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PCR Based Molecular Diagnostic Assays for Brucellosis: A Review

Vinay Kumar*, Nitish Bansal, Trilok Nanda, Aman Kumar, Rajni Kumari and Sushila Maan

Department of Animal Biotechnology, College of Veterinary Sciences, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar – 125004, India

**Corresponding author*

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A B S T R A C T

Brucellosis is a worldwide re-emerging zoonotic disease of public health and economic importance. It affects a large number of domestic as well as wild animals and results in heavy losses to the animal husbandry sector. The direct culture of bacteria and serological test are the gold standard for *Brucella* spp. identification in the clinical samples. However, these assays have various limitations therefore PCR can be a potential tool to address aforesaid limitations and can be used for early detection of causative agents in disease condition. In this review, we have tried to discuss most of the currently used PCR based methods for detection of *Brucella* at genus and species level in different biological samples. Now a day, these assays are becoming very important tools for the identification of *Brucella* at genus, species and biovar level.

Introduction

Brucellosis is widespread zoonosis that affect a large number of domestic as well as wild animals. It is caused by species of genus *Brucella* which belong to *Brucellaceae* family of α -2 subdivision of *Proteobacteria*. The genus is composed of eight terrestrial species and two marine species according to host preference and pathogenicity. Terrestrial species include *B. abortus, B. melitensis, B. suis. B. ovis, B. canis, B. neotomae, B. microti* and *B. inopinata. Brucella* species isolated from marine mammals are *B. ceti* and *B. pinnipedialis* (Cutler *et al.*, 2005). However, genetic studies indicate that the six classical species of *Brucella* are originated from a single genomospecies i.e. *B. melitensis*, based on DNA-DNA reassociation (Verger *et al.*, 1985) suggesting that the other *Brucella* species be described as biovars of *B. melitensis*. Based on DNA polymorphism at their outer membrane protein 2 (*omp2)* locus and host preference existence of two species that infect marine mammals can be explained (Cloeckaert *et al.*, 2001).

The gold standard for laboratory detection of brucellosis is largely based on serological tests or isolation of *Brucella* from clinical samples. However, these methods are time consuming and labour intensive and also have

reduced sensitivity in chronic cases. Isolation of *Brucella* is hazardous and resource intensive as it requires level 3 bio containment facilities (Yu and Nielsen, 2010) and highly skilled personnel to handle biological samples and live bacteria for eventual identification and biotyping. To overcome these disadvantages, now a day, methods based on nucleic acid amplification are commonly used for detection of *Brucella* spp. in clinical samples. In recent years, considerable efforts have been made to improve the sensitivity and specificity of these molecular assays with an aim of technical ease in performance and to lower costs. To date, several authors have published various reports on molecular identification and characterisation of *Brucella* spp. by PCR-based methods.

In this review, our main focus was to discuss most of the currently used PCR based molecular assays (conventional PCR, nested and semi nested PCR, multiplex PCR, real-time PCR and loop mediated isothermal amplification assay) using different target genes for detection of *Brucella* at genus as well as species level in different type of biological samples.

Conventional PCR

Polymerase chain reaction (PCR), invented by Kary Mullis in 1983, is a technique of molecular biology which is used to amplify single copy or a few copies of DNA into millions of copies of that particular DNA within hours. After its discovery, PCR is probably the most widely used technique in molecular biology for a broad variety of applications (Mullis *et al.,* 1986 and Bartlett and Sterling, 2003).

The implementation of PCR for diagnosis of Brucellosis was started in early 90's (Fekete *et al.,* 1990; Bricker and Halling, 1995 and Romero *et al.,* 1995). Then after, various target genes were used by researchers for the development of PCR based molecular assays for genus and species level detection of *Brucella.* Among these various target genes, *Brucella* cell surface protein (bscp) 31 (Baily *et al.,* 1992) is most used gene for development of genus specific identification of *Brucella* till date. Other target genes that have been used for identification of *Brucella* at genus level includes: outer membrane proteins (omp) 2 (Leal-Klevezas *et al.,* 1995), omp 2b, omp2a and omp25/omp31 (Vizcaino *et al.,* 2004 and Imaoka *et al.,* 2007); 16SrRNA (Romero *et al.,* 1995); 16S-23S intergenic transcribed spacers (ITS) (Rijpens *et al.,* 1996 and Bricker *et al.,* 2000); 16S-23S rDNA inter space (Keid *et al.,* 2007) and per (Lubek *et al.,* 2003 and Bogdanovich *et al.,* 2004). Some scientists (Fekete *et al.,* 1992) used arbitrary primers for identification of *Brucella* spp. in animals. The assays based on above discussed gene targets were found to be highly sensitive and specific in detection of *Brucella* spp. but their specificity and sensitivity vary according to the combination of primers. This can be better explained by the studies done by Navarro *et al.,* 2002; Baddour and Alkhalifa, 2008, who compared the sensitivity of 3 pairs of primers encoding *bcsp 31*, 16S rRNA of *B. abortus* and *omp2* gene. The results showed that the primers encoding *bcsp 31* were most sensitive followed by *omp2* gene based primers in detection of *Brucella* in clinical samples while, 16S rRNA based primer pair was found to be least sensitive. These results further indicates that the use of PCR assay based on more than one marker give increased sensitivity and higher specificity providing a better molecular diagnostic approach for screening of clinical samples in animals as well as humans.

