Molecular characteristics of Brucella melitensis isolates from humans in Qinghai Province, China

Zhijun Zhao

qinghai institute for endemic disease prevention and control

Jiquan Li

qinghai institute for endemic disease prevention and control

Li Ma

Qinghai institute for endemic disease prevention and control

Hongmei Xue

qinghai institute for endemic disease prevention and control

Xuxin Yang

qinghaii institute for endemic disease prevention and control

Yuanbo Zhao

qinghai institute for endemic disease prevention and control

Yuming qin

qinghai institute for endemic disease prevention and control

Xiaowen Yang

Centers for Disease Control and Prevention

Dongri Piao

china centre for disease prevention and control

Hongyan Zhao

china centre for disease prevention and control

Guozhong Tian

china centre for disease prevention and control

Qiang Li

qinghai institute for endemic disease prevention and control

Jianling Wang

qinghai institute for endemic disease prevention and control

Guang Tian

qinghai institute for endemic disease prevention and control

Hai Jiang

China centre for disease prevention and control

Liqing Xu (✉ qhxlq2006@163.com)

qinghaiinstitute for endemic disease prevention and control

Research Article

Abstract

Background

The prevalence of human brucellosis in Qinghai Province has been increasing rapidly, with confirmed cases distributed across 31 counties. However, the epidemiology of brucellosis transmission has not been fully elucidated. To characterize the two isolated strains, multiple locus variable-number tandem repeats analysis (MLVA) and whole-genome single-nucleotide polymorphism (SNP)-based approaches were employed.

Methods

Blood samples were isolated from two males that were confirmed *B. melitensis* positive following MLVA. Genomic DNA was extracted from these samples, and whole-genome sequencing (WGS) was performed. Next, SNP-based phylogenetic analysis was performed to compare the two strains to 94 *B. melitensis* strains (complete genome and draft genome) retrieved from online databases.

Results

The two *Brucella* isolates were identified as *B. melitensis* biovar 3 (QH2019001 and QH2019005) following conventional biotyping and were found to have differences in their variable number tandem repeats (VNTRs) using MLVA-16. Phylogenetic examination assigned the 96 strains to five genotype groups, with QH2019001 and QH2019005 assigned to the same group, but different subgroups. Moreover, the QH2019005 strain was assigned to a new subgenotype, IIj, within genotype II. These findings were then combined to determine the geographic origin of the two *Brucella* strains.

Conclusions

Utilizing a whole-genome SNP-based approach enabled differences between the two *B. melitensis* strains to be more clearly resolved, and facilitated the elucidation of their different evolutionary histories. This approach also revealed that QH2019005 is a member of a new subgenotype (IIj) with an ancient origin in the eastern Mediterranean Sea.

Background

Brucellosis, which is caused by bacteria in the *Brucella* genus, is one of the most important zoonoses worldwide and is considered a "forgotten, neglected zoonosis" by the WHO (World Health Organization 2014) [1]. This disease is endemic in regions within Africa, Asia, Latin America and other countries along the Mediterranean Sea [2, 3]. Human infections can occur due to the consumption of contaminated non-pasteurized milk or cheese, or by occupational exposure to infected animals or their carcasses, uterine

secretions, or aborted fetuses [4]. While the mortality rate of brucellosis is low, the morbidity rate is much higher. Worldwide, incidence of human brucellosis varies widely, from < 0.01 to > 200 per 100,000 people in endemic disease areas [5]. In mainland China, the total incidence rate of human brucellosis increased from 0.92 per 100,000 people in 2004 to 4.2 per 100,000 people in 2014 [6]. Recently, in Qinghai Province, the incidence rate has increased from 0.04 per 100,000 people in 2011 to 1.96 per 100,000 people in 2018, with confirmed cases distributed across 31 counties within the entire province. Thus, brucellosis is becoming a major public health problem that impacts human physical and mental health.

Brucella is a gram-negative, facultative, intracellular pathogen that induces cell toxicity by altering the plasma membrane and inducing cell apoptosis [7]. Currently, the *Brucella* genus contains 12 accepted species, with six of those, namely, *B. melitensis*, *B. abortus*, *B. suis*, *B. canis*, *B. ovis* and *B. neotomae*, considered "classical" species [8]. While various species differ in host preference, virulence and/or zoonotic potential, all *Brucella* species maintain a 97−99% genomic sequence identity [9].

In humans, *B. melitensis*, which contains three biovars (1, 2 and 3), is the most virulent species and has been the predominant strain associated with human brucellosis in China from 1953 to 2013 [10]. While all three biovars cause disease in small ruminants, their geographic distributions vary [11]. To ensure accurate epidemiological surveillance and to distinguish infected and vaccinated individuals, species identification and subtyping is essential [12]. Currently, *Brucella* identification and subtyping is performed using multiple loci variable-number tandem repeat analysis (MLVA), a genotyping tool for species identification and strains relatedness [13]. In a study examining *Brucella* in the Qinghai-Tibet Plateau region, none of the genotypes matched any of the sequences in the *Brucella* MLVA database (2012), possibly due to these strains having unique geographical characteristics or due to *B. melitensis* being the predominant species in that area [14]. Moreover, *Brucella* studies have also utilized whole-genome sequencing (WGS), which provides excellent genetic resolution, to resolve differences in closely related species [15]. In this study, the obtained *Brucella* samples were evaluated using MLVA and whole-genome single nucleotide polymorphism (SNP)-based approaches to elucidate strain characteristics and homologies for epidemiological purposes. It is hoped that these findings will provide further insight into *Brucella* epidemiology and enable improved control and prevention of brucellosis in Qinghai Province.

