Typing Discrepancy Between Phenotypic and Molecular Characterization Revealing an Emerging Biovar 9 Variant of Smooth Phage-Resistant B. abortus Strain 8416 in China

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A newly isolated smooth colony morphology phage-resistant strain 8416 isolated from a 45-year-old cattle farm cleaner with clinical features of brucellosis in China was reported. The most unusual phenotype was its resistance to two Brucella phages Tbilisi and Weybridge, but sensitive to Berkeley 2, a pattern similar to that of Brucella melitensis biovar 1. VITEK 2 biochemical identification system found that both strain 8416 and B. melitensis strains shared positive ILATk, but negative in other B. abortus strains. However, routine biochemical and phenotypic characteristics of strain 8416 were most similar to that of B. abortus biovar 9 except CO2 requirement. In addition, multiple PCR molecular typing assays including AMOS-PCR, B. abortus special PCR (B-ab PCR) and a novel sub-biovar typing PCR, indicated that strain 8416 may belong to either biovar 3b or 9 of B. abortus. Surprisingly, further MLVA typing results showed that strain 8416 was most closely related to B. abortus biovar 3 in the Brucella MLVA database, primarily differing in 4 out of 16 screened loci. Therefore, due to the unusual discrepancy between phenotypic (biochemical reactions and particular phage lysis profile) and molecular typing characteristics, strain 8416 could not be exactly classified to any of the existing B. abortus biovars and might be a new variant of B. abortus biovar 9. The present study also indicates that the present phage typing scheme for Brucella sp. is subject to variation and the routine Brucella biovar typing needs further studies.

Keywords: B. abortus, smooth phage-resistant (SPR), MLVA typing, unusual biochemical reactions

Brucellosis is one of the most common zoonotic infectious diseases, causing enormous economic loss in domestic animals and public health problems worldwide (Adone and Pasquali, 2013; Van der Henst et al., 2013). Transmission from animals to human occurs primarily through direct contact with infected animals and ingestion of raw milk or unpasteurized cheese. On the basis of obviously
different phenotypic characteristics, host preference, growth and biochemical characteristics including CO₂ requirement, substrate utilization and growth on dyes and agglutination with monospecific sera as well as Brucella phage lysis profiles, four main Brucella pathogenic species including Brucella melitensis (sheep and goat), B. suis (pigs), B. abortus (cattle), and B. canis (dogs), a taxonomic scheme can be defined and further divided into multiple biovars. For example, B. abortus is subdivided into eight biovars (biovar 1–7 and 9) (Van der Henst et al., 2013).

Because of unstable phenotypic characteristics among Brucella strains, it is somewhat difficult to define atypical strains into standard biovars. For instance, the susceptibility of smooth B. abortus strains to lysis by most of brucella phages, such as Tbilisi (Tb), Firenze (Fi), Weybridge (Wb), and Berkeley 2 (BK₂), is commonly regarded as one of the routine criteria to differentiate this organism from other Brucella species. However, the majority of B. abortus strains resistant to Brucella phage have been currently reported primarily due to variation from smooth to rough form during normal in vitro culture. Since the first smooth phage-resistant strain (SPR) of B. abortus isolated from bovine tissue was reported in 1973 (Corbel and Morris, 1974, 1975), a similar study describing SPR strains has not been reported yet. In this study, we report a newly isolated SPR strain, strain 8416 from a patient with brucellosis in the Inner Mongolia Autonomous Region of China on 2012. Actually, it is the only B. abortus strain among a total of 197 Brucella strains isolated and authenticated by Chinese CDC during this year. The Inner Mongolia Autonomous Region has the highest incidence, responsible for about more than 40% of reported cases in China (Zhang et al., 2010; Chen et al., 2013). Interestingly, the unique phenotypical characteristics of the B. abortus SPR strain 8416, determined by routine biotyping for the identification of Brucella species and biovars, did not completely fit into any of the recognized classification biovars, indicating the potential presence of a new variant of B. abortus biovar 3.

