

# Seroprevalence and Molecular Characterization of *Brucella abortus* from the Himalayan Marmot in Qinghai, China

Hongmei Xue<sup>1</sup>, Jiquan Li<sup>1</sup>, Li Ma<sup>1</sup>, Xuxin Yang<sup>1</sup>, Lingling Ren<sup>1</sup>, Zhijun Zhao<sup>1</sup>, Jianling Wang<sup>1</sup>, Yuanbo Zhao<sup>1</sup>, Zhongzhi Zhao<sup>1</sup>, Xuefei Zhang<sup>1</sup>, Zhiguo Liu<sup>2</sup>, Zhenjun Li<sup>2</sup>

<sup>1</sup>Department of Brucellosis Prevention and Control, Qinghai Institute for Endemic Disease Prevention and Control, Xining, Qinghai, People's Republic of China; <sup>2</sup>National Institute for Communicable Disease Control and Prevention, Chinese Center for Disease Control and Prevention, Beijing, People's Republic of China

Correspondence: Zhiguo Liu; Zhenjun Li, Email [wclblzg@126.com](mailto:wclblzg@126.com); [lizhenjun@icdc.cn](mailto:lizhenjun@icdc.cn)

**Objective:** Brucellosis is a serious public health issue in Qinghai (QH), China. Surveying the seroprevalence and isolation of *B. abortus* strains from marmots is key to understanding the role of wildlife in the maintenance and spread of brucellosis.

**Methods:** In this study, a set of methods, including a serology survey, bacteriology, antibiotic susceptibility, molecular genotyping (MLST and MLVA), and genome sequencing, were employed to characterize the two *B. abortus* strains.

**Results:** The seroprevalence of brucellosis in marmots was 7.0% (80/1146) by serum tube agglutination test (SAT); one *Brucella* strain was recovered from these positive samples, and another *Brucella* strain from a human. Two strains were identified as *B. abortus* bv. 1 and were susceptible to all eight drugs examined. The distribution patterns of the accessory genes, virulence associated genes, and resistance genes of the two strains were consistent, and there was excellent collinearity between the two strains on chromosome I, but they had significant SVs in chromosome II, including inversions and translocations. MLST genotyping identified two *B. abortus* strains as ST2, and MLVA-16 analysis showed that the two strains clustered with strains from northern China. WGS-SNP phylogenetic analysis showed that the strains were genetically homogeneous with strains from the northern region, implying that strains from a common lineage were spread continuously in different regions and hosts.

**Conclusion:** Seroprevalence and molecular clues demonstrated frequent direct or indirect contact between sheep/goats, cattle, and marmots, implying that wildlife plays a vital role in the maintenance and spread of *B. abortus* in the Qinghai–Tibet Plateau.

**Keywords:** *Brucella abortus*, marmots, isolated, MLST, MLVA, WGS-SNP, QH

## Introduction

Brucellosis is a zoonotic disease caused by *Brucella* spp., a gram-negative facultative intracellular coccobacillus.<sup>1</sup> These microorganisms can infect livestock, wildlife, and humans, causing significant public concern and substantial agricultural economic loss. Currently, at least six novel *Brucella* species have been identified: *Brucella pinnipedialis*, *Brucella ceti*,<sup>2</sup> *Brucella papionis*,<sup>3</sup> *Brucella microti*,<sup>4</sup> *Brucella inopinata*,<sup>5</sup> and *Brucella vulpis*.<sup>6</sup> However, *Brucella melitensis*, *Brucella abortus*, and *Brucella suis* remain the most important members of the genus because they are the strains largely responsible for human and animal diseases.<sup>7,8</sup> Although brucellosis is generally considered an occupational disease in developed countries, in many undeveloped regions of the world, including the Middle East, Africa, Latin America, Central Asia, and several regions of the Mediterranean, it is endemic.<sup>9</sup> In China, brucellosis has a high prevalence in humans and animals, and its geographic distribution is expanding annually, with a trend from northern to southern China.<sup>10,11</sup> *B. melitensis* is responsible for most human brucellosis cases, whereas fewer cases are caused by *B. abortus*<sup>12</sup> and *B. suis*.<sup>13</sup>

The Qinghai–Tibet Plateau region of China is a historic endemic region for human and animal brucellosis.<sup>14</sup> Yak brucellosis is a serious condition in this region, with a pooled prevalence rate of 8.39%,<sup>15</sup> along these lines, more than five *B. abortus* strains have been isolated from yaks in this area.<sup>16,17</sup> Meanwhile, *B. melitensis*–related sequences were

found in the guano of bats in India,<sup>18</sup> and *Brucella* DNA sequences were detected in the spleens of two different species of bats from Georgia that were co-infected with *Bartonella* and *Leptospira*.<sup>19</sup> In Africa, *B. abortus*, *B. melitensis*, *B. inopinata*, and *B. suis* were reported in wildlife; the pooled seroprevalence was 13.7% in buffalo, 7.1% in carnivores, and 2.1% in antelope.<sup>20</sup> The elk in the Paradise Valley is a maintenance host for *B. abortus* and transmits the bacteria to cattle in the northern portion of the Greater Yellowstone Ecosystem.<sup>21</sup> In Europe, more spillover events from wildlife to livestock, and possibly from wildlife to humans, are likely, particularly from the unrecognized wildlife reservoirs of *Brucella* spp.<sup>22</sup> In France, an investigation showed that a *B. melitensis* strain spills from wildlife to domestic ruminants and the sustainability of the infection in Alpine ibex.<sup>23</sup> Thus, wildlife reservoirs raise major concerns regarding brucellosis due to wild animals carrying or shedding *Brucella* strains, triggering a potential risk associated with transmission, persistence, and control in this population and domestic animals.<sup>24</sup>

Whole genome sequencing provides the most comprehensive collection of an individual's genetic variation.<sup>25</sup> A deeper comparative genetic analysis demonstrated important differences between the *Ochrobactrum* strains and different *Brucella* isolates.<sup>26</sup> Genome sequencing and comparative genomics of strains from wildlife can characterize the role of wildlife in the maintenance and spread of brucellosis and the genetic diversity of *B. abortus* strains.<sup>27,28</sup> Therefore, the purpose of this study was to characterize the seroprevalence and genetic profiles of two *B. abortus* strains isolated from marmots and humans in Qinghai, China.

