi *et al.,* 2007). The most common cause of endocarditis is *B. quintana*, followed by *B. henselae.* Differentiation between *B. quintana* endocarditis and *B. henselae* endocarditis is based on epidemiological factors such as possible route of infection (Maurin *et al*., 1997; Rolain *et al*., 2004). For instance, *B. henselae* is associated with exposure to cats, and *B. quintana* with homelessness and poor hygiene (Breathnach *et al.,* 1997).

Bartonella-caused endocarditis often requires valve replacement surgery due to the extensive damage caused by the bacilli. It is generally indolent and culture-negative, and delays in diagnosis contribute to a higher death rate than other forms of endocarditis. The selection of the appropriate treatment regimen is crucial in reducing *Bartonella*-related endocarditis mortality (Rolain *et al*., 2004).

1.6.7 Animal infections

Felids are the natural carriers of *Bartonella* spp. including *B. henselae, B. clarridgeiae, B. elizabethae*, *B. weissii,* and *B. koehlerae*, and the nature of *Bartonella* pathogenesis in these hosts is not clearly understood (O'Reilly *et al.,* 1999; Rolain *et al*., 2004). *B. henselae* or *B. clarridgeiae* bacteraemia is usually asymptomatic, and overt disease is strain dependant (Koehler *et al.,* 1994; Johnson *et al*., 2003; Chenoweth *et al.,* 2004). *Bartonella* spp. are responsible for chronic bacteraemia in kittens and adult cats. Prevalence of carriage ranges from 4% to 70% among apparently healthy cats (La *et al.,* 2004). Animals are not all prone to infection by all *Bartonella* spp. and infection is species specific; for example, it was reported that cats experimentally inoculated with *B. henselae* developed a persistent bacteraemia for 1 – 8 months, whereas *B. quintana*-inoculated cats did not develop bacteraemia at all (Kosoy *et al*., 2000). Cats have been found to remain bacteraemic for several months, or even years, without ever becoming symptomatic (Chomel *et al*., 1999).

Dogs have also been found to transmit *B. henselae* (Skerget *et al.,* 2003). There are several *Bartonella* spp. that contribute to the pathogenesis of a wide spectrum of diseases that are implicated in canine infection, and which may not be cause for concern in the felid population, for example*, B. clarridgeiae, B. elizabethae, B. vinsonii* subsp. *berkhoffii*, and B. *washoensis*, which may asymptomatically colonize feline counterparts. Canine diseases include polyarthritis, cutaneous vasculitis, endocarditis, myocarditis, epistaxis, peliosis hepatis, and granulomatous inflammatory disease (Chang *et al.,* 2001; Duncan *et al.,* 2007).

Perhaps the most significant reservoir for bartonellae is rodents. Rodent home ranges overlap our own and those of many other animals. Research has indicated that various *Bartonella* spp. occur naturally, are widely distributed, and highly prevalent in rodent communities of the southeastern United States (Kosoy *et al*., 1999). Wild-trapped cotton rats from natural populations, whether bacteraemic or not, showed no detectable antibody or only low antibody titers, measured by immunofluorescence using homologous antigens (Kosoy *et al*., 1997). In a study done by Kosoy *et al.* (1999), laboratory-bred cotton rats were experimentally infected with *Bartonella* spp. that occur naturally in this rodent host, in an attempt to describe the dynamics of the infection. It was observed that all inoculated rats became bacteraemic within the first two weeks

after inoculation although none illustrated any detectable illness and no rodent died following infection. In fact inoculated rats appeared indistinguishable from *Bartonella*-free control animals. The lack of antibodies to the bartonellae may be indicative of immune tolerance to the infection in the natural host, or an immunosuppressive mechanism or a mechanism of the bacteria to evade the host's immune system (Kosoy *et al*., 1999).

1.7 DIAGNOSIS AND TREATMENT OF *BARTONELLA* INFECTIONS

Different pathological responses to a *Bartonella* infections has made diagnosis tiresome and difficult (Slater *et al*., 1992; Rolain *et al.,* 2004). Clinical and historical information is important in making a diagnosis. Identification of CSD is based on five criteria: 1) the presence of a cutaneous inoculation site; 2) chronic lymphadenopathy without specific diagnosis; 3) cat scratches or history of contact with a cat/s; 4) a granuloma on histologic examination of lymph node tissue biopsy; or 5) a positive skin test (Maurin *et al.,* 1997; Riddler *et al.,* 2005). Due to the resolution of the point of inoculation or the patient not remembering the contact with the animal source, CSD is often missed as a possible cause of the infection. Misdiagnosis is often a major problem. There are various methods for the diagnosis of *Bartonella* infections. The conventional methods include culture (Johnson *et al*., 2003), enzyme-linked immunosorbent assay (ELISA), IgG and IgM immunofluorescent assays (IFA), western immunoblots (WB) and microscopy of tissue sections, when Warthin-Starry staining is especially useful. Molecular tests include polymerase chain reaction (PCR), real-time PCR (q-PCR) and nested PCR (n-PCR) (McCool *et al.,* 2008).