MATERIALS AND METHODS

Bacterial Isolation and Used Strains

The protocol for this study was approved by ethics committee of local disease control and Prevention Research Center of the Inner Mongolia Autonomous Region and Baotou Municipal Center for Disease Control and Prevention. In June 2012, two workers from a cattle farm in Sichuan province, presenting fever, night sweat and soreness of waist, arthralgia and muscle weakness, were admitted to one local hospital in the Inner Mongolia Autonomous Region. The serum samples from these two patients were strongly positive to Brucella by both Rose-Bengal-plate-agglutination-test (RBPT) and Serum Agglutination Test (SAT) with titers of 1/320 according to standard procedures. Moreover, the two serum samples were also confirmed by positive ELISA results with Brucella IgG (>150 U/ml) and IgM (>60 U/ml) (Brucella IgG and IgM ELISA kits, IBL Germany). At the same time, the blood culture of the two patients were inoculated in a dual-phase coloration blood culture bottle (BioMerieux Inc., Durham, USA) at 37°C for 2–3 weeks at the diagnostic laboratory of Baotou Municipal Center for Disease Control and Prevention, the Inner Mongolia Autonomous Region of China. However, only one blood sample from a 45-year-old male janitor yielded a positive culture result. The isolated strain 8416 displayed smooth, tiny, white, shiny and translucent colonies on solid agar after 3 days of incubation. The strain 8416 was sub-cultured on blood plate with 5% CO₂ and displayed typical colonies with small Gram-negative coccobacilli. The strain was sent to department of brucellosis, Chinese Communicable Disease Control and Prevention (Chinese CDC) for further analysis and identification. The reference strains including B. abortus biovar 1 to 7 and 9, strains: 544A (ATCC 23448), 86/8/59 (ATCC 23449), Tulya (ATCC 23450), 292 (ATCC 23451), B3196 (ATCC 23452), 870 (ATCC 23453), 63/75, and C68 (ATCC 23455), B. melitensis biovar 1 to 3, strains: 16M (ATCC 23456), 63/9 (ATCC 23457) and Ether (ATCC 23458)), B. suis biovar 1 to 5, strains: 13305 (ATCC 23444), Thomsen (ATCC 23445), 686 (ATCC 23446), 40 (ATCC 23447), and 513, B. neotomae RM6/66 (ATCC 23365), B. ovis 63/290 (ATCC 25840), and B. canis 5K33 (ATCC 23459) were used as controls for phenotype typing, biochemical and/or molecular analysis.

Analysis of Phenotypic Characteristics

At first, to exclude mixed cultures of different biovars and phage carrier state, the strain used in this study was subjected to a single cloned isolation for successive three times to confirm no variable colonial morphology as described by Jones et al. (1962). The strain was further characterized by using the classical Brucella phenotypic identification procedures, such as CO₂ requirement, H₂S production, dye sensitivity by basic fuchsin and thionin, agglutination with monospecific antisera, and phage typing as described by Alton GG (Alton et al., 1975). Brucella monospecific antisera to A, M, and R (rough) and Brucella phages Tb, Wb, and Bk₂ were used according to standard protocol of the Chinese CDC (Jiang et al., 2013) to characterize this strain. All of phenotypic characterizations in this study were repeated at least three times to make sure the results are repeatable.

Molecular Typing Identification

Brucella strains were inactivated by suspending one loop from a solid bacterial culture in 200 μl DNA storage buffer. Total genomic DNA was extracted using the DNeasy Blood & Tissue Kit (Qiagen China Ltd., Beijing, China) following the manufacturer's instruction. The PCR assay targeting bcsP31, was performed to confirm the Brucella genus as previously described (Bounadja et al., 2009), and species-level using the routine Abortus-Melitensis-Ovis-Suis PCR (AMOS-PCR) (Bricker and Halling, 1994). Furthermore, B. abortus B-ab PCR and a novel PCR to differentiate B. abortus biovar 3a, 3b, 5, 6, and 9 were performed as previously described (Ocampo-Sosa et al., 2005; Huber et al., 2009).

Multiple Locus Variable Number Tandem Repeat Analysis (MLVA) Genotyping

Multiple locus variable number tandem repeat analysis (MLVA) was performed as previously described by Le Fleche et al. (2006)
TABLE 1 | Comparison of phenotypic characteristics and Brucella phage lysis profiles of Brucella abortus strain 8416 and other Brucella reference strains.