## Materials and Methods

### *B. abortus* Strain Isolation and Phenotyping Identification

A total of 1446 serum samples of marmot were screened using the Rose Bengal plate test (RBPT) to select anti-*Brucella* serum-positive samples for further submission to bacteriological approaches. *Brucella* strains were isolated, and phenotypical characterization was performed in a biosafety level 3 laboratory (BSL-3), adhering to standard bacteriology and typing procedures.<sup>29,30</sup> Briefly, liver, and spleen tissue samples collected from marmots that were serologically positive for brucellosis were minced, homogenized, and placed on selective agar medium plates for *Brucella* spp. analysis (Oxoid Ltd., Basingstoke, Hampshire, UK) in a rigorous sterile manner. Approximately 5–10 mL of fresh blood from 69 suspected patients infected with *Brucella* spp. was injected into a biphasic culture flask (BioMerieux, Marcy-l'Étoile, France) in a biosafety cabinet. These plates (or flasks) were then incubated at 37°C for at least 2 weeks with and without CO<sub>2</sub>. Suspicious *Brucella* spp. clones were sub-cultivated on a *Brucella* medium plate and further subjected to conventional bio-typing procedures.<sup>30</sup> Gram and Kirschner staining were used to characterize the morphology of the strains. Biotyping reagents were purchased from the China Veterinary Drug Control Institute. Three vaccine strains, 104M (*B. abortus*), M5 (*B. melitensis*), and S2 (*B. suis*), were used as reference strains. All reagents were used in accordance with the manufacturer's instructions and within the expiration date.

### In vivo Antibiotics Susceptibility Analysis of Two Strains in This Study

In vivo, antibiotic susceptibility spectra of strains to nine first-line brucellosis treatment antibiotics were obtained based on the Clinical and Laboratory Standards Institute (CLSI) guidelines.<sup>31</sup> Minimal Inhibitory Concentration (MIC) values of rifampin (RIF), ciprofloxacin (CIP), levofloxacin (LVX), tetracycline (TCY), streptomycin (STR), doxycycline (DOX), minocycline (MON), sparfloxacin (SPX), ceftriaxone sodium (CRO), and sulfamethoxazole (SMZ) to two *B. abortus* strains were determined using the *E*-test strip method according to a previous study,<sup>32</sup> as well as testing procedure and results interpretation following the previous study,<sup>32</sup> and *B. abortus* 2308 was used as a reference strain.

### DNA Extraction and Molecular Identification of Two Strains

Subsequently, the genomic DNA of the two strains was extracted using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions, and the quality of the prepared DNA was detected using a fluorometer (Qubit<sup>®</sup> 2.0; Thermo Fisher Scientific, Waltham, MA, USA). The strains were further subjected to BCSP-31 PCR<sup>33</sup> and AMOS-PCR<sup>34</sup> for genus and species determination, respectively. The MLST genotypes were deduced from the WGS data using PubMLST platform resources (<https://pubmlst.org/organisms/brucella-spp>).<sup>35</sup> The MLVA-16

genotyping assay was conducted as previously described.<sup>36</sup> MLVA genotyping characterized the value of 111 strains previously identified within China (Table S1), which were collected for genetic comparison using BioNumerics version 8.0 software (Applied Maths NV, Sint-Martens-Latem, Belgium) with the unweighted pair group method using the arithmetic average.

## Library Construction, Sequencing, Assembly, and Annotation

The genome sequencing strategy of the strains was referenced as previously described.<sup>37</sup> Briefly, sequencing libraries were constructed using the NEBNext Ultra DNA Library Prep Kit for Illumina (NEB, Ipswich, MA, USA) according to the manufacturer's instructions. The whole genome of each strain was sequenced using the NovaSeq PE150 platform (Illumina, San Diego, CA, USA). Clean data were assembled using the SOAP Denovo 2.0 program,<sup>38</sup> and the assembly results were integrated using CISA software.<sup>39</sup> The GapCloser program<sup>40</sup> was used to fill the gaps in the preliminary assembly results. Next, genome component prediction and gene function annotation were completed as previously reported,<sup>41,42</sup> the GeneMarkS program,<sup>43</sup> Tandem Repeats Finder,<sup>44</sup> tRNAscan-SE version 2.0,<sup>45</sup> RNAmmer version 1.2, IslandPath-DIOMB version 0.2,<sup>46</sup> phiSpy version 2.3,<sup>47</sup> CRISPRdigger version 1.0,<sup>48</sup> and transposon PSI transposon traits program (<https://transposonpsi.sourceforge.net/>). In silico detection of AMR genes and virulence-associated determinants was performed using different databases, including the Virulence Factors of Pathogenic Bacteria Database (VFDB)<sup>49</sup> (accessed on September 12, 2019), and antimicrobial resistance genes were assessed by searching the CARD<sup>50</sup> and Resfinder version 4.0 databases.<sup>51</sup>

## Comparison of Genome and SNP Phylogenetic Analyses

A total of 76 *B. abortus* strains (two strains from the present study and 74 from GenBank) (Table S2) were used for comparative genomic analysis. Genomic alignment between the 74 sample genomes and the *B. abortus* 2308 reference genome (NC\_007618.1 and NC\_007624.1) was performed using the MUMmer,<sup>52</sup> and LASTZ<sup>53</sup> tools and genomic synteny were analyzed based on the alignment results. The gene family assignment was analyzed using the Cluster R package, and pan-genes (including core, specific, and dispensable genes) were analyzed using the CD-HIT rapid clustering of similar proteins software<sup>54</sup> (with a threshold of 50% pairwise identity and 0.7 length difference cutoff in amino acids). A Venn diagram and heatmap were then drawn to show the relationships among the samples. A phylogenetic tree was constructed using PhyML,<sup>55</sup> and bootstraps were set to 1000 with orthologous genes.

