Molecular Epidemiology Characteristics of *Brucella Abortus* Strains in China and Their Relationships With World Lineages

Xu-Ming Wang

Hainan Provincial People's Hospital: Hainan General Hospital

Zhongzhi Zhao

qinghai institute for endemic disease control and prevention

Miao Wang

ulanqab center for endemic disease prevention and control

Buyun Cui

china center for infection disease control and prevention

Zhiguo Liu (✉ wlcblzg@126.com)

national institute of infectious Disease Control and Prevention <https://orcid.org/0000-0002-4904-4878>

Zhenjun Li

china center for infection disease control and prevention

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Abstract

Background *Brucella abortus* is a facultative intracellular Gram-negative bacterium that causes chronic persistent infections in humans and livestock. In this study, conventional bio-typing, multiple-locus variable-number tandem repeat analysis (MLVA), and whole-genome sequencing-single-nucleotide polymorphism (WGS-SNP) were used to investigate the molecular epidemiology characteristics of *Brucella abortus* strains in China and their relationships to world lineages.

Results A total of 100 strains were collected from 1953 to 2013, suggesting that *B. abortus* circulated in China in the past five decades. Moreover, most strains were mainly distributed in the Northwest areas, suggest that provinces in the Northwest were a dominant epidemic area of this disease. During this period, seven biovars were found, indicating that *B. abortus* had a high diversity of biovars and it is also a potential reason for the disease ongoing spread in the Northern provinces. Strains have high genetic diversity, and *bruce07* is the most helpful locus for genotyping of this population. Moreover, 17 MLVA-11 genotypes were found; 13 of them are of known genotypes and four are unassigned genotypes, indicating that *B. abortus* in this study had several geographic origins. Still, strains from unassigned genotypes may originate from China. Many shared MLVA-16 genotypes were observed in strains from the same provinces in Northern China, which confirmed a *B. abortus* brucellosis outbreak within Northern regions. WGS-SNP analysis showed that eight Chinese strains formed a ladder-like phylogram (C. 8) with strains from nine countries, including Uganda, Iraq, Russia, Georgia, Spain, Italy, Egypt, Mongolia, and China; suggest that strains were introduced to these countries from a single source.

Conclusions Chinese *B. abortus* strains had high biovars and genetic diversity as well as represent characteristics of multiple geographic origins, and *B. abortus* strains from several mainly epidemic areas were closely related to strains from Russia and Mongolia; frequent animal (cattle) trade and exchanges may promote this process. We will provide new and valuable information to strengthening surveillance and control of *B. abortus* brucellosis in China.

Background

Brucellosis is an important zoonotic disease worldwide that can infect various domestic and wildlife animals, and also humans [1]. It is caused by species of the genus *Brucella*, they were small, nonencapsulated, nonmotile, facultatively intracellular coccobacilli, and clustered in two phylogenetic groups: classical and non-classical species [2]. The six classical species includes: *B. abortus*, *B. melitensis*, *B. suis*, *B. ovis*, *B. canis*, and *B. neotomae* [3], and six non-classical species comprised by *B. microti* [4], *B. ceti* and *B. pinnipedialis* [5], *B. inopinata* [6], *B. papionis* [7] and *B. vulpis* [8]. Of these, *B. abortus*, *B. melitensis*, and *B. suis* are the most vital agents for humans and animals brucellosis in China and worldwide [9, 10]. *Brucella spp.* enter the body main through the skin mucosa, digestive tract, and respiratory tract mucosa [11]. Human are contact with infected domestic and consumption of raw dairy products are the main infection way [12]. Human brucellosis is a systemic infectious disease with varying clinical manifestations, mainly display fever, fatigue, sweating, pain in the muscles and joints, pain in the

back, and/or loss of appetite, as well as resulting in significant public health risk [13]. In the infected animals, abortion, retained placenta, infertility, orchitis, epididymitis is common symptoms and due to reduces livestock production and reproduction cause hung economic loss [14].

