Multi-Locus sequence analysis reveals profound genetic diversity among isolates of the human pathogen Bartonella bacilliformis

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**Introduction**

Bartonellosis, or Carrion’s disease, caused by the bacterium *Bartonella bacilliformis*, has long been recognised in the Andean region of South America, particularly in the high valleys lining the western side of the cordillera in central Peru [1]. Bartonellosis may present in two markedly different clinical manifestations. Firstly, Oroya fever is characterised by fever, headache, pallor and myalgia, which progresses to a severe haemolytic anaemia. Mortality rates as high as 88% have been described in untreated patients with this manifestation. Alternatively, infection may provoke “ verruga peruana” characterised by angiogenic skin lesions akin to bacillary angiomatosis caused by *Bartonella henselae* and *Bartonella quintana*. Although the appearance of lesions may be dramatic, verruga peruana tends to be self-limiting and not life-threatening. The natural cycles of *Bartonella* species are characterised by mammalian reservoirs and arthropod vectors, and for *B. bacilliformis*, humans appear to be the sole reservoir host and sandflies (*Lutzomyia* spp.) are considered the most likely vectors. Asymptomatic and chronic infections of people living in areas where *B. bacilliformis* is endemic are thought to be common [2,3].

Monitoring of bartonellosis in Peru over the past two decades has revealed some dramatic epidemiological changes. The number of cases collated nationally by the Instituto Nacional de Salud rose from about 3,000 per annum in the 1990s to over 10,000 per annum between 2004 and 2006, before declining again over the past four years (http://www.ins.gob.pe/portal), and numerous new foci of bartonellosis have been identified in regions of the country where the disease was previously unknown [2,4–7]. The disease has also been reported in new locales in Colombia and Ecuador [5,8,9]. The ecological or anthropological bases for these changes are unknown, although it has been postulated that they may have resulted from warmer, wetter weather provoking increases in the population size and range of sandfly vectors [10].

Only very few studies exploring the genetic diversity of *B. bacilliformis* have been published [11,12]. These efforts employed a variety of different typing methods including pan-genomic approaches such as amplified fragment length polymorphism and infrequent restriction site polymerase chain reaction (PCR), and/or comparison of nucleotide sequence variation at loci including the citrate synthase gene (*cysA*) and Invasion-associated locus B gene (*ialB*) and, most frequently, the 16S-23S rDNA intergenic spacer region (ISR). All these approaches have delineated genotypes within the species, and have been useful in characterising the molecular epidemiology of bartonellosis [11,12].

Multi-locus sequence typing (MLST) is now established as a powerful approach to defining the population structures of bacterial species and to explore the evolutionary mechanisms that have shaped these population structures [13–15]. MLST schemes for *B. henselae* and *B. quintana* have already been described and have proven to be of value [16–21]. The aim of the present study was to develop a MLST scheme for *B. bacilliformis* then to exploit the

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**Abstract**

*Bartonella bacilliformis* is the aetiological agent of human bartonellosis, a potentially life-threatening infection of significant public health concern in the Andean region of South America. Human bartonellosis has long been recognised in the region but a recent upsurge in the number of cases of the disease and an apparent expansion of its geographical distribution have re-emphasized its contemporary medical importance. Here, we describe the development of a multi-locus sequence typing (MLST) scheme for *B. bacilliformis* and its application to an archive of 43 isolates collected from patients across Peru. MLST identified eight sequence types among these isolates and the delineation of these was generally congruent with those of the previously described typing scheme. Phylogenetic analysis based on concatenated sequence data derived from MLST loci revealed that seven of the eight sequence types were closely related to one another; however, one sequence type, ST8, exhibited profound evolutionary divergence from the others. The extent of this divergence was akin to that observed between other members of the *Bartonella* genus, suggesting that ST8 strains may be better considered as members of a novel *Bartonella* genospecies.
MLST isolates and growth conditions

Terminology

Materials and Methods

Results
Table 1. Characteristics of, and MLST data for, the 43 *B. bacilliformis* isolates studied.

