Microbiology Resource Announcements

| Bacteriology | Announcement

Complete genome sequence of *Brucella abortus* isolated from a human blood culture sample in Tanzania

Gershom A. Mbwambo,[1] Marco van Zwetselaar,[1] Tolbert Sonda,[1] AbdulHamid S. Lukambagire,[1,2] Judith S. Njau,[1] Boaz Wadugu,[1] Ignass P. Ignass,[1] Nelson B. Amani,[1] Ephrasia A. Hugho,[1,3] Matthew P. Rubach,[4] Philoteus Sakasaka,[1] Rose S. Oisso,[1] Nestory Mkenda,[5] Gabriel Shirima,[6] Roland T. Ashford,[7] Daniel T. Haydon,[8] Venance P. Maro,[9] Rudovick R. Kazwala,[10] Happiness H. Kumburu,[1,11,12] Blandina T. Mmbaga,[1,11] Jo E. B. Halliday[7]

AUTHOR AFFILIATIONS See affiliation list on p. 2.

ABSTRACT *Brucella abortus* causes infections in humans and livestock. Bacterial isolates are challenging to obtain, and very little is known about the genomic epidemiology of this species in Africa. Here, we report the complete genome sequence of a *Brucella abortus* isolate cultured from a febrile human in northern Tanzania.

KEYWORDS *Brucella*, molecular epidemiology, zoonoses

Brucellosis is a zoonosis caused by bacteria of the genus *Brucella*. *Brucella abortus* causes human illness and production impacts in livestock. *Brucella* species are challenging to isolate. Culture involves considerable infection risk for laboratory personnel, and high containment laboratory facilities are required for safe manipulation of isolates (1). *Brucella abortus* is endemic in Tanzania and has been isolated previously (2). Despite widespread distribution across Africa, there are very few published sequences of *Brucella abortus* from Africa and none from Tanzania specifically. We report a complete genome sequence of *Brucella abortus* isolated from a febrile human who presented for care in northern Tanzania in 2017 (3). A blood sample was collected and inoculated into Castañeda media (prepared at the Animal and Plant Health Agency Weybridge, UK). Culture bottles were incubated in a CO_2 incubator at 5%–10% CO_2 and 37°C. Bottles were examined for growth every 72 hours for up to 35 days. Isolates of Gram-negative *coccobacilli* with positive reactions for urease, catalase, and oxidase were classified as presumptive *Brucella* spp. and stored on Microbank beads (Pro-Lab Diagnostics, Bromborough, UK) at −70°C (3). Ethical approval for the study was granted by the Kilimanjaro Christian Medical Centre Ethics Committee (698), National Institute of Medical Research (NIMR), Tanzania (NIMR/HQ/R.8c/Vol. I/1140), and University of Glasgow College of Medical, Veterinary and Life Sciences Ethics Committee (200140149).

Isolate manipulation (culture and extraction) was done at the Kilimanjaro Clinical Research Institute-Biotechnology Laboratory. The thawed isolate was plated on sheep blood agar, incubated at 37°C and 5% CO_2 and observed for 2 days before selection of pure colonies morphologically consistent with *Brucella*. Extraction of genomic DNA was performed using the Quick-DNA HMW MagBead Kit (D6060, Zymo) and the QIAamp DNA Mini Kit (51306, Qiagen). Genomic DNA from both kits was assessed for quantity using a Qubit 4.0 fluorometer and for quality using a Nanodrop spectrophotometer. The input concentration was adjusted to approximately 53 ng/µL for MinION and 2.0 ng/µL for Illumina sequencing, respectively. Long reads were generated using a MinION Mk1B sequencer and R9.4.1 flow cell (Oxford Nanopore Technologies) after library preparation using the Rapid Barcoding Sequencing kit (SQK-RBK004, Oxford Nanopore Technologies). Short reads were generated using the Illumina Miseq sequencer and 2 × 250 bp paired-end protocol after library preparation using the Illumina Nextera XT protocol

Editor Julie C. Dunning Hotopp, University of Maryland School of Medicine, USA

Address correspondence to Jo E. B. Halliday, Jo.Halliday@glasgow.ac.uk.

