Advances in Animal and Veterinary Sciences. 2 (3S): 31 – 39
Special issue – 3 (Approaches in Diagnosis and Management of Diseases of Livestock and Poultry)
http://dx.doi.org/10.14737/journal.aavs/2014/2.3s.31.39

Review Article

Markers for the Molecular Diagnosis of Brucellosis in Animals

Vivek Kumar Gupta[1]*, Shivasharanappa Nayakwadi[1], Amit Kumar[2], Kumaresan Gururaj[1], Ashok Kumar[1], Rajveer Singh Pawaiya[1]

[1]Animal Health Division, Central Institute for Research on Goats, Makhdoom, PO. Farah, Mathura, Uttar Pradesh 281 122; [2]Department of Veterinary Microbiology, UP Pt. Deen Dayal Upadhayay Pashu Chikitsa Vigyan Vishwavidyalaya Evam Go–Anusandhan Sansthan. Mathura, Uttar Pradesh 281001.
*Corresponding author: gupta.drvivek@gmail.com

ARTICLE HISTORY

Received: 2014–02–01
Revised: 2014–02–27
Accepted: 2014–02–28

Key Words: Brucellosis, Diagnosis, Molecular markers, Brucella spp

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.

ARTICLE CITATION: Gupta VK, Nayakwadi S, Kumar A, Gururaj K, Kumar A, Pawaiya RS (2014). Markers for the molecular diagnosis of brucellosis in animals. *Adv. Anim. Vet. Sci.* 2 (3S): 31 – 39.

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 infecxted infected animal (Foster et al., 2007; Munoz et al., 2010).

There are several methods for diagnosis of *Brucell*a 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 animalshas been proved to be 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.

ISSN: 2307–8316 (Online); ISSN: 2309–3331 (Print)

Molecular Genetics of Brucella Spp

For tracing the brucella infection biovar differentiation is an important parameter. Biovar/srain 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 16M. (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*–941, *B. suis*–1330 and *B. melitensis*16 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).

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 (Fekete *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.

	Molecular markers/Antigenic composition	Organism	Assay	Reference
Genus level (is useful for e.g. diagnosis of human brucellosis, contamination of food products)	43kDa Omp19	*B abortus* S19	PCR	Fekete et al. (1990).
	16S rRNA gene	*B abortus*	PCR	Herman and De Ridder, (1992)
	rRNA operon	*Brucella*	PCR	Romero et al. (1995)
	BCSP31 antigenic, periplasmic protein	Conserved in all species and biovars of *Brucella*	PCR	Baily et al. (1992)
Species level (is useful in eradication and epidemiological trace back)	IS711 (IS6501)	Differentiate *B. abortus* biovars 1,2&4; *B. melitensis*, *B. ovis* and *B.suis* biovar 1	AMOS–PCR	Bricker and Halling, (1994)
	Omp2 (36kDa outer membrane protein)	Highly conserved across *Brucella* species but significant polymorphisms discovered.	PCR	Ficht et al., 1989
	Omp2A	*B. abortus* biovars 1,2&4 contained 115pb deletion in omp2A gene	PCR	Fichtet al., 1989
	Omp2A and omp2B	*B abortus*	RFLP–PCR	Cloeckaert et al., 1995)
	Omp25 and dnaK gene	Differentiate *B. melitensis* and *B. ovis* from other biovars	RFLP–PCR	Cloeckaert et al., 1995, 1996)

ISSN: 2307–8316 (Online); ISSN: 2309–3331 (Print)

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.

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

Sr. No.	Species specific primers	Primer Sequence (5'–3')	Size of amplicon (bp)
1.	*B. abortus*	F:GACGAACGGAATTTTTCCAATCCC R:TGCCGATCACTTAAGGGCCTTCAT	498
2.	*B. melitensis*	F:AAATCGCGTCCTTGCTGGTCTGA R:TGCCGATCACTTAAGGGCCTTCAT	731bp
3.	B. *ovis*	F:CGGGTTCTGGCACCATCGTCG R:TGCCGATCACTTAAGGGCCTTCAT	976bp
4.	*B. suis*	F:GCGCGGTTTTCTGAAGGTTCAGG R:TGCCGATCACTTAAGGGCCTTCAT	285bp
Additional oligonucleotides for Vaccine strain differentiation			
5.	RB51/2308	F:CCCCGGAAGATATGCTTCGATCC R:TGCCGATCACTTAAGGGCCTTCAT	364–bp for strains 2308 and RB51, and 498–bp for other *B. abortus*
6.	eri primers	F: GCGCCGCGAAGAACTTATCAA R: CGCCATGTTAGCGGCGGTGA	178bp eri

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

Molecular targets	Primer Sequence (5'–3')	Size of amplicon (bp)
Glycosyltransferase, gene wboA	F: ATC–CTA–TTG–CCC–CGA–TAA–GG R: GCT–TCG–CAT–TTT–CAC–TGT–AGC	1682
Immunodominant antigen, gene bp26	F: GCG–CAT–TCT–TCG–GTT–ATG–AA R: CGC–AGG–CGA–AAA–CAG–CTA–TAA	450
Outer membrane protein, gene omp31	F: TTT–ACA–CAG–GCA–ATC–CAG–CA R: GCG–TCC–AGT–TGT–TGT–TGA–TG	1071
Outer membrane protein OMP–2	F: GCGCTCAGGCTGCCGACGCAA R: ACCAGCCATTGCGGTCGGTA	193
Polysaccharide deacetylase	F: ACG–CAG–ACG–ACC–TTC–GGT–AT R: TTT–ATC–CAT–CGC–CCT–GTC–AC	794
Erythritol catabolism, gene eryC (Derythrulose–1–phosphate dehydrogenase)	F: GCC–GCT–ATT–ATG–TGG–ACT–GG R: AAT–GAC–TTC–ACG–GTC–GTT–CG	587
ABC transporter binding protein	F: GGA–ACA–CTA–CGC–CAC–CTT–GT R: GAT–GGA–GCA–AAC–GCT–GAA–G	272
Ribosomal protein S12, gene rpsL	F: CAG–GCA–AAC–CCT–CAG–AAG–C R: GAT–GTG–GTA–ACG–CAC–ACC–AA	218
Transcriptional regulator, CRP family	F: CGC–AGA–CAG–TGA–CCA–TCA–AA R: GTA–TTC–AGC–CCC–CGT–TAC–CT	152