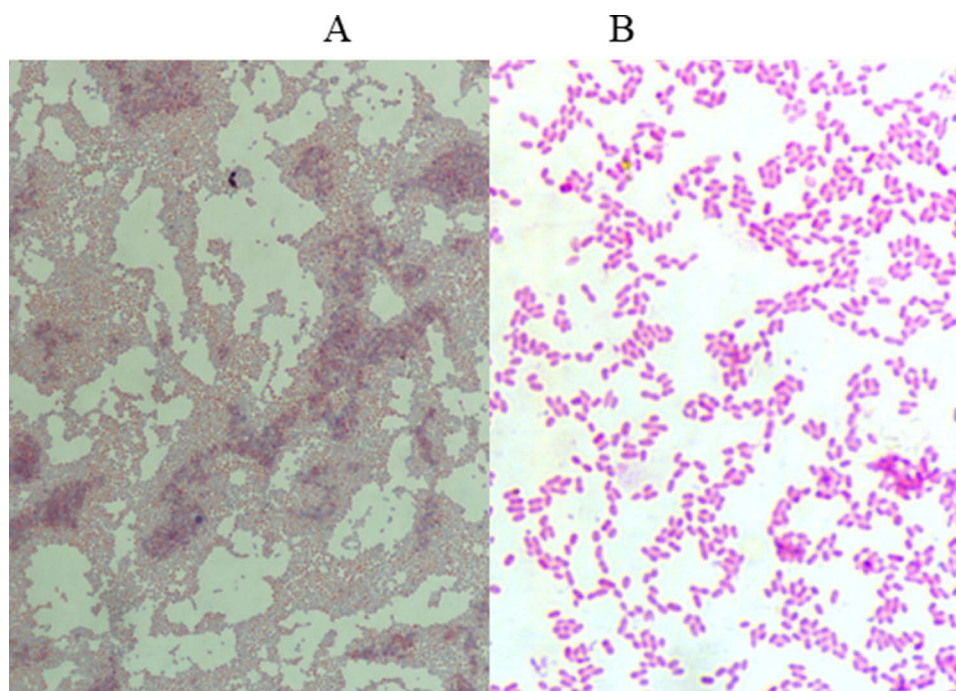
## Results

### Seroprevalence, Species/biovar Identified, and Drug Susceptibility of *B. abortus* Strains

The seroprevalence of brucellosis in marmots was 7.0% (80/1146) according to the SAT. XHM5 was obtained from these positive samples; XHM22 was isolated from one screened patient with brucellosis out of 69 from passive surveillance in outpatients who had a definite history of contact with animals and livestock and exhibited classical clinical manifestations, including fever, muscle, and joint pain, fatigue, and sweat. Colonies of suspicious strains appeared colorless, transparent, round, and slightly uplifted with smooth and homogeneous surfaces. Based on the staining results, gram-negative bacteria were observed (Figure 1A), and the strains were stained red using Kirschner staining (Figure 1B). According to the bio-typing tests, the two strains were identified as *B. abortus* bv.1 (Table 1). Special 223-bp and 498-bp bands were observed for BCSP-31 PCR and AMOS-PCR, respectively (Figure S1). The *E*-test results showed that the two strains were susceptible to all nine examined antibiotics, and no resistance phenotypes were detected (Table S3).

### Genome Component and Comparison Genome Analysis of *B. abortus* Strains

The sequencing profiles of both strains are shown in Table S4. The genome size (bp), gene number (#), and GC% values of the two strains (XHM5 and XHM22) were 3,256,796 and 3,256,846 bp, 3258 and 3258, and 57.24 and 57.23, respectively. The gene components of the two strains were similar, including scattered repeat sequences, tandem repeats, ncRNAs, gene islands, and prophages (Table 2). The prophage number and total length were different between the strains; the number and length values were 2 and 73,903 bp, respectively, in XHM5 versus 5 and 203,525 bp,



**Figure 1** Gram staining (A) and Koch staining (B) features of the strains in the present study.

respectively, in XHM22 (Table 2). The SNP numbers for XHM5 and XHM22 were 1387, and 1408, respectively (Table 2). Compared with *B. abortus* 2308, multiple genome SVs were observed, including inversions, translocations, and indels, especially on chromosome II (Figure 2 and Table 2). Based on core pan-genome analysis, there were 1658 core genes among them (Figure S2), and the number of special genes was three in XHM5 and four in XHM5 (Figure S2). Meanwhile, the patterns and numbers of accessory genes and gene families of the two strains were consistent (Figure 3, marked in orange; Figure S3, marked in bold).