| Isolate designation | geographic location where infection was acquired (endemic/new focus) | year | age/sex | disease | MLST allelic profile | ST |
|---------------------|---------------------------------------------------------------|------|---------|---------|----------------------|----|
| KC583               | Huarochiri, Lima (End)                                        | 1960s| NK      | NK      | 1 1 1 1 1 1 1 1     |    |
| KC584               | Churcampa, Huancavella (End)                                  | 1960s| NK      | NK      | 1 1 1 1 1 1 1 1     |    |
| T2                  | Huaraz, Ancash (End)                                          | 1999 | NK      | OF      | 1 1 1 1 1 1 1 1     |    |
| Hua-Rub             | Huarochiri, Lima (End)                                        | 1999 | 23 M    | OF      | 1 1 1 1 1 1 1 1     |    |
| Sih-ism             | Sihuas, Ancash (End)                                          | 1999 | 28 M    | OF      | 1 1 1 1 1 1 1 1     |    |
| Hua-Chu             | Huarochiri-Quiripa, Lima (End)                                | 1999 | 16 M    | OF      | 1 1 1 1 1 1 1 1     |    |
| Hua-Mar             | Huarochiri-Puellucanchi-Lima (End)                            | 1999 | 2 M     | OF      | 1 1 1 1 1 1 1 1     |    |
| Hua-Nol             | Huarochiri-Quinti, Lima (End)                                 | 1999 | 50 M    | OF      | 1 1 1 1 1 1 1 1     |    |
| Alca                | Caraz-Ancash (End)                                            | 1999 | 17 M    | OF      | 1 1 1 1 1 1 1 1     |    |
| Cas                 | Caraz-Ancash (End)                                            | 1999 | 43 M    | AS      | 1 1 1 1 1 1 1 1     |    |
| Quillay             | Huaraz, Ancash (End)                                          | 2003 | NK      | NK      | 1 1 1 1 1 1 1 1     |    |
| Quispe              | Huancoco -Huaracuchuco-(end)                                  | 2002 | 33 M    | OF      | 1 1 1 1 1 1 1 1     |    |
| Fili                | Yungay, Ancash (End)                                          | 2004 | 2 M     | NK      | 1 1 1 1 1 1 1 1     |    |
| Vega                | Comas, Lima (End)                                             | 2005 | 17 M    | NK      | 1 1 1 1 1 1 1 1     |    |
| Sot                 | Huaraz, Ancash (End)                                          | 2005 | 47 M    | NK      | 1 1 1 1 1 1 1 1     |    |
| Bon                 | San Martin (NK)                                               | 2006 | 23 M    | NK      | 1 1 1 1 1 1 1 1     |    |
| DB06-P154           | Canete, Lima (NK)                                             | 2006 | 11 M    | OF      | 1 1 1 1 1 1 1 1     |    |
| DB07-P219           | Huaral, Lima (NK)                                             | 2007 | 26 M    | OF      | 1 1 1 1 1 1 1 1     |    |
| DB07-P207           | Huaral, Lima (NK)                                             | 2007 | 14 M    | OF      | 1 1 1 1 1 1 1 1     |    |
| FBC-220             | Huaraz, Ancash (End)                                          | 2006 | NK      | NK      | 1 1 1 1 1 1 1 1     |    |
| CONDO44             | Huaylas, Ancash (End)                                         | 1997 | 10 F    | AS      | 2 3 2 2 2 1 1 2     |    |
| NCTC12134           | NK                                                              | 1949 | NK      | NK      | 1 2 2 3 1 2 2 3     |    |
| NCTC12135           | NK                                                              | 1941 | NK      | NK      | 1 2 2 3 1 2 2 3     |    |
| CON600-1            | Huaylas, Ancash (End)                                         | 1997 | 8 F     | AS      | 1 2 2 3 1 3 2 4     |    |
| Olivares            | Llumpe, Ancash (End)                                          | 2003 | 5 M     | NK      | 1 2 2 3 1 3 2 4     |    |
| Luna                | Chimbote, Ancash (End)                                        | 2003 | 17 M    | NK      | 1 2 2 3 1 3 2 4     |    |
| Mul                 | NK                                                              | 2005 | NK      | NK      | 1 2 2 3 1 3 2 4     |    |
| DB06-P162           | Huarochiri, Lima (End)                                        | 2006 | NK M    | NK      | 1 2 2 3 1 3 2 4     |    |
| FBC-186             | Huaraz, Ancash (End)                                          | 2006 | NK      | NK      | 1 2 2 3 1 3 2 4     |    |
| FBC-196             | Huaraz, Ancash (End)                                          | 2006 | NK      | NK      | 1 2 2 3 1 3 2 4     |    |
| Mor                 | Ancash (NK)                                                   | 2006 | NK      | NK      | 1 2 2 3 1 3 2 4     |    |
| 150-01              | Huaraz, Ancash (End)                                          | 2006 | NK      | NK      | 1 2 2 3 1 3 2 4     |    |
| Agui                | Huarochiri, Lima (End)                                        | 2006 | NK      | NK      | 1 2 2 3 1 3 2 4     |    |
| Gan                 | NK                                                              | 2006 | NK      | NK      | 1 2 2 3 1 3 2 4     |    |
| CUSCO5              | Urubamba, Cusco (NF)                                          | 1998 | 5 M     | OF      | 3 1 1 1 1 4 1 5     |    |
| CUSCO407            | Urubamba, Cusco (NF)                                          | 1998 | NK      | AS      | 3 1 1 1 1 4 1 5     |    |
| Cusco-Ana           | Urubamba, Cusco (NF)                                          | 1998 | 10 F    | OF      | 3 1 1 1 1 4 1 5     |    |
| CUSCO8              | Urubamba-Pallata, Cusco (NF)                                  | 1998 | 28 M    | OF      | 3 1 1 1 1 4 1 5     |    |
| ER-Cha              | Luya, Amazonas (NF)                                           | 1999 | 16 M    | OF      | 3 1 1 1 4 1 5 1     |    |
| ER-Yal              | Luya, Amazonas (NF)                                           | 1999 | NK      | OF      | 3 1 1 1 4 1 5 1     |    |
| ER-Tej              | Luya, Amazonas (NF)                                           | 1999 | 38 M    | OF      | 3 1 1 1 4 1 5 1     |    |
| LA6.3               | Bolognesi, Ancash (End)                                       | 1990 | NK      | OF      | 4 4 3 5 3 6 3 8     |    |
| Luc-Uba             | Mariscal Luzuriaga, Ancash (End)                              | 1999 | 4 F     | OF      | 4 4 3 5 3 6 3 8     |    |