Due to the variability in clinical manifestations of *Bartonella*-induced disease, no single treatment has yet been identified for all cases, and treatment is uniquely adapted to each clinical situation (Rolain *et al.,* 2004). Chloramphenicol (50 mg/kg/day up to 3 g/day) has traditionally been the recommended treatment for Oroya fever; however, due to drug failure in recent times, chloramphenicol has been replaced with ciprofloxacin (500mg orally for 10 days in adults and 250 mg orally for 10 days in children under 15 years) as the drug of choice (Rolain *et al*., 2004). For the severe anaemia associated with Oroya fever, packed red blood cell transfusions are required for treatment. Verruga peruana is often treated with oral rifampicin (10 mg/kg/day) for 2-3 weeks; however oral

erythromycin, ciprofloxacin, and azithromycin had also been reported to be effective in treating verruga peruana (Walker *et al.,* 2006). Antibiotic treatment for CSD has little or no effect on the course of infection, although azithromycin may be prescribed to treat lymphadenopathy. In uncomplicated CSD the immune system is responsible for controlling the infection. The majority of antibiotics are merely bacteriostatic, whereas gentamycin is bactericidal against *B. henselae*. Oral doxycyline (100 mg twice daily) or erythromycin (0.5 – 1.0 g four times a day) for 2 to 3 months are used to treat bacillary angiomatosis, although oral tetracycline, minocycline, azithromycin, clarithromycin, and chloramphenicol have been reported to have successful results (Raoult *et al*., 2003; Walker *et al*., 2006). Endocarditis caused by bartonellae often requires valve replacement and intravenous administration of antibiotics (Walker *et al*., 2006).

1.8 EPIDEMIOLOGY

Since the initial reclassification and characterization of bartonellae, vast interest in this genus has lead to a variety of epidemiologic studies around the globe. These studies have looked at the impact of *Bartonella* infections in both human and animal populations.

It has been reported that primates, including humans, are the only species found to be made profoundly ill by *Bartonella* infection. In acute, untreated co-infections with salmonellosis, shigellosis, malaria, toxoplasmosis, histoplasmosis, or pneumocystosis, a 40 – 88 % mortality rate has been reported (Lydy *et al*., 2008).

1.8.1 Global seroprevalence of *Bartonella* infections

To determine the real incidence of *Bartonella* infections, the seroprevalence of the general population, principal reservoirs and vectors of infection have been studied. In a study conducted in Catalonia, Spain, between 2004 and 2005, the seroprevalence of *Bartonella* infections in HIV-positive patients was evaluated. Of the 340 HIV-positive patients enrolled in the study, none had a past history of exposure or any symptoms of *Bartonella* spp. infections. Serological immunofluorescent assays (IFA) were carried out and a high prevalence (22%) of *Bartonella* antibodies was detected in the study group (Pons *et al*., 2008).

Early serological studies in the USA have reported *B. henselae* seroprevalence in domestic cats as high as 54% (Jameson *et al.,* 1995; Muñana *et al.,* 2001). General epidemiological studies conducted in North America have indicated serological incidence from 4 to 81%, and bacteremia up to 40% (Koehler *et a*l., 1994; Childs *et al*., 1995; Chomel *et al*., 1995; Gurfield *et al*., 2001). In 2006 in the USA, the overall *Bartonella* spp. DNA prevalence rates in cats (n=92) was 47.8% and 62% in their fleas. The *B. henselae* DNA prevalence rates in cats (34.8%) was higher than that of *B. clarridgeiae* (20.7%), whereas the *B. clarridgeiae* prevalence (50%) in fleas was greater than that of *B. henselae* (40.2%) (Lappin *et al.,* 2006; Lappin *et al.,* 2008). Studies have been conducted on other species within the cat family. The seroprevalence of *B. henselae* was investigated in serum samples taken from 28 free-ranging Florida panthers (*Puma concolor coryi*) and 7 mountain lions from Texas (*P. concolor stanleyana*) living in southern Florida (USA), between 1997 to 1998. Samples were tested for the presence of antibodies to *B. henselae* and 20% (7/35) sero-reactivity was observed overall. A prevalence of 18% (5/28) for panthers and 28% (2/7) for the mountain lions was reported (Rotstein *et al.,* 2000).