ISSN: 2307–8316 (Online); ISSN: 2309–3331 (Print)

Table 4: The molecular targets employed in routine Brucella PCR assay

Molecular targets	Primer Sequence (5'–3')	Size of amplicon (bp)
Outer membrane protein OMP–2 of *Brucella spp.*	F: GCGCTCAGGCTGCCGACGCAA R: ACCAGCCATTGCGGTCGGTA	193
Single step PCR for *Brucella abortus* protein BCSP31	F:TGGCTCGGTTGCCAATATCAA R: CGCGCTTGCCTTTCAGGTCTG	223
16S rRNA Detection of *Brucella abortus*	F: TCGAGCGCCCGCAAGGGG R: ACCATAGTGTCTCCACTAA	905
B. abortus vaccine RB51 detection		
For *wboA* gene	F:TTAAGCGCTGATGCCATTTCCTTCAC R:GCCAACCAACCCAAATGCTCACAA	~1300 bp (RB51), approx 400 bp (all other *Brucella* spp. with intact *wboA* gene)
For *wboA* gene with part of IS711	F:TTTAGTTTGCCGTAATATAGGTCTAGAACCT GTC R: GCCAACCAACCCAAATGCTCACAA	900
Real–time PCR of *Brucella abortus*	F: CCATTGAAGTCTGGCGAGC R:CGATGCGAGAAAACATTGACCG	196

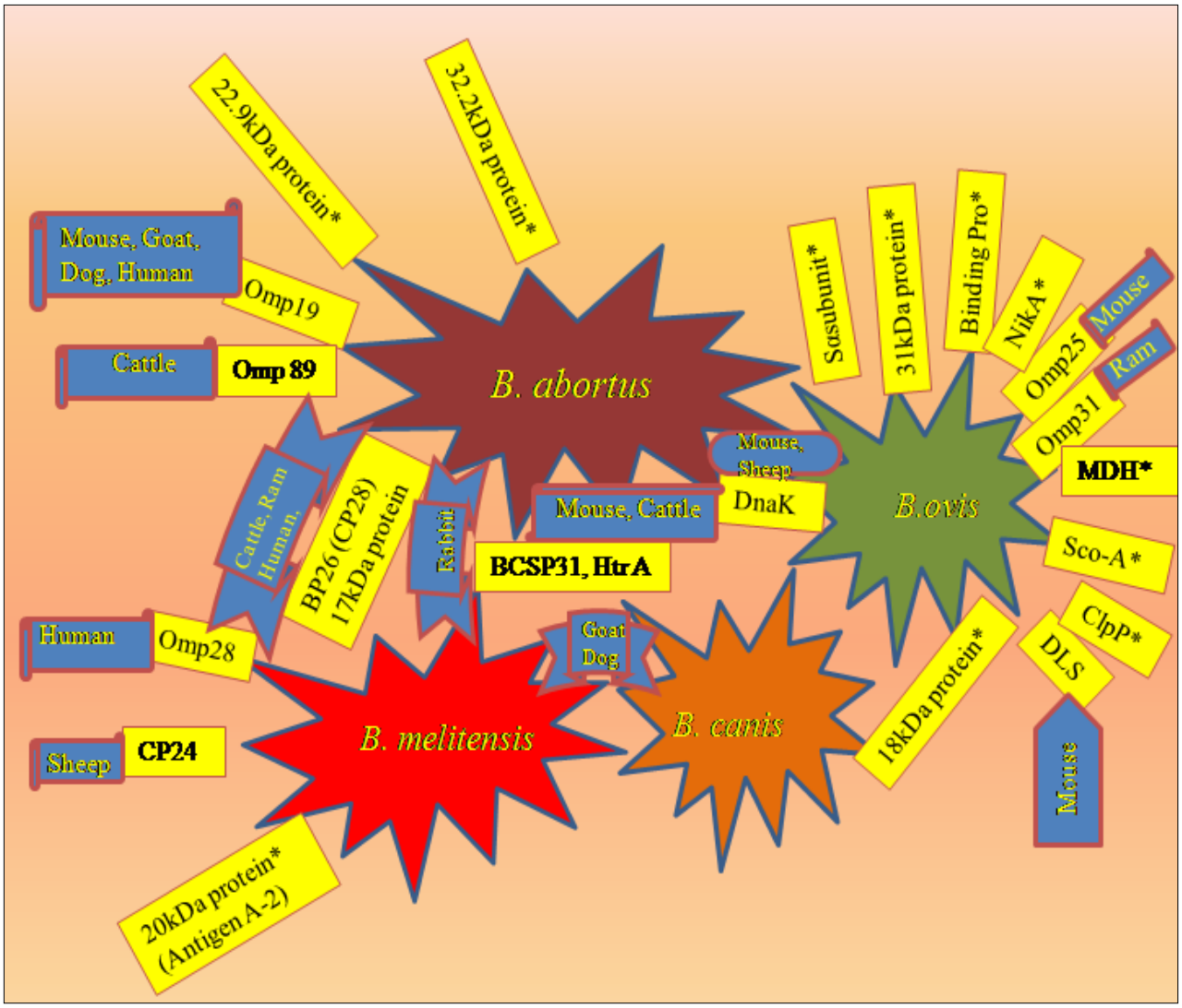

Figure 1: B cell inducing brucella Antigens as molecular signatures in different species; DLS– Dilrydrolipoamide succinyltransferase,MDH– Malate Dehydrogenase, Sco–A– Succimylcoenzyme A, Sosubunit– Synthetasealpha submit, Binding Pro–Leu/Lle/Val–binding protein precursor; Note: many of the antigens have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

ISSN: 2307–8316 (Online); ISSN: 2309–3331 (Print)

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 Manganous ion. In initial reaction conditions the calcein fluorescence is quenched by manganous 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 naphthol 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 10^3 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). I addition to LPS, the outer membrane is also a rich source of several major proteins.

