Review Article

Markers for the Molecular Diagnosis of Brucellosis in Animals

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ABSTRACT

Brucellosis is a reemerging zoonotic disease, which acquire high significance because it's worldwide prevalence and threat to human health. So far, isolation of the organism is the gold standard for the confirmation of the disease. However, the biosafety concern limits the isolation without costly equipment and skilled technical staff. Under such scenario diagnosis is to be performed by the best available methods with minimum possibility of biohazards. These methods involved serological and molecular detection of antigens/antibodies and nucleic acids. Serological methods are not so prompt for species specific identification and need differentiation of vaccinated to infected animals. Whereas molecular methods are less time consuming and more sensitive and specific for genus and species identification in the same reaction. Hence present review discusses all the possible molecular targets with antigenic signatures presently being used for the genus and species identification of the Brucella. These molecular targets are the base for the confirmatory diagnosis at species and biovars levels directly from the samples without going for the isolation of the organism.


INTRODUCTION

Brucellosis, an important zoonotic disease, is a major threat to human and animal health with worldwide prevalence. Brucella spp, causative agent of brucellosis has a wide range of hosts which includes wild animals. This disease resulted into abortion, still birth and subsequent infertility (Alton 1988). A number of (nine) brucella spp are known till date are classified based on host and antigenic variation. These are B. melitensis (host: Sheep and goats), B. abortus (host: cattle), B. ovis (host: Sheep), B. suis (host: Pigs), B. neotomae (host: Wood rats), B. canis (host: Dogs), and B. microti (host: Common voles) (Cutler and Whatmore, 2003). Some species of Brucella isolated from marine animals viz: B. pinnipedialis and B. ceti (Munoz et al., 2010). The B abortus, B. melitensis and B. suis are categorized as classical Brucella and comprised of seven, three and five biovars respectively. Other Brucella species are not been differentiated into biovars. (Verger et al., 1987). The clinical picture of the disease usually comprised of retained placenta, orchitis and epididymitis, arthritis, with excretion of the Brucella spp in discharges and milk of Brucella infected infected animal (Foster et al., 2007; Munoz et al., 2010).

There are several methods for diagnosis of Brucella spp infection but the gold standard test still remains the culture isolation of the organism. (Alton et al., 1988; Lulu et al., 1988). The contaminated vaginal discharges, organs of aborted fetuses such as lymph nodes, stomach content, milk secretions of infected animals has been proved to be an important source of isolation. Phage typing has been a very handy tool for species and biovars characterization alongwith biochemical tests (Godfroid et al., 2002, Singh et al., 2014)

There are many brucellosis tests have been published to determine accurate diagnosis of brucellosis. Different serological tests have been developed by keeping various goals in mind but the validation of all these tests is still an issue, the combination of different serological tests with appreciable specificity and sensitivity values can be utilized to know the status of animals (Ariza et al., 1992; Weynants et al., 1996). It is imperative to use both direct and indirect methods for accurate and reliable diagnosis of brucellosis (Carmichael and Greene, 1990; Wanke, 2004).

Many laboratories across the world are involved in developing sensitive and specific assays based on the molecular markers of Brucella spp in order to eradicate menace of brucellosis. This present review describes the different molecular markers which can be used for the development of molecular diagnostics along with the identification and characterization of Brucella to develop a reliable assay for the eradication of the brucellosis from animals and human population.
Molecular Genetics of Brucella spp

For tracing the brucella infection biovar differentiation is an important parameter. Biovar/s strain differentiation is required in many instances specially in the areas where many biotypes are circulating in the population. For this, there is a steady progress towards development of many differential assays despite of high level of conservation among Brucella species and strains. In the recent past, genomic data for comparison studies of B. suis, B. melitensis and B. abortus have been utilized. These studies revealed that each of this species have the average genome of 2.37 x 10^9 daltons. A total of 3198 ORFs have been detected in the B. melitensis strain l6M. (Del Vecchio et al., 2002).

There are many unique and variable genes are reported from the 3100 genes available from B. melitensis, which may be used as potential diagnostic markers for quick and reliable discrimination among different Brucella species. The availability of full-genome sequencing data of three Brucella biovars (B. abortus–914, B. suis–1330 and B. melitensis16 M) has given a flip for comparison of closely related Brucella spp. The identified unique genes or “differentiating genes” that has been successfully exploited as markers or targets to differentiate among Brucella strains by applying specific PCR assays are described (Del Vecchio et al., 2002; Ratushna et al., 2006).