NF = New foci, End = Endemic, NK = Not Known, OF = Oroya Fever, AS = Asymptomatic.

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7, only 37 variable positions (1.3%) among the 2951 bp of sequence data were observed, whereas among STs 1–8 this number rose to 157 (5.3%) (Table 3). Phylogenetic analysis, inferred from alignment of concatenated sequence data for all seven loci (2951 bp) confirmed the profound divergence of isolates belonging to ST8 from all other B. bacilliformis isolates studied (Figure 2). This dendrogram, inferred using splits decomposition analysis, is also characterised by a network structure that suggests recombination has influenced the divergence of B. bacilliformis STs.

This suggestion is supported by the results of a phi test, which also indicated significant evidence for recombination (P = 0.042).

We further explored the extent of divergence between ST8 and other B. bacilliformis sequence types by assessing the phylogenetic distance between them relative to inter-species divergence across the Bartonella genus. We assembled concatenated sequences from ftsZ, flaA, rpoB and rpoB data available for all valid Bartonella species, and inferred phylogeny from a 1323 bp alignment of these sequences (Figure 3). These data suggest that the divergence observed between ST8 and other B. bacilliformis STs is as great as, and occasionally exceeds, that separating Bartonella species.

**Molecular epidemiology**

All isolates belonging to ST1, ST2, ST3 and ST4 were obtained from patients living in the region of Peru where bartonellosis has long been considered endemic. ST3 comprised solely of isolates associated with a large outbreak of bartonellosis in Urubamba, Cusco, in 1999. The three ST6 and ST7 isolates were associated with a new focus of bartonellosis in Pisquria, Amazonas. The two ST8 isolates were obtained from patients living in the same region of Peru where ST1 to 4 were encountered.