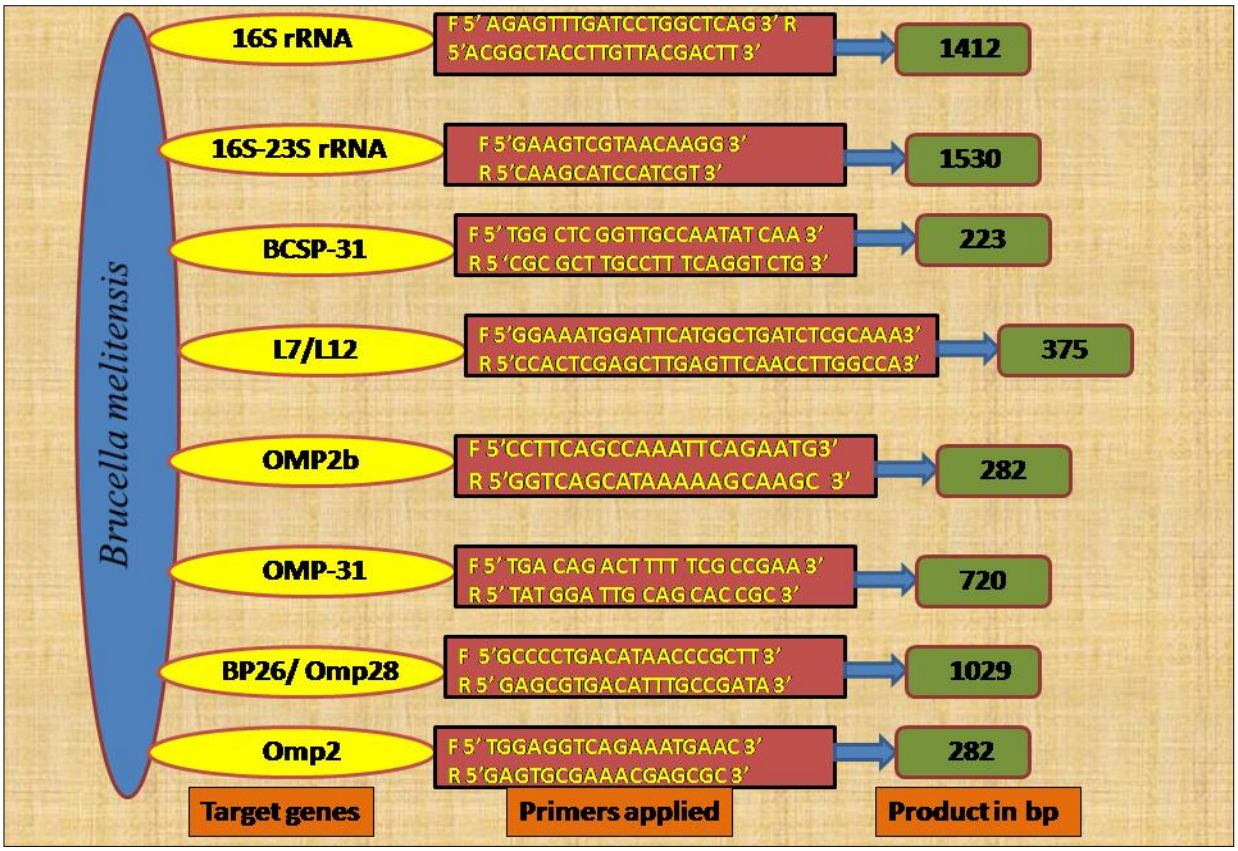

Figure 2: Molecular markers for molecular diagnosis of *Brucella melitensis*

ISSN: 2307–8316 (Online); ISSN: 2309–3331 (Print)

It is well known that due to presence of cross reacting epitopes lipopolysaccharide (LPS) has its limitation as potential diagnostic reagent. Because of these drawbacks with the anti–LPS antibodies, 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 comparises of Omp2a and Omp2b (36 to 38 kDa) and group 3 which comparises of mainly Omp25 and Omp31 (25 to 27 and 31 to 34 kDa). Omp31 was initially cloned from *B. melitensis*16M, and found to possess significant homology (34% identity) with Brucella Omp25 (Vizcaíno 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–releasingfactor (CP24), Heat shock protein(HtrA, DnaK), Lumazinesynthase (18–kDaprotein in *B.ovis and B.canis*), periplasmic or cytoplasmic protein (BP26(CP28)), Dihydrolipoamide Succinyltransferase, Malate dehydrogenase, SuccinylcoenzymeA, Synthetasealpha subunit ABC–typetransporter, Leu–Ile–Val–binding– protein precursor, Stress protein (ClpP) 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

