Step I: White Paper Application

Application Guidelines

1. *The application should be submitted electronically per requirements via the web site of any of the NIAID Genomic Sequencing Centers for Infectious Diseases. Include all attachments, if any, to the application.*
2. *There are no submission deadlines; white papers can be submitted at anytime.*
3. *GSC personnel at any of the three Centers can assist / guide you in preparing the white paper.*
4. *Investigators can expect to receive a response within 4-6 weeks after submission.*
5. *Upon approval of the white paper, the NIAID Project Officer will assign the project to a NIAID GSC to develop a management plan in conjunction with the participating scientists.*

White Paper Application

Project Title: Characterization of Pathogenicity and Ecology of *Bartonella* Species Through Whole Genome Sequence Analysis

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1. Executive Summary *(Please limit to 500 words.)*

Bartonella are a ubiquitous cause of chronic bacterial infection with infectious prevalence exceeding 50% in many mammals. Importantly, these vector-borne agents are increasingly recognized cause of human illness. Human disease manifestations include acute and chronic fever, lymph node swelling, heart valve infection, encephalitis, blindness, arthritis, and bacterial-induced vascular tumors.

To address gaps in our knowledge about this genus, the aim of this proposal is to obtain full genome sequence for 15 *Bartonella* species and 11 additional Bartonella strains that are predicted to give significant insights into *Bartonella* infectious potential. Sequence data will be used in support of the following overall goals:

1) Identify mechanisms of pathogen-host interaction. Sequence data will be used to identify potential virulence factors through homology searches with the existing database. As a complementary approach, highly divergent genes (more divergent than the rest of the genome for each *Bartonella* species) will be identified as they may represent critical points of pathogen-host or pathogen-vector interaction (i.e., virulence factors) – and as such will be under increased selective pressure and show larger divergence. Putative virulence genes identified by homology and sequence analysis will later be studied using classical bacterial pathogenesis techniques, e.g., gene knockout experiments, to determine their contribution to pathogenesis.

2) Determinants of human infection. Certain strains among *Bartonella* species demonstrate distinct pathogenic capabilities, e.g., some strains infect humans, while others do not. Comparison of the genomes of these strains will likely efficiently identify genomic content responsible for these strain-specific properties. We therefore propose sequencing additional strains of *B. melophagi*, *tamiae, arupensis, washoensis, elizabethae* and *quintana* with contrasting pathogenic

potential. The role and importance of strain specific elements will then be examined and validated by correlation of their presence with clinical and ecological associations in the world's largest *Bartonella* collection at the CDC-Fort Collins and through subsequent testing in experimental models.

3) Groundwork for diagnostics. There is thought to be a large burden of undiagnosed human *Bartonella* infection. Through analysis of the proposed genome sequence, we plan to identify ideal targets across the *Bartonella* genus that will establish the basis for alternative and much more powerful molecular diagnostic and serological assays. These new assays would allow for in depth field studies in animals and epidemiological surveys in humans to understand more fully the global impact of these organisms.

2. Justification

Provide a succinct justification for the sequencing or genotyping study by describing the significance of the problem and providing other relevant background information.

This section is a key evaluation criterion.

1. *State the relevance to infectious disease for the organism(s) to be studied; for example the public health significance, model system etc.*

Public Health Importance. *Bartonella* are Gram negative bacteria that are likely the most prevalent cause of systemic, chronic infection among mammals. In humans, *Bartonella henselae,* the *Bartonella* species thus far most frequently detected in human infections, causes a prolonged febrile illness, known as cat scratch disease, associated with swollen lymph nodes, low red cell counts, and liver damage. Some patients go on to infection related disease of the brain (encephalopathy), joints (arthritis), and eyes (blindness). *Bartonella quintana* similarly causes a prolonged febrile illness. Fascinatingly, in immunocompromised patients these and other *Bartonella* species cause new blood vessel formation, i.e., angiogenesis. These proliferating blood vessels form tumor-like masses, which through replacement of normal tissue may lead to organ dysfunction and death. These species are important causes of heart valve infection (endocarditis).