Methods

Ethics statement

This research was conducted according to the principles of the Declaration of Helsinki, and the study protocols were approved by the Ethics Committees of the National Institute for Communicable Disease Control and Prevention (Beijing, China). Informed consent was obtained from the two patients who received a brucellosis diagnosis at the Qinghai Institute for Endemic Disease Prevention (Qinghai, China), with diagnoses based on the Diagnosis for Brucellosis Standards (WS 269-2019) used in China.

Bacterial strains

Blood cultures were examined for brucellosis at the Qinghai Institute for Endemic Disease Prevention and Control in Qinghai Province, China, and deemed *Brucella*-positive. The samples were identified as *B. melitensis* strains based on morphology and conventional identification methods according to standard biotyping procedures, including CO_2 requirement, inhibition of growth by basic fuchsin and thionin, agglutination with monospecific antisera (A,M) and phage typing (Bk_2,Tb). Total genomic DNA was extracted from the two *B. melitensis* strains, QH2019001 and QH2019005, using a sodium dodecylsulfate (SDS)-based method [16]. The obtained DNA was then assessed via agarose gel electrophoresis and quantified using a Qubit 2.0 Fluorometer (Thermo Scientific, USA).

Brucella MLVA-16 genotyping scheme

MLVA-16 was performed and the loci analyzed as previously described [17]. Briefly, 16 gene loci were examined and divided into three groups as follows: panel 1 with eight loci (bruce06, bruce08, bruce11, bruce12, bruce42, bruce43, bruce45 and bruce55), panel 2A with three loci (bruce18, bruce19 and bruce21) and panel 2B with five loci (bruce04, bruce07, bruce09, bruce16 and bruce30). For panel 1, the loci amplicons were analyzed via gel electrophoresis. The panel 2 loci PCR amplicons were denatured and resolved via capillary electrophoresis on an ABI Prism 3130 automated fluorescent capillary DNA sequencer (Applied Biosystems), with the sequenced amplicons then compared to strains from Qinghai Province. All data were analyzed using BioNumerics (version 5.1; Applied Maths, Sint-Martens-Latem, Belgium). Clustering analysis was based on the categorical coefficient and the unweighted pair group method using the arithmetic averages (UPGMA) method.

Whole-genome sequencing and single-nucleotide polymorphism (SNP) analyses

Sequencing libraries were generated using a NEBNext® Ultra™ DNA Library Prep Kit for Illumina (NEB, USA) following the manufacturer's recommendations. Briefly, DNA samples were fragmented by sonication to a size of 350 bp. The DNA fragments were then end-repaired, adenylated and ligated prior to PCR amplification and Illumina sequencing. Finally, PCR products were purified using an AMPure XP system (Beckman Coulter, USA), and library size distributions were analyzed using an Agilent 2100 Bioanalyzer and quantified using real-time PCR. WGS for the QH2019001 and QH2019005 strains was performed by Novogene Bioinformatics Technology Co., Ltd. (Beijing, China) using an Illumina NovaSeq 6000 (PE 150 bp).

The obtained genomic sequences were then deposited in the Microbial Pathogens Database (http://data.mypathogen.org.; accession numbers: ICDC-20200117-135823-300 and ICDC-20200117-140311-357).

Genomic alignments between a sample genome and reference genome (or among more than two sample genomes) were performed using the MUMmer [18] and LASTZ[19, 20] tools. Additionally, 94 *B. melitensis* genomes 〖Supplementary table 1〗were retrieved from GenBank and used for comparison and preliminary phylogenetic analyses. A phylogenetic tree was constructed using TreeBeST and PHYML (maximum-likelihood based, with 1000 bootstrap replicates utilized).

Results

Identification of *Brucella* strains

The *Brucella* strains QH2019001 and QH2019001 were identified as *B. melitensis* biovar 3 using a conventional biotyping method (Table 1).

MLVA-16 genotyping results

The MLVA-16 assay was used to determine the genotypes for *B. melitensis* strains QH2019001 (1- 4 - 3 - 13 - 2 - 2 - 3 - 2 - 4 - 40 - 8 - 6 - 4 - 3 - 18 - 5) and QH2019005 (1 - 5 - 3 - 13 - 3 - 2 - 3 - 2 - 4 - 38 - 8 - 5 - 4 - 7 - 16 - 5), with the two strains showing a 99.1% genotype similarity. For the two *B. melitensis* strains, variable number tandem repeat (VNTR) differences were present in bruce08 and bruce42 (panel 1) and bruce19, bruce21, bruce04, bruce09 and bruce16 (panels 2A and 2B). Furthermore, the two *B. melitensis* strains were also similar to the other strains associated with Qinghai Province. When combining these findings with epidemical data, *B. melitensis* strain QH2019001 appears to have been imported from Anhui Province, while the QH2019005 strain appears to be native to Qinghai Province (Fig. 1).

Genomic characteristic and phylogenetic analysis based on whole-genome SNPs

The two strains comprised 24 contigs, with N50 contig size of 297 and 202 bp (QH2019001) and 332 and 914 bp (QH2019005). The total contig sequence lengths were 3,291,786 bp for QH2019001 and 3,289,996 bp for QH2019005, with both having a GC content of 57.24%.