Sr. No.	Name of Assay	References	Remrks
1.	First PCR–based for Genus Brucella	Fekete et al. 1990;	Genus specific PCR
2.	Genus specific PCR	Baily et al., 1992; Leal–Klevezaset al., 1995; Da Costa et al., 1996; Rijpenset al., 1996; Bricker, 2002; Morataet al., 2003; Bogdanovichet al., 2004; Mukherjee et al., 2005; O'Leary et al., 2006	31 kDa BCSP, omp2, 16S rRNA, IS711 and other gene markers
3.	Genus specific PCR	Feketeet al., 1992; Leal–Klevezaset al., 1995; Amin et al., 2001; Leylaet al., 2003; O'Leary et al., 2006, Singh et al., 2013.	Field samples based assays
4.	Species specific PCR–based diagnostic assay	Ewalt and Bricker, 2000; Bardensteinet al., 2002; Probertet al., 2004; Mukherjee et al., 2005; Ferrao–Beck et al., 2006, Singh et al., 2014.	strain typing based on locus–specific variations
5.	Species specific PCR–based diagnostic assay	Bricker and Ewalt, 2006; Le Fleche et al., 2006	variable tandem repeats
6.	*Brucella* AMOS PCR assay	Bricker et al., 1994; Bricker and Halling, (1994)	For the discrimination of four Brucella species
7.	multiplex Brucella PCR assay	Kang et al., 2011; Schmoock et al., 2011; Bricker et al., 1995	For the discrimination of brucella species and strain
8.	Real–time PCR assay	Redkaret al., 2001; Probertet al., 2004; Navarro et al., 2004; Queipo–Ortuno et al., 2005; Queipo–Ortuño et al., 2008; Winchell et al., 2010	Tissue based quantitative assay
9.	Hybridization/in–situ hybridization assay	Doganay and Doganay, 2013; Wellinghausen et al., 2006; Fernández–Lago et al., 2000	Oligonuclotide based fluorescence assay
10.	Microarray assay	Tian et al., 2013; Schmoock et al., 2011; Viadas et al., 2009	Gene based assays
11.	Biosensor assay	Doganay and Doganay, 2013; Lee et al., 2000; Edelstein et al., 2000;	Gene based assays
12.	LAMP test	Soleimani et al., 2013; Chen et al., 2013; Lin et al., 2011; Song et al., 2012; Ohtsuki et al., 2008	Detection of specific gene sequence in colorimetric assay

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 amplify different molecular markers for the diagnosis of B *melitensis* infectionin 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 electrophoresis are used for the confirmation of *B. melitensis.*

ISSN: 2307–8316 (Online); ISSN: 2309–3331 (Print)

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 Ridder, 1992; Romero et al., 1995;; O'Leary et al., 2006), and the bcsp31 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 these 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. Controll 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.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the support and facilities provided by the Director, CIRG, Mathura and Vice Chancellor, DUVASU, Mathura.

CONFLICT OF INTEREST

There is no conflict of interest among authors.

REFERENCES

Adone R, Francia M and Ciuchini F (2008). Evaluation of *Brucella melitensis* B115 as rough–phenotype vaccine against *B. melitensis* and *B. ovis* infections. Vaccine 26: 4913–4917.

Alton GG, Jones LM, Angus RD and Verger JM (1988). Techniques for the Brucellosis Laboratory. Paris: Institute National DeLa Recherche Agronomique.

Amin AS, Hamdy ME andIbrahim AK (2001). Detection of *Brucella melitensis* in semen using the polymerase chain reaction assay. Vet(Vet Microbiol)83: 37–44.

Ariza J, Pellicer T, Pallares R, Foz A, Gudiol F (1992) Specific antibody profile in human brucellosis. Clinicalectious Diseases (clinc infect diseases)14: 131–140.

Baily GG, Krahn JB, Drasar BS, Stocker NG (1992). Detection of *Brucella melitensis* and abortus by DNA amplification. Journal of Tropical Medicine and Hygine.(J Trop Med Hygene) 95: 271–275.

Bardenstein S, Mandelboim M, Ficht TA, Baum M, Banai M (2002) Identification of the *Brucella melitensis* vaccine strain Rev 1 in animals and humans in Israel by PCR analysis of the Pst 1 site polymorphism of its Omp 2 gene. Journal of Clinical Microbiology(J Clic Microbiol). 40: 1475–1480.

Bogdanovich T, Skurnik M, Lubeck PS, Ahren P, Hoorfar J (2004) Validated 5' nuclease PCR assay for rapid identification of the genus *Brucella*. Journal of Clinical Microbiology (J Clinc Microbiol)42: 2261–2263.

Bricker BJ (2002) PCR as a diagnostic tool for brucellosis. Veterinary Microbiology (Vet Microbiol)90: 435–446.

Bricker BJ, Ewalt DR (2006) Hoof prints: Brucella strain typing by PCR amplification of multilocus tandem–repeat polymorphisms. Methods of Molecular Biology(Methods Mol Microbiol) 345: 141–173.

Bricker BJ, Ewalt DR, Halling SM (2003) Brucella 'HOOF–Prints': Strain Typing by Multi–Locus Analysis of Variable Number Tandem Repeats (VNTRs). BMC Microbiology. 3: 15.

Bricker BJ, Ewalt DR, Olsen SC (2003) Evaluation of the Brucella abortus species specific PCR, an improved version of brucella AMOS PCR assay for cattle. Journal of Veterinary Diagnostic Investigation(J Vet Diagnos Invest) 15: 374–378.

Bricker BJ, Halling SM (1994) Differentiation of Brucella abortus bv. 1, 2, and 4 Brucella melitensis, Brucella ovis, and Brucella suis bv. 1 by PCR. Journal of Clinical Microbiology (J Clinc Microbiol)32: 2660–2666.

Bricker BJ, Halling SM (1995) Enhancement of the Brucella AMOS PCR assay for differentiation of Brucella abortus vaccine strains S19 and RB51. Journal of Clinical Microbiology(J Clinic Microbiol) 33:1640.

Carmichael LE, Greene CE (1990) Canine Brucellosis In: Infectious diseases of the Dog and Cat. Philadelphia: WB. Saunders Company. pp. 573–584.