| Strain | Growth characteristics | Mono specific phage at Brucella MLVA16 | Interpretation |
|--------|------------------------|----------------------------------------|----------------|
| B. abortus biovar 9 variant 544A | – + – + + – + + 4 5 4 1 22233 | 5 2 1 8 3 5 3 453 | |
| B. abortus biovar 3 870 | – – + + + – + + + 3 3 6 5 3 3 7 3 28322 | 2 3 3 | |
| B. abortus biovar 6 C68 | – + + + – + + + + 6 3 6 5 3 3 1 28322 | 2 3 | |
| B. abortus biovar 9 16M | – – + + ++ – – + 3 4 2 1 3 4 2 1 8 5 3 1 8 6 2 5 8 3 6 | 5 | |
| B. melitensis biovar 1 Ether | – – + + + + – – + 7 5 1 2 3 4 3 2 2 4 5 3 2 1 9 9 6 6 5 53 | 3 | |
| B. melitensis biovar 3 1330S | – + + + – + – – + + 2 3 6 1 0 4 1 5 2 4 1 9 9 6 6 5 5 3 | 2 | |
| B. suis biovar 1 | | | |

and by Jiang et al. (2013), respectively. The 16 primer pairs comprised three main groups: panel 1 including bruce06, 08, 11, 12, 42, 43, 45, and 55 for species identification, panel 2A (bruce18, 19, and 21), and panel 2B (bruce04, 07, 09, 16, and 30) for further subspecies differentiation were used.

**Biochemical Identification by VITEK 2 System**

A total of 47 biochemical reactions of the Brucella strains were analyzed using the standard Gram-negative bacteria identification card on automatic VITEK 2 system according to the manufacturer's instructions.

**RESULTS**

**Routine Phenotypic Typing Characteristics**

According to routine phenotypic analysis, strain 8416 was anti-R negative and H₂S positive, agglutination with anti-M serum but not anti-A serum and grew in the presence of thionine and fuchsin dyes (Table 1). Moreover, it was not lysed by Tb and Wb phages both in 1× RTD (Routine Test Dilution) and 10²× RTD, but lysed by BK₂ phage both in 1× RTD and 10³× RTD (Figure 1A). Thus, the particular phenotypic profiles of the strain 8416 were more similar to that of the classic characteristics of B. abortus biovars 9.

**Biochemical Identification of Automatic VITEK 2 System**

Four biochemical indicators ProA (L-pyrroldonyl-arylamidase), TyrA (tyrose arylamidase), URE (urease), and GlyA could be used to distinguish Brucella species. All of eight B. abortus reference strains and 21 field strains were positive in ILAT_k (L-lactate alkalization), but it was negative in strain 8416, three B. melitensis reference strains and 92 field strains (Cui BuYun's unpublished data). This result indicated that strain 8416 showed special biochemical characteristics distinct from that of B. abortus strains.

**Molecular Typing Identification**

Strain 8416 was identified as B. abortus by the combination of bcsp31 PCR (223-bp, data not shown) and B-ab PCR (370-bp) (Figure 1B) but not as biovar 1, 2, and 4 of B. abortus according to AMOS-PCR (Figure 1C). The novel PCR assay was used to compare strain 8416 to B. abortus biovar 3b, 5, 6, and 9, and found that the PCR product of 1.7 kb from strain 8416 was similar to B. abortus biovar 3b, 5, 6, and 9, but not to other B. abortus biovars (Figure 1D).

**MLVA Genotyping**

According to Brucella MLVA typing database (Grissa et al., 2008), 16 loci of MLVA matching results displayed that strain 8416 was closely related to B. abortus biovar 3 (Jiang et al., 2013), but primarily different in four variable loci, bruce04, bruce07, bruce11, and bruce55 (Table 2).
Finally, based on these typing results, strain 8416 might be a new variant of *B. abortus* biovar 9.

**DISCUSSION**

Until now, the phage resistance mechanism from *Brucella* SPR strains was poorly understood. In this study, a natural SPR strain of *B. abortus* isolated from a patient in China was identified. Although SPR strains of *B. abortus* were rarely isolated from patients, a SPR strain was isolated from a *B. abortus* phage sensitive parent strain 544 in 1974 and a SPR variant of *B. abortus* strain 19 was identified in 1976 through the manipulation of laboratory cultures (Corbel and Morris, 1974; Corbel and Thomas, 1976). Compared to the parent strain 544, the SPR strain FS showed no differences in virulence, morphological, cultural, biochemical or metabolic, and serological reactions, but with an altered phage resistance profile (Corbel and Morris, 1974). The potential mechanism of the phage resistance may be due to its failure to penetrate the FS cell wall since the strain FS is more resistant to lysis by phage lysozymes than that of the phage-sensitive parent strain 544 (Corbel and Morris, 1975). Strain 544-FS showed a complete resistance to lysis by many *Brucella* phages except Bk2 at 1× RTD and 104× RTD. Subsequently, another *B. abortus* SPR strain with resistance to phage Tb, was isolated from a supramammary lymph node of a cow and it is virulent to guinea-pigs (Harrington et al., 1977). Interestingly, these *B. abortus* SPR strains mentioned above belonging to *B. abortus* biovar 1 were identified. However, strain 8416 was significantly different from all of *B. abortus* biovars by using
TABLE 2 | Comparison of Brucella MLVA typing results of B. abortus strain 8416 and the most closely related B. abortus biovar 3 field strains in the Brucella MLVA database.