Isolates belonging to ST1 were collected as long ago as the “early” 1960s and as recently as 2007, suggesting its continued circulation in the “endemic region” of Peru for at least 40 years. One of the four isolates obtained from the asymptomatic patients was the only member of ST2, however the other three isolates from asymptomatic patients belonged to STs that also included isolates obtained from patients with overt disease (Oroya fever). For 20 patients, no information about their disease manifestation was available.

**Discussion**

B. bacilliformis remains an enigmatic pathogen; despite being identified over 100 years ago and continuing to pose a significant public health threat, our knowledge of its ecology and pathogenicity, and the epidemiology of the infections it causes, remains very incomplete. This shortfall is particularly unsatisfactory as, given its limited geographic distribution and its apparent specific adaptation to humans, eradication of B. bacilliformis infections using

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**Table 2. Primers used for the amplification and sequencing of the seven loci evaluated for the B. bacilliformis MLST scheme.**

| locus | putative gene product | product size (bp) | position | forward primer (5’-3’) | reverse primer (5’-3’) | reference |
|-------|-----------------------|-------------------|----------|------------------------|------------------------|-----------|
| bvrR  | regulatory protein    | 486               | 1385452–1385937 | GACCAGGATTTTGGGACCTC | GCATCCGACTAAAGCCACGTAC | [16]      |
| ribC  | riboflavin synthase alpha subunit | 349 | 652816–653164 | GATATCGGTTGTGTTAAGGA | AAAGGCCGCTAAGTTC | [20]      |
| ftsZ  | cell division protein | 497               | 969686–970182 | CTCAGTGAGGCTCTGGTA | CCAATTGACTCTCTGTTTAC | this study |
| groEL | heat shock protein    | 442               | 1211811–1212252 | CAACAGGATGAAAGGAAAA | TAGAATCCACCTCCGCCCATT | this study |
| flaA  | flagellin A           | 517               | 1076953–1077409 | TTCACTGAAGCTGCTGTAA | CTTGATTTGTAAGTGCTTA | this study |
| rnpB  | RNA subunit of endoribonuclease RNase P | 297 | 988378–988674 | CGGGATCCGAGGGAAGAGAG | CGGAATTCTAAGGCCAGAC | [21]      |
| rpoB  | RNA polymerase beta subunit | 363 | 579639–580001 | ACCTGGAGGTGCTCAAATAT | CTTACAGAGGACTGTCAT | this study |

1 Corresponding to the complete genome sequence of B. bacilliformis strain KC583 Genbank accession number CP000524.

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**Table 3. Characteristics of the seven loci evaluated for the B. bacilliformis MLST scheme.**

| locus | isolates belonging to STs 1-8 | isolates belonging to STs 1-7 |
|-------|-------------------------------|-------------------------------|
|       | number of alleles | number (%) of variable sites | number of alleles | number (%) of variable sites |
| ftsZ  | 4 | 41 (8.2) | 3 | 5 (1.0) |
| flaA  | 4 | 32 (6.2) | 3 | 12 (2.3) |
| ribC  | 3 | 17 (4.9) | 2 | 3 (0.9) |
| rnpB  | 5 | 10 (3.4) | 4 | 3 (1.0) |
| rpoB  | 3 | 11 (3.0) | 2 | 5 (1.4) |
| bvrR  | 6 | 19 (3.9) | 5 | 5 (1.0) |
| groEL | 3 | 27 (6.1) | 2 | 4 (0.9) |

