

## Emergence of a Clonal Lineage of *Brucella abortus* Biovar 3 in Clinical Cases in Spain<sup>∇</sup>

*Brucella abortus* biovar 3 is an uncommon cause of human brucellosis in Spain, where *Brucella melitensis* has accounted for 97.5% of all cases. *B. abortus* biovar 3 was detected in specimens from four patients in Cáceres (west-central Spain) with a febrile syndrome associated with arthralgia and myalgia and from one patient in Lugo (northwestern Spain) with orchepididymitis (March 2004 and April 2005). For tracing, these isolates were studied by biotyping, analysis of 16S rRNA genes, *rpoB*, *gyrA*, *parC*, and housekeeping genes, multilocus variable-number tandem-repeat analysis (MLVA-16), and analysis of hypervariable octameric oligonucleotide fingerprints (HOOF-prints).

Isolates were identified as *B. abortus* biovar 3 (3b) by their CO<sub>2</sub> requirements, H<sub>2</sub>S production, agglutination in anti-A-positive and anti-M-negative monospecific sera, and growth on thionin and basic fuchsin (20 µg/ml). They showed a single 16S rRNA gene and *rpoB* sequences (6, 9). The latter gene harbored substitutions (243-GAC, 268-ACT, and 340-GAA) with respect to the *B. melitensis* 16 M sequence under GenBank accession no. AE009516 (9). The replacement at codon 268 is thought to be a *B. abortus* marker because of its presence in reference biovars. The *rpoB* sequence showed a 100% match with the sequence of *B. abortus* biovar 7 strain 63/75 (GenBank accession no. DQ086138) (9). The authenticity of *B. abortus* biovar 7, however, has been questioned for many years (2, 5) because the reference strain was a mixture of *B. abortus* biovars 3 and 5. Further work showed the obtained *rpoB* gene differs at position 340 from the

sequence of *B. abortus* biovar 7 reference strain Tulya (GenBank accession no. AY562180) (9). The Tulya strain and other African *B. abortus* biovar 3 isolates show distinct genetic patterns versus European isolates of the same biovar when analyzed with different genetic markers. The division of *B. abortus* biovar 3 into two groups has been proposed: 3a, containing the Tulya and African field isolates; and 3b, containing the European isolates (including those we analyzed) (10). Therefore, analyses of our five *B. abortus* biovar 3 isolates differed from those for the Tulya strain (9).

The *gyrA* and *parC* amplifications and sequencing were performed with the primers *Bru\_gyrA* + 4651 (5'-TGCAGCGG TCTTATCTTGATT-3'), *Bru\_gyrA*-5589 (5'-CAAACGAGG TCTGCAAAGG-3'), *Bru\_parC* + 2040 (5'-CAAGCTGACC GAGCTTGAA-3'), and *Bru\_parC*-2871 (5'-CACGAAGGCC GTCAGTATATC-3') to provide polymorphisms related to the bacterial identification (7). All showed one *gyrA* (5285-T → C) mutation and one *parC* [2600-Ala(GCT) → Val(GTT)] mutation compared to *B. melitensis* 16 M. The *gyrA* change appears in *B. abortus* biovars and in *Brucella suis*, while the *parC* mutation is seen only in *B. abortus* biovars. The isolates showed the same sequence type, ST5 (*gap-2 aroA-1 glk-1 dnaK-2 gyrB-1 trpE-4 cobQ-1 omp25-1 int-hyp-1*) (11), identical to the corresponding sequence of the *B. abortus* biovar vaccine isolates S19 and RB51. The Spanish isolates differed at two (*glk* and *trpE*) and seven (*gap*, *aroA*, *glk*, *dnaK*, *gyrB*, *trpE*, and *cobQ*) of the nine housekeeping genes from those of the *B.*