Detection of Brucella spp by PCR

PCR based assays can be more handy in detection of Brucella spp. from pure microbial cultures. However, when dealing with suspected field samples, there may be decrease in the efficiency due to the presence of inhibitory substances like fat, nucleases, high concentration of divalent calcium ions, which would be directly interfering in the polymerase activity, thereby affecting the DNA amplification (Rossen et al., 1992; Wilson, 1997).

<table>
<thead>
<tr>
<th>Molecular markers/Antigentic composition</th>
<th>Organism</th>
<th>Assay</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genus level (is useful for e.g. diagnosis of human brucellosis, contamination of food products)</td>
<td>B. abortus S19</td>
<td>PCR</td>
<td>Feke et al. (1990).</td>
</tr>
<tr>
<td>43kDa Omp19</td>
<td>B. abortus</td>
<td>PCR</td>
<td>Herman and De Radler, (1992)</td>
</tr>
<tr>
<td>16S rRNA gene</td>
<td>B. abortus</td>
<td>PCR</td>
<td>Romero et al. (1995)</td>
</tr>
<tr>
<td>rRNA operon</td>
<td>Brucella</td>
<td>PCR</td>
<td></td>
</tr>
<tr>
<td>BCSP1 antigenic, periplasmic protein</td>
<td>Conserved in all species and biovars of Brucella</td>
<td>PCR</td>
<td>Baily et al. (1992)</td>
</tr>
<tr>
<td>Species level (is useful in eradication and epidemiological trace back)</td>
<td>Differentiate B. abortus biovars 1,2,4 &amp; B. melitensis, B. ovis and B. suis biovar 1</td>
<td>AMOS-PCR</td>
<td>Bricker and Halling, (1994)</td>
</tr>
<tr>
<td>IS711 (IS6301)</td>
<td>Highly conserved across Brucella species but significant polymorphisms discovered.</td>
<td>PCR</td>
<td>Ficht et al., 1989</td>
</tr>
<tr>
<td>Omp2 (36kDa outer membrane protein)</td>
<td>B. abortus biovars 1,2,4 contained 115pb deletion in omp2A gene</td>
<td>PCR</td>
<td>Ficht et al., 1989</td>
</tr>
<tr>
<td>Omp2A</td>
<td>B. abortus</td>
<td>PCR</td>
<td></td>
</tr>
<tr>
<td>Omp2A and omp2B</td>
<td>B. abortus</td>
<td>PCR</td>
<td></td>
</tr>
<tr>
<td>Omp25 and dnaK gene</td>
<td>Differentiate B. melitensis and B. ovis from other biovars</td>
<td>RFLP-PCR</td>
<td>Cloeckaert et al., 1995</td>
</tr>
<tr>
<td></td>
<td>B. abortus</td>
<td>RFLP-PCR</td>
<td>Cloeckaert et al., 1995</td>
</tr>
</tbody>
</table>

Genus-specific PCR for identification of brucella are proved to be simple and adequate. The diagnostic PCRs assays so far introduced in field animals for direct screening since the first application of PCR for Brucella diagnosis (Feke et al., 1990; Rijpens et al., 1996; Amin et al., 2001; Leyla et al., 2003; O’Leary et al., 2006) various molecular marker gene viz; 16s rRNA, BCSP31, omp2, omp19, BP26, IS711 based assays are reported for genus specific identification of Brucella which are summarized in table 1. For achieving better sensitivity some real time PCR assays have also been described (Queipo-Ortuno et al., 2005; Probert et al., 2004). For distinction between strain and biotypes and to ascertain the tandem repeats several assays are described. (Ewalt and Bricker, 2000; Bardenstein et al., 2002; Probert et al., 2004; Mukherjee et al., 2005; Ferrao-Beck et al., 2006; Bricker and Ewalt, 2006; Le Fleche et al., 2006) (Table 1).