Our previous study showed that *B. melitensis* is a latent "travel bacterium" that spread and expanded from North China to South China [15]. However, study on the molecular epidemiology characteristics of *B. abortus* countrywide is rare [16]. In Northern China, where the traditional agropastoral areas with more developed animal breeding industries are located, the *B. abortus* brucellosis seroprevalence was greater than 10%; the seroprevalence of brucellosis in Southern China reached only 5.5% [17]. Despite the relatively low seroprevalence (2.8%) in a serological survey of yaks, the estimated economic losses were substantial in Tibet. With no control program for brucellosis in yaks, the total annual economic loss in the study area was estimated at US\$521, 043, mainly resulting from abortions and reduction of the milk and meat yield [18]. Therefore, the molecular epidemiology of brucellosis caused by *B. abortus* at the national level is an urge.

Although bio-typing was useful for *Brucella* strain identification and species differentiation, it is time-consuming and difficult to interpret due to a lack of standardization of the typing reagents [19]. Moreover, multi-locus variable-number tandem repeat assays (MLVA) can providing a fine-scale resolution among isolates and allowing the determination of the source of infection, geographical origin, and spread pattern of disease [20, 21]. The whole-genome sequencing- single-nucleotide polymorphism (WGS-SNP) is used to establish phylogenetic relationships of *Brucella* isolates [22, 23]. In this study, 100 strains were obtained from samples collected between 1953 and 2013 from various host animals throughout the countrywide in China. Our study was aimed to determine the distribution regions, genotyping characteristics, geographic origins, and phylogenetic relationships of *B. abortus* strains at the global level.

Methods

Bacterial source and identified

The MLVA-16 characteristics, panel1, MLVA-11 genotypes, species/biovars, hosts, location, and year of *B. abortus* strains from China were collected, and Microsoft Excel (Microsoft, Redmond, CA, U.S.) was used for data cleaning. A total of 100 strains were collected from 14 different provinces from animals and humans, which of 65 were found in cattle, 20 in humans, 7 in sheep, 4 in yak, 3 in goat, and for 1 strain the host is unknown. Moreover, 36 strains previously published from Inner Mongolia [24] were involved in this study. Species/biovars of all 100 strains were identified based on standard bio-typing procedures [25], the specific identification methods were identical to previously published [26, 27]. Furthermore, AMOS-PCR (*B. abortus* bv. 1, 2, and 4, *B. melitensis*, *B. ovis*, and *B. suis* bv. 1) [28] was used to verify further *B. abortus* bv. 1, 2, and 4.

Brucella MLVA-16 genotyping scheme

MLVA-16 genotyping in all strains was conducted as previously described [29, 30]. The 16 primer pairs were sorted into three panels, panel 1, panel 2A, and panel 2B. Panel 1 including eight loci (MLVA-8: *bruce06*, *bruce08*, *bruce11*, *bruce12*, *bruce42*, *bruce43*, *bruce45*, and *bruce55*), and often used for species identification; panel 2A (three loci including *bruce18*, *bruce19*, and *bruce21*), and panel 2B including five loci (*bruce04*, *bruce07*, *bruce09*, *bruce16*, and *bruce30*), and used for genotype comparison. Moreover, MLVA-11 comprised of panels 1 and 2A and used to trace back the geographic origin of strains, and MLVA-16 (panels 1, 2A, and 2B). PCR amplification, products analysis, and repeat unit numbers comparison were performed as the same with previous studies [31, 32].

Analysis of the MLVA data

Hunter-Gaston diversity index (HGDI) [33] was applied to evaluate the genetic diversity of *B. abortus* strains as well as the resolution of each locus in the MLVA approach in this study. MLVA-16 data (Table S1) in all strains were analyzed by the BioNumerics version 5.1 software (Applied math, Belgium) based on the categorical coefficient and unweighted pair group methods. MLVA-11 was used to investigate the geographical origins between our isolates [34, 35] and 1,482 isolates from the MLVA bank (<http://microbesgenotyping.i2bc.paris-saclay.fr/databases>) (Table S2). Subsequently, the minimum spanning tree was constructed by the goeBURST algorithm using the Phyloviz software v 2.0 [36]. Investigation on genetically related among strains from our reported (n = 100) (Table S1) and other regions of worldwide (n = 1,525) based on the MLVA-16 data was performed (Table S3). Moreover, phylogenetic analyses of 62 *B. abortus* (Table S4) genomes retrieved from GenBank were performed, the phylogenetic tree was constructed based on the PHYL (PHYlogenetic inferences using Maximum Likelihood) software with parameter default values [37].