| Strain name | Distance | BaseView | Strain | Host | Isolated_in | Species-biovar | Contact | Group | Year | MLVA8 | MLVA11 | MLVA16 | MLVA17 | MLVA18 | MLVA19 |
|-------------|----------|----------|--------|------|-------------|----------------|---------|-------|------|-------|-------|-------|-------|-------|-------|
| 8416        |          | 8416     | Human  | Inner Mongolia, China | B. abortus biovar 3 | Buyun Cui | 2012   | 4     | 5    | 4    | 12    | 2     | 2     | 3     | 2     | 6     | 42    | 8     | 6     | 7     | 3     | 3     |
| 2013Jiang#096 | 0       | Brucelia2013 | Human  | Inner Mongolia, China | B. abortus biovar 3 | Buyun Cui | 2012   | 4     | 5    | 4    | 12    | 2     | 2     | 3     | 2     | 6     | 42    | 8     | 6     | 7     | 3     | 3     |
| 2013Garofolo_9263 | 3     | Brucelia_ITALIA_1 | Bufalo | Albanella, Italy | B. abortus biovar 3 | Giuliano | 2011   | 36    | 72   | 12    | 2     | 2     | 3     | 1     | 6     | 42    | 8     | 5     | 6     | 7     | 3     | 3     |
| 2013Jiang#108 | 3       | Brucelia2013 | NM1068 | Cattle | Inner Mongolia, China | B. abortus biovar 3 | Buyun Cui | 1985   | 36    | 117  | 4     | 5     | 3     | 12    | 2     | 2     | 3     | 6     | 42    | 8     | 6     | 4     | 4     | 3     | 3     |
| 2013Jiang#105 | 3       | Brucelia2013 | NM1065 | Cattle | Inner Mongolia, China | B. abortus biovar 3 | Buyun Cui | 1988   | 36    | 117  | 4     | 5     | 3     | 12    | 2     | 2     | 3     | 6     | 42    | 8     | 6     | 4     | 4     | 3     | 3     |
| 2013Jiang#131 | 3       | Brucelia2013 | NM1158 | Cattle | Inner Mongolia, China | B. abortus biovar 3 | Buyun Cui | 1990   | 36    | 117  | 4     | 5     | 3     | 12    | 2     | 2     | 3     | 6     | 42    | 8     | 6     | 4     | 7     | 3     | 3     |
| 2013Jiang#140 | 3       | Brucelia2013 | NM1175 | Cattle | Inner Mongolia, China | B. abortus biovar 3 | Buyun Cui | 1990   | 36    | 117  | 4     | 5     | 3     | 12    | 2     | 2     | 3     | 6     | 42    | 8     | 6     | 4     | 7     | 3     | 3     |
| 2013Garofolo_3636 | 3     | Brucelia_ITALIA_1 | 3636   | Cattle | Monte San Giacomo, Italy | B. abortus biovar 3 | Giuliano | 2011   | 36    | 72   | 12    | 2     | 2     | 3     | 1     | 6     | 42    | 8     | 4     | 6     | 7     | 3     | 3     |
| 2013Garofolo_3916 | 3     | Brucelia_ITALIA_1 | 3916   | Cattle | Monte San Giacomo, Italy | B. abortus biovar 3 | Giuliano | 2011   | 36    | 72   | 12    | 2     | 2     | 3     | 1     | 6     | 42    | 8     | 4     | 6     | 7     | 3     | 3     |
| 2013Garofolo_3920 | 3     | Brucelia_ITALIA_1 | 3920   | Cattle | Teggiano, Italy | B. abortus biovar 3 | Giuliano | 2011   | 36    | 72   | 12    | 2     | 2     | 3     | 1     | 6     | 42    | 8     | 4     | 6     | 7     | 3     | 3     |
| 2013Garofolo_4363 | 3     | Brucelia_ITALIA_1 | 4363   | Cattle | Laurenzana, Italy | B. abortus biovar 3 | Giuliano | 2011   | 36    | 72   | 12    | 2     | 2     | 3     | 1     | 6     | 42    | 8     | 4     | 6     | 7     | 3     | 3     |
| 2013Garofolo_12183 | 3     | Brucelia_ITALIA_1 | 12183  | Cattle | Corleto Monforte, Italy | B. abortus biovar 3 | Giuliano | 2011   | 36    | 72   | 12    | 2     | 2     | 3     | 1     | 6     | 42    | 8     | 4     | 6     | 7     | 3     | 3     |