Rudovick R. Kazwala passed away on 3 April 2023 during the preparation of this manuscript.

The authors declare no conflict of interest.

See the funding table on p. 3.

Received 14 November 2023
Accepted 7 January 2024
Published 30 January 2024

TABLE 1 Characteristics of the *B. abortus* sequence

Characteristic	Isolate data
Study ID	PRJEB66434
Year of isolation	2017
Country of origin	Tanzania
Source	Human blood culture
Number of ONT reads	66,790
ONT average read length (bp)	7,849
Number of sequenced bp, ONT	524,253,445
Genome coverage, ONT	160×
Number of Illumina reads (pairs)	251,458
Number of sequenced bp, Illumina	107,559,286
Genome coverage, Illumina	33×
Genome size after hybrid assembly (bp)	3,280,926 (2,118,235 + 1,162,691)
GC content after hybrid assembly (%)	57.2%
Number of genes	3,238
Number of CDS	3,115
Number of rRNA/tRNA/tmRNA/ncRNA	9/54/1/59
MLST	ST32
Genome accession number	GCA_963555505

(Illumina, Inc.). Long reads were basecalled and demultiplexed with Guppy (v6.4.2) using the "sup" model. Long-read assembly was done with Flye (v2.9.2-16) in "nano-hq" configuration using five polishing iterations (4). The resulting draft assembly consisted of two complete circular contigs and was polished with Medaka (v1.9.1) using the "r941_min_sup_g507" model (5). Short reads were then used to polish the long-read assembly using Polypolish (v0.5.0) with default settings. The resulting complete genome assembly was annotated with Prokka (v1.14.6) and EMBLmyGFF3 (v2.3)(6). Table 1 gives details of the sequence obtained.

ACKNOWLEDGMENTS

This work was supported by the Biotechnology and Biological Sciences Research Council project African Skills Training for health Research And Learning (ASTRAL): A University of Glasgow One Health training programme for Tanzania (BB/R020280/1). The isolate was obtained through project BB/L018845. The sequencing and bioinformatics work were funded by the UK Department of Health and Social Care through the Fleming Fund SeqAfrica Regional Grant.

AUTHOR AFFILIATIONS

[1]Kilimanjaro Clinical Research Institute, Moshi, Tanzania
[2]EcoHealth Alliance, New York, New York, USA
[3]Institute of Public Health, Kilimanjaro Christian Medical University College, Moshi, Tanzania
[4]Department of Medicine, Division of Infectious Disease and International Health, Duke Global Health Institute, Duke University School of Medicine, Durham, North Carolina, USA
[5]Endulen Hospital, Arusha, Tanzania
[6]Nelson Mandela Africa Institute of Science and Technology, Arusha, Tanzania
[7]Department of Bacteriology, Animal and Plant Health Agency, Weybridge, United Kingdom

[8]School of Biodiversity, One Health & Veterinary Medicine, College of Medical Veterinary and Life Sciences, University of Glasgow, Glasgow, United Kingdom
[9]Department of Internal Medicine, Kilimanjaro Christian Medical University College, Moshi, Tanzania
[10]Department of Veterinary Medicine and Public Health, College of Veterinary Medicine and Biomedical Sciences, Sokoine University of Agriculture, Morogoro, Tanzania
[11]Kilimanjaro Christian Medical University College, Moshi, Tanzania
[12]Kilimanjaro Christian Medical Centre, Moshi, Tanzania

AUTHOR ORCIDs

Jo E. B. Halliday http://orcid.org/0000-0002-1329-9035

FUNDING

Funder	Grant(s)	Author(s)
UKRI | Biotechnology and Biological Sciences Research Council (BBSRC)	BB/R020280/1, BB/L018845	Matthew P. Rubach Nestory Mkenda Gabriel Shirima Roland T. Ashford Daniel T. Haydon Venance P. Maro Rudovick R. Kazwala Blandina T. Mmbaga Jo E. B. Halliday

