# Identification of *Brucella melitensis* Using a Multiplex PCR Bruce-Ladder System

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## ABSTRACT

Several PCR-based assays have been used for identification of *Brucella* to increase the effectiveness of diagnosis. This study uses Bruce-ladder PCR was used to identify the exact *Brucella* species for better understanding of the epidemiology of brucellosis. Nine isolates of *Brucella melitensis,* isolated from several local goat farms were subjected to the test. This study indicates that Bruce-Ladder PCR is useful in identification of *Brucella* melitensis.

## INTRODUCTION

Brucellosis is a zoonotic disease caused by *Brucella sp.*, a Gram-negative, facultative and intra cellular bacterium. The disease is characterised by abortion leading to a major economic losses and a barrier factor for animal transportation and exportation. The genus *Brucella* shows a wide range of species specific and has six classical species. They are are *Brucella melitensis, Brucella abortus, Brucella canis, Brucella neotomae, Brucella suis* and *Brucella ovis*. Recently, Mohandoss et al., (2011) concluded that the *Brucella spp.* are not really a host specific and they may cross-infect other than the natural host. For example, cattle are not only infected by *B.abortus*, but also by *B.suis* and *B.melitensis* (Cook and Noble, 1984). Similarly, *B.abortus* and *B.melitensis* have been isolated in swine (Verger 1985). The conventional method of species and biotyping identification are time consuming and require trained personnel for interpretation. Therefore, the study used the rapid and easy to perform Bruce-ladder PCR to help in characterization and differentiation of all local isolates of *Brucella* species.

## MATERIALS AND METHOD

*Bacterial isolates*

A total of nine *Brucella* field isolates from several local goat farms that were maintained at Histopathology Laboratory, Faculty of Veterinary Medicine, Universiti Putra Malaysia were used in this study. A references strain of *Brucella melitensis* (16M) was also included in this study.

*DNA extraction from Brucella culture*

Brucella isolates were grown in Brucella selective agar at 37ºC before the DNA was extracted by mixing 1µl of genomic DNA isolation reagent (DNAzol) with one pick of colony and incubated for 15min.

*Brucella Ladder PCR*

PCR was carried out in a 25µl reaction with 6.25 pmol of each primer in cocktail of eight primer sets (Table1), 2.5µl PCR buffer 10x, 400µM dNTPs, 3.0mM $Mg^{2+}$, 1.5U TaqDNA polymerase (Fermentas), 13.2µl distilled $H_2O$ and 1µl of template. The PCR amplification was performed in a thermocycler (Vapo Protect, Eppendorf). The samples were subjected to amplification as follows: initial denaturation step at 95ºC, for 7 minutes, followed by denaturation for 35 s at 95ºC, annealing temperature was set up at 64ºC for 45 s and 3 minutes of extension stage at 72ºC for 25 cycles, respectively and a final elongation phase at 72ºC for 6 min. Three microliters of samples were loaded in 1% agarose gel and

electrophoresed for 30 minute at 90V in 1x TBE buffer through GelRed (GelRed™). A 100 bp molecular size marker (RBC, Real-Biotech) was run concurrently. The gel was examined under UV light and photographed by using a Kodak EDAS 290 gel document system for documentation and determination of expected band.