| 2013Garofolo_12185 | 3     | Brucelia_ITALIA_1 | 12185  | Cattle | San Rudo, Italy | B. abortus biovar 3 | Giuliano | 2011   | 36    | 72   | 12    | 2     | 2     | 3     | 1     | 6     | 42    | 8     | 4     | 6     | 7     | 3     | 3     |
| 2013Garofolo_21571 | 3     | Brucelia_ITALIA_1 | 21571  | Cattle | Monte San Giacomo, Italy | B. abortus biovar 3 | Giuliano | 2011   | 36    | 72   | 12    | 2     | 2     | 3     | 1     | 6     | 42    | 8     | 4     | 6     | 7     | 3     | 3     |
| 2013Garofolo_21675 | 3     | Brucelia_ITALIA_1 | 21675  | Cattle | Monte San Giacomo, Italy | B. abortus biovar 3 | Giuliano | 2011   | 36    | 72   | 12    | 2     | 2     | 3     | 1     | 6     | 42    | 8     | 4     | 6     | 7     | 3     | 3     |
| 2013Garofolo_22839 | 3     | Brucelia_ITALIA_1 | 22839  | Bufalo | Albanella, Italy | B. abortus biovar 3 | Giuliano | 2011   | 36    | 72   | 12    | 2     | 2     | 3     | 1     | 6     | 42    | 8     | 4     | 6     | 7     | 3     | 3     |
| 2013Garofolo_22842 | 3     | Brucelia_ITALIA_1 | 22842  | Cattle | Teggiano, Italy | B. abortus biovar 3 | Giuliano | 2011   | 36    | 72   | 12    | 2     | 2     | 3     | 1     | 6     | 42    | 8     | 4     | 6     | 7     | 3     | 3     |
| 2013Garofolo_5362 | 3     | Brucelia_ITALIA_1 | 5362   | Bufalo | Monte San Giacomo, Italy | B. abortus biovar 3 | Giuliano | 2011   | 36    | 72   | 12    | 2     | 2     | 3     | 1     | 6     | 42    | 8     | 4     | 6     | 7     | 3     | 3     |
| 2013Garofolo_8980 | 3     | Brucelia_ITALIA_1 | 8980   | Cattle | Teggiano, Italy | B. abortus biovar 3 | Giuliano | 2011   | 36    | 72   | 12    | 2     | 2     | 3     | 1     | 6     | 42    | 8     | 4     | 6     | 7     | 3     | 3     |
| 2013Garofolo_21571 | 3     | Brucelia_ITALIA_1 | 21571  | Cattle | Monte San Giacomo, Italy | B. abortus biovar 3 | Giuliano | 2011   | 36    | 72   | 12    | 2     | 2     | 3     | 1     | 6     | 42    | 8     | 4     | 6     | 7     | 3     | 3     | (Continued)
| Strain name | Distance | BaseView | Strain | Host | Isolated_in | Species-biovar | Contact | Group | Year | MLVA8 | MLVA11 | MLVA16 | Bruce6-122 | Bruce6-1134 | Bruce12-73 | Bruce2-124 | Bruce2-247 | Bruce6-233 | Bruce6-239 | Bruce8-324 | Bruce8-1954 | Bruce8-1543 | Bruce8-1290 | Bruce8-548 | Bruce8-1505 |
|-------------|----------|----------|--------|------|-------------|----------------|---------|-------|------|-------|-------|-------|-----------|-------------|--------|----------|--------|---------|-----------|---------|-----------|---------|-----------|---------|-----------|
| 2013Garofolo_8984 | 3 | Brucella_ITALIA_1 | 8984 | Cattle | Sassano, Italy | B. abortus biovar3 | Giuliano Garofolo | 2011 | 36 | 72 | 4 | 5 | 3 | 12 | 2 | 2 | 3 | 1 | 6 | 42 | 8 | 4 | 6 | 7 | 3 | 3 |
| 2013Jiang#093 | 3 | Brucella2013 | 2011166 | Human | Chongqing, China | B. abortus biovar3 | Buyun Cui | 2011 | 36 | 4 | 5 | 3 | 12 | 2 | 2 | 3 | 1 | 6 | 42 | 8 | 4 | 6 | 7 | 3 | 3 |
| 2013Jiang#094 | 3 | Brucella2013 | YLQ | Human | Zhejiang, China | B. abortus biovar3 | Buyun Cui | 2006 | 36 | 4 | 5 | 3 | 12 | 2 | 2 | 3 | 1 | 6 | 42 | 8 | 4 | 6 | 7 | 3 | 3 |