To gain insight into the geographical distributions of the 96 examined *B. melitensis* strains, a phylogenetic analysis was performed using the whole-genome SNPs. The strains were divided into five major genotypes as follows: genotype I, Bruc048 and its related strains; genotype II, UK3/06 and its related strains; genotype III, strains of African origin; genotype IV, B115 and its related strains; and genotype V, 16 M and its related strains (Fig. 2). The QH2019001 and QH2019005 strains were both associated with the genotype II group. Furthermore, several clades and subclades were isolated within the lineages and were associated with geographic attributes. The QH2019001 strain was assigned to subgenotype IIh, while the QH2019005 strain was assigned to subgenotype IIj (a new subgroup).

Discussion

Due to the high mutation rate in tandem DNA repeats loci, the MLVA-16 scheme provides the ability to distinguish field strains [21] and associate isolates with their geographic origins [22]. In China, MLVA genotyping provides valuable insight when examining an epidemiological linkage or trace-back investigation during a brucellosis outbreak [10, 23, 24]. Furthermore, previous studies have shown that MLVA can be used as an epidemiological tool to distinguish relapses from reinfections in brucellosis patients [25]. In this study, the QH2019001 and QH2019005 strains did exhibit VNTR difference, but most of the genetic loci for these strains were similar. However, due to the high genomic homology of the *B.*

melitensis genus, the MLVA approach can be limited based on the nature of VNTRs and the technique itself [26]; thus the exact origins of the two strains could not be determined.

In one study that utilized only an MLVA approach, of the 63 patients that were examined, only 52 genotypes could be distinguished. However, when a whole-genome SNP-based approach was utilized, all of the patients could be distinguished from each other, thus demonstrating a higher discriminatory power [27]. Furthermore, WGS-based analysis has been shown to distinguish very closely related *B. melitensis* strains (up to a six gene or seven SNP difference), while MLVA profiling may struggle to provide enough resolution to accurately predict phylogenetic relationships [28]. Thus, utilizing SNP data can enable the development of a novel high-resolution molecular typing technique that can discriminate inter- and intraspecies relationships in pathogenic microorganisms.

Following phylogenetic clustering, all the *B. melitensis* strains (n = 96) were divided into five genotypes [23], with genotype I forming the earliest diverging clade. Most of the Asian isolates of *B. melitensis* clustered into genotype II, while genotype III was associated with Africa, and genotypes IV and V were associated with Europe and the Americas, respectively, which is consistent with previous findings [29].

The genotype II strains had the widest geographical distribution after diverging from genotype I. The diversification of this genotype may have taken place in the second half of the third millennium BC, approximately 5,270 years ago [30]. The most important land routes connecting Eastern Europe with India on the western part of the Silk Road went through Central Asia or the Caucasus and Iranian highlands; thus the Arabs played a key role in Europe's trade with Southeast Asia and China. Moreover, genotype II includes nine previously described subgenotypes (IIa–IIi) [29, 30].

Following WGS, the two strains, QH2019001 and QH2019005, were both assigned to genotype II, but to different subgroups. Furthermore, *B. melitensis* strain QH2019005 was assigned to genotype IIj (a new subtype), which is closely associated with subgenotype IIa that contained only a single strain, UK3/06. The QH2019001 strain was assigned to subgenotype IIh. The differences between the QH2019001 and QH2019005 strains suggest that the nucleotide variation may be attributed to changes in geographic distribution. It is also possible that subgenotype IIh diversification may have occurred during the second half of the XIV century A.D., and it may have been driven by the relatively isolated and more unique environment of the Qinghai-Tibet Plateau, with its extremely high altitudes. Due to the environment, it is difficult for lowland livestock breeds or wild animals to survive; hence, livestock exchange between Qinghai Province and other regions would be limited [11]. The QH2019005 strain was the most similar to the UK3/06 strain that was isolated from the Near East, thus suggesting that this strain and its new subgenotype have ancient origins. The QH2019001 strain was the most similar to the I-349 strain that was isolated from Central Asia (Russia: Republic of Tuva) [31], thus suggesting that this strain shares a common origin. Furthermore, active trade between Russia and China could have promoted the transmission of *B. melitensis* strain QH2019001 to other regions (Anhui Province) in China (Fig. 3).

Conclusion

In the present study, the molecular characteristics of two human *Brucella* strains isolated from Qinghai Province were examined to gain a further understanding of the epidemiology of brucellosis. The two strains were found to have different origins and evolutionary histories, with the native strain, QH2019005, assigned to a new subgenotype with an ancient origin in the eastern Mediterranean region. Additionally, this study further highlighted that utilizing a whole-genome SNP-based approach can enable intraspecies relationships between *B.* melitensis to be more fully examined.

Abbreviations

whole-genome sequencing WGS

multiple loci variable-number tandem repeat analysis MLVA

single nucleotide polymorphism SNP

serum agglutination tests SAT

variable number tandem repeat VNTR

Declarations

Ethics approval and consent to participate

This research was conducted according to the principles of the Declaration of Helsinki, and the study protocol was approved by the Ethics Committees of the National Institute for Communicable Disease Control and Prevention (Beijing, China). Informed consent was obtained from the two patients who received a brucellosis diagnosis at the Qinghai Institute for Endemic Disease Prevention and Control based on the Diagnosis for Brucellosis Standards (WS 269-2019) used in China. Obtained patient history/data were anonymized for the purpose of this study. Blood samples were obtained and Brucella strains were isolated.