AUTHOR CONTRIBUTIONS

Gershom A. Mbwambo, Investigation, Methodology, Writing – original draft, Writing – review and editing | Marco van Zwetselaar, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Supervision, Writing – original draft, Writing – review and editing | Tolbert Sonda, Data curation, Investigation, Writing – review and editing | AbdulHamid S. Lukambagire, Conceptualization, Investigation, Resources, Supervision, Writing – review and editing | Judith S. Njau, Investigation, Methodology, Writing – review and editing | Boaz Wadugu, Investigation, Methodology, Writing – review and editing | Ignass P. Ignass, Investigation, Methodology, Writing – review and editing | Nelson B. Amani, Investigation, Methodology, Writing – review and editing | Ephrasia A. Hugho, Investigation, Methodology, Writing – review and editing | Matthew P. Rubach, Conceptualization, Resources, Supervision, Writing – review and editing | Philoteus Sakasaka, Investigation, Methodology, Supervision, Writing – review and editing | Rose S. Oisso, Investigation, Methodology, Writing – review and editing | Nestory Mkenda, Conceptualization, Investigation, Writing – review and editing | Gabriel Shirima, Conceptualization, Funding acquisition, Investigation, Writing – review and editing | Roland T. Ashford, Conceptualization, Investigation, Methodology, Supervision, Writing – original draft, Writing – review and editing | Daniel T. Haydon, Conceptualization, Funding acquisition, Writing – review and editing | Venance P. Maro, Conceptualization, Funding acquisition, Writing – review and editing | Rudovick R. Kazwala, Conceptualization, Funding acquisition | Happiness H. Kumburu, Methodology, Supervision, Writing – review and editing | Blandina T. Mmbaga, Conceptualization, Funding acquisition, Supervision, Writing – review and editing | Jo E. B. Halliday, Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing – original draft, Writing – review and editing

DATA AVAILABILITY

This project has been deposited in the European Nucleotide Archive under accession number PRJEB66434. Raw reads are under accessions ERR12080966 (ONT) and ERR12080967 (MiSeq). The genome assembly has GenBank accession GCA_963555505 and chromosome accessions OY741352 and OY741353.

REFERENCES

1. Yagupsky P, Morata P, Colmenero JD. 2019. Laboratory diagnosis of human brucellosis. Clin Microbiol Rev 33:e00073-19. https://doi.org/10.1128/CMR.00073-19
2. Mathew C, Stokstad M, Johansen TB, Klevar S, Mdegela RH, Mwamengele G, Michel P, Escobar L, Fretin D, Godfroid J. 2015. First isolation, identification, phenotypic and genotypic characterization of *Brucella abortus* biovar 3 from dairy cattle in Tanzania. BMC Vet Res 11:156. https://doi.org/10.1186/s12917-015-0476-8
3. Bodenham RF, Lukambagire AS, Ashford RT, Buza JJ, Cash-Goldwasser S, Crump JA, Kazwala RR, Maro VP, McGiven J, Mkenda N, Mmbaga BT, Rubach MP, Sakasaka P, Shirima GM, Swai ES, Thomas KM, Whatmore AM, Haydon DT, Halliday JEB. 2020. Prevalence and speciation of brucellosis in febrile patients from a pastoralist community of Tanzania. Sci Rep 10:7081. https://doi.org/10.1038/s41598-020-62849-4
4. Sanderson ND, Kapel N, Rodger G, Webster H, Lipworth S, Street TL, Peto T, Crook D, Stoesser N. 2023. Comparison of R9.4.1/Kit10 and R10/Kit12 oxford nanopore flowcells and chemistries in bacterial genome reconstruction. Microb Genom 9:mgen000910. https://doi.org/10.1099/mgen.0.000910
5. Fàbregas N, Pérez D, Viñes J, Fonticoba R, Cuscó A, Migura-García L, Ferrer L, Francino O. 2022. Whole-genome sequencing and de novo assembly of 67 *Staphylococcus pseudintermedius* strains isolated from the skin of healthy dogs. Microbiol Resour Announc 11:e0003922. https://doi.org/10.1128/mra.00039-22
6. Dainat J, Viklund J, Gourlé H. 2023. Brillet-Guéguen. Available from: NBISweden/EMBLmyGFF3: EMBLmyGFF3-v2.3. Zenodo