In a study conducted in the Philippines, the sera of 107 cats were serologically tested by IFA and enzyme immunoassay (EIA) for the prevalence of antibodies to 2 *Bartonella* spp., *B. henselae* and *B. clarridgeiae*. It was reported that approximately 58% of the 107 sera tested were simultaneously positive for *B. henselae* and *B. clarridgeiae*, 7.5% for *B. clarridgeiae* only, and 10.3% positive for *B. henselae* only (Chomel *et al*., 1999). A study carried out to assess the carriage of *Bartonella* spp. in exotic pet mammals imported into Japan without quarantine, reported a *Bartonella* prevalence of 26% (142/546) (Inoue *et al.,* 2009).

Cats in Israel have had a 40% seroprevalence reported (Baneth *et al*., 1996; Gurfield *et al*., 2001), whereas Egyptian cats were reported to have a 12% seroprevalence (Childs *et al*., 1995; Gurfield *et al*., 2001).

In the Netherlands, domestic cats (n=163) were reported to have a seroprevalence of between 50% and 56% (Bergmans *et al*., 1997; Gurfield *et al*., 2001). A French survey of 94 stray cats in Nancy illustrated a 53% seroprevalence (Heller *et al*., 1997).

1.8.2 National prevalence of *Bartonella* spp. in human and animal studies

The prevalence of *Bartonella* spp. is largely unknown in South Africa. There are some case reports of BA caused by *Bartonella* spp. in HIV-positive patients (Frean *et al.*, 2002) and some work on prevalence of *B. henselae* in domestic and wild felines in southern Africa published (Kelly *et al.,* 1996).

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.*, 2006). A non-random pilot survey of outpatients of 3 Johannesburg HIV clinics has been done on a relatively small sample group (n = 188 patients). This study showed a 10% prevalence of *B. henselae* in blood of HIV-positive patients, determined by nested polymerase chain reaction (PCR) (Frean *et al.*, 2002).

Kelly *et al.* (1996) reported evidence that domestic cats are the principal reservoirs, and the aetiological agents of human diseases including cat-scratch disease, bacillary angiomatosis, bacillary peliosis and a febrile bacteraemia syndrome. Feline sera from South Africa and Zimbabwe were tested by indirect fluorescent antibody assays and the overall seroprevalance for South Africa and Zimbabwe collectively was reportedly 23% (39/171) *B. henselae* prevalence (Kelly *et al.,* 1996).

1.9 GEOGRAPHICAL STUDY AREA

Gauteng is the province of South Africa with the smallest geographic area and the highest population per km^2 (Figure 1.5). There are approximately 10.5 million people (21.5% of the total population) in Gauteng as reported in July 2008 by STATS SA and the province has a population density of approx. 467 people/km^2 (Statistics South Africa, 2008).

Gauteng lies in the highveld of the country at subtropical altitude, although the climate is comparatively cooler than would be expected for its latitude. Johannesburg, the main city in Gauteng, is at an altitude of 1694 m above sea level with low relative humidity. Gauteng experiences summer rainfall with average temperatures in mid-summer at 28°C

maximum and 17℃ minimum. In midwinter the average maximum is 19℃ and the average minimum is 5℃. The annual precipitation is approx. 700 mm^3 and snow is very rare, but has occurred on some occasions in the Johannesburg metropolitan area (South African weather service, 2009).

When the South African climate and environment is compared to other *Bartonella*-endemic countries many similarities exist, yet it is unknown whether *Bartonella* poses the same significant medical burden as it does in other endemic regions sharing a similar climate and latitude.

Figure 1.5 Political map of South Africa (source: http://www.nationsonline.org/oneworld/map/za_provinces_map.htm)

1.10 AIMS AND OBJECTIVES

The deficit in South African knowledge about the prevalence of *Bartonella* spp. in both human and animal populations has been the rationale for this study. The general aim is to study aspects of the epidemiology of *Bartonella* infections in Gauteng, South Africa.

My specific objectives for both human and animal populations in this study were:

i. To determine the prevalence of *Bartonella* infections in HIV-positive patients presenting for treatment at a Gauteng HIV-clinic treating a large part of the Gauteng population.

ii. To determine to what extent bartonellae affect the general healthy population in terms of current or past infection.

iii. To determine the seroprevalence of *Bartonella henselae* and *Bartonella quintana* antibodies in HIV-negative antenatal patient sera taken from various maternity units in Gauteng public hospitals.

iv. To investigate cats and dogs in Johannesburg for carriage of bartonellae.

v. To investigate commensal and wild rodents for carriage of bartonellae.