Consent for publication

Not applicable.

Availability of data and materials

All of the relevant data have been provided within the manuscript or as a supporting file.

Competing interests

The authors have no competing interests to declare

Funding

Author Contributions

Conceptualization: Hai Jiang and Liqing Xu

Formal analysis: Zhijun Zhao and Jiquan Li

Investigation: Zhijun Zhao, Jiquan Li, Li Ma, Hongmei Xue, Xuxin Yang, Yuanbo Zhao, Yumin Qin、Guang Tian 、Jianling Wang and Qiang Li

Methodology: Dongri Piao, Hongyan Zhao, Guozhong Tian and Xiaowen Yang

Project administration: Hai Jiang and Liqing Xu

Supervision: Liqing Xu

Writing−original draft: Zhijun Zhao and Jiquan Li

Writing−review & editing: Hai Jiang

Acknowledgments

Not applicable.

References

1. World Health Organization. 2014. The control of neglected zoonotic diseases. In: NZD4 organising committee, editor. WHO conference report. Geneva: WHO Press. pp. 23−35.

2. Felipe F T, Regina B G, Natacha C. Human-to-human transmission of *Brucella* - a systematic review. Tropical Medicine and International Health , 2017，22:539-546.doi: 10.1111/tmi.12856

3. Rubin B, Band JD, Wong P, et al. Person-to-person transmission of *Brucella melitensis*. Lancet, 1991 ; 1:14−15. doi: 10.1155/1995/909404.PMCID: PMC3327908

4. Alcina V C N , Juliana PS M, Mariana N X , Tatiane A, Andrey P L , Renato L S . Pathogenesis of bovine brucellosis. The Veterinary Journal, 2010， 184:146−155.

5. Hanot MD , Boarbi S , Michel P , et al. Imported human brucellosis in Belgium: bio and molecular typing of bacterial isolates, 1996−2015. Plos one. 2017;12:e0174756.

6. S Lai, H Zhou, W Xiong e, t al. Changing epidemiology of human brucellosis, China , 1955-2014. Emerging Infectious Diseases,2017， 23:184−194

7. Garrity, G.M . 2001. Bergey's Manual of Systematic Bacteriology, second ed. Springer, New York. 721 pp.

8. Gamal W, Falk M, Herbert T, Uwe R , Heinrich N. Detection of *Brucella* abortus DNA in aborted goats and sheep in Egypt by real-time PCR. BMC Res Notes ,2015⬚8:212. Doi: 10.1186/s13104-015-1173-1

9. Marcela SE, Kate S. B, Nazareth RV, Gabriela HM,Elıas BC, et al. *Brucella* genetic variability in wildlife marine mammals populations relates to host preference and ocean distribution. Genome Biol. Evol.2017⬚9:1901–1912. doi:10.1093/gbe/evx137

10. Guo-Zhong T, Bu-Yun C, Dong-Ri P, Hong-Yan Z, Lan-Yu L, Xi L,et al .Multi-locus variable-number tandem repeat analysis of Chinese Brucella strains isolated from 1953 to 2013. Infectious Diseases of Poverty⬚2017⬚6:89.doi:10.1186/s40249-017-0296-0

11. Sofia C, Evridiki B,Antonios Z, Eleni M, Vassilios S, Anna P ,et al . Novel diagnostic approach on the identification of Brucella melitensis Greek endemic strains-discrimination from the vaccine strain Rev.1 by PCR-RFLP assay. Veterinary Medicine and Science ,2018⬚ 4:172–182

12. Hai J, Pengcheng D, Wen Z, Heng W, Hongyan Z, Dongri P⬚et al .Comparative genomic analysis of *Brucella melitensis* vaccine strain M5 provides insights into virulence attenuation .Plos one ,2013,8:e70852

13. A. C. Ferreira, R. Dias, M. I. C. de S´a, R. Tenreiro. Whole genome mapping reveals a large chromosomal inversion on Iberian *Brucella suis* biovar 2 strains. Veterinary Microbiology, 2016⬚ 192:220-225

14. JunYing M, Hu W, XueFei Z, LiQing X, GuiYing H, Hai J, et al .MLVA and MLST typing of Brucella from Qinghai, China. Infectious Diseases of Poverty 2016⬚4: 1-8.doi:10.1186/s40249-016-0123-z

15. Sankarasubramanian J, Vishnu US, Sridhar J, Gunasekaran P, Rajendhran J. A genome-wide SNP based phylogenetic analysis distinguishes different biovars of Brucella suis. Infect Genet Evol 2016; 41: 213–17.

16. Lim H J , Lee E.-H, Yoon Y , et al. Portable lysis apparatus for rapid single-step DNA extraction of Bacillus subtilis. J Applied Microbiology, 2016⬚ 120(2):379-387

17. Le FP, Jacques I, Grayon M, Al DS, Bouchon P, Denoeud F, Nockler K, Neubauer H, Guilloteau LA, Vergnaud G: Evaluation and selection of tandem repeat loci for a Brucella MLVA typing assay. BMC Microbiol 2006⬚6:9.

18. Arris R S. Improved pairwise alignment of genomic DNA[M]. ProQuest, 2007.

19. Chiaromonte F, Yap V B, Miller W. Scoring pairwise genomic sequence alignments[C]/ /Pacific Symposium on Biocomputing. 2001, 7: 115.

20. Altschul S F, Gish W, Miller W, et al. Basic local alignment search tool[J]. Journal of molecular biology, 1990, 215(3): 403-410.