Table 1: Primers for Bruce-Ladder

| **Primer** | **Sequence (5'-3')** | **Amplification size** |
|---|---|---|
| BMEI0998f | ATC CTA TTG CCC CGA TAA GG | 1682 |
| BMEI0998r | GCT TCG CAT TTT CAC TGT AGC | |
| BMEI10843f | TTT ACA CAG GCA ATC CAG CA | 1071 |
| BMEI10843r | GCG TCC AGT TGT TGT TGA TG | |
| BMEI1436f | ACG CAG ACG ACC TTC GGT AT | 794 |
| BMEI1435r | TTT ATC CAT CGC CCT GTC AC | |
| BMEI10428f | GCC GCT ATT ATG TGG ACT GG | 587 |
| BMEI10428r | AAT GAC TTC ACG GTC GTT CG | |
| BMEI10535f | GCG CAT TCT TCG GTT ATG AA | 450 |
| BMEI10536r | CGC AGG CGA AAA CAG CTA TAA | |
| BR0963f | GGA ACA CTA CGC CAC CTT GT | 272 |
| BR0963r | GAT GGA GCA AAC GCT GAA G | |
| BMEI10752f | CAG GCA AAC CCT CAG AAG C | 218 |
| BMEI10752r | GAT GTG GTA ACG CAC ACC AA | |
| BMEI10987f | CGC AGA CAG TGA CCA TCA AA | 152 |
| BMEI10987r | GTA TTC AGC CCC CGT TAC CT | |

## RESULTS

Bruce-ladder revealed similar banding pattern for all isolates of *B.melitensis*. The results showed amplification of 1320 bp, 1071 bp, 794 bp, 587 bp, 450 bp and 152 bp bands, which confirmed that the isolates belonged to *B. melitensis* (Figure 1).

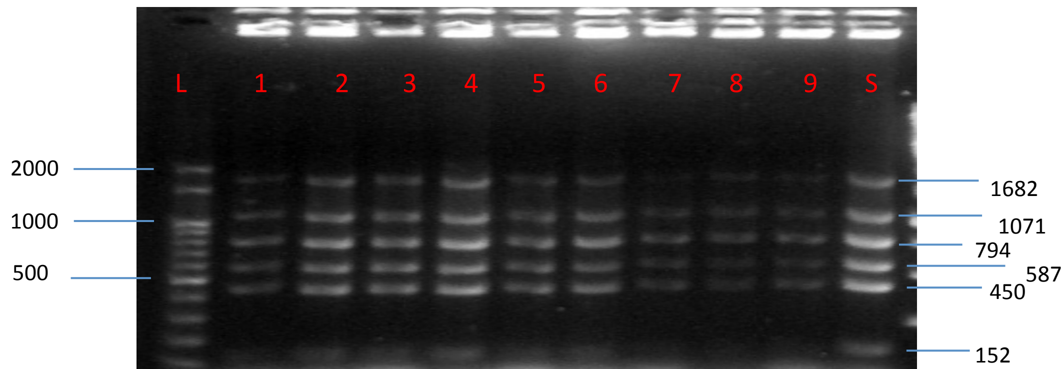


Figure 1.Bruce-Ladder PCR for *Brucella* field isolates. Lane L 100 bp-ladder, Lane 1-9 *Brucella melitensis*, Lane S *B.melitensis* 16M

## DISCUSSION

Brucellosis is one of the important zoonotic diseases, known as Malta fever in human that leads to high economic losses in animal (Al Dahouk *et al*., 2004). *Brucella* sp. can expand its host horizon with new species emerging from marine mammals. Therefore, it is beneficial to understand beyond the level of genus to have an appreciation of the disease. Multiplex Bruce-Ladder PCR assay has been employed to characterize the species typing of *Brucella.* Bruce-Ladder is species specific and was based on the sizes of eight products amplified by PCR which are 1682 bp, 1071 bp, 794 bp , 587 bp, 450 bp, 272 bp, 218 bp and 152 bp in size. In this study, Bruce-Ladder PCR has successfully been used to determine the species of *Brucella* from Malaysian field isolates. Furthermore, the major advantage of using Bruce-Ladder is that it can identify for the first time all of the *Brucella* species in the same test (López-Goňi *et al*., 2008).

Although *Brucella* was claimed to have cross-species infection, studies concluded that there was no evidence of occurrence in Malaysia. Hence, more samples should be tested in order to assess the situation in this country. Moreover, PCR based identification methods are very sensitive and this multiplex PCR Bruce-ladder system is very useful for identification of *Brucella*.

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