Chen S, Li X, Li J, Atwill ER (2013) Rapid detection of Brucella spp. using loop–mediated isothermal amplification (LAMP). Methods of Molecular Biology(Methods Mol Biol) 1039:99–108

Cherwonogrodzky JW, Nielsen KH (1988) Brucella abortus1119–3 0–chain polysaccharide to differentiate sera from B. abortus S–19–vaccinated and field–strain–infected cattle by agar gel immunodiffusion. Journal of Clinical Microbiology(J Clinic Microbiol) 26:1120–1123.

Cloeckaert A, Verger JM, Grayon M, Grepinet O (1995). Restriction site polymorphism of the genes encoding the major 25kDa and 36kDa outer membrane proteins of Brucella. Microbiology(Microbiol) 141: 2111–2121

Cloeckaert A, Verger JM, Grayon M, Vizcaíno N (1996). Molecular and immunological characterization of the major outer membrane proteins of Brucella. FEMS Microbiology Letters 145:1–8.

Cloeckaert A, Vizcaíno NJ, Paquet Y, Bowden RA, Elzer PH (2002) Major outer membrane proteins of Brucella spp. past, present and future. Veterinary Microbiology(Vet Microbiol) 90:229–247.

Corbel MJ (1989) Microbiology of the Genus Brucella. In: Young Edward J, Corbel Michael J Brucellosis: Clinical Laboratory Aspects. CRC Press, Inc., Boca Raton, Florida. Pp. 53 – 72.

Cutler S, Whatmore A (2003) Progress in understanding brucellosis. Veterinary Record (Vet Rec)153: 641–642.

Da Costa M, Guillou JP, Garin–Bastuji B, Thie´baud M, Dubray G (1996) Specificity of six gene sequences for the detection of the genus Brucella by DNA amplification. Journal of Applied Bacteriology (J App Bacteriol)81: 267–275.

Del Vecchio VG, Kapatral V, Redkar RJ, Patra G, Mujer C, Los T, Ivanova N, Anderson I, Bhattacharyya A, Lykidis A, Reznik G, Jablonski L, Larsen N, D'Souza M, Bernal A, Mazur M, Goltsman E, Selkov E, Elzer PH, Hagius S, O'Callaghan D, Letesson JJ, Haselkorn R, Kyrpides N, Overbeek R (2002) The genome sequence of the facultative intracellular pathogen Brucella melitensis. Proceedings of National Academy of Sciences (Proc Ntional Acad Sci)99: 443–448.

Doganay GD, Doganay M (2013). Brucella as a potential agent of bioterrorism. Recent Pat Anti infections Drug Discovery 8(1):27–33

Edelstein RL, Tamanaha CR, Sheehan PE, Miller MM, Baselt DR, Whitman LJ, Colton RJ (2000) The BARC biosensor applied to the detection of biological warfare agents. Biosensors and Bioelectronics 14: 805–813.

Ewalt DR, Bricker BJ (2000) Validation of the Abbreviated Brucella AMOS PCR as a Rapid screening method for differentiation of Brucella abortus field strain isolates and the vaccine strains, 19 and RB51. Journal of Clinical Microbiology (J Clinic Microbiol)38(8): 3085–3086.

Fekete A, Bantle JA, Halling SM (1992a) Detection of brucella by polymerase chain reaction in bovine fetal and maternal tissues. Journal of Veterinary Diagnostic Investigation(J Vet Diagnos Invest) 4: 79–83.

Fekete A, Bantle JA, Halling SM, Sanborn MR (1990). Priliminary development of a diagnostic test for Brucella using Polymerase chain reaction. Journal of Applied Bacteriology (J Appl Bacteriol)69: 216–227.

Fekete A, Bantle JA, Halling SM, Stich RW (1992b) Amplification fragment length polymorphism in Brucella strains by use of polymerase chain reaction with arbitrary primers. Journal of Bacteriology (J App Bacteriol)174: 7778–7783.

Fernández–Lago L, Vallejo FJ, Trujillano I, Vizcaíno N (2000) Fluorescent whole–cell hybridization with 16S rRNA–targeted oligonucleotide probes to identify Brucella spp. by flow cytometry. Journal of Clinical Microbiology (J Clinic Microbiol)38:2768–2771.

Ferrao–Beck L, Cardoso R, Munoz PM, de Miguel MJ, Albert D, Ferreira AC, Marin CM, Thiebaud M, Jacques I (2006). Development of a multiplex PCR assay for polymorphism analysis of Brucella suis biovars causing brucellosis in swine. VeterinMicrobiology (Vet Microbiol)115: 269–277.

ISSN: 2307–8316 (Online); ISSN: 2309–3331 (Print)

Ficht TA, Bearden SW, Sowa BA, Adams LG (1989). DNA sequence and expression of the 36–kilodalton outer membrane protein gene of Brucella abortus. Infections and Immunity (Infect Immun)57:3281–3291.

Foster G, Osterman BS, Godfroid J, Jacques I, Cloeckaert A, (2007) Brucella ceti sp. nov. and Brucella pinnipedialis sp. nov. for Brucella strains with cetaceans and seals as their preferred hosts. International Journal of Systemic Evolutionary Microbiology(Int J Syst Evol Microbiol) 57: 2688–2693.

Garcia–yoldi D, Marin CM, De Miguel PM, Munoz PM, Vizmanos JL, Lopez–goni I (2006) Multiplex PCR assay for the identification and differentiation of all Brucella species and the vaccine strains Brucella abortus S19 and RB51 and Brucella melitensis Rev1. Clinical Chemistry(Clinic Chem) 52: 779–781.

Godfroid J, Saegerman C, Wellemans V, Walravens K, Letesson JJ, Tibor A, McMillan A, Spencer M, Sanna S, Bakker D, Pouillot R, Garin–Bastuji B (2002) How to substantiate eradication of bovine brucellosis when a specific serological reactions occur in the course of brucellosis testing. Veterinary Microbiology(Vet Microbiol) 90: 461–477.

