

## Complete Genome Sequence of *Bartonella ancashensis* Strain 20.00, Isolated from the Blood of a Patient with Verruga Peruana

Jun Hang,<sup>a</sup> Kristin E. Mullins,<sup>b,c</sup> Robert J. Clifford,<sup>a</sup> Fatma Onmus-Leone,<sup>a</sup> Yu Yang,<sup>a</sup> Ju Jiang,<sup>c</sup> Mariana Leguia,<sup>d</sup> Matthew R. Kasper,<sup>d</sup> Ciro Maguiña,<sup>e</sup> Emil P. Lesho,<sup>a</sup> Richard G. Jarman,<sup>a</sup> Allen L. Richards,<sup>c</sup> David Blazes<sup>b</sup>

Walter Reed Army Institute of Research, Silver Spring, Maryland, USA<sup>a</sup>; Uniformed Services University of the Health Sciences, Bethesda, Maryland, USA<sup>b</sup>; Naval Medical Research Center, Silver Spring, Maryland, USA<sup>c</sup>; U.S. Naval Medical Research Unit No. 6, Lima, Peru<sup>d</sup>; Universidad Peruana Cayetano Heredia, Lima, Peru<sup>e</sup>

J.H., K.E.M., and R.J.C. contributed equally to this work.

Here we present the complete genome sequence of *Bartonella ancashensis* strain 20.00, isolated from the blood of a Peruvian patient with verruga peruana, known as Carrion's disease. *Bartonella ancashensis* is a Gram-negative bacillus, phylogenetically most similar to *Bartonella bacilliformis*, the causative agent of Oroya fever and verruga peruana.

Received 3 September 2015 Accepted 25 September 2015 Published 5 November 2015

Citation Hang J, Mullins KE, Clifford RJ, Onmus-Leone F, Yang Y, Jiang J, Leguia M, Kasper MR, Maguiña C, Lesho EP, Jarman RG, Richards AL, Blazes D. 2015. Complete genome sequence of *Bartonella ancashensis* strain 20.00, isolated from the blood of a patient with verruga peruana. Genome Announc 3(6):e01217-15. doi:10.1128/genomeA.01217-15. Copyright © 2015 Hang et al. This is an open-access article distributed under the terms of the Creative Commons Attribution 3.0 Unported license.

Address correspondence to Kristin E. Mullins, kristin.e.mullins3.ctr@mail.mil.

Members of the genus *Bartonella* are Gram-negative bacilli that are found worldwide and are associated with animal and human diseases (1). The most common diseases associated with *Bartonella* species are trench fever, cat scratch disease, and the biphasic illness Carrion's disease, which are caused by *B. quintana*, *B. henselae*, and *B. bacilliformis*, respectively (2–4). More recently, *B. henselae* and *B. quintana* have been associated with bacillary angiomatosis, and more than eight *Bartonella* species have been associated with either febrile illnesses or endocarditis (2, 4, 5).

In a 2003 study involving 127 patients with verruga peruana in the Peruvian Ancash mountain area, two patients were found to be infected with a *Bartonella* species distinct from any known *Bartonella* species. This determination was based on the sequences of the *gltA*, *rpoB*, *ftsZ*, *groEL*, and *rrs* genes and the 16s-23s intergenic internal transcribed spacer (ITS) region. The genetic differences and culture characteristics of the strain meet the taxonomic criteria to classify this non-*B. bacilliformis* pathogen as a new species, designated *Bartonella ancashensis*. The nomenclature has been accepted by the *International Journal of Systematic and Evolutionary Microbiology*, and *B. ancashensis* strain 20.00 was accepted by both ATCC and DSMZ for archiving and distribution for research use (6–8).

The genome of *B. ancashensis* strain 20.00 was sequenced using Roche 454 next-generation sequencing technology (Roche 454 Life Sciences, Branford, CT). A total of 198,990 filtered reads consisting of 57.4 Mb of data were assembled into 94 contigs with average sequence coverage of 41.7× using GS Assembler software (Newbler) version 2.5.3. Final assembly was verified using a whole-genome AfIII restriction map generated with the Argus system (OpGen, Gaithersburg, MD) (9). *B. ancashensis* strain 20.00 has a circular genome of 1,466,048 bp and has a G+C content of 38.4%, which is similar to the genome of the *Bartonella* prototype strain, *B. bacilliformis* KC583 (NC\_008783.1), which is 1,445,021 nucleotides in length with a G+C content of 38.2%.

Whole-genome annotation was performed using RAST (Rapid

Annotation using Subsystem Technology) (http://www.nmpdr .org/FIG/wiki/view.cgi/FIG/RapidAnnotationServer); structural and functional annotation was completed using the IGS Annotation Engine (http://ae.igs.umaryland.edu/cgi/index.cgi). Genome annotations were reviewed and finalized using Genome Viewer (http://www.nmpdr.org/FIG/wiki/view.cgi/FIG/GenomeViewer) and Manatee (http://manatee.sourceforge.net/) (10, 11). The B. ancashensis strain 20.00 genome contains 1,346 putative protein-encoding genes, of which 79.1% are found to have homologs in B. bacilliformis. Like B. bacilliformis, B. ancashensis strain 20.00 encodes flagellar proteins. However, unlike B. bacilliformis, B. ancashensis strain 20.00 encodes VirB/D4 type IV secretion system proteins most similar to those of B. clarridgeiae and B. rochalimae, both of which have been implicated in human disease. Further, B. ancashensis possesses a family of virulencemodulating proteins which are present only in B. australis and human-pathogenic Leptospira species (12-16).