21. Shevtsova E, Shevtsov A, Mukanov K, Filipenko M, Kamalova D, Sytnik I, et al. Epidemiology of brucellosis and genetic diversity of Brucella abortus in Kazakhstan. Plos one⬚2016⬚ 11 (12): e0167496. doi:10.1371/journal. pone.0167496

22. Zhi-guo L, Li-jun W, Dong-ri P, Miao W, Ri-hong L, Hong-yan Z, et al. Molecular investigation of the transmission pattern of Brucella suis 3 from Inner mongolia, China. Frontiers in Veterinary Science ,2018，5:271

23. Fengbo Z, Zhiwei L, Xiaolin L, Xiumin M, Yaoxin Z, Ping J, et al. Multiple-locus variable-number tandem-repeat analysis of Brucella isolates from patients in Xinjiang China. Int J Clin Exp Med，2015;8(9):15716-15723

24. Hai J, Heng W, Liqing X, Guiying H, Junying M, et al. MLVA genotyping of Brucella melitensis and Brucella abortus isolates from different animal species and humans and identification of Brucella suis vaccine strain S2 from cattle in China. Plos one,2013，8: e76332

25. Mireille M. Kattar,Rola F. Jaafar, George F. Araj, Philippe Le Fle`che, Ghassan M. Matar, et al .Evaluation of a multilocus variable-number tandem-repeat analysis scheme for typing human Brucella isolates in a region of brucellosis endemicity .J Clinical Microbiology 2008，46(12):3935-3940.doi:10.1128/JCM.00464-08

26. Garofolo G, Ancora M, Di Giannatale E. MLVA-16 loci panel on Brucella spp. using multiplex PCR and multicolor capillary electrophoresis. J Microbiol Methods ，2013，92:103–107. https://doi.org/10.1016/j.mimet .2012.11.007.

27. Enrico G, Mathias CW, Marie-Theres P, Bernd HN , Lesca M, et al. Whole genome sequencing of Brucella melitensis isolated from 57 patients in Germany reveals high diversity in strains from Middle East. PLoS ONE ,2017，12(4): e0175425.doi: 10.1371/journal.pone.0175425.PMCID: PMC5384748

28. Anna Janowicz, Fabrizio De Massis, Massimo Ancora, Cesare Cammà, Claudio Patavino,et al .Core Genome multilocus sequence typing and single nucleotide polymorphism analysis in the epidemiology of Brucella melitensis infections. Journal of Clinical Microbiology, 2018， 56 ，9，: e00517-18

29. Sarwar Azam,Sashi Bhushan Rao,Padmaja Jakka, Veera NarasimhaRao,Bindu Bhargavi, et al .Genetic characterization and comparative genome analysis of Brucella melitensis Isolates from India. International Journal of Genomics,2016，Article ID 3034756, 13 pages .doi: 10.1155/2016/3034756 .PMCID: PMC4976149

30. Sergey V. P, Dmitry A. K, Anna S. V , Dmitry G. P, Diana V. R, et al .Global evolution and phylogeography of Brucella melitensis strains. BMC Genomics ，2018，19:353 .doi: 10.1186/s12864-018-4762-2.PMCID: PMC5946514

31. Cynthia C. Encyclopedia of world trade: from ancient times to the present. Armonk: Sharpe Reference; 2005.

Tables

Table 1. Biotyping identification results for the two *B. melitensis* strain

Strain IDs	CO$_2$ Requirement	Sensitivity to Dyes		Serum agglutination		RTD	
		Fuchin 1:25 000	Thionin1:50 000	A	M	BK2	Tb
QH2019001	-	+	+	+	+	+	+
QH2019005	-	+	+	+	+	+	+