| 2013Jiang#104 | 4 | Brucella2013 | NM1061 | Cattle | Inner Mongolia, China | B. abortus biovar3 | Buyun Cui | 1984 | 112 | 4 | 5 | 3 | 12 | 2 | 2 | 3 | 1 | 6 | 42 | 8 | 5 | 4 | 7 | 3 | 3 |
| 2013Jiang#092 | 4 | Brucella2013 | 2011165' | Human | Chongqing, China | B. abortus biovar3 | Buyun Cui | 2011 | 36 | 4 | 5 | 3 | 12 | 2 | 2 | 3 | 1 | 6 | 42 | 8 | 4 | 7 | 7 | 3 | 3 |
| 2012Ferreira#146 | 4 | Brucella2012 | LNV-328Ba20-06 | Alentejo, Portugal | B. abortus 3 | Cristina Fereira | B. abortus | 2006 | 36 | 72 | 4 | 5 | 3 | 12 | 2 | 2 | 3 | 1 | 6 | 42 | 8 | 4 | 7 | 7 | 3 | 3 |
| 2013Garofolo_3272 | 4 | Brucella_ITALIA_1 | 3272 | Cattle | Apricena, Italy | B. abortus biovar3 | Giuliano Garofolo | 2011 | 4 | 5 | 3 | 12 | 2 | 2 | 3 | 1 | 6 | 42 | 8 | 7 | 6 | 3 | 3 | 3 |
| 2013Garofolo_18061 | 4 | Brucella_ITALIA_1 | 18061 | Cattle | Apricena, Italy | B. abortus biovar3 | Giuliano Garofolo | 2011 | 4 | 5 | 3 | 12 | 2 | 2 | 3 | 1 | 6 | 42 | 8 | 7 | 6 | 3 | 3 | 3 |
| 2006LeFlèche#119 | 4 | Brucella2012 | BCONH99-98 | Mongolia | B. abortus 7 | Gilles Vergnaud | B. abortus | 1999 | 36 | 72 | 4 | 5 | 3 | 12 | 2 | 2 | 3 | 1 | 6 | 42 | 8 | 5 | 6 | 4 | 3 | 3 |
| 2013Jiang#089 | 4 | Brucella2013 | 2011165 | Cattle | Hebei, China | B. abortus biovar3 | Buyun Cui | 2011 | 36 | 4 | 5 | 3 | 12 | 2 | 2 | 3 | 1 | 6 | 42 | 8 | 4 | 5 | 7 | 3 | 3 |
| 2013Jiang#090 | 4 | Brucella2013 | 2011165' | Cattle | Hebei, China | B. abortus biovar3 | Buyun Cui | 2011 | 36 | 4 | 5 | 3 | 12 | 2 | 2 | 3 | 1 | 6 | 42 | 8 | 4 | 5 | 7 | 3 | 3 |
| 2006LeFlèche#112 | 4 | Brucella2012 | BCONH94-18 | Cattle | Limoges, France | B. abortus 3 | Gilles Vergnaud | B. abortus | 1994 | 36 | 72 | 4 | 5 | 3 | 12 | 2 | 2 | 3 | 1 | 6 | 42 | 8 | 4 | 5 | 7 | 3 | 3 |
| 2013Jiang#100 | 4 | Brucella2013 | NM1051 | Cattle | Inner Mongolia, China | B. abortus biovar3 | Buyun Cui | 1984 | 36 | 4 | 5 | 3 | 12 | 2 | 2 | 3 | 1 | 6 | 42 | 8 | 6 | 4 | 8 | 3 | 3 |
| 2006LeFlèche#005 | 4 | Brucella2012 | REF 292 | Cattle | England | B. abortus 4 | Gilles Vergnaud | B. abortus | 30 | 78 | 4 | 5 | 4 | 12 | 2 | 2 | 3 | 1 | 6 | 42 | 8 | 3 | 4 | 3 | 3 | 5 |
| 2009Her#004 | 4 | Brucella2012 | KRef04 | Cattle | England | B. abortus 4 | Moon Her | B. abortus | 30 | 78 | 4 | 5 | 4 | 12 | 2 | 2 | 3 | 2 | 6 | 42 | 8 | 3 | 4 | 3 | 3 | 5 |
| 2012Ferreira#213 | 4 | Brucella2012 | REF 292 | Cattle | England | B. abortus 4 | Cristina Fereira | B. abortus | 30 | 78 | 4 | 5 | 4 | 12 | 2 | 2 | 3 | 2 | 6 | 42 | 8 | 3 | 4 | 3 | 5 | 5 |
| 2013Jiang#127 | 4 | Brucella2013 | NM1147 | Cattle | Inner Mongolia, China | B. abortus biovar3 | Buyun Cui | 1988 | 117 | 4 | 5 | 3 | 12 | 2 | 2 | 3 | 2 | 6 | 42 | 8 | 5 | 4 | 3 | 3 | 3 |
| 2013Jiang#130 | 4 | Brucella2013 | NM1156 | Sheep | Inner Mongolia, China | B. abortus biovar3 | Buyun Cui | 1988 | 36 | 4 | 5 | 3 | 12 | 2 | 2 | 3 | 1 | 6 | 42 | 8 | 6 | 4 | 4 | 3 | 3 |
| 2013Jiang#125 | 4 | Brucella2013 | NM1140 | Cattle | Inner Mongolia, China | B. abortus biovar3 | Buyun Cui | 1988 | 36 | 4 | 5 | 3 | 12 | 2 | 2 | 3 | 1 | 6 | 42 | 8 | 5 | 4 | 7 | 3 | 3 |
| Strain name  | Distance | BaseView | Strain | Host | Isolated_in | Species-biovar | Contact | Group | Year | MLVA8 | MLVA11 | MLVA16 | Bruc e e98-1322 | Bruc e e98-1134 | Bruc e12-73 | Bruc e4-424 | Bruc e4-379 | Bruc e5-233 | Bruc e5-2066 | Bruc e18-339 | Bruc e16-324 | Bruc e18-1250 | Bruc e04-1543 | Bruc e21-239 | Bruc e09-588 | Bruc e16-548 | Bruc e30-1505 |
