Deletion in the gene BruAb2_0168 of *Brucella abortus* strains: diagnostic challenges

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Abstract

Three *Brucella abortus* strains were isolated from joint hygromas from cows in northern Togo. Two deletions in the 5′ side of the gene BruAb2_0168 were identified. As this gene is used for species identification, these deletions have consequences for diagnostic procedures. Multiple locus variable number of tandem repeat (VNTR) analysis was therefore performed for species identification. The strains showed unique VNTR profiles, providing some of the first genotypic data from West Africa. More molecular and epidemiological data are needed from the region, in order to better understand transmission patterns and develop suitable diagnostic assays.

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Although brucellosis is one of the world's most common zoonoses, there is a lack of data from sub-Saharan Africa [1,2]. Brucellosis impacts on human and animal health [3] and has important economic consequences [4]. This report describes the isolation and genetic characterization of *Brucella abortus* strains from Togo, which posed a diagnostic challenge due to deletions in a gene targeted by PCR and the inability to grow on selective medium.

During a brucellosis serosurvey in Togo (2011–2012) [5], joint fluid was aspirated aseptically from nine seropositive cows from five herds with hygromas in the carpi/hocks. Sterile swab tips were soaked in the fluid and sealed in liquid Amies medium. After storage and transportation at 4°C according to international biosafety standards, they were cultivated in Switzerland on tryptic soy agar plates supplemented with 5% sheep blood (BD, Allschwil, Switzerland) and on *Brucella*-medium base agar supplemented with 5% inactivated horse serum and modified *Brucella* selective supplement (Oxoid, Basingstoke, UK). The plates were incubated for 10 days at 37°C in 5% CO_2. All culture manipulations were performed under Biosafety Level 3 containment. Although there was no growth on *Brucella* selective medium, mixed cultures grew on blood agar. *Brucella*-like colonies were purified and isolates were recovered from three cows from three different herds.

Phenotypic testing of the strains was performed. The strains did not require CO_2 for growth and grew in the presence of thionin (0.04 mg/mL and 0.02 mg/mL) and basic fuchsine (0.02 mg/mL). They produced H_2S, as detected by hydrogen sulphide test strips (Sigma Aldrich, Buchs, Switzerland), and were urease positive (Oxoid). These results indicated *B. abortus* biovar 3 [6].

Molecular identification of the genus *Brucella* was performed by real-time PCR, as previously described [7]. As a species-specific signal for *abortus* or *melitensis* could not be obtained but *B. abortus* was suspected, it was decided to amplify a larger DNA segment of the target locus of the real-time PCR, which was specific for the species *abortus*, the BruAb2_0168 gene (BAbS19_II01580 in vaccine strain S19). The primer pair used was 0168_Babo_1F and 0168_Babo_1R. The PCR products were sequenced (primers in Table 1). Two deletions of 2678 and 263 base pairs were identified, from nucleotide positions 984-3662 and 4295-4558 of the sequence of *B. abortus* strain S19 (Fig. 1) (GenBank accession number: KC847095).

The isolated strains displayed features that complicated diagnosis. Firstly, none grew on *Brucella* selective medium. As some *B. melitensis* and *B. abortus* strains are sensitive to the antibiotic concentrations in modified *Brucella* selective supplement, it is therefore advisable to concurrently inoculate a standard growth medium or modified Thayer-Martin plate [8]. Secondly, the deletions in the BruAb2_0168 gene suggest that this may not be a suitable target for an *abortus* species-specific PCR. Samples for which a species-specific signal cannot be obtained should always be tested for a genus-specific signal to ensure that *Brucella* sp is not missed. False negatives due to deletions in targets of diagnostic assays can have important

TABLE 1. Primers used for the amplification and sequencing of the BruAb2_0168 gene

Primers	Sequences (5′ to 3′)
0168_Babo_1F	GGCGTGTATGTTGTTGGTAA
0168_Babo_2F	AACATATCGGCGACGCAGTA
0168_Babo_3F	GTGACGGGGACGGGTTGGAC
0168_Babo_4F	TGCGGATAATAATCTGGGTGA
0168_Babo_5F	CGGTTAATGGCACGCTTGAA
0168_Babo_6F	CCTCAATGGTGCGTGGGACAA
0168_Babo_7F	CGGTTCTGGGTGGCACGGTTA
0168_Babo_8F	GCACGGGCAGTCTGACGAAG
0168_Babo_9F	GGCGGCACGACGACGGTTGATG
0168_Babo_11F	GGATACTGACGGCACGCTTGA
0168_Babo_14F	GAACTTCATATCGGTACTGGTG
0168_Babo_15F	CTTGGTGATGACAATTCCAAGA
0168_Babo_16F	GTCTTTTGTGCTCAAGAACAATC
0168_Babo_17F	TGTCGTCAATGGCGGGCGATGGA
0168_Babo_18F	GGGAGTGCAGGCAATCACAG
0168_Babo_19F	CAGGCACGCTGACGCTGA
0168_Babo_20F	ATACACTGACGCTTCAGAACA
0168_Babo_1R	ATCGCCACCAACCATCAGC
0168_Babo_2R	CGCCGTCAGACTGCCCTCCA
0168_Babo_3R	ACCGTCGTTGCGCCCGTA
0168_Babo_4R	AAGTGCCGCCGTTAAACGTCA
0168_Babo_5R	GTGCCTGTGCCGCTCTTCA
0168_Babo_6R	AATTATTCGCCGCATCCTCA