all loci - 157 (5.3) - 37 (1.3)
vaccination should be a realistic goal. Perhaps the most significant finding of the current study is that *B. bacilliformis* may not be a single species; we use MLST data to provide clear evidence that a minority of isolates recovered from patients with haemolytic anaemia (Oroya fever) have diverged from other *B. bacilliformis* isolates to a degree akin to that observed between other *Bartonella* species. These isolates, belonging to ST8, are therefore likely to belong to a novel *Bartonella* genospecies, although further (polyphasic) characterization of these isolates is needed to support their formal taxonomic reclassification. The two ST8 isolates were not epidemiologically linked, being obtained nine years apart, and from locations that lie 150 km from one another. Two further isolates that are also potential members of ST8/a new genospecies have been described elsewhere [28]; the partial gldA and ISR sequences of these isolates are indistinguishable from those of LA6.3 and Luc-Uba, the two ST8 isolates included in the current study. The two further isolates were both obtained from Caraz, a town in Ancash where bartonellosis has long been recognized, which lies at the heart of the region where bartonellosis is considered endemic [28]. At present, we have no insight into the ecological basis for the divergence of ST8 from other *B. bacilliformis* STs; on a broad geographical scale at least, the distribution of ST8 overlaps with those of STs1-4. To what extent the genetic divergence of ST8 from other *B. bacilliformis* STs is reflected in phenotypic differences is, as yet, unclear; the growth requirements for ST8 isolates are, apparently indistinguishable from those of other *B. bacilliformis* strains (i.e., unusually for *Bartonella* species, they grow at 30°C in the absence of CO₂), and their colonial and microscopic morphology is the same. Furthermore, antiserum from a patient infected with a “likely ST8” strain (Vega) reacted strongly with antigens prepared from other *B. bacilliformis* isolates including EC-01, an isolate which bore genotypic similarity to ST1 strains [28]. These shared phenotypic traits are significant from a diagnostic perspective as laboratory confirmation of infection status relies primarily on microscopic examination of blood smears, bacterial isolation and, albeit less frequently, demonstration of specific antibodies [2–4,11,28]. These approaches would appear to be as suitable for ST8 strains as for less genetically divergent *B. bacilliformis* strains.

In general, the delineation of *B. bacilliformis* using MLST matches that inferred using other typing methods. MLST-defined STs are akin to the genotypes identified in an earlier study on the
basis of AFLP analysis and comparison of ISR sequences. That MLST has delineated more genotypes than either AFLP or ISR-based typing is the result of MLST, but not other schemes, differentiating between strains associated with a new focus of bartonellosis in Luya province, and segregating Cond044 from ST1 strains. The first of these differences was the result of a single nucleotide polymorphism in the \( bbr\) locus, and was verified by repeat amplification and sequencing of this locus. Thus, even in locations where bartonellosis has only recently been recognized, genotypically distinct strains are in circulation. The second of these differences is also noteworthy as the allelic profile of Cond044 was very different (only 2/7 shared alleles) from that of ST1, with which it clustered using AFLP and ISR-based typing [11]. Indeed, among the non-ST18 sequence types, ST2, of which Cond044 was the sole representative, was the most divergent. Thus there appears to be marked incongruence between MLST and an approach involving AFLP and ISR sequence comparison in determining the position of Cond044 within the genotypic spectrum of the species. Closer re-examination of AFLP data suggests that although Cond044 clustered with ST1 strains, it was the outlier of the cluster, however its ISR sequence was indistinguishable from that of ST1 isolates. Amongst the MLST loci, ST1 and ST2 shared the same \( bbr\) and \( groEL\) alleles, and sequence dissimilarity at the other five loci ranged from 0.7% (\( rnpB\)) to 2.3% (\( flaA\)), emphasizing the existence of markedly different levels at variation in different parts of the genome and hence the benefits of using MLST approach.

Complete genome sequences are currently available for six \( Bartonella\) species, \( B. henselae\), \( B. quintana\), \( B. tribocorum\), \( B. bacilliformis\), \( B. grahamii\) and \( B. claridgeiae\) [29–32], with more in draft [32]. Comparative analysis of four of the complete genome sequences has revealed that diversity between them is primarily shaped by significant expansions (due to lateral gene transfer and gene duplication) and reductions (due to gene decay and deletion) in their accessory genomes [33]. Recently, high recombination frequencies and large variations in genome size have been reported in \( B. grahamii\) [34], and recombination has been identified as playing a dominant role in the diversification of four rodent-associated \( Bartonella\) species [35]. Our analysis suggests that recombination has also had a strong influence in shaping.

\( B. bacilliformis\) genomes. Although splitstree analysis suggested that the extent of recombination between STs was greater than that reported for \( B. henselae\) [18], quantification of the relative rate of recombination in \( B. bacilliformis\) compared to other \( Bartonella\) genomes was not attempted.