Gupta VK (2010) Annual Report. Central Institute for research on goats (CIRG). Makhdoom, PO. Farah, Mathura, Uttar Pradesh 281 122.

Gupta VK, Kumari R, Vohra J, Singh SV, Vihan VS (2010) Comparative evaluation of recombinant BP26 protein for serological diagnosis of Brucella melitensis infection in goats. Small Ruminant Research(Small Ruminant Res) 93: 119–125.

Gupta VK, Rout PK, Vihan VS (2007b) Induction of immune response in mice with a DNA vaccine encoding outer membrane protein (OMP31) of Brucella melitensis 16M. Research in Veterinary Sciences(Res Vet Sci) 87: 305–313.

Gupta VK, Verma DK, Rout PK, Singh SV, Vihan VS (2006a) Polymerase chain reaction (PCR) for detection of Brucella melitensis in goat milk. Small Ruminant Research (Small Ruminant Res)65: 79–84.

Gupta VK, Verma DK, Singh K, Kumari R, Singh SV, Vihan VS (2006b) Single–step PCR for detection of Brucella melitensis from tissue and blood of goats. Small Ruminant Research(Res) 66: 169–174.

Gupta VK, Verma DK, Singh SV, Vihan VS (2007a) Serological diagnostic potential of recombinant outer membrane protein (Omp31) from Brucella melitensis in goat and sheep brucellosis. Small Ruminant Research (Res)70: 260–266

Herman L, De Ridder H (1992) Identification of Brucella spp. by suing the polymerase chain reaction. Applied Environmental Microbiology(App Envir Microbiol) 58: 2099–2101.

Jumas–Bilak E, Michaux–Charachon S, Bourg G, Ramuz M, Allardet–Servent A (1998) Unconventional genomic organization in the alpha–subgroup of the Proteobacteria. Journal of Bacteriology(J Bacteriol) 180: 2749–2755.

Jumas–Bitlak E, Maugard C, Michaux–Charachon, S, Allardet–Servent A, Perrin A, O'Callaghan D (1995) Study of the organization of the genomes of Escherichia coli, Brucella melitensis and Agrobacterium tumefaciens by insertion of a unique restriction site. Microbiology(Microbiol) 141: 2425–2432.

Kang SI, Her M, Kim JW, Kim JY, Ko KY, Ha YM, Jung SC (2011) Advanced multiplex PCR assay for differentiation of Brucella species. Applied Environmental Microbiology (App Envir Microbiol)77:6726–6728.

Kittelbeger R, Diack DS, Vizcaino N, Zygmunt MS, Cloeckaert A (1998) Characterization of an immuno–dominant antigen in Brucella ovis and evaluation of its use in an enzyme– linked Immunosorbent assay. Veterinary Microbiology (Vet Microbiol)59: 213–227.

Ko J, Splitter GA (2003). Molecular host–pathogen interaction in brucellosis: current understanding and future approaches to vaccine development for mice and humans. Clinical Microbiology Review(Clinic Microbiol Rev) 16: 65–78.

Le Fleche F, Jacques I, Grayon M, Al Dahouk S, Bouchon P, Denoeud F, Nöckler K, Neubauer H, Guilloteau LA, Vergnaud G (2006) Evaluation and Selection of Tandem Repeat Loci for a Brucella MLVA Typing Assay. BMC Microbiology(Microbiol) 6:9.

Le Flèche P, Jacques I, Grayon M, Dahouk Sal, Bouchon P, Denoeud F, Nöckler K, Neubauer H, Guilloteau LA, Vergnaud G (2006) Evaluation and selection of tandem repeat loci for a Brucella MLVA typing assay. BMC Microbiology (Microbiol)6:9.

Leal–Klevezas DS, Martinez VIO, Lopez MA, Martinez SJP (1995) Single step PCR for detection of Brucella spp. from blood and milk of infected animals. Journal of Clinical Microbiology(J Clinic Microbiol) 3: 3087–3090.

Lee WE, Thompson HG, Hall JG, Bader DE (2000) Rapid detection and identification of biological and chemical agents by immunoassay, gene probe assay and enzyme inhibition using a silicon–based biosensor. Biosensors and Bioelectronics 14:795–804.

Leyla G, Kadri G, Umarn O (2003) Comparison of polymerase chain reaction and bacteriological culture for the diagnosis of sheep brucellosis using aborted fetus samples. Veterinary Microbiology(Vet Microbiol) 93: 53–61.

Lin GZ, Zheng FY, Zhou JZ, Gong XW, Wang GH, Cao XA, Qiu CQ (2011) Loop–mediated isothermal amplification assay targeting the omp25 gene for rapid detection of Brucella spp. Molecular Cell Probes(Mol Cell Probes) 25:126–129.

Lulu AR, Araz GF, Khatib MI, Mustafa MY, Yusuf AR, Fenech FF (1988) Human brucellosis in Kuwait: a prospective study of 400 cases. Quaternary Journal of Medicine (J Med)66: 39–54.

Manish K, Chand P, Rajesh C, Teena R, Sunil K (2013) Brucellosis: An updated review of the disease. Indian Journal of Animal Sciences(Indian J Anim Sci) 83: 3–16.

Mantur BG, Amarnath SK (2008) Brucellosis in India a review. Journal of Biosciences(J BioSci) 33:539–547.

Michaux S, Paillisson J, Carles–Nurit MJ, Bourg G, Allardet–Servent A, Ramuz M (1993) Presence of two independent chromosomes in the Brucella melitensis 16M genome. Journal of Bacteriology (J Bacteriol)175: 701–705.

Morata P, Queipo–Ortuno M.I, Reguera JM, Garcia–Ordonez MA, Cardenas A, Colmenero JD (2003) Development and evaluation of a PCR–enzyme linked immunosorbent assay for diagnosis of human brucellosis. Journal of Clinical Microbiology(J Clinic microbial) 41:144–148.