public health consequences. In Sweden, a deletion of 377 base pairs in the target of a commercial PCR for the diagnosis of *Chlamydia trachomatis* was identified following a decrease in the number of human cases detected [9]. Genomes can undergo modifications such as deletions, insertions or rearrangements, and this aspect of bacterial evolution must always be considered when performing PCR assays for detection and/or identification. Ideally, more than one target should be tested and unexpected results investigated.

Loci with a variable number of tandem repeats (VNTR) can be used as markers to identify species belonging to the *Brucella* genus (panel 1 markers) and to discriminate among *Brucella* strains (panel 2A and 2B markers). Molecular typing by multiple locus VNTR analysis (MLVA) over 16 loci can be used as a molecular epidemiology tool to assess brucellosis transmission patterns [10,11].

MLVA was performed on the three culture lysates and on *B. melitensis* strain 16M^T. For most loci, sequencing confirmed the exact PCR product length. However, five loci could not be sequenced (bruce06, bruce07, bruce09, bruce30, bruce55) and their length was determined by comparing the PCR product bands with those of *B. melitensis* 16M^T on an agarose gel, and by the Agilent 2100 Bioanalyzer using a DNA 1000 LabChip kit (Agilent Technologies, Waldbronn, Germany) followed by comparison with the results of De Santis *et al.* [12]. The MLVA data were analysed using the *Brucella* aggregated database on MLVAnet (http://mlva.u-psud.fr/) hosted by Université Paris-Sud [13]. This database compares queried strains with described strains and performs a clustering analysis using the categorical coefficient and unweighted pair group method with arithmetic mean (UPGMA). The resultant Newick strings were imported into R statistical software Version 2.12.2 (http://www.R-project.org) and a dendogram was drawn using the package 'ape' (http://ape-package.ird.fr/). The three strains showed distinct VNTR profiles (Table 2) clustering with African strains of *B. abortus* biovar 3 (Fig. 2), confirming the phenotypic results. There is only one other genotyped strain of *B. abortus* reported from West Africa, isolated from the Gambia [14]. Furthermore, the three strains were obtained from a small geographical zone, the sampling sites being only 13–42 km from one another. This demonstrates the diversity of the circulating strains. Given the importance of semi-nomadic herd management and cross-border trade in Togo [15], livestock movements are likely to play a role in this genetic diversity.

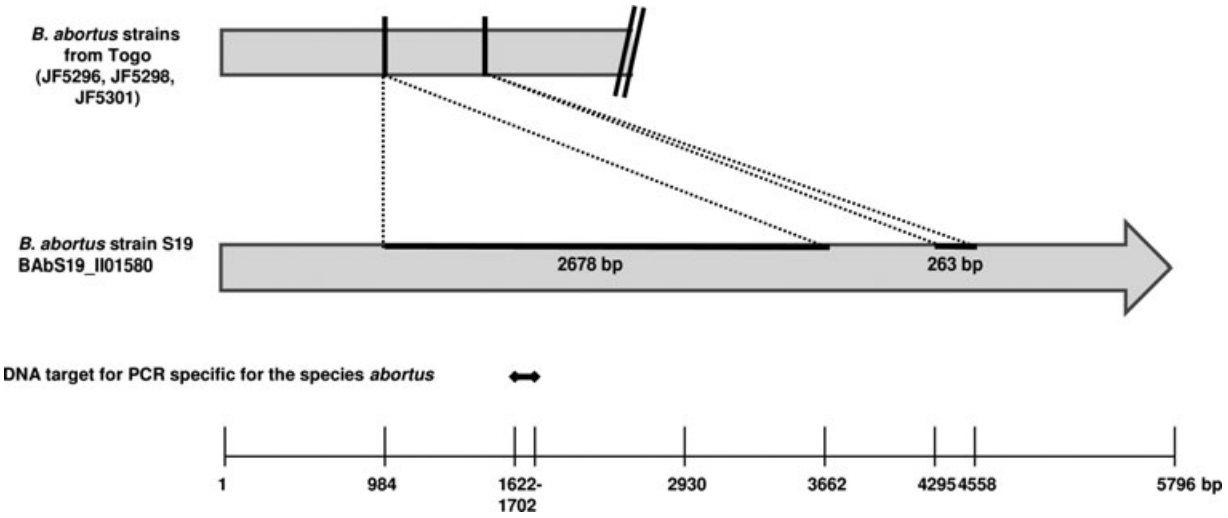

FIG. 1. Location of two deletions in the BruAb2_0168 gene of *Brucella abortus* strains from Togo compared with *B. abortus* vaccine strain S19. The DNA target for the PCR specific for the *abortus* species is shown to fall in the range of the first deletion.