Isolation and identification of *Brucella melitensis* from aborted ruminants

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Abstract

Brucellosis is a contagious zoonotic disease caused by gram negative bacteria. *Brucella abortus*, *B. melitensis* and *B. suis* are the most perilous species affecting cattle, pig, sheep and goats respectively. *Brucella* causes epididymitis in male whereas abortion, placentitis and infertility in female animals. The objectives of the present study was to isolate and characterize *Brucella* organisms from a total of five hundred and twenty five samples comprising milk (245), blood (145), aborted foetuses (15) and vaginal swabs (120) of ruminants population which were recently aborted. Isolation of *Brucella melitensis* was done on Brucella selective media subsequently isolates was characterized by conventional biotyping methods, while molecular typing was done by AMOS polymerase chain reaction (AMOS-PCR). Three isolates of *Brucella melitensis* were confirmed both by biochemical and molecular methods.

Keywords: *Brucella melitensis*, abortion, isolation, molecular characterization, sheep and goat

Introduction

Brucellosis is a highly contagious gram negative bacterial disease of zoonotic importance causing significant reproductive loss due to high rates of abortion and infertility (Wareth, et al., 2017) [8]. The genus *Brucella* was named after David Bruce, who first isolated the organism in 1887 from the spleen of a soldier suffering from a disease that was called Malta fever (Nicoletti et al., 2002). The disease has been reported in cattle, buffaloes, sheep, goats, and camels, and *Brucella* spp. has been isolated from Nile cat fish and carrier hosts such as dogs and cats. The zoonotic nature of brucellosis was demonstrated in 1905 by isolating Brucella from goat milk (Nicoletti et al., 2002). This zoonosis causes a severely debilitating illness characterized by intermittent fever, chills, sweats, weakness, myalgia, osteo-articular or obstetrical complications and endocarditis (Mesureur, et al., 2018) [9].

Currently, the *Brucella* genus consists of eleven recognized species plus several isolates that have not yet been officially designated. The major zoonotic species are *B. melitensis*, *B. abortus* and *B. suis* which are subdivided into biosvars by a set of phenotypic characteristics including lipopolysaccharide (LPS) epitopes, phage sensitivity, dye sensitivity and a battery of biochemical tests. These three species are also the most common in domestic livestock (Garofolo, et al., 2017) [10].

Detection of *Brucella* spp. DNA in semen of seronegative bulls has previously been reported and in milk of seronegative cows. *Brucella* spp. has been recovered from milk came from seronegative cows and from blood, bone marrow, lymph nodes and vaginal exudates of seronegative cattle and goats (Lindahl-Rajala et al., 2017) [8].

The aim of this study was to isolate *Brucella melitensis* from milk, blood, aborted fetuses, and vaginal swabs of ruminants and to characterize these isolates using the (AMOS) PCR, and also to ascertain biovar of *Brucella melitensis*, employing biochemical tests, growth in the presence of thionin and fuschin dyes.

Materials and Methods

A total of five hundred and twenty five samples comprising milk (245), blood (145), aborted foetuses (15) and vaginal swabs (120) from ruminant population which were recently aborted from different regions of Tamil Nadu (Table 1).
Table 1: Details of samples collected from different species with history of abortion

<table>
<thead>
<tr>
<th>Area</th>
<th>Species</th>
<th>Blood</th>
<th>Milk</th>
<th>Vaginal Swab</th>
<th>Aborted Fetus</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chennai</td>
<td>Ovine</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Bovine</td>
<td>10</td>
<td>102</td>
<td>0</td>
<td>1</td>
<td>113</td>
</tr>
<tr>
<td></td>
<td>Caprine</td>
<td>20</td>
<td>0</td>
<td>16</td>
<td>4</td>
<td>40</td>
</tr>
<tr>
<td>Kancheepuram</td>
<td>Caprine</td>
<td>78</td>
<td>98</td>
<td>103</td>
<td>6</td>
<td>285</td>
</tr>
<tr>
<td>Thiruvallur</td>
<td>Caprine</td>
<td>36</td>
<td>45</td>
<td>0</td>
<td>3</td>
<td>84</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>145</td>
<td>245</td>
<td>120</td>
<td>15</td>
<td>525</td>
</tr>
</tbody>
</table>

The samples collected were inoculated in Trypticase soy broth followed by incubation at 37 °C for 48–72 hours. Subsequently the samples were inoculated in Brucella selective media.