Results

The species/biovars, region distribution, isolated years, and host profile of *B. abortus*

A total of seven biovars were observed in this population, including *B. abortus* bv. 1 (n = 24), *B. abortus* bv. 2 (n = 2), *B. abortus* bv. 3 (n = 60), *B. abortus* bv. 5 (n = 1), *B. abortus* bv. 6 (n = 2), *B. abortus* bv. 7 (n = 1), and *B. abortus* bv. 9 (n = 10). *B. abortus* bv. 3 was the dominant species in China (Table 1). These strains were collected from 14 provinces (cities and autonomous regions) from 1953 to 2013, including 36 strains in Inner Mongolia, 14 in Xinjiang, 11 in Hebei, 10 in Heilongjiang, 6 in Gansu, and 5 in Zhejiang; the remaining regions contained 1 to 4 strains, 85% strains was distributed in Northern, China. A total of 100 *B. abortus* samples were collected from 1953 to 2013, 11 in the 1950s, 9 in the 1960s, 5 in the 1970s, 38 in the 1980s, 1 in 1990, 4 in 2000s, and 32 in 2010s. Moreover, 100 strains were obtained from five hosts, 65 in cattle, 65%, followed by 20 in humans, 7 in sheep, 4 in yak, 3 in goat, and 1 in an unknown host (Table 1).

Table 1

Location, numbers, percentages (%), species, panel1, MLVA-11, hosts, and year of 100 *Brucella abortus* in this study.

Provinces	No.	Species-biovar	Panel1	MLVA-11	Host	Year
Anhui	1	<i>B. abortus</i> bv. 2	28	82	Cattle	1955
Liaoning	1	<i>B. abortus</i> bv. 3	36	72	Cattle	1990
Beijing	2	<i>B. abortus</i> bv. 1, 3	28, 36	82, 72	Cattle	1953, 1959
Ningxia	2	<i>B. abortus</i> bv. 3	Ba8-3	Ba11-1, 2	Human, cattle	1973, 1979
Shanghai	2	<i>B. abortus</i> bv. 1	28, 37	181, 77	Cattle	1954, 1964
Tianjin	2	<i>B. abortus</i> bv. 1	28, 37	82, 77	Human	1957
Chongqing	4	<i>B. abortus</i> bv. 3	36, 117	72, 328	Human	2011
Sichuan	4	<i>B. abortus</i> bv. 1, 2	28, 37	83, 77, 82	Yak, Human	1963, 1981
Zhejiang	5	<i>B. abortus</i> bv. 3, 7	36, 112	72, 326	Human	2006, 2008, 2013
Gansu	6	<i>B. abortus</i> bv. 9	112, 117	210, 327	Cattle, sheep, Yaks, Goat	1980
Heilongjiang	10	<i>B. abortus</i> bv. 3, 5, 6	36, Ba8-2	72, Ba11-4	Cattle	2010, 2012
Hebei	11	<i>B. abortus</i> bv. 3	36, 117, Ba8-1	72, 328, Ba11-3	Cattle	2011
Xinjiang	14	<i>B. abortus</i> bv. 1, 3, 9	28, 36, 112, 117	72, 82, 210, 328	Cattle, Human	1960, 1961, 1980, 2011
Inner Mongolia	36	<i>B. abortus</i> bv. 1, 3, 6	28, 30, 36, 37 112, 116, 117	71, 72, 75, 78, 82 210, 326, 328, 331	Cattle, sheep, Human, Goat	1956–1988

The genetic diversity profile of *B. abortus* based on the HGDI

Based on HGDI analysis, three loci (*bruce06*, *08*, and *12*) showed no diversity (HGDI = 0.0000) and *bruce55* showed the highest diversity in all loci of panel 1, with a value of HGDI of 0.6515. The lowest value of HGDI in panel 2B was *bruce16* (HGDI = 0.2576), the HGDI value in other four loci were > 0.5705,

and the HGDI value of panel1, MLVA-11, and MLVA-16 was 0.7820, 0.8196, and 0.9853, respectively (Table 2).