MLST analysis on our isolate archive confirmed the earlier observation, based on AFLP and ISR sequence comparison, that genotypes associated with new foci of bartonellosis were distinct from those present in the region where bartonellosis is considered endemic, with STs 5, 6 and 7 being encountered only outside this region. This finding contrasts with work reported by others in which such geographic delineation of \( B. bacilliformis\) isolates was not observed. Hambuch and colleagues (2004) used infrequent restriction site PCR in combination with partial \( fla\) and \( ialB\) sequence comparison to explore genotypic relationships among isolates from patients in Caraz (endemic region) and isolates associated with the outbreak of disease in Urubamba in 1998 (epidemic) [12]. They did not detect significant differences in variation between the two populations or distinguished one population from the other. Why these results should contrast with those of the current and other previous studies [11] is unclear. However, more recently, Lydy and colleagues (2008) have also reported the presence of isolates similar to those belonging to ST5 (i.e. associated with the Urubamba outbreak) in Caraz and elsewhere in the endemic zone [28]. Thus, it appears increasingly likely that, due to its relatively small size and the opportunistic nature of collection, our archive is limited in terms of its representation of the diversity of genotypes circulating in Peru. This shortfall can only be accurately addressed with systematic surveys, which will require considerable resources, although MLST appears to be an appropriate genotyping method to employ when such a study is instigated.
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References