## Characteristics of Virulence-Associated Genes and Resistance Genes

The distribution patterns of virulence associated and resistance genes of the two strains were consistent. A total of 66 virulence-associated genes were observed, but *bmaA* and *btaE* were not observed (Figure S4). Moreover, the distribution profile of virulence-associated genes in the strains was identical to that of the majority of the compared strains from GenBank, but different from that of *B.ab.clpP*, *B.ab.RB51*, *AHVLA*, *B.ab.NI352*, *B.ab.NI649*, and *B. ab. MC* (Figure S4). In addition, *Brucella\_suis\_mprF* resistance genes were detected in both strains in the present study (Figure S5); the resistance gene profiles of the two studied strains were similar to those of the other strains, except for the *B. ab. clpP* strain. A resistance gene, *APH* (3')-la, was found in the *B. ab. clpP* strain, but not in the other strains (Figure S5).

**Table 1** Bio-Typing Profile of the Two *B. abortus* Strains in This Study

| Key             | Strains    | Growth Feature  |                  |    |    | Antiserum Factors |   |   | Phage Lysis Tests |                 |    | No. | Interpretation               |
|-----------------|------------|-----------------|------------------|----|----|-------------------|---|---|-------------------|-----------------|----|-----|------------------------------|
|                 |            | CO <sub>2</sub> | H <sub>2</sub> S | BF | TH | A                 | M | R | Tb                | BK <sub>2</sub> | Wb |     |                              |
| Control strains | I04M       | -               | +                | +  | -  | +                 | - | - | CL                | CL              | CL | 1   | <i>B. abortus</i> vaccine    |
|                 | M5         | -               | -                | +  | +  | -                 | + | - | NL                | CL              | NL | 1   | <i>B. melitensis</i> vaccine |
|                 | S2         | -               | ++               | -  | +  | +                 | - | - | NL                | CL              | CL | 1   | <i>B. suis</i> vaccine       |
| Tested strains  | XHM5 XHM22 | -               | +                | +  | -  | +                 | - | - | CL                | CL              | CL | 2   | <i>B. abortus</i> bv.1       |

**Abbreviations:** BF, basic fuchsin; TH, threonine; CL, complete lysis; NL, not lysis; +, positive; -, negative.

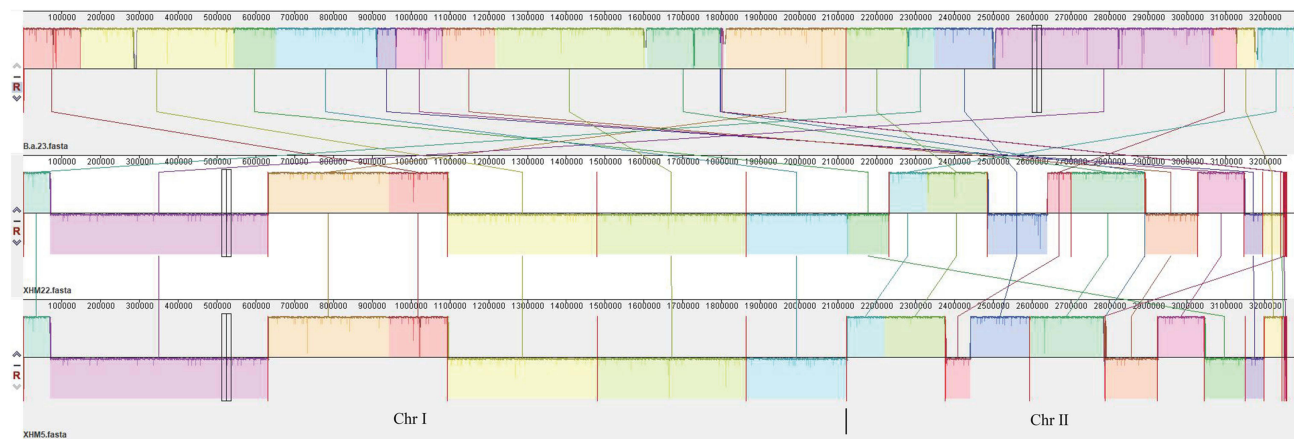
**Table 2** Gene Components of Two Strain in This Study