Table 2
Allelic types and HGDI of *B. abortus*
for 16 loci in this study.

Panel and Locus	HGDI values
Bruce06	0.0000
Bruce08	0.0000
Bruce11	0.4034
Bruce12	0.0000
Bruce42	0.0776
Bruce43	0.1139
Bruce45	0.0396
Bruce55	0.6515
Bruce18	0.1701
Bruce19	0.2204
Bruce21	0.0396
Bruce04	0.6392
Bruce07	0.7905
Bruce09	0.7719
Bruce16	0.2576
Bruce30	0.5705
Panel 1	0.7820
MLVA-11	0.8196
MLVA-16	0.9835

MLVA-16 Genotyping Characteristics

In panel 1, 10 genotypes were observed, of which 7 are known, including 116 (n = 1), 30 (n = 4), 37 (n = 5), 112 (n = 13), 117 (n = 15), 28 (n = 17), and 36 (n = 39). The remaining three unassigned genotypes were *Ba8-1* (n = 2) (4-5-3-12-2-1-3-2), *Ba8-2* (n = 2) (4-5-3-1-2-3-1-3-2), and *Ba8-3* (n = 2) (4-5-3-1-2-3-1-5-2) (Fig. 1). In MLVA-11, 17 genotypes were found, 13 of which were of known genotypes, namely, 71 (n = 1),

83 (n = 1), 181 (n = 1), 210 (n = 9), 331 (n = 1), 327 (n = 3), 75 (n = 4), 78 (n = 4), 326 (n = 4), 77 (n = 5), 82 (n = 11), 328 (n = 12), 72 (n = 38), and 4 novel genotypes: *Ba11-1* (n = 1), *Ba11-2* (n = 1), *Ba11-3* (n = 2), and *Ba11-4* (n = 2) (Fig. 1). Genotype 72 was distributed in seven regions, the higher diversity of MLVA-11 genotype was observed in strain from Inner Mongolia, Xinjiang Province, Hebei Province, and Sichuan Province (Table 1). However, the novel MLVA-11 genotype was found in Ningxia, Heilongjiang Province, and Hebei Province (Table 1). Using the complete MLVA-16 loci, 100 strains were sorted into four groups (I–IV) and 65 MLVA-16 genotypes (G.T.) had a 50% similarity coefficient. Both biovar 1 and 2 were fell into group I; the other five biovars 3, 5, 6, 7, and 9 were clustered in other three groups (II, III, and IV), of which group I contained six subgroups (a–f) (Fig. 1). The 54 strains consisted of 19 shared genotypes that contained 2–8 strains, and 46 strains of an independent genotype, each present a single strain. Eighteen out of 19 strain-shared genotypes comprised strains from the same province; only one shared genotype (GT48) contained five strains obtained from four provinces (Fig. 1).

Geographic origin features of Chinese *B. abortus* strains on a global scale

Among 17 MLVA-11 genotypes, seven were shared genotypes (72, 75, 82, 83, 181, 210, and 328) comprising strains from 2 to 10 countries, and the remaining 10 genotypes were single genotypes that were exclusively found in Chinese strains. The genotype 72 (n = 38) was the predominant population and accounted for 38% (38/100), 12 in genotype 328, 11 in genotype 82, and 9 in genotype 210. MLVA-11 genotype 72 was shared by strains from six countries, including France, Germany, Italy, Kazakhstan, Portugal, and China. Genotype 82 comprised strains from 10 countries, including Brazil, Costa Rica, France, Germany, Italy, Kazakhstan, Portugal, South Korea, the United States, and China. Genotype 210 consisted of strains from four countries, Costa Rica, Italy, United States, and China. Genotype 78 was shared by strains from England, the United States, and China. Genotype 83 was shared by strains from Brazil, Costa Rica, England, and China. Genotype 75 included strains from Brazil and China. Genotype 181 consisted of strains from Portugal and China. However, another 10 single genotypes were exclusive of strains from China (Fig. 2).