AMOS PCR Assay for Brucella:

The identification of brucella was precisely performed with various PCR assay. However, the need was to have an assay that can discriminate and different species in a same reaction. Based on five primers Bricker and Halling (1994) described an assay (Table 2) to identify selected biovars of four species of genus Brucella (AMOS– abortus, melitensis, ovis and suis). The assay was able to differentiate B. abortus (biovars 1, 2, and 4); B. melitensis (all three biovars), B. suis (biovars 1) and B. ovis (all biovars). Six bacterial species which are close to brucella viz Agrobacterium radiobacter, Agrobacterium rhizogenes, Ochrobactrum anthropi, Rhizobium leguminosarum, Rhizobium meliloti and Rhodospirillum rubrum were also differentiated based on this assay.

Table 1: Genus and Species level identification of molecular markers in different biovars of Brucella spp.
However, still the issue to discriminate vaccine strain was an issue. To achieve this in a single reaction Amos PCR assay was updated and updated AMOS assay (Bricker and Halling, 1995) was developed to differentiate two vaccine strains of Brucella abortus (strains S19 and RB51) with the addition of three new oligonucleotide primers (Table 2) and assay was designated as multiplex Brucella AMOS PCR assay (Bricker and Halling, 1995).

**Multiplex PCR for one-step Identification of Brucella spp (Bruce–Ladder)**

For rapid and one-step identification of Brucella, a novel multiplex PCR assay (Bruce–ladder) has been developed (Garcia–Yoldi, et al., 2006). This multiplex PCR assay has the cutting edge advantage compared to the previously described PCR assays, for identification and differentiation of most Brucella spp. including the vaccine strains in a single tube. The detail of molecular markers employed in this assay has been given in the table 3. Other than this many assays have been used for the detection of Brucella organisms (Table 4).

**Loop Mediated Isothermal Amplification PCR for Brucella spp**

Point-of-care diagnostics were applied in molecular diagnosis of Brucella spp. for its fast, reproducible, efficient, and highly sensitive results. The LAMP based diagnostic assay has been used in the diagnosis of Brucella spp., to harvest all the advantages in a molecular diagnostic coupled with point-of-care diagnosis. The LAMP protocol involves the use of Bst DNA polymerase with strand displacement activity and specially designed four primers identifying six regions in the gene (Notomi et al., 2000; Tomita et al., 2008). The strand displacement activity of this enzyme attributes to a special property to this PCR–based assay viz. isothermal amplification managed using water–bath without need for any costly sophisticated equipment like thermocycler and gel–documentation. The total assay time in LAMP PCR was reduced to 30–60 minutes against the conventional PCR which takes around 2–3 hours, making a better candidate for a diagnostic assay. The specific primers identifying multiple regions in the gene increases the specificity of the assay whereas the use of additional loop primers enhances the sensitivity. The important determinant of this assay that makes it more appealing for its quality as a point–of–care diagnostic is the visual detection of results.

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**Table 2: The molecular markers employed in Brucella AMOS PCR assay**

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Species specific primers</th>
<th>Primer Sequence (5'–3')</th>
<th>Size of amplicon (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><em>B. abortus</em></td>
<td>F: GACGAACCGGAATTTTTCCATCAGTTCAATCCTGCGCCGTACATTAAAGGGCCCTTCAT</td>
<td>498</td>
</tr>
<tr>
<td>2.</td>
<td><em>B. melitensis</em></td>
<td>F: AAATCGGCTGTTGCTATTCAATCCTGCGCCGTACATTAAAGGGCCCTTCAT</td>
<td>736 bp</td>
</tr>
<tr>
<td>3.</td>
<td><em>B. ovis</em></td>
<td>F: CGGGTTCCTGGCACCACCTAGTCGTCGCGCCGTACATTAAAGGGCCCTTCAT</td>
<td>976 bp</td>
</tr>
<tr>
<td>4.</td>
<td><em>B. suis</em></td>
<td>F: GCCGGGTTTTTCTGAAAGGTTTCAGGGCGCCGTACATTAAAGGGCCCTTCAT</td>
<td>285 bp</td>
</tr>
<tr>
<td>5.</td>
<td>RB51/2308</td>
<td>F: CCCCCGGAGATATGCTTCCGATCC</td>
<td>364–bp for strains 2308 and RB51, and 498–bp for other <em>B. abortus</em></td>
</tr>
<tr>
<td>6.</td>
<td>eri primers</td>
<td>F: GCGGCCGCGAAGAAGCTTATACAA</td>
<td>174 bp</td>
</tr>
</tbody>
</table>