Moriyon I, Grillo MJ, Monreal D, Gonzalez D, Marin C, Lopez–Goni I, Mainar–Jaime RC, Moreno E, Blasco JM (2004). Rough vaccines in animal brucellosis: structural and genetic basis and present status. Veterinary Research(Vet Res) 35: 1–38.

Mukherjee F, Jain J, Grillo MJ, Blasco JM, Nair M (2005) Evaluation of Brucella abortus S19 vaccine strains by bacteriological tests, molecular analysis of ery loci and virulence in BALB/c mice. Biologicals 33, 153–160.

Mukherjee F, Jain J, Patel V, Nair M (2007) Multiple genus–specific markers in PCR assays improve the specificity and sensitivity of diagnosis of brucellosis in field animals. Journal of Medical Microbiology (J Med Microbiol)56:1309–1316.

Muñoz PM, Mariana B, Arnal M, J de Miguel M, Revilla M, Martínez D, Vicente J, Acevedo P, Oleaga A, Ruiz–Fons F, Marín CM, Prieto JM, de la Fuente J, Barral M, Barberán M, de Luco DF, Blasco JM, Gortázar C (2010) Spatial distribution and risk factors of Brucellosis in Iberian wild ungulates. BMC Infectious Diseases(Infect Disesses) 10: 46.

Navarro E, Casao MA, Solera J (2004) Diagnosis of human brucellosis using PCR. Expert Review of Molecular Diagnosis(Exp Rev Mol Diagn)4:115–123.

Notomi T, Okayama H, Masubuchi H, Yonekawa T, Watanabe K, Amino N, Hase T (2000) Loop–mediated isothermal amplification of DNA. Nucleic Acids Research(Res) 28, E63.

O'Leary S, Sheahan M, Sweeney T (2006) Brucella abortus detection by PCR assay in blood, milk and lymph tissue of serologically positive cows. Research in Veterinary Sciences (Res Vet Sci)81: 170–176.

Ohtsuki R, Kawamoto K, Kato Y, Shah MM, Ezaki T, Makino SI (2008) Rapid detection of Brucella spp. by the loop–mediated isothermal amplification method. Journal of Applied Microbiology (J App Microbiol)104:1815–23

OIE (2009) Manual of standards for diagnostic tests and vaccines. Paris: Office international des epizooties(OIE).

Pan W, Wang JY, Shen HY, Zhao MQ, Ju CM, Dong XY, Yi L, Chen JD (2011) Development and application of the novel visual Loop–mediated isothermal amplification of Omp25 sequence for rapid detection of Brucella sp. Journal of Animal and Veterinary Advances (J Anim Vet Adv)10: 2120–2126.

Paulsen IT, Seshadri R, Nelson KE, Eisen JA, Heidelberg JF, Read TD, Dodson RJ, Umayam L, Brinkac LM, Beanan MJ (2002) The Brucella suis genome reveals fundamental similarities between animal and plant pathogens and symbionts. Proceedings of national Academy of Sciences(Proc National Acad Sci). 99:13148–13153.

Probert WS, Schrader KN, Khuong NY, Bystrom SL, Graves MH (2004) Real–time Multiplex PCR Assay for Detection of Brucella spp., B. abortus, and B. melitensis. Journal of Clinical Microbiology(J Clinic Microbiol) 42: 1290–1293.

Queipo–Ortuño MI, Colmenero JD, Bravo MJ, García–Ordoñez MA, Morata P (2008) Usefulness of a quantitative real–time PCR assay using serum samples to discriminate between inactive, serologically positive and active humanbrucellosis. Clinical Microbiology of Infections(Clinic Microbiol Infect) 14:1128–1134.

Queipo–Ortuno, MI, Colmenero JD, Baeza G, Morata P (2005) Comparison between Light cycler real–time polymerase chain reaction (PCR) assay with serum and PCR enzyme–linked immunosorbent assay with whole blood samples for the diagnosis of human brucellosis. Clinical Infectious Diseases (Clinic Infect Diseases)40: 260–264.

ISSN: 2307–8316 (Online); ISSN: 2309–3331 (Print)

Radostits OM, Blood DC, Gay CC (editors) (1994) A text book of the diseases of cattle, sheep, pigs, goats and horses, 8th edn. Veterinary Medicine(Vet Med). Baillie`reTindall.789–812.

Ratushna VG, Sturgill DM, Ramamoorthy S, Reichow SA, He Y, Lathigra R, Sriranganathan N, Halling SM, Boyle SM, Gibas CJ (2006). Molecular targets for rapid identification of Brucella spp. BMC Microbiology(Microbiol) 6:13.

Redkar R, Rose S, Bricker B, DelVecchio, V (2001). Real–time detection of Brucella abortus, Brucella melitensis, and Brucella suis. Molecular Cellular Probes(Mol Cellu Probes) 15:43–52.

Rigby CE, Fraser AD (1989) Plasmid transfer and plasmid–mediated genetic exchange in Brucella abortus. Canadian Journal of Veterinary Research(CJ Vet Res) 53: 326–330.

Rijpens NP, Jannes G, Van Asbroeck M, Rossau R, Herman LM (1996) Direct detection of Brucella spp. in raw milk by PCR and reverse hybridization with 16S–23S rRNA spacer probes. Applied Environmental Microbiology (App Envir Microbiol)62: 1683–1688.

Romero C, Gamazo C, Pardo M, Lopez–Goñi I (1995a) Specific detection of Brucella DNA by PCR. Journal of Clinical Microbiology (J Clinic Microbiol)33: 615–617.

Romero C, Pardo M, Grilló MJ, Díaz R, Blasco JM, Lopez–Goñi I (1995b) Evaluation of PCR and indirect enzyme–linked immunosorbent assay on milk samples for diagnosis of brucellosis in dairy cattle. Journal of Clinical Microbiology(J Clinic Microbiol) 33: 3198–3200.