|-------------|----------|----------|--------|------|-------------|----------------|---------|-------|------|-------|-------|-------|-----------------|----------------|------------|-----------|----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
| 2013Jiang#126 | 4 | Brucella2013 | NM1146 | Cattle | Inner Mongolia, China | B. abortus biovar 3 | Buyun Cui | 1988 | 36 | 4 | 5 | 3 | 12 | 2 | 2 | 3 | 1 | 6 | 42 | 8 | 5 | 4 | 7 | 3 | 3 |
| 2013Jiang#128 | 4 | Brucella2013 | NM1148 | Cattle | Inner Mongolia, China | B. abortus biovar 3 | Buyun Cui | 1988 | 36 | 4 | 5 | 3 | 12 | 2 | 2 | 3 | 1 | 6 | 42 | 8 | 5 | 4 | 7 | 3 | 3 |
| 2013Jiang#141 | 4 | Brucella2013 | NM1176 | Cattle | Inner Mongolia, China | B. abortus biovar 3 | Buyun Cui | 1990 | 36 | 4 | 5 | 3 | 12 | 2 | 2 | 3 | 1 | 6 | 42 | 8 | 5 | 4 | 7 | 3 | 3 |
| 2013Jiang#150 | 4 | Brucella2013 | NM1215 | Cattle | Inner Mongolia, China | B. abortus biovar 3 | Buyun Cui | 1994 | 36 | 4 | 5 | 3 | 12 | 2 | 2 | 3 | 1 | 6 | 42 | 8 | 5 | 4 | 7 | 3 | 3 |
| 2013Jiang#151 | 4 | Brucella2013 | NM1218 | Cattle | Inner Mongolia, China | B. abortus biovar 3 | Buyun Cui | 1995 | 36 | 4 | 5 | 3 | 12 | 2 | 2 | 3 | 1 | 6 | 42 | 8 | 5 | 4 | 7 | 3 | 3 |
| 2013Jiang#146 | 4 | Brucella2013 | NM1185 | Cattle | Inner Mongolia, China | B. abortus biovar 3 | Buyun Cui | 1990 | 36 | 4 | 5 | 3 | 12 | 2 | 2 | 3 | 1 | 6 | 42 | 8 | 6 | 4 | 5 | 3 | 3 |
| 2013Jiang#152 | 4 | Brucella2013 | NM1219 | Cattle | Inner Mongolia, China | B. abortus biovar 3 | Buyun Cui | 1995 | 36 | 4 | 5 | 3 | 12 | 2 | 2 | 3 | 1 | 6 | 42 | 8 | 5 | 5 | 7 | 3 | 3 |
| 2013Jiang#153 | 4 | Brucella2013 | NM1075 | Cattle | Inner Mongolia, China | B. abortus biovar 3 | Buyun Cui | 1985 | 117 | 4 | 5 | 3 | 12 | 2 | 2 | 3 | 2 | 8 | 42 | 8 | 5 | 6 | 3 | 3 | 3 |
| 2006LeFlinch#135 | 4 | Brucella2012 | BFR 95 | Mouse | ? | B. abortus 1 | Gilles Vergnaud | B. abortus | 28 | 82 | 4 | 5 | 4 | 12 | 2 | 2 | 3 | 3 | 6 | 42 | 8 | 3 | 6 | 3 | 3 | 5 |
| 2009Her#011 | 4 | Brucella2012 | KRef15 | Cattle | USA | B. abortus 1 | Moon Her | B. abortus | 28 | 82 | 4 | 5 | 4 | 12 | 2 | 2 | 3 | 3 | 6 | 42 | 8 | 3 | 6 | 3 | 3 | 5 |
| 2013Jiang#083 | 4 | Brucella2013 | Xinjiang, China | Cattle | Xinjiang, China | B. abortus biovar 3 | Buyun Cui | 2011 | 36 | 4 | 5 | 3 | 12 | 2 | 2 | 3 | 1 | 6 | 42 | 8 | 4 | 6 | 5 | 3 | 3 |
| 2013Garofolo_3921 | 4 | Brucella_ITALIA_1 | 3921 | Cattle | San Gregorio Magno, Italy | B. abortus biovar 3 | Giuliano Garofolo | 2011 | 36 | 72 | 4 | 5 | 3 | 12 | 2 | 2 | 3 | 1 | 6 | 42 | 8 | 4 | 6 | 6 | 3 | 3 |
| 2013Garofolo_5007 | 4 | Brucella_ITALIA_1 | 5007 | Cattle | San Gregorio Magno, Italy | B. abortus biovar 3 | Giuliano Garofolo | 2011 | 36 | 72 | 4 | 5 | 3 | 12 | 2 | 2 | 3 | 1 | 6 | 42 | 8 | 4 | 6 | 6 | 3 | 3 |
phenotypic and molecular typing method. However, it shared the same phage lysis profile to that of *B. melitensis* biovar 1. In conclusion, strain 8416 is the only SPR strain isolated from the infected human thus far with a similar phage lysis pattern with *B. melitensis* 16 M. However, despite same resistant to phage Tb, we could not comprehensively compare with phage lysis profiles of the three reported SPR strains due to different *Brucella* phages tested among them.