Worldwide Phylogenetic Analysis Based On Both *Mlva*-16 And *Wgs*-snp

In present study, 1,625 strains were sorted into three groups (A–C), and Chinese strains fell into group A and clustered together with strains from Italy, Kazakhstan, and Brazil, but had no sharing of the MLVA-16 genotype was observed (Fig. 3). Whole-genome SNP analysis showed that 65 *B. abortus* strains were grouped into seven clades (I–VII) (Fig. 4); 16 Chinese strains were grouped into five clades (Clade I, II, III, IV, and V). Clade I was composed of two Chinese strains and one strain from Kenya; clade II included strains from China, Poland, and Spain; clade III included strains from China, South Korea, and USA; clade VI included strains from China, Italy, and Greece. Clade VII was composed of 19 strains from nine countries, including Uganda, Iraq, Russia, Georgia, Spain, Italy, Egypt, Mongolia, and China. These strains formed a

ladder-like phylogram. However, most strains (n = 8) from China were found in clade I and were the most similar to strains from Russia and Mongolia (Fig. 4).

Discussion

B. abortus infection was highly endemic in dairy herds in China [17]. The comprehensive molecular epidemiological and phylogenetic analysis of the *B. abortus* strains nationwide are useful for improving the surveillance and control program to determine the risk factors associated with brucellosis in humans. In this study, 100 *B. abortus* were collected from 1953 to 2013, indicating that *B. abortus* strains have circulated in China for the past several decades, but the strain numbers were lower than those of *B. melitensis* [38]. This observation is in agreement with a report that *B. abortus* was a secondary pathogen agent for brucellosis in China [9]. The seven biovars were observed in this study, and *B. abortus* bv. 3 was dominant species in China, suggesting that *B. abortus* in China has had a high diversity of species/biovars, which poses a potential challenge for animal vaccination. The 85% strains were distributed in traditional and domestic animals breeding developed provinces, providing helpful information for disease control and prevention. However, the distribution range of this species is different from that of the *B. melitensis* strains, which cover all mainland regions in China [39]. Similarly, the most frequently recorded *B. abortus* biovar is biovar 3 in the Republic of Kazakhstan. More than 90% of the overall *B. abortus* samples were isolated from the northern regions of East and West Kazakhstan [40]. Five kinds of hosts were observed in this population, and 65% of strains were from cattle. This indicated a narrower host spectrum than that of *B. melitensis* [41], which is a significant impact factor for strains with similar geographic distribution and is closely related to the low virulence of *B. abortus* strains. In Italy, the strains identified from cattle showed a high prevalence of *B. abortus* bv. 3 isolates (84.5%), followed by *B. melitensis* bv. 3 (9.9%) and *B. abortus* biovars 1 and 6 (5.5% and 0.1%, respectively) [42]. In Africa, *B. abortus* bv. 3 is the most commonly isolated strains in cattle [43]. Moreover, there were no strains from swine and wild animals; further pathogen surveillance in other hosts is a priority.

The complete MLVA-16 approach exhibited excellent discriminatory power to *B. abortus* with a high HGDI value (0.953). Notably, five panel 2B markers had a high Hunter-Gaston index, and *bruce07* is the most helpful locus for genotyping *B. abortus* in China. This result was consistent with *B. canis* data in China; there were at least two alleles for each of the 16 loci investigated by the MIVA approach [20]. However, this feature is different from the results of studies of *B. melitensis* diversity in China. The majority of loci from panel 1 and panel 2A showed low diversity, and the *bruce04* locus displayed the highest diversity (HGDI = 0.841) [44]. MLVA-16 panel has a high discriminatory power to *B. abortus* from Italy, with polymorphism levels of 0.939 based on the HGDI, compared to 0.380 and 0.380 for the MLVA-8 and MLVA-11 panels, respectively; and the highest variability was detected in the 2B panel for the *bruce09* locus [45]. HGDI analysis of *B. abortus* bv. 3 from West Africa showed that the highest diversity indices were observed with markers composing panel 2 with three to nine alleles, especially at *bruce16*, which is known as one of the most variable loci [46]. Betsy J et al. reported that, historically, approximately 85% of *B. abortus* infections in the U.S. were caused by biovar 1, which would result in an HGDI $\approx$ 0.2 [47]. The variability of HGDI features among strains from different regions may reveal different microevolution

patterns of the *Brucella* population [48], suggesting that the strains described in this study were introduced from multiple geographic regions.