Figures

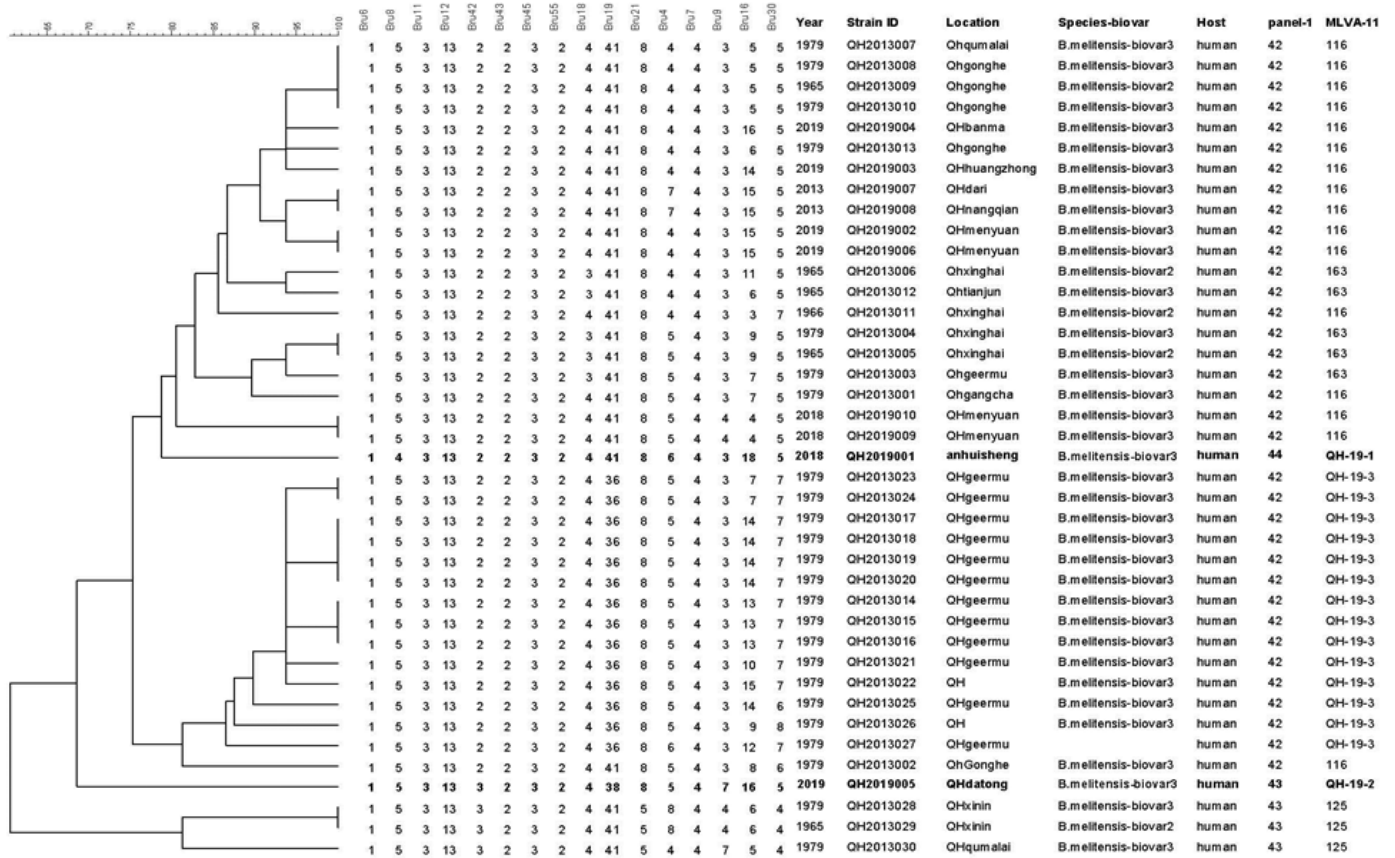| Bru6	Bru8	Bru11	Bru12	Bru42	Bru43	Bru45	Bru55	Bru18	Bru19	Bru21	Bru4	Bru7	Bru9	Bru16	Bru30	Year	Strain ID	Location	Species-biovar	Host	panel-1	MLVA-11
1	5	3	13	2	2	3	2	4	41	8	4	4	3	5	5	1979	QH2013007	Qhqumalai	B.melitensis-biovar3	human	42	116
1	5	3	13	2	2	3	2	4	41	8	4	4	3	5	5	1979	QH2013008	Qhgonghe	B.melitensis-biovar3	human	42	116
1	5	3	13	2	2	3	2	4	41	8	4	4	3	5	5	1965	QH2013009	Qhgonghe	B.melitensis-biovar2	human	42	116
1	5	3	13	2	2	3	2	4	41	8	4	4	3	5	5	1979	QH2013010	Qhgonghe	B.melitensis-biovar3	human	42	116
1	5	3	13	2	2	3	2	4	41	8	4	4	3	16	5	2019	QH2019004	QHbanma	B.melitensis-biovar3	human	42	116
1	5	3	13	2	2	3	2	4	41	8	4	4	3	6	5	1979	QH2013013	Qhgonghe	B.melitensis-biovar3	human	42	116
1	5	3	13	2	2	3	2	4	41	8	4	4	3	14	5	2019	QH2019003	QHhuangzhong	B.melitensis-biovar3	human	42	116
1	5	3	13	2	2	3	2	4	41	8	7	4	3	15	5	2013	QH2019007	QHdari	B.melitensis-biovar3	human	42	116
1	5	3	13	2	2	3	2	4	41	8	7	4	3	15	5	2013	QH2019008	QHnangqian	B.melitensis-biovar3	human	42	116
1	5	3	13	2	2	3	2	4	41	8	4	4	3	15	5	2019	QH2019002	QHmenyuan	B.melitensis-biovar3	human	42	116
1	5	3	13	2	2	3	2	4	41	8	4	4	3	15	5	2019	QH2019006	QHmenyuan	B.melitensis-biovar3	human	42	116
1	5	3	13	2	2	3	2	3	41	8	4	4	3	11	5	1965	QH2013006	Qhxinghai	B.melitensis-biovar2	human	42	163
1	5	3	13	2	2	3	2	3	41	8	4	4	3	6	5	1965	QH2013012	Qhtianjun	B.melitensis-biovar3	human	42	163
1	5	3	13	2	2	3	2	4	41	8	4	4	3	3	7	1966	QH2013011	Qhxinghai	B.melitensis-biovar2	human	42	116
1	5	3	13	2	2	3	2	3	41	8	5	4	3	9	5	1979	QH2013004	Qhxinghai	B.melitensis-biovar3	human	42	163
1	5	3	13	2	2	3	2	3	41	8	5	4	3	9	5	1965	QH2013005	Qhxinghai	B.melitensis-biovar2	human	42	163
1	5	3	13	2	2	3	2	3	41	8	5	4	3	7	5	1979	QH2013003	Qhgeermu	B.melitensis-biovar3	human	42	163
1	5	3	13	2	2	3	2	4	41	8	5	4	3	7	5	1979	QH2013001	Qhgangcha	B.melitensis-biovar3	human	42	116
1	5	3	13	2	2	3	2	4	41	8	5	4	4	4	5	2018	QH2019010	QHmenyuan	B.melitensis-biovar3	human	42	116
1	5	3	13	2	2	3	2	4	41	8	5	4	4	4	5	2018	QH2019009	QHmenyuan	B.melitensis-biovar3	human	42	116