Here we have reported the first complete genome sequence for the new pathogen *B. ancashensis*. Further *B. ancashensis* genome studies and comparisons will elucidate factors involved in virulence and pathogenicity of not only *B. ancashensis*, but also the *Bartonella* genus as a whole.

**Nucleotide sequence accession number.** This whole-genome shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession number CP010401.

## ACKNOWLEDGMENTS

J.H., M.L., E.P.L., R.G.J., D.B., and A.L.R. are military service members or employees of the U.S. Government and this work was prepared as part of their official duties.

This work is supported by the Global Emerging Infections Surveillance and Response System, a Division of the Armed Forces Health Surveillance Center. The views expressed in this article are those of the author and do not necessarily reflect the official policy or position of the Department of the Army, the Department of the Navy, the Department of Defense, nor the United States. Government.

## REFERENCES

- Birtles RJ, Harrison TG, Saunders NA, Molyneux DH. 1995. Proposals to unify the genera *Grahamella* and *Bartonella*, with descriptions of *Bartonella talpae* comb. nov., *Bartonella peromysci* comb. nov., and three new species, *Bartonella grahamii* sp. nov., *Bartonella taylorii* sp. nov., and *Bartonella doshiae* sp. nov. Int J Syst Bacteriol 45:1–8. http://dx.doi.org/ 10.1099/00207713-45-1-1.
- Harms A, Dehio C. 2012. Intruders below the radar: molecular pathogenesis of *Bartonella* spp. Clin Microbiol Rev 25:42–78. http://dx.doi.org/ 10.1128/CMR.05009-11.
- Kaiser PO, Riess T, O'Rourke F, Linke D, Kempf VAJ. 2011. Bartonella spp.: throwing light on uncommon human infections. Int J Med Microbiol 301:7–15. http://dx.doi.org/10.1016/j.ijmm.2010.06.004.
- Rolain JM, Brouqui P, Koehler JE, Maguina C, Dolan MJ, Raoult D. 2004. Recommendations for treatment of human infections caused by *Bartonella* species. Antimicrob Agents Chemother 48:1921–1933. http:// dx.doi.org/10.1128/AAC.48.6.1921-1933.2004.
- Mosepele M, Mazo D, Cohn J. 2012. *Bartonella* infection in immunocompromised hosts: immunology of vascular infection and vasoproliferation. Clin Dev Immunol 2012:612809. http://dx.doi.org/10.1155/2012/ 612809.
- Blazes DL, Mullins K, Smoak BL, Jiang J, Canal E, Solorzano N, Hall E, Meza R, Maguina C, Myers T, Richards AL, Laughlin L. 2013. Novel Bartonella agent as cause of verruga peruana. Emerg Infect Dis 19: 1111–1114. http://dx.doi.org/10.3201/eid1907.121718.
- Mullins KE, Hang J, Jiang J, Leguia M, Kasper MR, Maguiña C, Jarman RG, Blazes DL, Richards AL. 2013. Molecular typing of "Candidatus *Bartonella ancashi*," a new human pathogen causing verruga peruana. J Clin Microbiol 51:3865–3868. http://dx.doi.org/10.1128/JCM.01226-13.
- 8. Mullins KE, Hang J, Jiang J, Leguia M, Kasper MR, Ventosilla P, Maguina C, Jarman RG, Blazes D, Richards AL. 2015 Jul 1. Description of *Bartonella ancashensis* sp. nov., isolated from the blood of two patients

with verruga peruana. Int J Syst Evol Microbiol. http://dx.doi.org/ 10.1099/ijsem.0.000416.

- Onmus-Leone F, Hang J, Clifford RJ, Yang Y, Riley MC, Kuschner RA, Waterman PE, Lesho EP. 2013. Enhanced de novo assembly of highthroughput pyrosequencing data using whole genome mapping. PLoS One 8:e61762. http://dx.doi.org/10.1371/journal.pone.0061762.
- Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formsma K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNeil LK, Paarmann D, Paczian T, Parrello B, Pusch GD, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O. 2008. The RAST Server: rapid annotations using subsystems technology. BMC Genomics 9:75. http://dx.doi.org/10.1186/ 1471-2164-9-75.
- Galens K, Orvis J, Daugherty S, Creasy HH, Angiuoli S, White O, Wortman J, Mahurkar A, Giglio MG. 2011. The IGS Standard operating procedure for automated prokaryotic annotation. Stand Genomic Sci 4:244–251. http://dx.doi.org/10.4056/sigs.1223234.
- Dehio C. 2008. Infection-associated type IV secretion systems of *Barto-nella* and their diverse roles in host cell interaction. Cell Microbiol 10: 1591–1598. http://dx.doi.org/10.1111/j.1462-5822.2008.01171.x.
- 13. Eicher SC, Dehio C. 2012. *Bartonella* entry mechanisms into mammalian host cells. Cell Microbiol 14:1166–1173. http://dx.doi.org/10.1111/j.1462 -5822.2012.01806.x.
- Chamberlin J, Laughlin L, Gordon S, Romero S, Solorzano N, Regnery RL. 2000. Serodiagnosis of *Bartonella bacilliformis* infection by indirect fluorescence antibody assay: test development and application to a population in an area of bartonellosis endemicity. J Clin Microbiol 38: 4269–4271.
- Benson LA, Kar S, McLaughlin G, Ihler GM. 1986. Entry of Bartonella bacilliformis into erythrocytes. Infect Immun 54:347–353.
- Scherer DC, DeBuron-Connors I, Minnick MF. 1993. Characterization of *Bartonella bacilliformis* flagella and effect of antiflagellin antibodies on invasion of human erythrocytes. Infect Immun 61:4962–4971.