Bartonella bacilliformis, the agent of Carrion's disease, is an emerging threat which classically causes a biphasic human-specific vascular disease that initially presents as a potentially fatal hemolytic anemia (Oroya fever) followed by chronic cutaneous lesions known as verruga peruana. In endemic regions, severe Oroya fever is rarely seen except in children, but is more common in cases occurring in non-endemic areas. The basis for this difference is thought to be differences in host immunity or in pathogenicity between *B. bacilliformis* strains. Asymptomatic bacteremia has also been observed, suggesting that humans are the disease reservoir.

With more robust detection methods it is now clear that many other *Bartonella* species infect people, leading to debilitating chronic symptoms[1-3]. A recent study found that 5% of patients from the Southwestern US with unexplained chronic fever, fatigue, anemia, and liver abnormalities show seroconversion to *Bartonella* antigens from previously unidentified *Bartonella* species found in local rodent populations[4]. Recently studies by Michael Kosoy (co-investigator) and colleagues have shown widespread and previously unexpected human infection with *Bartonella tamiae* in Southeast Asia[5].

Bartonella also cause chronic bloodstream infections in animals as diverse as whales, kangaroos, bats, dogs, rodents, deer, cattle, sheep, squirrels, and turtles. Remarkably, prevalence of infection of up to 50% has been observed in populations of prairie dogs, rodents, deer, and cattle in surveys taken around the world, including the United States. *Bartonella* are generally passed among hosts by blood sucking arthropod vectors, e.g., fleas, mite, ticks, midges, and flies. Humans in turn become infected through contact with animals or arthropod vectors. Clearly, the burden of *Bartonella* infection and potential for transmission to humans is very large.

Based on increasing recognition of *Bartonella* as serious human and veterinary pathogen, the National Institutes of Allergy and Infectious Diseases (NIAID) has recently classified *Bartonella* as priority emerging infectious agents. The Centers for Disease Control and Prevention (CDC) also established a laboratory in Fort Collins, Colorado specifically devoted to characterizing new *Bartonella* species, and determining the disease associations in humans and animals around the world (Michael Kosoy, Bartonella Laboratory Chief, Co-investigator).

2. *Are there genome data for organisms in the same phylum / class / family / genus? What is the status of other sequencing / genotyping projects on the same organism including current and past projects of the NIAID GSC? Provide information on other characteristics (genome size, GC content, repetitive DNA, pre-existing arrays etc.) relevant to the proposed study. Have analyses been performed on the raw data already generated/published? If additional strains are proposed for a species, please provide a justification for additional strains?*

Genomes of several *Bartonella* species have already been sequenced and these species are listed in Table I below. Investigated *Bartonella* genomes ranged from 1.4 to 2.6 megabases in size[6] with a GC content of approximately 40%. Repetitive DNA elements are present, especially in regions containing putative host adaptability genes. *B. bacilliformis* was the only species thusfar sequenced as part of a prior NIAID GSC effort. Several analyses of genomes already sequenced have been published, most prominently by Siv Andersson, an advisor for this project and principal investigator for Uppsala projects listed in Table I[6, 7]. Through this analysis key evolutionary relationships were determined between *Brucella* and *Bartonella*, and among *B. henseale*, *quintana* and *bacilliformis*.

Table I. Summary of Active and Completed *Bartonella* genome projects

Strain	Status	Site	Project ID
B. bacilliformis	In progress	Uppsala Univ. and Univ. Montana	13486
B. bacilliformis KC583	Complete	TIGR/NCBI	16249
B. henselae str. Houston-1	Complete	Uppsala Univ.	196
B. quintana str. Toulouse	Complete	Uppsala Univ.	44
B. tribocorum CIP105476	Complete	Penn State	28109
B. tribocorum	In progress	Max Planck Institute	16098
B. grahamii as4aup	Complete	Uppsala Univ.	34873
B. bartlesii	(not publically available)	Pasteur Institute	
B. clarridgeaie	Complete? (not publically available)	Greg Dash (CDC) and Christoph Dehio	
B. australis	Complete (not publically available)	Uppsala Univ.	
B. bovis	In progress – (not publically available)	Uppsala Univ.	
B. vinsonii berkoffii	In progress (not publically available)	Uppsala Univ.	