| Scattered repeat sequence | Sample ID | Type               | Number (#)            | Total Length (bp)   | In Genome (%)     | Average length | Sample ID    | Type               | Number (#)          | Total Length (bp) | In Genome (%)     | Average length |
|---------------------------|-----------|--------------------|-----------------------|---------------------|-------------------|----------------|--------------|--------------------|---------------------|-------------------|-------------------|----------------|
|                           | XHM5      | LTR                | 14                    | 1223                | 0.0376            | 87             | XHM22        | LTR                | 15                  | 1304              | 0.04              | 87             |
|                           | XHM5      | DNA                | 9                     | 561                 | 0.0172            | 62             | XHM22        | DNA                | 9                   | 561               | 0.0172            | 62             |
|                           | XHM5      | LINE               | 2                     | 126                 | 0.0039            | 63             | XHM22        | LINE               | 2                   | 126               | 0.0039            | 63             |
|                           | XHM5      | SINE               | 7                     | 463                 | 0.0142            | 72             | XHM22        | SINE               | 7                   | 463               | 0.0142            | 72             |
|                           | XHM5      | RC                 | 0                     | 0                   | 0                 | 0              | XHM22        | RC                 | 0                   | 0                 | 0                 | 0              |
|                           | XHM5      | Unknown            | 1                     | 98                  | 0.003             | 98             | XHM22        | Unknown            | 1                   | 98                | 0.003             | 98             |
|                           | XHM5      | Total              | 33                    | 2471                | 0.0759            | 76             | XHM22        | Total              | 34                  | 2552              | 0.0784            | 76             |
| Tandem repeat             | Sample ID | Type               | Number (#)            | Repeat Size (bp)    | Total Length (bp) | In Genome (%)  | Sample ID    | Type               | Number (#)          | Repeat Size (bp)  | Total Length (bp) | In Genome (%)  |
|                           | XHM5      | TR                 | 83                    | 5~825               | 5839              | 0.1793         | XHM22        | TR                 | 80                  | 5~339             | 5445              | 0.1672         |
|                           | XHM5      | Minisatellite DNA  | 62                    | 12~33               | 2363              | 0.0726         | XHM22        | Minisatellite DNA  | 62                  | 12~33             | 2327              | 0.0714         |
|                           | XHM5      | Microsatellite DNA | 1                     | 5~5                 | 26                | 0.0008         | XHM22        | Microsatellite DNA | 1                   | 5~5               | 26                | 0.0008         |
| ncRNA                     | Sample ID | Type               | Number (#)            | Average length (bp) | Total length (bp) | Sample ID      | Type         | Number (#)         | Average length (bp) | Total length (bp) |                   |                |
|                           | XHM5      | tRNA               | 51                    | 78                  | 3994              | XHM22          | tRNA         | 51                 | 78                  | 3994              |                   |                |
|                           | XHM5      | 5s (denovo)        | 1                     | 114                 | 114               | XHM22          | 5s (denovo)  | 1                  | 114                 | 114               |                   |                |
|                           | XHM5      | 16s (denovo)       | 1                     | 1473                | 1473              | XHM22          | 16s (denovo) | 1                  | 1473                | 1473              |                   |                |
|                           | XHM5      | 23s (denovo)       | 1                     | 2909                | 2909              | XHM22          | 23s (denovo) | 1                  | 2909                | 2909              |                   |                |
|                           | XHM5      | sRNA               | 3                     | 80                  | 242               | XHM22          | sRNA         | 0                  | 0                   | 0                 |                   |                |
| Gene Island               | Sample ID | GIs number (#)     | GIs total length (bp) | Average length (bp) |                   |                |              |                    |                     |                   |                   |                |
|                           | XHM5      | 5                  | 59,162                | 11,832              |                   |                |              |                    |                     |                   |                   |                |
|                           | XHM22     | 5                  | 57,904                | 11,581              |                   |                |              |                    |                     |                   |                   |                |

(Continued)

Table 2 (Continued).

|            |                |                       |                         |                    |                 |                   |                |                |               |               |            |           |
|------------|----------------|-----------------------|-------------------------|--------------------|-----------------|-------------------|----------------|----------------|---------------|---------------|------------|-----------|
| Prophage   | Sample_ID      | Prophage_Num          | Total_Length            | Average_Length     |                 |                   |                |                |               |               |            |           |
|            | XHM5           | 2                     | 73,903                  | 36,951.5           |                 |                   |                |                |               |               |            |           |
|            | XHM22          | 5                     | 203,525                 | 40,705             |                 |                   |                |                |               |               |            |           |
| Transposon | Sample name    | Match Number          | Match length            | Average length     |                 |                   |                |                |               |               |            |           |
|            | B.ab.23        | 5                     | 2373                    | 474                |                 |                   |                |                |               |               |            |           |
|            | XHM5           | 5                     | 2373                    | 474                |                 |                   |                |                |               |               |            |           |
|            | XHM22          | 5                     | 2373                    | 474                |                 |                   |                |                |               |               |            |           |
| Hcluster   | Species        | #Total genes          | #Genes in families      | #Unclustered genes | #Families       | #Unique families  |                |                |               |               |            |           |
|            | XHM5           | 3258                  | 3254                    | 4                  | 2677            | 0                 |                |                |               |               |            |           |
|            | XHM22          | 3258                  | 3253                    | 5                  | 2673            | 0                 |                |                |               |               |            |           |
| Ortholog   | #Species       | Single-copy orthologs | Multiple-copy orthologs | Unique paralogs    | Other orthologs | Unclustered genes |                |                |               |               |            |           |
|            | XHM5           | 1194                  | 831                     | 0                  | 1229            | 4                 |                |                |               |               |            |           |
|            | XHM22          | 1194                  | 833                     | 0                  | 1226            | 5                 |                |                |               |               |            |           |
| InDel      | Reference Name | Sample ID             | Start codon ins         | CDS inside ins     | Stop codon ins  | Start codon del   | CDS inside del | Stop codon del |               |               |            |           |
|            | B.ab.23        | XHM5                  | 0                       | 18                 | 0               | 0                 | 11             | 0              |               |               |            |           |
|            | B.ab.23        | XHM22                 | 0                       | 19                 | 0               | 0                 | 11             | 0              |               |               |            |           |
| Mutation   | Reference Name | Sample ID             | Frame-shifted           | Start codon        | Stop codon      | Premature stop    | Effect         | CDS with indel | All CDS       |               |            |           |
|            | B.ab.23        | XHM5                  | 15                      | 0                  | 0               | 2                 | 11             | 28             | 3106          |               |            |           |
|            | B.ab.23        | XHM22                 | 16                      | 0                  | 1               | 2                 | 10             | 29             | 3106          |               |            |           |
| SNP        | Reference Name | Sample_name           | Start_syn               | Stop_syn           | Start_nonsyn    | Stop_nonsyn       | Premature_stop | Synonymous     | Nonsynonymous | Total_CDS_SNP | Intergenic | Total_SNP |
|            | B.ab.23        | XHM5                  | 0                       | 0                  | 2               | 1                 | 8              | 484            | 674           | 1169          | 218        | 1387      |
|            | B.ab.23        | XHM22                 | 0                       | 0                  | 3               | 2                 | 8              | 489            | 676           | 1178          | 230        | 1408      |