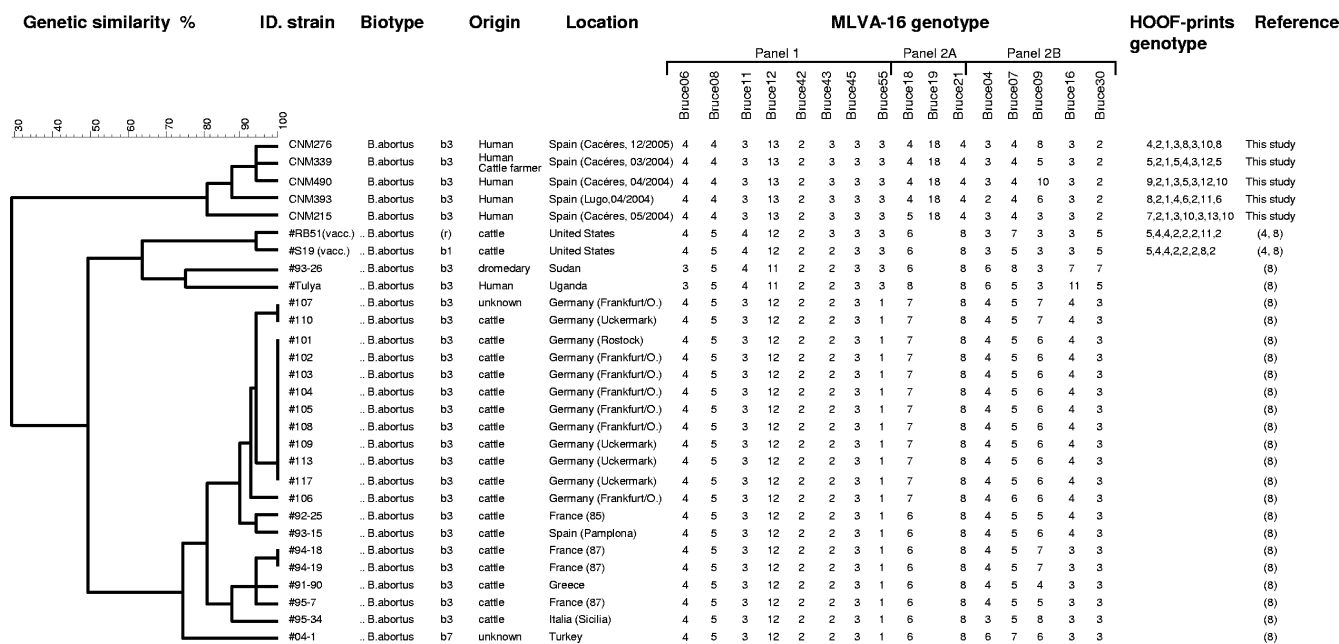


FIG. 1. Dendrogram of clustered MLVA-16 genotypes of the Spanish *B. abortus* biovar 3 human isolates (see the *Brucella* 2007 MLVA database [http://mlva.u-psud.fr]), the European *B. abortus* biovar 3 (3b) isolates, the African *B. abortus* biovar 3 (3a) Tulya strain, and the *B. abortus* biovar 1 RB51 and S19 vaccine isolates (8), generated using the categorical coefficient and UPGMA analysis (unweighted-pair group method using arithmetic averages). MLVA-16 information for Bruce09 was not available for these previously studied strains (8).

*abortus* biovar 3 5/93 strain and the Tulya strain, respectively (11).

Five MLVA-16 (1) (genetic similarity range, 80% to 95%) and five HOOOF-print (4) (35% to 65%) genotypes were obtained. In a comparison of those genotypes to other MLVA-15 genotypes (8), the Spanish cluster split from other European and African *B. abortus* biovar 3 isolates (similarity index, <40%), suggesting a different clonal lineage (Fig. 1).

In summary, we described the first reported cases of *B. abortus* biovar 3 causing human brucellosis in Spain. MLVA and HOOOF-print genotyping revealed a close genetic relationship between the detected emergent isolates; this is further supported by their identical 16S rRNA gene, *rpoB*, *gyrA*, *parC*, and housekeeping gene sequences.

**Nucleotide sequence accession numbers.** GenBank has assigned the following accession numbers: for 16S rRNA, EF192470 to EF192474; for *gyrA*, EF420872; for *parC*, EF420873; and for *rpoB*, EU623594.

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