**Table 3: The molecular markers employed in multiplex PCR assay (Bruce–ladder)**

<table>
<thead>
<tr>
<th>Molecular targets</th>
<th>Primer Sequence (5'–3')</th>
<th>Size of amplicon (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Outer membrane protein OMP–2</td>
<td>F: GCGCTACGGTGGGACGCCAAG</td>
<td>193</td>
</tr>
</tbody>
</table>
Table 4: The molecular targets employed in routine Brucella PCR assay

<table>
<thead>
<tr>
<th>Molecular targets</th>
<th>Primer Sequence (5′–3′)</th>
<th>Size of amplicon (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Outer membrane protein OMP-2 of Brucella spp.</td>
<td>F: GCGCTCAGGCTGCGGAGCGAA</td>
<td>193</td>
</tr>
<tr>
<td></td>
<td>R: ACCAGCCATTGCGGTCCGTA</td>
<td></td>
</tr>
<tr>
<td>Single step PCR for Brucella abortus protein BCSP31</td>
<td>F: TGGCCTGGTGGCCAAATATCAA</td>
<td>223</td>
</tr>
<tr>
<td></td>
<td>R: GGGCTTGCCTTCTCAGGTCG</td>
<td></td>
</tr>
<tr>
<td>16S rRNA Detection of Brucella abortus</td>
<td>F: TCGAGCGCCGGCAAGGGG</td>
<td>905</td>
</tr>
<tr>
<td></td>
<td>R: ACCATAGGTCTCCACTAA</td>
<td></td>
</tr>
</tbody>
</table>

**B. abortus vaccine RB51 detection**

- For **whoA gene**
  - F: TTAAGGCGCCATGCATGCTTTCCTCAC
  - R: GCAACCAACCCCAATGCTCACAA
  - Size: -1300 bp (RB51), approx 400 bp (all other Brucella spp. with intact whoA gene)

- For **whoA gene with part of IS711**
  - F: TTATTGTTGCCGTAATATAGGTTCTAGAAC<br>GTC
  - R: GCCCAACCAACCCCAATGCTCACAA
  - Size: 900 bp

- Real-time PCR of **Brucella abortus**
  - F: CCATTGAAGTCTGCCGAGC
  - R: CGATGCGAGAAAACATTGACCG
  - Size: 196 bp

Figure 1: B cell inducing brucella antigens as molecular signatures in different species; DLS- Dilhydrolipoamide succinyltransferase, MDH- Malate Dehydrogenase, Sco-A- Succinylcoenzyme A, Sosubunit- Synthetase alpha subunit, Binding Pro-Leu/Lle/Val-binding protein precursor; Note: many of the antigens have not been established for serum host. Established serum host have been depicted in boxes.

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This has been made possible due to the large accumulation of pyrophosphate molecules along with the DNA product, and that was exploited by the addition of fluorophore dyes like Calcein and Manganese ion. In initial reaction conditions the calcein fluorescence is quenched by manganese ion, which in the later part binds to the accumulating pyrophosphate molecules, making the calcein to fluoresce, and that was augmented by its binding with divalent Magnesium ions, ideally captured by naked eye or hand-held UV source. Colorimetric detection is also possible by the addition of dyes like Hexa napthol blue (HNB), which gives a violet color in negative samples and turns distinct sky blue in positive samples. The first report on LAMP PCR for detection of Brucella was from Ohtsuki et al., (2008), in which a BCSP31 gene based LAMP PCR assay was developed that could detect six Brucella species spanning across 22 strains, with a sensitivity of 10fg of brucella DNA detected from spiked samples, the assay conditions were 63°C for 35 minutes. For detection of Brucella spp., including B abortus, B melitensis and B ovis, an OMP25 gene based LAMP assay was developed which was reported to have a very high sensitivity detecting Brucella as low as 1.3 x 10³ CFU/ml in spiked milk samples with up to 10pg of genomic DNA per tube (Pan et al., 2011). The specificity in these tests were validated using DNA from other non–Brucella species, and were invariably found to be negative.