**Figure 2** Collinearity profiles of the genomes of the two strains in this study.

## MLST and MLVA-16 Genotyping Profiles of *B. abortus* Strains

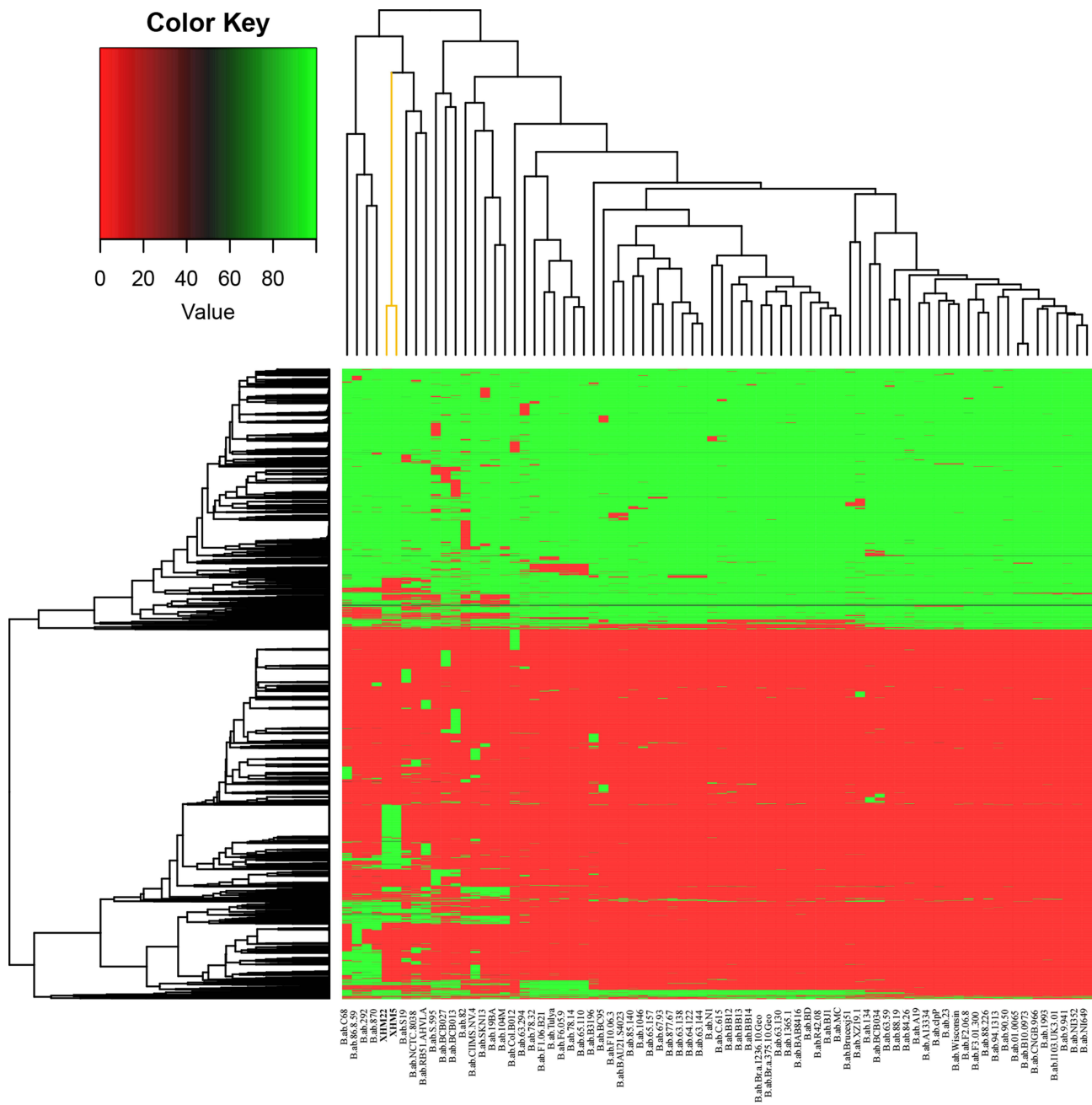
MLST (9) genotyping showed two strains belonging to ST2; ST is the same as strains isolated previously in QH (Ma10 and 64) (Figure 4). In MLVA-16, two new MLVA-8 and MLVA-11 genotypes were observed, and further comparison analysis of 111 strains (Table S1) from China found no completely matched MLVA-16 genotypes. However, the two strains were clustered in the same cluster and subdivided into two subclusters (SC I and II) (Figure 2). Furthermore, XHM5 and XHM22 were genetically related to strains from Inner Mongolia (GCA\_001043295), Xinjiang (2013jiang#084, 86–90), and Hebei Province (2013jiang#085) (Figure 2).

## Phylogenetic Analysis of Two *B. abortus* Strains from Qinghai on a Global Scale

Phylogenetic analysis showed that 76 strains were divided into eight clades (A–H) (Figure 5), and strains from China formed an independent clade and were placed in clade C; furthermore, two strains could be sorted into two sub-clades, namely C I (XHM5) and C II (XHM22). XHM5 (C I) was isolated from marmot and clustered with strains from Heilongjiang Province, while XHM22 (C II) was grouped with strains from Inner Mongolia, Hebei, Beijing, and Gansu Province (Figure 5). In addition, a close genetic relationship was observed between the strains from the present study and those from Russia.