A total of 17 MLVA-11 genotypes were observed; the higher diversity of MLVA-11 genotype was observed in strains from Inner Mongolia, Xinjiang Province, Hebei Province, and Sichuan Province, suggesting that animal trade often occurred among these regions in the past. The seven shared MLVA-11 genotypes (72, 75, 82, 83, 181, 210, and 328) comprising strains from 2 to 10 countries suggest that the strains from each shared genotype had a common source, indicating that the *B. abortus* from this study had multiple geographic, cross-border transmission, probably due to exchange and trade of infected animal. Trade ties and livestock exchange between countries have a long history; archeological findings unraveled trade relations between the nomadic people who inhabited Central Asia and China long before the Common Era (C.E.) [49]. MLVA-11 genotype 72 was shared by strains from six countries, including France, Germany, Italy, Kazakhstan, Portugal, and China. MLVA-16 comparison analysis showed that Chinese strains were clustered together with Italy, Kazakhstan, and Brazil. A previously reported that, 4,500 years ago, seasonal nomadic pastoralist routes were formed from modern Southern Kazakhstan to Xinjiang Uygur Autonomous Region, covering more than 70% of the high-mountain route of the silk road [50]. However, there is no trade-in live cattle between Kazakhstan and China in modern history, but actively developing livestock products [51]. Furthermore, four strains from Sichuan Province had three MLVA-11 genotypes (83, 77, and 82), two of which were shared (82 and 83) with strains from other regions, and one (77) is exclusive of strains from Sichuan Province. Although it was a historical area of *B. abortus* brucellosis, it had no shared genotype 72 [9]. The investigation of serology and bacteriology of *B. abortus* infection in this region is a priority. However, 10 single genotypes were exclusively found in Chinese strains, indicating that these strains originated from China lineages, but further investigation on the origin of the strains is recommended.

In this study, the 19 shared MLVA-16 genotypes included 54 strains, with each genotype representing 2–8 strains, and 18 out of 19 shared genotypes consisted of strains of the same province. Only one shared genotype (GT48) was present in five strains, and these were obtained from four different provinces, suggesting that the majority of brucellosis cases were outbreak epidemic within respective regions. This conclusion is in agreement with the geographic distribution of the strains and outbreak cases mainly focus on Northern China. Moreover, the remaining 46 independent genotypes of each single strain indicated that 46% (46/100) cases had epidemiology unrelated or sporadic characteristics [31]. Strengthening the surveillance of *B. abortus* animal infection applied serology, bacteriology, molecular assays in northern regions, China helps better understand the epidemiology of brucellosis.

The sixteen Chinese strains were divided into five clades by whole-genome SNP analysis, confirming that strains from this study had multiple ancestor strains. Remarkably, clade Ⅱ formed a ladder-like phylogram and consisted of strains from nine countries, including Uganda, Iraq, Russia, Georgia, Spain, Italy, Egypt, Mongolia, and China from a single ancestor. The *B. abortus* isolates from Italy are substantially different from those found in Europe and North America and are more closely related to strains from the Middle East and Asia [52]. Furthermore, most Chinese strains (n = 8) were the most similar to Russia and

Mongolia; they were geographically close. They revealed that strains from a common ancestor were continuously circulating among these countries. Human brucellosis seroprevalence among rural people in Mongolia is high [53], and Mongolian *B. melitensis* isolates had high genetic similarity with Chinese strains, likely due to geographical proximity [54]. Moreover, some strains from Mongolia had closely related MLVA genotypes to strains from Russia [55]. In the North Caucasian Federal District, the largest number of new human brucellosis cases was notified in the Republic of Dagestan (59.3%) and the Stavropol Territory (27.4%), and the true prevalence of brucellosis greatly exceeds the official statistical data [56]. The frequent spread of these lineages from one country to another due to long-term trading partnerships between the three countries is a likely explanation for the data. Otherwise, due to the uncontrolled introduction of the agents via humans, infected animals, semen, and vectors have a high risk of *B. abortus* infection [57]. In endemic countries, combining the serodiagnosis and bacteriology, and molecular diagnosis for surveillance of brucellosis is essential. Molecular genotyping should be systematically applied to support control plans for control of brucellosis in China.