3. *If analyses have been conducted, briefly describe utility of the new sequencing or genotyping information with an explanation of how the proposed study to generate additional data will advance diagnostics, therapeutics, epidemiology, vaccines, or basic knowledge such as species diversity, evolution, virulence, etc. of the proposed organism to be studied.*

Although several *Bartonella* genomes have been sequenced, extensive gaps in our knowledge about this genus remain. It should be pointed out there is extensive diversity within this genus, even within species, evident in the genome data already available. *Bartonella* species appears to have evolved in part through acquisition and loss of virulence factors and host adaptability genes within large numbers of mobile genetic elements. Therefore, we believe that sequencing of additional members of the genus will provide unique and critical information for addressing fundamental unanswered questions in our field.

Specifically, as yet little to nothing is known about: (a) adaptation of *Bartonella* to specific vertebrate hosts and arthropod vectors; (b) the identity and function of virulence factors; (c) how the organisms are able to sustain chronic infection in natural hosts; (d) the biology underlying *Bartonella*'s unique ability to cause blood vessel proliferation; (e) evolutionary relationship amongst species; and (f) elements that confer the ability to infect and cause specific disease manifestations in humans. Furthermore, as mentioned in previous section, the disease burden in

humans is underappreciated and under-investigated through lack of adequate diagnostic methods. The additional sequencing effort in this proposal we believe critical for identifying optimal diagnostic targets that will allow us to detect that range of *Bartonella*-associated illness in humans. We have canvassed prominent researchers in the *Bartonella* field (a number of whom are advisors for this project) and are confident that data generated will be widely used for studies in each of the above categories.

3. Rationale for Strain Selection

4. *Provide the rationale behind the selection of strains and the number of strains proposed in the study. The focus of the program is on potential agents of bioterrorism or organisms responsible for emerging or re-emerging infectious diseases. Non-select agents or non-pathogenic organisms will be considered when they can provide insight into these scientific areas.*

Bartonella are emerging and incredibly successful pathogens as judged by their ability to chronically infect a wide range of mammals with prevalence often exceeding 50% in studied populations. Genomic information to date indicates both conservation and acquisition of unique elements that appear to confer to the ability to adapt to new host environments. Understanding the diversity within this genus through sequencing of representative genomes will give us a much better understanding of how *Bartonella* has expanded its host range and acquired new pathogenic capabilities. Note, available genomic information indicates that *Bartonella* has evolved from *Brucella*, a CDC Category B agent. Indeed, several clinical and ecological features are shared by these two genera - their ability to cause chronic systemic infection often complicated by heart valve infection and their ability to infect a wide variety and overlapping set of mammals. Based on use of similar hosts, continued genetic exchange between these two species is possible. Furthermore, it is likely that both genera share at least some pathogenic strategies. For example, *Bartonella* and *Brucella* (and also predicted for *Coxiella)* make use of specialized type IV secretion systems to alter host cells and establish infection[8-10]. The availability of 10 sequenced *Brucella* genomes[11]) will expand the potential for comparative genomic investigations between these two genera. Taken together, the proposed sequencing effort will therefore give us critical insights into an emerging pathogen and also likely give insights into the pathogenesis of several related select agents.

Unlike most human bacterial pathogens, *Bartonella* cannot be isolated by standard cultures techniques, hampering our ability to detect human illness. Recent work using the limited molecular diagnostic tools available (e.g., studies by our project advisors Michael Kosoy and Ed Breitschwerdt) have provided mounting evidence

there is a significant burden of undetected, chronic human illness resulting from infection by a diverse range of *Bartonella* species -- many heretofore not previously associated with human disease. Therefore, the availability of genomic information from the diversity of the genus will be critical to further efforts for detecting and defining the range of *Bartonella*-induced human illness.

To these ends, we propose sequencing the genomes of 13 previously unsequenced *Bartonella* species. In combination with the genomes already sequenced or in progress, this sequencing effort will thereby make available to the scientific community, genomes of the most prevalent *Bartonella* species including nearly all thus far associated with human illness.