Other methods of PCR based identification of Brucella include a multi locus analysis of genome regions with a variable number of tandem repeats (MLVA) (Bricker et al., 2003) and multi locus sequencing of genome regions of the bacterial isolate (MLSA) (Fleche et al., 2006). These methods are based on the quantifying the number of tandem repeats in a particular locus of bacterial genome and are used for Brucella genotyping not only at the level of genus and species, but also biovars.

**Antigens of Brucella Spp. as Molecular Signature**

Many antigenic components of Brucella have been characterized from all the species. However, commonly used immunodominant antigen from brucella is the lipopolysaccharide (LPS). A number of other antigens like outer and inner membranes, cytoplasmic, and periplasmic proteins have also been characterized and are potential targets for diagnostic tests (Gupta et al., 2006a, 2006b). Some B cell response inducer *Brucella* antigens are depicted in figure 1.

*Brucella* consists of an outer layer of lipopolysaccharide–protein about 9 nm thick as an outer layer (Corbel, 1989). On culture media *Brucella* usually grow as either smooth or rough colony, with sometime mucoid type with some strains (Schurig et al., 2002). In addition to LPS, the outer membrane is also a rich source of major proteins.

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**Figure 2:** Molecular markers for molecular diagnosis of *Brucella melitensis*

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It is well known that due to presence of cross reacting epitopes like lipopolysaccharide (LPS) has its limitation as potential diagnostic reagent. Because of these drawbacks workers have renewed their interest in searching more specific antigens like OMPs and cytoplasmic proteins. Cloeckaert et al., 2002 classified outer membrane proteins of Brucella in group 2 which comprises of Omp2a and Omp2b (36 to 38 kDa) and group 3 which comprises of mainly Omp25 and Omp31 (25 to 27 and 31 to 34 kDa). Omp31 was initially cloned from B. melitensis (Vizzaino et al., 1996; Cloeckaert et al., 2002). Due to 25–kb chromosomal deletion comprising omp31 and other genes Omp31 is not expressed in B. abortus. Some differences have been reported between Omp31 from B. melitensis and Omp31 from B. ovis (Cherwonogrodzky et al., 1988; Kittelbeger et al., 1998).

The antigens which provide the base for molecular signature of the bacteria in particular species have specific cellular and molecular function characteristic to that antigen. These mainly include outer membrane proteins (omp), Ribosome–releasefactoring (CP24), Heat shock protein(HtrA, DnaK), Lumazinesynthase (18–kDa protein in Bovis and Beantis), periplasmic or cytoplasmic protein (BP26(CP28)), Dihydrolipoamide Succinyltransferase, Malate dehydrogenase, SuccinyloenzymeA, Synthetelipase subunit ABC–tptransporter, Leu–Ile–Val–binding– protein precursor, Stress protein (CIPP) and Nickeltransport (NikA) as expressed in figure 1. Although the protection studies with these antigenic markers revealed the protection only with Omp25 in mice against B. ovis and Omp31 in ram against B. ovis (Ko and Splitter, 2003).