Currently, MLVA has been mainly used for tracking the variances of the bacterial genus with a high homology, such as *Brucella* genus (Haguenoer et al., 2011). The MLVA-16 (panel 1, 2A and 2B) assay was widely used for molecular typing of a larger collection of isolates at both species and biovars level. The panel 1 comprised eight minisatellite markers for species identification (Le Fleche et al., 2006) and the panel 2 markers were found with a higher biovar discriminatory power. Surprisingly, the MLVA-16 typing results showed that strain 8416 was clustered into the Chinese *B. abortus* biovar 3 strains (jiang et al., 2013) with four variable loci (bruce04, 07, 11, and 55). Actually, among the four known panel 1 genotypes (28, 30, 112, 116), strain 8416 (genotype 30) was distinct from other 65 Chinese *B. abortus* biovar 3 strains isolated previously from different geographic origins, suggesting that more *B. abortus* strains phenotypically identified as biovar 3 are required for the comparison. The MLVA assay confirmed that *B. abortus* biovar 3 is a heterogeneous group (Le Fleche et al., 2006), and in agreement with the *B. abortus* biovar 3 divided into two sub-biovar 3a and 3b (Huber et al., 2009).

In this study, an atypical *B. abortus* strain displaying a phage lysis profile similar to *B. melitensis* biovars 1 was identified. Most importantly, the lysis pattern by bacteriophages observed in this newly uncovered *B. abortus* SPR strain. Although phage typing in general can successfully classify *Brucella* species, our research calls for attention as to conclusions on SPR strains. Further investigation focusing on the strain 8416's whole genomic variations associated with phage resistance is needed.

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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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