More specifically, species (and specific strains within this species) were chosen based on the following criteria:

a. Will add to the understanding of genomic diversity within the *Bartonella* species.

b. Desire to sample species infecting different mammalian hosts and vectors.

c. Prioritization given to species for which research models have been established or presumably could be because of susceptible mammalian hosts that might serve as tractable research models.

d. Prioritization given to those species and strains which have been found to cause disease in humans and/or livestock.

e. Strains must be able to be deposited in BEI Resources in concert with this proposal.

We have also proposed sequencing strains from six additional species: *B. tamiae*, *melophagi*, *arupensis, washoensis, elizabethae, and quintana* (these are starred in Table II). The six species were chosen for this additional analysis based on the divergent ability of different strains among these species to cause human pathologies and/or demonstration of different pathological traits. We believe that pair-wise comparison of the genomes of the divergent strains within these species groups will lead to further insights into genomic content important for human and/or animal infection.

Specifically, *B. melophagi* strains were chosen based on differential isolation from sheep and humans respectively -- a distinction associated with a phenotypic difference in genetic elements, one example of which is that the former expresses flagella and the latter does not[12]. The human infecting type strain of *B. elizabethae* (isolated from a human endocarditis case in Massachusetts), which does not cause blood stream infection in rats, will be compared to an Asian rat isolate that consistently causes blood stream infection in rats. Likewise, *B. vinsonii arupensis* isolates showing similar properties will be compared. (Note, despite the "subspecies" designation, *B. vinsoniii* susps. *arupensis* is a completely distinct species from the previously sequenced *B. vinsonii susp. berkhoffii* – the original "subspecies" designation was a historical inaccuracy). Two human cases were caused by *B. washoensis* (one was associated with cardiac illness in Nevada[13] and another with meningitis in California[14]). The strain obtained from the Californian case will be compared to one of the numerous strains from CDC

collections, which widely circulate among ground squirrels without any evident pathology in natural hosts. Two strains of *B. tamiae* isolated from Thai patients associated with asymptomatic vs. severe clinical disease will be compared[5]. Five strains of *B. quintana* (a species which is believed only to infect humans) were chosen: one is the most widely distributed amongst *Bartonella* researchers (JK13); the second is of interest because of its association with widespread cutaneous bacillary angiomatosis disease in a patient in concert with rearrangement of bacterial cell surface proteins; and the third is phenotypically distinct, and was associated with distinct pathology, i.e., bacillary angiomatosis-associated bone infection. The last two strains (JK56 and JK63) were isolated from the same patient 14 months apart. Comparison of these two strains will likely give important insights into how the bacteria adapts to its human host during chronic infection. Taken together, we predict this comparative genomic analysis of strains in this proposal will inform our understanding of focal points of pathogen-host interaction and thereby contribute to development of new therapeutic strategies.

Isolates of *B. bacilliformis* have been shown to vary by AFLP, PFGE, MLST and IGR typing. Studies that genetically classify *B. bacilliformis* isolates using MLST show isolates from traditionally non-endemic areas (Cusco and Amazonas, Peru) cluster separately from isolates originating from traditional endemic regions. Some strains presenting with clinical symptoms associated only with *B. bacilliformis* infection appear divergent enough to be considered as candidates for new species or subspecies.

We therefore propose sequencing of eleven isolates of *B. bacilliformis* in order to:

1) determine the breadth of the species by sequencing three of the outlying strains.

2) determine whether isolates from non-endemic regions are unique to those regions or could have originated from endemic areas by sequencing three isolates from Cusco and Amazonas.

3) sample the extent of the diversity within *B. bacilliformis* by sequencing isolates from endemic regions that are classified into different groups by MLST and IGR typing (~7 isolates which overlap with #1 and #2) and to compare them to strains from epidemic regions.

4a. Approach to Data Production: Data Generation

5. *State the data and resources planned to be generated. (e.g draft genome sequences, finished sequence data, SNPs, DNA/protein arrays generation, clone generation etc.)*

As we wish to identify novel genetic elements that are potential unique to certain species, we will generate draft *de novo* genome sequences for all proposed strains using the Illumina platform. Some *Bartonella* species have significant numbers of repetitive elements. To overcome this problem, we will use large

jumping library insert sizes (~5 kb) which should enable us to scaffold over the repeated elements.