## Discussion

In the present study, a preliminary serology survey and molecular characterization of *B. abortus* strains from marmots and humans were conducted to provide novel insights into the epidemiology of brucellosis in wildlife. A high seroprevalence of brucellosis was detected in the marmots' population, which demonstrates that marmots frequently come in direct or indirect contact with sheep/goats, cattle, or wildlife such as yaks. Marmots were contacted with abortion products, and their secretions of livestock (sheep/goats) and their consumption of aborted fetuses are potential reasons. In addition, animal husbandry is the main source of income for the local economy in Qinghai, and farmers and herders also frequently skin and eat marmots,<sup>56</sup> creating opportunities for *Brucella* to jump hosts. *B. abortus* strains were isolated from sheep and yaks in northwest China,<sup>16</sup> and *B. abortus* biovar 4 (XZ19-1) was first isolated from yaks in Tibet, China.<sup>57</sup> *B. abortus* was also isolated from Himalayan marmots in the Xinjiang Uygur Autonomous Region and had similar MLVA-16 genotypes with strains from yaks and humans in Qinghai Province.<sup>58</sup> These data verify that the ongoing distribution of *B. abortus* strains occurs in northern China. These areas have not only the same nomadic culture but also the same adjacency and shared grassland, thus providing a potential opportunity for the genetic homogeneity of strains. The most reported *Brucella* species in wildlife was *B. abortus*, with the highest prevalence rate recorded in the American bison, *Bison bison* (39.9%), followed by the Alpine ibex, *Capra ibex* (33%).<sup>59</sup> The persistence of brucellosis in wildlife reservoirs poses a risk to the reintroduction of *Brucella* into domestic livestock and humans. The present study

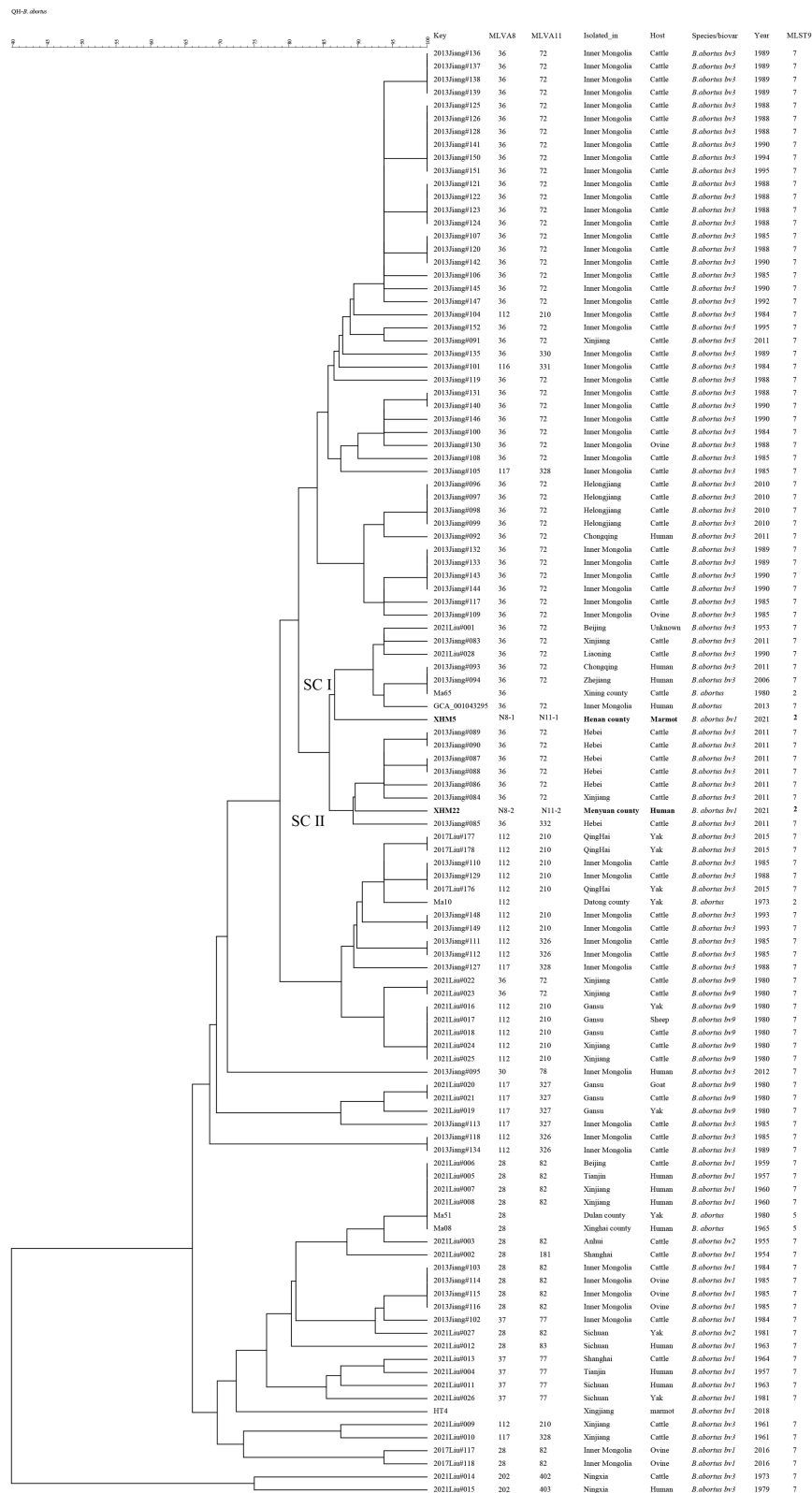


**Figure 3** Cluster analysis of accessory genes in *B. abortus* from this study and GenBank. **Note:** Strains from this study are marked in yellow in the upper-right part of the image.