A. Presumptive growth in the plates

B. Biochemical test for H₂S Production

C. Urease Test

D. Fuschin dye test

E. Fuschin dye test

Brucella broth and Brucella selective medium (Himedia) with supplement was used for isolating B. melitensis. Isolation and identification of Brucella melitensis was carried out as detailed in OIE (2000) [12]. Biochemical characteristics test for hydrogen sulphide production, growth in the presence of basic fuchsin dyes (20µg/ml) (Meyer and Shaw, 1984) [10] were carried out. Identification of B.melitensis from milk, blood, aborted fetuses, and vaginal swabs of ruminants and characterization using AMOS- PCR (Table 2) were carried out (Bricker and Halling, 1994) [2].
The PCR products were detected by electrophoresis in 1.7 per cent agarose gel in TAE buffer (1x). The PCR product (5 µl) was loaded into the respective well along with the molecular weight marker (100 bp DNA ladder, Gene DireX, Inc), positive and negative control. The electrophoresis was carried out at 100V for 30 minutes or until the tracking red dye migrated more than two third of the length of the gel tray in the buffer.

**Results and Discussion**

In the present study, five hundred and twenty five samples comprising milk (245), blood (145), aborted foetuses (15) and vaginal swabs (120) from ruminant population which were recently aborted. The cultured samples showed colonies were rounding, convex with smooth margin, translucent and pale yellow in colour on Brucella selective media (Figure A). Biochemical tests showed positive for urease and thionin but variation was seen in the strength of reaction; some isolates showed strong reaction, and others, weak reaction (Figure C, D and E) (Corbel, 1991, Banai et al., 1990) [3, 1]. Negative for H2S production mean that bacteria did not break sulphides to H2S, where it was characterized by the absence of black colour on TSI media. The negative urease means that the bacteria did not own an urease enzyme that have capability hydrolysing urea which changed yellow-coloured alkaline become pink acid by using methyl red indicator in (Figure B) (Koestanti, et al., 2018) [4]. In the present study Brucella melitensis (3) isolated from aborted foetus and vaginal swab which is consistent with Teixeira-Gomes et al., (2000) [13] and Leyla et al., (2003) [7] also isolated Brucella melitensis from vaginal swabs and aborted foetuses in sheep and goats. The DNA extracted from the samples was subjected to AMOS-PCR for species identification showed amplification with the size of 731 bp was observed in positive samples for AMOS PCR (Figure F) which is similar to findings of Ewalt and Bricker (2000) [5] who reported that Brucella AMOS-PCR was in 100% agreement with the conventional biochemical identification procedures in identifying the Brucella isolates tested.

**Table 2:** List of primers along with sequence (5’ to 3’), target gene, and size (in base pair) used for AMOS PCR.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Gene target</th>
<th>Sequence (5’-3’)</th>
<th>Amplicon size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bru-AMOS-Ab</td>
<td>alpha-ketoglutarate-dependent dioxygenase</td>
<td>GAC GAA CGG AAT TTT TCC AAT CCC</td>
<td>498</td>
</tr>
<tr>
<td>Bru-AMOS-Me</td>
<td>Glycosyltransferase, gene wboA hypothetical protein</td>
<td>AAA TCG CGT CCT TGC TGG TCT GA</td>
<td>731</td>
</tr>
<tr>
<td>Bru-AMOS-Ov</td>
<td>TRAP transporter solute receptor, TAXI family protein</td>
<td>CCG GGT CTG GCA CCA TCG TCG</td>
<td>976</td>
</tr>
<tr>
<td>Bru-AMOS-Su</td>
<td>indole-3-glycerol phosphate synthase</td>
<td>GCG CGG TTT TCT GAA GGT TCA GG</td>
<td>285</td>
</tr>
<tr>
<td>Bru-AMOS-IS711</td>
<td>IS711</td>
<td>TGC CGA TCA CTT AAG GGC CTT CAT</td>
<td></td>
</tr>
</tbody>
</table>

Ab: B. abortus; Me: B. melitensis; B. ovis. and B. suis

**References**