4b. Approach to Data Production: Data Analysis

6. *Briefly describe the analysis (value-add) envisioned to be performed subsequently by the community and the potential to develop hypotheses driven proposals given the datasets and resources produced by this work.*

We believe that the sequencing information will be embraced and transformative for the entire *Bartonella* community – the genomic sequencing information will provide an unprecedented resource to further ongoing investigations currently limited by available sequence information. A large number of hypothesis driven proposals will be spawned from the genomic data. For example, both the Kosoy and Kirby laboratories have developed murine infection models using two species in this proposal. With genomic information available, they would then be able to take a candidate gene approach to address the potential role of factors identified by homology and divergence searches. Numerous other pathogenesis investigators in the field would use sequencing information for similar ends.

Of particular interest and an expected outcome is the identification of lateral gene transfer associated with new pathogenic potential. For example, comparison of *B. henselae* with *Brucella* has led to the hypothesis that the *Bartonella* arose from remodeling of a common ancestor through both loss of genetic material and gain of new host adaptability genes. Further comparisons including the diversity of *Bartonella* sequences is likely to identify new host adaptability factors, refine evolutionary models, and give further insights into the natural history of both *Bartonella* and *Brucella*.

We expect that Siv Andersson's group would combine analysis of new sequence data with previous data sets under study to validate their intriguing hypothesis on the role of run off transcription of host adaptability genes in evolution of the genus and adaptation to new hosts[6]. Furthermore, large-scale comparative genomic hybridization analysis would be performed similar to studies underway with sequenced species[6, 15-18] to investigate micro-evolution within species across geographic and host boundaries. Use of genomic data will allow relationships among species to be drawn and give new insights into steps that must occur concomitantly with spread into new host ranges. Dr. Kosoy will, for example, specifically use these data as part of an ongoing effort to understand the spread and adaptation of rat-associated *Bartonella* species during migration of host

species between the Old and New worlds. Finally, we expect that several groups will also use sequencing data to develop new diagnostic assays and apply them to new and ongoing field studies to define the implications of *Bartonella* infection in human and animal populations.

5. Community Support and Collaborator Roles:

7. *Provide evidence of the relevant scientific community's size and depth of interest in the proposed sequencing or genotyping data for this organism or group of organisms. Please provide specific examples.*

Depth of Interest of Scientific Community. There is exceptional interest amongst the *Bartonella* community in this sequencing effort. Many of the most prominent investigators in the field have joined in our efforts to obtain and prioritize strains for this proposal.

Size of Scientific Community. The number of *Bartonella* studies continues to grow. There were 155 articles published in 2009 concerning this genus, reflecting a wide range of research interests, from basic and comparative biology, to diagnostics, and medical impact. There is deep interest in the *Bartonella* field amongst the scientists studying *Rickettsia*, *Brucella*, *Francisella*, *Anaplasma*, and *Ehrlichia,* as these organisms are also zoonotic, vector borne, and/or cause chronic blood stream infections. In some cases (*Rickettsia*, *Ehrlichia, Anaplasma*), an important relationship similarly exists with endothelial cell or blood cells. There are several forums in which *Bartonella* researchers exchange ideas and information in addition to the published literature. Examples include both national and international microbiology meetings; the 6th International Meeting on *Bartonella* as Medical and Veterinary Pathogens which was held in the United Kingdom in June 2009 (organized by Richard Birtles, project advisor); and the American Society of Rickettsiology meetings that occur approximately ever other year. In 2008, *B. bacilliformis* was the subject of an international conference sponsored by the Ministry of Health of Peru to determine the need and informational limitations for development of a vaccine to augment vector control efforts.