Moreover, our study has some limitations. First, the strains were collected from previous studies that might have been influenced by many aspects, such as the local economic situation and the diagnosis and surveillance status of brucellosis. Second, due to variability in the number of strains collected among different provinces and for different years in this study, a genome analysis of strains from more regions and hosts is recommended. Third, no related epidemiology data were collected, and analysis of animals moves, and exchange is lacking. Therefore, a whole-province survey on human and animal infections with *B. abortus* should be initiated.

Conclusion

In this study, a nationwide molecular analysis of *B. abortus* strains were performed. Our research showed that there was a high diversity of biovar and MLVA-11 genotypes among this population. *B. abortus* strains exhibited multiple geographic origin characteristics and the predominant genotype was shared with strains from many countries. Some strains from main epidemic areas were similar to strains from Russia and Mongolia. These have been traditionally countries of cattle and sheep breeding industries. Our work contributes to a better understanding of the epidemiology of *B. abortus* brucellosis in China and also provides invaluable information that could be helpful to devise control strategies for the disease.

Abbreviations

PCR: Polymerase chain reaction

MLVA: Multiple-locus variable-number tandem repeat analysis

VNTR: Variable-number tandem repeat analysis

HGDI: Hunter-Gaston discrimination index

SNPs: Single-nucleotide polymorphisms

WGS: Whole-genome Sequencing

MST: Minimum-spanning tree

Declarations

Ethics approval and consent to participate

This study was conducted according to the principles of the Declaration of Helsinki. This study is a retrospective investigation of historical strain collections using modern molecular approach. The Ethics Committees approved the research protocol of the National Institute for Communicable Disease Control and Prevention and the Chinese Centers for Disease Control and Prevention. All *B. abortus* strains (human and animals) used in this study were collected from published academic articles found in PubMed, Chinese life science databases (e.g., Wanfang and CNKI databases), and MLVA bank (<http://microbesgenotyping.i2bc.paris-saclay.fr/databases>).

Consent to publish

Not applicable

Availability of data and materials

All data generated or analyzed during this study are included in this published article and its supplementary information files.

Competing interests

The authors have no conflicts of interest.

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Authors' Contributions

LZG and ZZZ performed strains collected and analysis, WXM and LZG performed genotyping and cluster analysis and drafted the manuscript; WM conducted epidemiological investigations and data analysis; CBY and LZG participated in the design of the study and critically reviewed the manuscript; WXM and LZJ participated in the design of the study and managed the project. All authors read and approved the final version of the manuscript.

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Authors' Information

Affiliations

Hainan Provincial People's Hospital, Haikou, 570311, China

Xuming Wang

Qinghai Institute for Endemic Diseases Prevention and Control, Xining, 811602, PR China

Zhaozhi Zhao

Ulanqab Centre for Endemic Disease Prevention and Control, Jining, 012000 Inner Mongolia, PR China

Miao wang

State Key Laboratory for Infectious Disease Prevention and Control, National Institute for Communicable Disease Control and Prevention, Chinese Center for Disease Control and Prevention, 155 Chang bai Road, Chang ping, Beijing 102206, PR China

Buyun Cui, Zhiguo Liu & Zhenjun Li

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Figures

Figure 1

Dendrogram based on the MLVA-16 genotyping assay (UPGMA method) showing the relationships among the 100 *B. abortus* isolates. The columns show the identification numbers (Key), MLVA-16 genotypes (GT), MLVA-11 (panels 1 and 2A) genotypes, species biovars, hosts, geographical locations, and the year each strain was isolated.

Figure 2

Geographic origins of Chinese *B. abortus* strains based on MLVA-11.

Figure 3

Minimum spanning tree for *B. abortus* using MLVA-16 data with Chinese isolates (blue).

Figure 4

WGS-SNP phylogenetic analysis of *B. abortus* at a global level.

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