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

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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

rucellosis is a zoonosis caused by bacteria of the genus Brucella. Brucella abor-B tus 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 CO₂ incubator at 5%-10% CO₂ 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% CO₂ 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

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

The authors declare no conflict of interest

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 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.

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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

- 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
- 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
- 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
- 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
- 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
- Dainat J, Viklund J, Gourlé H. 2023. Brillet-Guéguen. Available from: NBISweden/EMBLmyGFF3: EMBLmyGFF3-v2.3. Zenodo