Readiness of Scientific Community to Use Data. The *Bartonella* community has already exploited the available genomic information for several research projects and is therefore well prepared to use the data generated from this proposal. For example, the four sequenced, publically available genomes have been used to deduce preliminary evolutionary relationships. This analysis led to the discovery of the lateral transfer of type IV secretions associated with different pathogenic capabilities and to the discovery of the ancestral relationship

between *Brucella* and *Bartonella*[6, 7]. In addition, subsequent study using microarray comparative genomic hybridization based on the genome of the rodent-associated *Bartonella grahamii* led to the discovery of the loss and gain of Type IV secretion systems and surface proteins amongst different geographically distinct isolates, contributing to an understanding of the plasticity of the genus[19]. Furthermore, the field has a long tradition of studying relationships among strains through genetic analysis (RFLP, intergenic spacer analysis, and sequence based strain typing)[20]. Related to potential use in diagnostics, the CDC's Bartonella Laboratory in Fort Collins (Chief - Michael Kosoy) in collaboration with the University of Texas Medical Branch (PI: Vladimir Motin) and the Department of Computer Science at the University of Houston (PI: Yuri Fofanov) developed highly specific primers for 'host-blind' detection and identification of *Bartonella* species in several mammalian background organisms based on genomic information. Whole-genome screening conducted during this study identified a total of 98 potential primers sets predicted to only amplify Bartonella spp; while cross-referencing the primers commonly used in the diagnostics were shown having high cross-reactivity both to potential Bartonella hosts and to bacterial species that could inhabit similar ecological niches[21]. We expect that the genome sequencing proposed will allow much more powerful diagnostic techniques to be developed and applied that will identify the range of *Bartonella* species that may infect humans.

8. List all project collaborators and their roles in the project

Management of the Sequencing Project. The sequence project will be managed by the laboratories of James Kirby, Michael Kosoy, and the GSC of the Broad Institute. Futhermore, a wide variety of *Bartonella* experts have contributed their expertise toward prioritizing organisms for sequencing, serve on the proposed project's advisory committee, and will contribute to subsequent analysis and research.

James Kirby and Michael Kosoy will be the principal investigators for the biological side of the project. Dr. Kirby's laboratory will culture the organisms, and prepare genomic DNA for sequencing. Dr. Kosoy maintains the largest collection of *Bartonella* strains in the world (>>1000) at CDC in Fort Collins, and will provide the strains used in this sequencing proposal.

Advisory Committee:

United States

Michael Kosoy, Ph.D. and Co-Investigator, Chief of Bartonella Laboratory, Division of Vector-Borne Diseases, CDC, Fort Collins. Dr. Kosoy has 15 years experience working in the *Bartonella* field. His laboratory at CDC has several projects relevant to proposed sequencing effort: (1) to determine the regional diversity of *Bartonella* strains among wild and domestic animals; (2) to develop new laboratory techniques and approaches for detecting *Bartonella*

species in clinical and environment specimens; (3) to identify zoonotic reservoirs and arthropod vectors for this family of human pathogens; and (4) to evaluate the causative relationship between *Bartonella* species and specific clinical manifestations, including febrile illness and infective endocarditis in humans and animals.

James Kirby, MD and Co-Investigator. Director, Clinical Microbiology, Beth Israel Deaconess Medical Center and Harvard Medical School. His laboratory has performed experimental work on many *Bartonella* organisms, developed several novel *in vitro* models for analysis of *Bartonella*-host interaction and *Bartonella*-induced angiogenesis [22, 23], and recently developed an immunocompromised murine model of *Bartonella taylorii* infection that mimics pathological features of human disease[24].

Ed Breitschwerdt, **Ph.D.** Professor of Internal Medicine, and Director Vector Borne Disease Laboratory, School of Veterinary Medicine, North Carolina State University. Adjunct Associate Professor of Medicine, Duke University Medical Center. Dr. Breitschwerdt established new techniques for *Bartonella* detection and has performed extensive surveys of *Bartonella* infection in vertebrates. He also has performed extensive work documenting the role of a myriad of *Bartonella* species in previously unrecognized, chronic human infection.