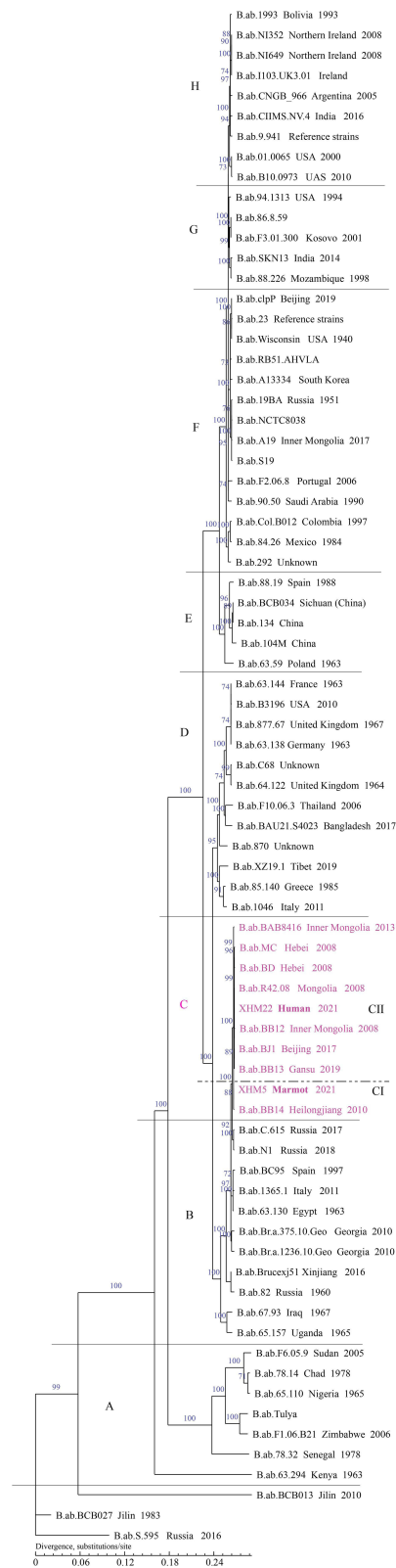
highlights that *B. abortus* infection events cannot be ignored, and strengthening wildlife surveillance and supervision is needed.

The distribution patterns of the AMR and virulence genes in the two *B. abortus* strains were consistent with the absence of classical AMR genes and virulence-associated factors. Similarly, a study showed that there was no clear difference in the distribution of AMR and virulence genes among both resistant and sensitive *B. abortus* and *B. melitensis* strains, even for those recovered from different hosts.<sup>60</sup> The most identified pathogenicity-associated genes are involved in LPS production and type IV secretion systems, which play vital roles in cell entry, intracellular trafficking, and intracellular survival.<sup>61–63</sup> These virulence-associated factors contribute to the pathogenesis of infections in humans and domestic animals, further triggering clinical manifestations. In addition, *Brucella\_suis\_mprF* resistance genes were detected in two strains, which





**Figure 4** MVA-16 dendrogram of 111 strains from this study. **Note:** The two strains from this study are marked in bold in the figure.



**Figure 5** WGS-SNP phylogenetic analysis of *B. abortus* strains.  
**Note:** Strains from this study are marked in pink.

potentially increased the virulence and resistance of methicillin-resistant *Staphylococcus aureus* (MRSA) and other pathogens to cationic host defense peptides and antibiotics.<sup>64</sup> Similarly, a *B. abortus* strain isolated from marmots exhibited amikacin resistance and obtained amikacin resistance genes from *Salmonella* spp. through Tn3 family transposons.<sup>65</sup> Although resistance genes are not always consistent with the strains' displayed resistance, they exist and spread among strains, further posing a challenge to human brucellosis treatment. Further investigations should focus on phenotypic resistance mechanisms at the proteomic and transcriptomic levels. Prophages can contribute many biological properties to their bacterial hosts, such as virulence, biosynthesis, toxin secretion, genomic divergence, and evolution.<sup>66</sup> There are differences in the prophage number and length between the two strains; that is, the number of prophages in strains from marmots is less than that in strains from humans, and they have significant genome SVs on chromosome II, which may reflect the different evolution patterns among strains. Further comparative analysis is necessary.

Both MLVA and WGS-SNP analyses highlighted that the two strains were genetically similar to strains from northern China, implying that the strains from a common lineage were spread continuously in different regions and hosts. Human activity has created a channel between livestock and wildlife for the spread and transmission of diseases. The control of human brucellosis mainly depends on the surveillance and control of the disease in domestic animals and livestock, and greater attention to farmers and butcher-related occupational populations is needed. Control measures are necessary to block the transmission of *Brucella* spp. among domestic and wild animals, and continuous and targeted molecular monitoring of wildlife is important to curb brucellosis in this region.

## Conclusions

In the present study, survey data showed that there was frequent direct or indirect contact between sheep and goats, cattle, and marmots through livestock production activities, which represents a new challenge in controlling brucellosis in humans and livestock. Therefore, strengthening pathogen genome surveillance and collecting more strains from different hosts, as well as conducting more detailed epidemiological investigations and etiological analysis, will be helpful in determining the risk factors and infectious chains of brucellosis in Qinghai.

## Abbreviations

WGS-SNP, Whole-genome sequencing single-nucleotide polymorphism; MLVA, Multilocus variable-number tandem repeat analysis.

## Data Sharing Statement

The datasets used and/or analyzed in the current study are available in the manuscript and [Supplementary Files](#). Moreover, genome sequence data have been deposited in the NCBI database (PRJNA930323 and PRJNA930316).

## Ethics Approval and Consent to Participate

This study was conducted in accordance with the principles of the Declaration of Helsinki. The study protocol was approved by the Ethics Committee of the Institute for Endemic Disease Control and Prevention, Qinghai, China (No. 2022006). Informed consent was obtained from all patients, and *Brucella* spp. was isolated as a diagnostic approach for brucellosis. The animal sample collection plan was also checked and approved by the Ethics Committee of the Institute for Endemic Disease Control and Prevention, Qinghai (No. 2022006).

## Consent for Publication

All authors have approved and agreed to the publication of this manuscript.

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## Disclosure

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as potential conflicts of interest.

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