**Table 5: Established molecular markers based assays for the Brucellosis**

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Name of Assay</th>
<th>References</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>First PCR–based diagnostic assay for Genus Brucella</td>
<td>Fekete et al. 1990; 31 kDa BCSP, omp2, 16S rRNA, 16S ribosomal RNA precursor, Stress protein (CIPP)</td>
<td>Field samples based assays</td>
</tr>
<tr>
<td>2.</td>
<td>Genus specific PCR</td>
<td>Bally et al., 1992; Leal‐Klevezaset al., 1995; Da Costa et al., 1996; Rippenset et al., 1996; Bricker, 2002; Morataet al., 2003; Bogdanovichet al., 2004; Mukherjee et al., 2005; O’Leary et al., 2006; 34% identity with B. melitensis</td>
<td>Strain typing based on locus-specific variations</td>
</tr>
<tr>
<td>3.</td>
<td>Genus specific PCR</td>
<td>Fekete et al., 1992; Leal‐Klevezaset al., 1995; Amin et al., 2001; Leylaet al., 2003; O’Leary et al., 2006, Singh et al., 2013.</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>Species specific PCR–based diagnostic assay</td>
<td>Ewalt and Bricker, 2000; Bardensteiniet al., 2002; Probert et al., 2004; Mukherjee et al., 2005; Ferrao–Becket al., 2006, Singh et al., 2014.</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>Species specific PCR–based diagnostic assay</td>
<td>Bricker and Ewalt, 2006, Le Fleche et al., 2006</td>
<td>Variable tandem repeats</td>
</tr>
<tr>
<td>6.</td>
<td>Brucella AMOS PCR assay</td>
<td>Bricker et al., 1994; Bricker and Halling, (1994)</td>
<td>For the discrimination of four Brucella species</td>
</tr>
<tr>
<td>7.</td>
<td>multiplex Brucella PCR assay</td>
<td>Kang et al., 2011; Schmook et al., 2011; Bricker et al., 1995</td>
<td>For the discrimination of brucella species and strain</td>
</tr>
<tr>
<td>8.</td>
<td>Real‐time PCR assay</td>
<td>Redkaret al., 2001; Probertet al., 2004; Navarro et al., 2004; Queipo‐Ortuno et al., 2005; Schmook et al., 2011; Winchell et al., 2009</td>
<td>Tissue based quantitative assay</td>
</tr>
<tr>
<td>10.</td>
<td>Microarray assay</td>
<td>Tian et al., 2013; Schmook et al., 2011; Viadas et al., 2009</td>
<td>Gene based assays</td>
</tr>
<tr>
<td>11.</td>
<td>Biosensor assay</td>
<td>Doganay and Doganay, 2013; Lee et al., 2000; Edelstein et al., 2000</td>
<td>Gene based assays</td>
</tr>
<tr>
<td>12.</td>
<td>LAMP test</td>
<td>Soleimani et al., 2013; Chen et al., 2013; Lin et al., 2011; Song et al., 2012; Ohitsuki et al., 2008</td>
<td>Detection of specific gene sequence in colorimetric assay</td>
</tr>
</tbody>
</table>

**Role of Molecular Diagnosis and Recombinant Proteins against Brucella Melitensis**

Out of seven species of genus brucella, *Brucella melitensis* is mainly responsible for the zoonoses. Studies have been carried out regarding different recombinant proteins of *Brucella melitensis*. Evaluation of recombinant BP26 protein in different serological tests for diagnosis of *Brucella melitensis* infection in goats is reported (Gupta, et al., 2010). A DNA vaccine encoding outer membrane protein (OMP31) of *Brucella melitensis* 16M has been found protective against *B. melitensis* challenge in mice (Gupta, et al., 2007, 2007b).

These recombinant proteins have been successfully applied to improve specificity and sensitivity of the serological diagnostic methods. Moreover, Polymerase Chain Reaction assay has been standardized to amply different molecular markers for the diagnosis of *B. melitensis* infection in goats (Figure 2). These genes can be employed for the molecular epidemiological investigation also (Gupta, et al., 2010). The primers designed vary upon the target and specific size amplicon products elucidate in electrophoreses are used for the confirmation of *B. melitensis*. 

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Various Methods Applied for the Molecular Detection of Brucella Spp.

The molecular targets/signatures of Brucella spp. is largely based on the genomic variations in different biovars. Although, the differentiating genes and conserved targets can be used for future diagnostics but it requires further evaluation in domestic animals. The gold standard test for Brucellosis still is isolation of Brucella spp. from infected animal. Different molecular markers based assay have been developed and established for rapid, confirmatory and precise diagnosis of brucellosis in clinical samples with minimum time (Table 5).

PCR-based methods that identify these molecular markers are more useful and practical as other assays are still in validation process and will take time to be an established assay for brucellosis. PCR-based methods that are simple, quick, less hazardous and possess high sensitivity (Bricker, 2002, Singh et al., 2013) for Brucella detection, especially those using the 16S rRNA as targets (Herman and De Riddler, 1992; Romero et al., 1995; O’Leary et al., 2006), and the bscp31 genes (Baily et al., 1992; Singh et al., 2014), which are highly conserved in the genus Brucella.

CONCLUSION

Most of the markers explained herein are in context of PCR assay for the diagnosis. But those markers may be potential candidate genes for developing recombinant proteins for the diagnostics and vaccines. Most of the new methods for Brucella spp. identification and typing are still in the process of development and still await validation for use with clinical samples. Control and eradication of animal brucellosis in countries like India requires serious effort to provide infrastructure to provide awareness among livestock owners, farmers, animal husbandry workers.

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CONFLICT OF INTEREST

There is no conflict of interest among authors.

REFERENCES