Gregory Dasch, Ph. D., Rickettsial and Bartonella Team Leader, Rickettsial Zoonoses Braanch and Director, CDC WHO/PAHO Collaborating Center for Reference and Research on Rickettsial and Bartonella-Associated Diseases, National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, GA. Adjunct Professor, Population Biology Ecology and Evolution Program, Emory School of Graduate Studies, Emory University. Dr. Dasch has studied the biochemistry, physiology, and genetics of several *Bartonella* agents, described two new species of *Bartonella*, and developed several diagnostic and genetic typing assays for *Bartonella*. He consults on the diagnosis, epidemiology, and treatment of human bartonelloses with national and state public health laboratories. He has worked extensively on the genomics of *Rickettsia* and *Orientia.*

Laura Hendrix, Ph.D. Assistant Professor of Microbial and Molecular Pathogenesis, College of Medicine, Texas A&M Health Science Center. Dr. Hendrix has described a contact-dependent hemolysin in *Bartonella bacilliformis* and currently is studying the identity and function of *B. bacilliformis* proteins that bind human erythrocyte membranes. She also works on the identification of immunogenic proteins of vaccinogenic and diagnostic importance in *Coxiella burnetii.*

International

Siv G. E. Andersson, Ph.D. Department of Molecular Biology, Biomedical

Center, Uppsala University, Uppsala, S-751 24, Sweden. Her laboratory has sequenced, analyzed, and published data on several *Bartonella* genomes. Data from this proposal will be used to supplement her current analysis of *Bartonella* genomic evolution.

Richard Birtles, Ph.D. Infection Biology Group, Department of Veterinary Pathology, University of Liverpool, UK (organizer of 6th and most recent international Bartonella meeting). His group has performed extensive population studies of *Bartonella* infection in mammalian hosts throughout the world including discovery of a number of species in the proposed sequencing project. Dr Birtles has a long held interest in the ecology of *Bartonella* species and the interaction between bartonellae and their reservoir hosts and vectors at a population and individual level. He produced early work on the evolutionary relationships between *Bartonella* species and, more recently has further explored their diversity and their potential role as zoonotic agents. He has worked extensively in Peru on the epidemiology and ecology of human bartonellosis.

Bioinformatics Consultants

Mark Boguski, M.D., Ph.D. Center for Bioinformatics, Harvard Medical School and Department of Pathology, BIDMC. Dr. Buguski was the former Vice President and Global Head of Genome and Proteome Sciences, Novartis Institute for Biomedical Research. He is an expert in bioinformatic genomic analysis[25].

Ramy Arnaout, M.D., Ph.D. Former Marshall Scholar in Mathematical Biology, currently in the Department of Pathology at Beth Israel Deaconess Medical Center. Former member of the Siv Andersson laboratory. His research laboratory uses bioinformatics and deep sequencing for analysis of human immunity[26].

9. *List availability of other funding sources for the project.*

None currently available. Many of the investigators associated with the project plan to apply for additional funds to perform supplementary investigations of the sequencing data.

6. Availability & Information of Strains:

10. *Indicate availability of relevant laboratory strains and clinical isolates. Are the strains/isolates of interest retrospectively collected, prepared and ready to ship?*

Note: If samples are prospectively prepared the GSC can provide protocols and recommendation based on the Centers past experiences. The samples must however meet minimum quality standards as established by the Center for the optimal technology platform (sequencing/ genotyping) to be used in the study.

The strains have all been retrospectively collected and available in strains collections of collaborators in this project. Genomic DNA will be prepared according to GSC recommendations for preparation.

11. *Attach relevant information, if available in an excel spreadsheet for multiple samples: e.g.*
    - *Name*
    - *Identifier*
    - *Material type (DNA/RNA/Strain)*
    - *Genus*
    - *Species*
    - *Specimen / Strain*
    - *Isolation source*
    - *Isolated from*
    - *Select agent status*
    - *International permit requirement*
    - *BEIR/ATCC repository accession number*
    - *Other public repository location*
    - *Other public repository identifier*
    - *Sample provider's name*
    - *Sample provider's contact*

12. *What supporting metadata and clinical data have been collected or are planned on being collected that could be made available for community use?*

Table II.