1	**4**	**3**	**13**	**2**	**2**	**3**	**2**	**4**	**41**	**8**	**6**	**4**	**3**	**18**	**5**	**2018**	**QH2019001**	**anhuisheng**	**B.melitensis-biovar3**	**human**	**44**	**QH-19-1**
1	5	3	13	2	2	3	2	4	36	8	5	4	3	7	7	1979	QH2013023	QHgeermu	B.melitensis-biovar3	human	42	QH-19-3
1	5	3	13	2	2	3	2	4	36	8	5	4	3	7	7	1979	QH2013024	QHgeermu	B.melitensis-biovar3	human	42	QH-19-3
1	5	3	13	2	2	3	2	4	36	8	5	4	3	14	7	1979	QH2013017	QHgeermu	B.melitensis-biovar3	human	42	QH-19-3
1	5	3	13	2	2	3	2	4	36	8	5	4	3	14	7	1979	QH2013018	QHgeermu	B.melitensis-biovar3	human	42	QH-19-3
1	5	3	13	2	2	3	2	4	36	8	5	4	3	14	7	1979	QH2013019	QHgeermu	B.melitensis-biovar3	human	42	QH-19-3
1	5	3	13	2	2	3	2	4	36	8	5	4	3	14	7	1979	QH2013020	QHgeermu	B.melitensis-biovar3	human	42	QH-19-3
1	5	3	13	2	2	3	2	4	36	8	5	4	3	13	7	1979	QH2013014	QHgeermu	B.melitensis-biovar3	human	42	QH-19-3
1	5	3	13	2	2	3	2	4	36	8	5	4	3	13	7	1979	QH2013015	QHgeermu	B.melitensis-biovar3	human	42	QH-19-3
1	5	3	13	2	2	3	2	4	36	8	5	4	3	13	7	1979	QH2013016	QHgeermu	B.melitensis-biovar3	human	42	QH-19-3
1	5	3	13	2	2	3	2	4	36	8	5	4	3	10	7	1979	QH2013021	QHgeermu	B.melitensis-biovar3	human	42	QH-19-3
1	5	3	13	2	2	3	2	4	36	8	5	4	3	15	7	1979	QH2013022	QH	B.melitensis-biovar3	human	42	QH-19-3
1	5	3	13	2	2	3	2	4	36	8	5	4	3	14	6	1979	QH2013025	QHgeermu	B.melitensis-biovar3	human	42	QH-19-3
1	5	3	13	2	2	3	2	4	36	8	5	4	3	9	8	1979	QH2013026	QH	B.melitensis-biovar3	human	42	QH-19-3
1	5	3	13	2	2	3	2	4	36	8	6	4	3	12	7	1979	QH2013027	QHgeermu		human	42	QH-19-3
1	5	3	13	2	2	3	2	4	41	8	5	4	3	8	6	1979	QH2013002	QhGonghe	B.melitensis-biovar3	human	42	116
1	**5**	**3**	**13**	**3**	**2**	**3**	**2**	**4**	**38**	**8**	**5**	**4**	**7**	**16**	**5**	**2019**	**QH2019005**	**QHdatong**	**B.melitensis-biovar3**	**human**	**43**	**QH-19-2**
1	5	3	13	3	2	3	2	4	41	5	8	4	4	6	4	1979	QH2013028	QHxinin	B.melitensis-biovar3	human	43	125
1	5	3	13	3	2	3	2	4	41	5	8	4	4	6	4	1965	QH2013029	QHxinin	B.melitensis-biovar2	human	43	125
1	5	3	13	3	2	3	2	4	41	5	4	4	7	5	4	1979	QH2013030	QHqumalai	B.melitensis-biovar3	human	43	125