Rossen L, Norskov P, Holmstrom K, Rasmussen OF (1992) Inhibition of PCR by components of food samples, microbial diagnostic assays and DNA–extraction solutions. International Journal of Food Microbiology (Int J Food Microbiol)17: 37–45.

Sanchez DO, Zandomeni RO, Cravero S, Verdun RE, Pierrou E, Faccio P, Diaz G, Lanzavecchia S, Aguero F, Frasch AC (2001) Gene discovery through genomic sequencing of Brucella abortus. Infection and Immunity(Infect Immun) 69: 865–868.

Schmoock G, Ehricht R, Melzer F, Elschner M, Tomaso H, Neubauer H, Al Dahouk S (2011) Development of a diagnostic multiplex polymerase chain reaction micro array assay to detect and differentiate Brucella spp. Diagnostic Microbiologyogy of Infectious Diseases(Diagnos Infect Diseases) 71:341–53

Scholz HC, Hubalek Z, Sedlacek I, Vergnaud G, Tomaso H, Al Dahouk S, Melzer F, Kampfer P, Neubauer H, Cloeckaert A, Maquart M, Zygmunt MS, Whatmore AM, Falsen E, Bahn P, Gollner C, Pfeffer M, Huber B, Busse HJ, Nockler K (2008) Brucella microti sp. nov., isolated from the common vole Microtus arvalis. International Journal of Systemic and Evolutionary Microbiology (Int J Syst Evol Microbiol)58: 375–382.

Schurig GC, Sriranganathan N, Corbel MJ (2002) Brucellosis vaccines: past, present and future. Veterinary Microbiology(Vet Microbiol) 90: 479–496.

Singh A, Gupta VK, Kumar A, Singh VK, Shivasaranappa N (2013). 16S rRNA and Omp31gene based molecular characterization of field strains of *B. melitensis* from aborted foetus of goats in India. The Scientific World Journal. 2013, Article no. 160376, 7 pages.

Singh A, Gupta VK, Kumar A, Singh VK, Shivasaranappa N (2014) Omp31gene based molecular detection of B. melitensis from serum samples of goats. Indian Journal of Animal Sciences (Indian J Anim Sci)(In Press).

Soleimani M, Shams S, Majidzadeh–A K. 2013. Developing a real–time quantitative loop–mediated isothermal amplification assay as a rapid and accurate method for detection of Brucellosis. Journal of Applied Microbiology (J App Microbiol)115:828–834.

Song L, Li J, Hou S, Li X, Chen S (2012) Establishment of loop–mediated isothermal amplification (LAMP) for rapid detection of Brucella spp. and application to milk and blood samples. Journal of Microbiology Methods(J Microbiol Methods) 90:292–297.

Sreevatsan S, Bookout JB, Ringpis F, Perumaalla VS, Fitch TA, Adams LG, Haguis SD, Elzer PH, Bricker BJ (2000) A multiplex approach to molecular detection of Brucella abortus and/or Mycobacterium B. ovis infection in cattle. Journal of Clinical Microbiology(J clinic Microbiol) 38: 2602–2610.

Tian M, Qu J, Han X, Zhang M, Ding C, Ding J, Chen G, Yu S. 2013. Microarray–based identification of differentially expressed genes in intracellular Brucella abortus within RAW264.7 cells. PLoS One. 8:e67014.

Tomita N, Mori Y, Kanda H, Notomi T (2008) Loop–mediated isothermal amplification (LAMP) of gene sequences and simple visual detection of products. Natural Protocols 3: 877– 882.

Verger JM, Grimont F, Grimont PA, Grayon M (1987) Taxonomy of the genus Brucella. Ann. Inst. Pasteur Microbiology (Microbiol)138: 235–238.

Viadas C, Rodríguez MC, García–Lobo JM, Sangari FJ, López–Goñi I (2009) Construction and evaluation of an ORFeome–based Brucella whole–genome DNA microarray. Microbes Pathology(Pathol). 47:189–195.

Vizcaíno N, Cloeckaert A, Zygmunt MS, Dubray G (1996) Cloning, nucleotide sequence, and expression of the Brucella melitensis omp31 gene coding for an immunogenic major outer membrane protein. Infection and Immunity(Infect Immun) 64:3744–3751.

Wanke MM (2004). Canine brucellosis. Animal Reproduction Science (Anim Prod Sci)82/83: 195–207.

Wellinghausen N, Nöckler K, Sigge A, Bartel M, Essig A, Poppert S (2006) Rapid detection of Brucella spp. in blood cultures by fluorescence in situ hybridization. Journal of Clinical Microbiology (J Clinic Microbiol)44:1828–1830.

Weynants V, Tibor A, Denoel PA, Saegerman C, Godfroid J, Thiange P, Letesson JJ (1996) Infection of cattle with Yersinia enterocolitica O: 9 a cause of the false positive serological reactions in bovine brucellosis diagnostic tests. Veterinary Microbiology(Vet Microbiol) 48: 101–112.

Wilson IG (1997). Inhibition and facilitation of nucleic acid amplification. Applied Environmental Microbiology(App Envir Microbiol) 63: 3741–3751.

Winchell JM, Wolff BJ, Tiller R, Bowen MD, Hoffmaster AR (2010) Rapid identification and discrimination of Brucella isolates by use of real–time PCR and high–resolution melt analysis. Journal of Clinical Microbiology (J Clinic Microbiol)48:697–702.

Yagupsky P. (1994) Detection of Brucella melitensis by BACTEC NR660 blood culture system. Journal of Clinical Microbiology (J Clinic Microbiol)32: 1899–1901..

ISSN: 2307–8316 (Online); ISSN: 2309–3331 (Print)