1. Herrer A (1990) Epidemiologia de la verruga peruana. Prihaceb, Lima, Peru.
2. Kosek M, Lavarello R, Gilman RH, Delgado J, Maguina C, et al. (2000) Natural history of infection with Bartonella bacilliformis in a nonendemic population. J Infect Dis 182: 865–872.
3. Chamberlin J, Laughlin LW, Romero S, Solorzano N, Gordon S, et al. (2002) Epidemiology of endemic Bartonella bacilliformis: A prospective cohort study in a Peruvian mountain valley community. J Infect Dis 186: 983–990.
4. Ellis BA, Rotz LD, Leake JAD, Samalvides F, Bernable J, et al. (1999) An outbreak of acute bartonellosis (Oroya fever) in the Urubamba region of Peru, 1998. Am J Trop Med Hyg 61: 344–349.
5. Maguina C, Gotuzzo E (2000) Bartonellosis - New and old. Infect. Dis. Clin. North Am 14: 1–22.
6. Alexander B (1995) A review of Bartonellosis in Ecuador and Colombia. Am J Trop Med Hyg 52: 354–359.
7. Cooper P, Guaderian R, Paredes W, Daniels R, Perez A, et al. (1996) Bartonellosis in Zamora Chinchipe province in Ecuador. Trans R Soc Trop Med Hyg 90: 241–243.
8. Zhou J, Lau WKM, Laughlin LW, Masuoka PM, Andre RC, et al. (2002) The effect of regional climate variability on outbreak of epidemics of bartonellosis in Peru. Third Symposium on Environmental Applications:Facilitating the Use of Environmental Information. pp 123–126.
9. Birtles RJ, Fry NK, Ventaila P, Caceres AG, Sanchez E, et al. (2002) Identification of Bartonella bacilliformis genotypes and their relevance to epidemiological investigations of human bartonellosis. J Clin Microbiol 40: 3606–3612.
14. Maiden MCJ, Byraves JA, Feil E, Morelli G, Russell JE, et al. (1998) Multilocus sequence typing: A portable approach to the identification of clones within populations of pathogenic microorganisms. Proc Natl Acad Sci U S A 95: 3140–3145.
15. Platunov AE, Shipulin GA, Platunova OV (2000) Multilocus sequence typing: A new method and the first results in the genotyping of bacteria. Russ J Genet 36: 481–487.
16. Arvand M, Feil EJ, Giladi M, Boulouis HJ, Viezens J (2007) Multi-Locus Sequence Typing of Bartonella henselae Isolates from Three Continents Reveals Hypervirulent and Feline-Associated Clones. Plos One 2: e1346.
17. Arvand M, Raoult D, Feil EJ (2010) Multi-Locus Sequence Typing of a Geographically and Temporally Diverse Sample of the Highly Clonal Human Pathogen Bartonella quintana. PLoS One 5: e9765.
18. Iredell J, Blanckenberg D, Arvand M, Grauling S, Feil EJ, et al. (2003) Characterization of the natural population of Bartonella henselae by multilocus sequence typing. J Clin Microbiol 41: 5071–5079.
19. Lindroos H, Vinnere O, Mira A, Repsilber D, Naslund K, et al. (2006) Genome rearrangements, deletions, and amplifications in the natural population of Bartonella henselae. J Bacteriol 188: 7426–7439.
20. Mietze A, Morick D, Kohler H, Harrus S, Dehio C, et al. (2010) Combined MLST and AFLP typing of Bartonella henselae isolated from cats reveals new sequence types and suggests clonal evolution. Vet Microbiol doi:10.1016/j.vetmic.2010.08.012.
21. Yanagihara M, Tsunoka H, Hoshizaki S, Ishida E, Umeda A, et al. (2010) Molecular typing of Bartonella henselae DNA extracted from human clinical specimens and cat isolates in Japan. FEMS Immunol Med Microbiol 60: 44–48.
22. Struelens MJ (1996) Consensus guidelines for appropriate use and evaluation of microbial epidemiologic typing systems. Clin Microbiol Infect 2(1): 2–11.
23. Bereswill S, Hinkelmann S, Kist M, Sander A (1999) Molecular analysis of riboflavin synthesis genes in Bartonella henselae and use of the ribC gene for differentiation of Bartonella species by PCR. J Clin Microbiol 37: 3159–3166.
24. Pinille C, Streich C, Brown JW, Breitschwerdt EB (2002) Investigation of the phylogenetic relationships within the genus Bartonella based on comparative sequence analysis of the rmpB gene, 16S rDNA and 23S rDNA. Int J Syst Evol Microbiol 52: 2075–2080.
25. Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol Biol Evol 24: 1596–1599.
26. Jolley KA, Feil EJ, Chan MS, Maiden MCJ (2001) Sequence type analysis and recombinational tests (START). Bioinformatics 17: 1230–1231.
27. Hussin DH, Bryant D (2006) Application of phylogenetic networks in evolutionary studies. Mol Biol Evol 23: 254–267.
28. Lydy SL, Eremeeva ME, Amis D, Paddock CD, Nicholson WL, et al. (2008) Isolation and characterization of Bartonella bacilliformis from an expatriate Ecuadorian. J Clin Microbiol 46: 627–637.
29. Alouk SM, Frank AC, Karlberg EO, Legault BA, Ardell DH, et al. (2004) The louse-borne human pathogen Bartonella quintana is a genomic derivative of the zoonotic agent Bartonella henselae. Proc Natl Acad Sci U S A 101(26): 9716–9721.
30. Saenz HL, Engel P, Stoeckli MC, Lanz C, Raddatz, G, et al. (2007) Genomic analysis of Bartonella identifies type IV secretion systems as host adaptability factors. Nat Genet 39(12): 1469–1476.
31. Berglund EC, Frank AC, Calteau A, Vinnere Pettersson O, Granberg F, et al. (2009) Run-off replication of host-adaptability genes is associated with gene transfer agents in the genome of mouse-infecting Bartonella hgrahamii. PLoS Genet 5(7): e1000546.
32. Engel P, Salzburger W, Liesch M, Chang, CC, Maruyama S, et al. (2011) Parallel Evolution of a Type IV Secretion System in Radiating Lineages of the Host-Restricted Bacterial Pathogen Bartonella grahamii. PLoS Genet 7(2): e1001296.
33. Engel P, Dehio C (2009) Genomics of Host-Restricted Pathogens of the Genus Bartonella. In: DeReuse H, Bereswill S, eds. Microbial Pathogenicomics. pp 158–169.
34. Berglund EC, Ellegaard K, Granberg F, Xia ZP, Maruyama, S, et al. (2010) Rapid diversification by recombination in Bartonella grahamii from wild rodents in Asia contrasts with low levels of genomic divergence in Northern Europe and America. Mol Ecol 19(11): 2241–2255.
35. Paziewska A, Harris PD, Zwoinski L, Bajer A, Sinski E (2011) Recombination Within and Between Species of the Alpha Proteobacterium Bartonella Infecting Rodents. Microb Ecol 61(1): 134–143.