Figure 1

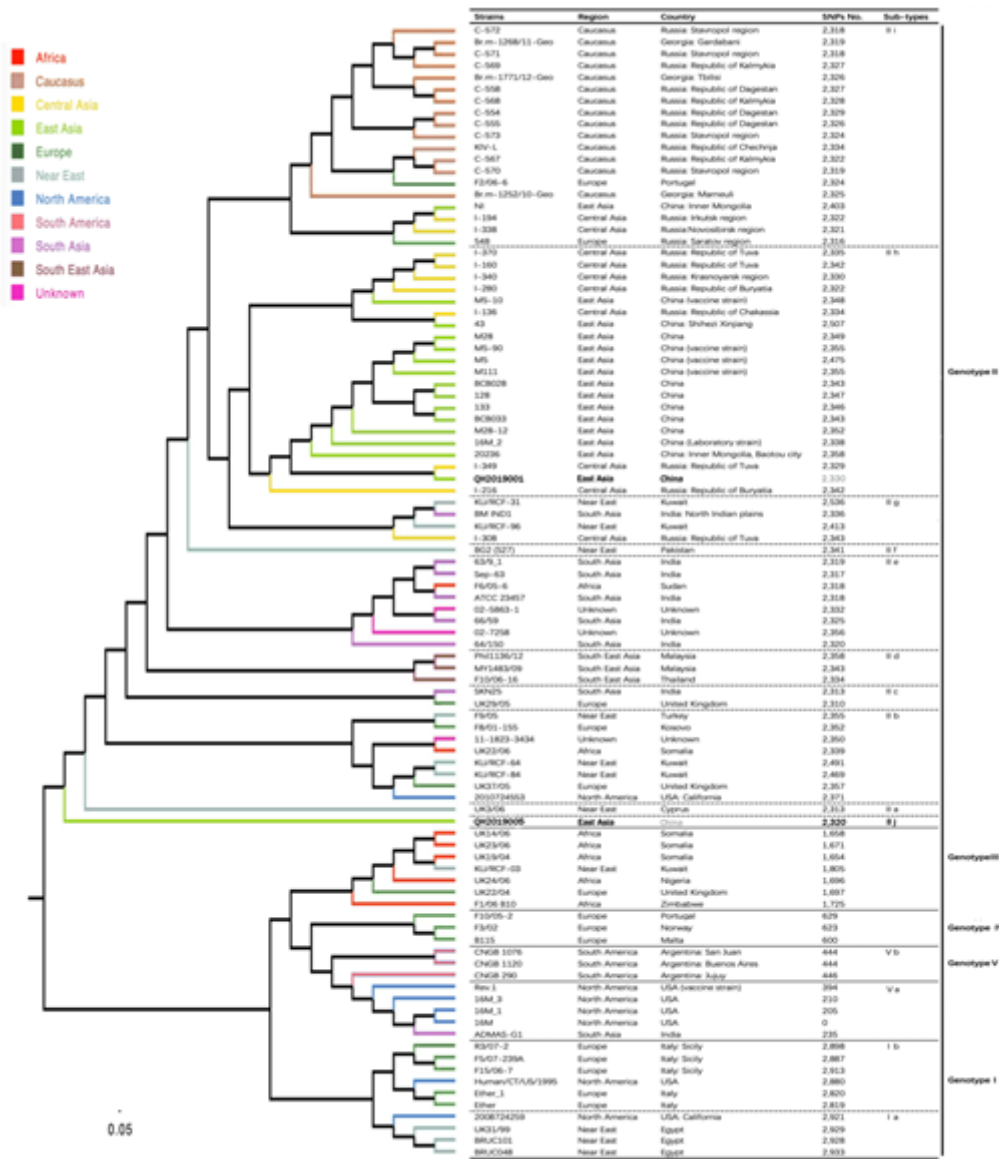Dendrogram based on MLVA-16 genotyping.

Figure 2

Phylogenetic tree showing the evolutionary relationships between 96 B. melitensis strains. Terminal branch colors correspond to the region where a given strain was isolated
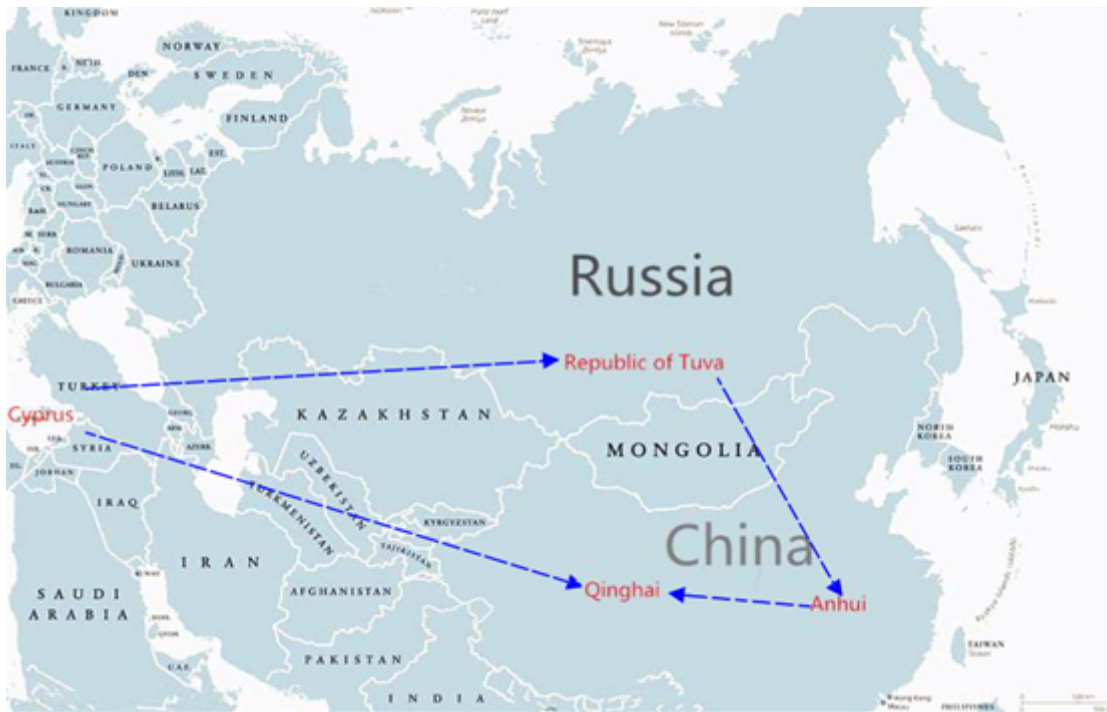
Figure 3

Possible transmission routes of B. melitensis strains QH2019001 and QH2019005. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- ListofB.melitensisgenomesinformationsupplement.xls