	Species	Strain Designation Original	ATCC #	Host	Human Infection	Rationale
1*	*B. tamiae*	a) Pathogenic strain Th239[5] b) Non-pathogenic strain Th307	BAA-1343	Detected in ticks and chigger mites from rodents) [5, 27 28, 29]	Fever, Anemia, Rash	Human infection, murine infection model (unpublished)
2*	*B. vinsonii subsp arupensis*	a) Human isolate that does not infect mice b) murine isolate		Deer Mice[30, 31]	Fever[30], endocarditis[32]	Comparison of human and murine strains
3	*B. taylorii*	MAC-36 or BR-WM9		Mice, bank voles[33]; flea vector		Ubiquitous rodent species, understanding diversty

4.	*B. birtlesii*	LL-WM9		Wood mice[34], voles[35]		Extensively studied murine infection models[24, 36-39]; understanding species diversity
5	*B. rochalimae*	BMGH	BAA-1498	Dogs, grey fox [40], red fox, cayotes,raccoons[41]. Rat (Rattus norvegicus)[42]	Endocarditis[40], Bacillary Angiomatosis[43]	Human
6*	*B. melophagi*	a)K-2C or alternative strains isolated directly from sheep per Dr. Kosoy b) Human isolate from Dr. Ed Breitschwerdt	BAA-1500	Sheep and Sheep Ked	Bacteremia[12]	Live stock
7*	*B. washoensis*	a) Strain Sb944nv from ground squirrel b) human isolate		Ground squirrel, Dog Endocarditis [13,31], Squirrel fleas associated vector	Cardiac disease[31], Meningitis[14]	Human
8	*B. koehlerae*	C-29	7006d93	Cats[44]; Dogs[45],; rodent fleas[46]	Endocarditis[47]	Human infection, closely related to *B. henselae*, predominant human pathogen.
9	*B. alsatica*	CIP 105477		Rabbit (Alsace, France)[48] and flea from wildcat[49]	Endocarditis[50, 51], lymph node infection[52]	Unique mammalian host (rabbit). Rabbit contact implicated in human infection.
10*	*B. elizabethae*	a) Human isolate b) Rat isolate	ATCC 49927	rats	endocarditis	Human and rodent infection
11	*B. rattimassiliensis*	*AY515122*	CIP 107705	rats[53, 54]; world-wide distribution		Wide-spread rodent infection, species diversity
12	*B. capreoli*	CIP 106691		Roe Deer (Capreolus capreolus), France[55]		Livestock infection

13	*B. doshiae*	700133	NCTC 12862	Microtus agrestis (Vole) United Kingdom; fleas Afghanistan[46]	[56]	Remaining old world rodent associated species; diversity of genus
14	*Novel shrew associated species, as yet unnamed*			Shrew[57]		Host range limited only to shrew[57] yet related to B. clarridgeae;implications for host specificity
15*	*B. quintana**	Strains JK13, JK19, JK31, JK56, and JK63		Human only mammalian host, louse vector	Trench fever, bacillary angiomatosis, endocarditis	Human disease
16	*B. bacilliformis*	11 strains		Human only mammalian host, sandfly vector	Carrion's disease, Oroya fever, verruga peruana	Human Disease

7. Compliance Requirements:

7a. Review NIAID's Reagent, Data & Software Release Policy:

NIAID supports rapid data and reagent release to the scientific community for all sequencing and genotyping projects funded by NIAID GSC. It is expected that projects will adhere to the data and reagent release policy described in the following web sites.

http://www3.niaid.nih.gov/research/resources/mscs/data.htm

http://grants.nih.gov/grants/guide/notice-files/NOT-OD-08-013.html

<Each Center to include their website that describes/points to the guidelines>

Once a white paper project is approved, NIAID GSC will develop with the collaborators a detailed data and reagent release plan to be reviewed and approved by NIAID.

Accept ☒ Decline ☐

7b. Public Access to Reagents, Data, Software and Other Materials:

13. *State plans for deposit of starting materials as well as resulting reagents, resources, and datasets in NIAID approved repositories. Sequencing projects will not begin until the strain is deposited into NIAID funded BEI repository (http://www.beiresources.org/). This includes web based forms are completed by the collaborator and received by the NIAID BEI (http://www.beiresources.org/).*

The strains will be deposited in BEI repository in conjunction with the sequencing project and relevant clinical information and literature citations will be made available. Note, many of the strains used in this proposal have been described in the literature cited in the attached bibliography.

7c. Research Compliance Requirements

Upon project approval, NIAID review of relevant IRB/IACUC documentation is required prior to commencement of work. Please contact the GSC Principal Investigator(s) to ensure necessary documentation are filed for / made available for timely start of the project.

Investigator Signature:

Investigator Name: **Date:**

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