Imaoka *et al.,* (2007) designed a combinatorial PCR to detect *Brucella* spp. at

the genus level by using four pairs of primers based on bcsp31 and outer membrane proteins (omp2b, omp2a, omp31). These four pair of primers was used in different combinations in four individual PCRs to identify *B. abortus, B. melitensis, B. canis,* and *B. suis*. This combinatorial PCR was found to be an ideal method for diagnosis of human brucellosis. A novel PCR assay was developed by Hinic *et al.,* (2008) for the rapid identification and differentiation of six recognized spp. of *Brucella* genus (except *B. microti*) in seven different PCR reactions. The assay was found to be highly efficient and specific, and was found suitable for both conventional and real-time PCR formats.

There are many biovars of *Brucella* spp. and the prevalence of these biotypes varies according to geographic areas means one species of *Brucella* may be more prevalent in particular geographical areas than others. For identification and differentiation of these biovars of *Brucella* spp. some researchers have developed PCR assays based on either polymorphism arising from species-specific localization of the genetic element IS711 in the *Brucella* chromosome (Bricker and Halling, 1994 and Nashwa *et al.,* 2007) or use of primers specific to IS711with AMOS-ERY PCR primer cocktail (Ocampo-sosa *et al.,* 2005). Leal-Klevezas *et al.,* (2000) also described one such PCR based on omp2 gene that can differentiate *B. abortus* biovars 1, 2, and 4 from other *Brucella* species.

At various Farms and in field conditions *Brucella abortus* strain S19 and RB51 are used as vaccines for cattle and buffaloes. Therefore, these vaccine strains must be differentially diagnosed from pathogenic *Brucella abortus* strains to avoid misdiagnosis. One such PCR assay based on DNA polymorphism at the ERY locus was described by Sangari and Aguero (1994) to detect and differentiate S19 strain from field strains. While, Vemulapalli *et al.,* 1999 identify an IS711 genetic element interrupting the wboA gene of *Brucella abortus* vaccine strain RB51 and on the basis of this they developed RB51-specific PCR which can differentiate vaccine strain RB51 from other *Brucella* species.

In continuation, Nan *et al.,* (2014) described a duplex PCR for differentiation of the vaccine strain of *Brucella suis* S2 and *Brucella suis* biovar1 from other field strains of *Brucella* spp. In this study they designed the transcriptional regulator IclR primers based on a 25bp deletion in the *Brucella suis* S2 genome, for the specific detection of *Brucella suis* S2. While, for detection of field strain of *Brucella suis* biovar1 they used IS711 primers, selected from the *Brucella abortus-melitensis-ovis-suis* (AMOS) PCR (Bricker and Halling, 1994). Recently Amenov *et al.,* (2017) developed a Rapid Xtreme Chain Reaction (XCR) assay for the detection of brucellosis in cattle targeting host specific antigen gene and IS711 repeats from the transposase gene of *Brucella*. The XCR is a quick and highly sensitive PCR variant for target DNA amplification.

Nested and semi-nested PCR

The nested PCR is a variant of PCR in which two different set of primers are used to reduce non-specific binding in products due to the amplification of unexpected primer binding sites. The first set of primers is designed to anneal the sequences upstream of the second set of primers and is used in an initial PCR reaction. The initial PCR reaction generates a reaction product that is used as the template for the second round of amplification using a set of primers internal to the first one (Carr *et al.*, 2010). Same as nested, semi-nested PCR has two different pairs of PCR primers, but the second pair of primers has one primer identical to the first pair (Seah *et al.,* 1995).

The nested PCR and semi-nested PCR have also been used by many researchers in developing diagnostic assays with an aim of easy and early detection of human as well as animal brucellosis. Henault *et al.,* (2000) developed and validated a nested-PCR based on the IS6501/711 sequence for the detection of *Brucella* in animal samples. Two such studies (Al Nakkas *et al.,*2002; 2005) on development and validation of single tube nested PCR assays, based on IS711, have been performed for the diagnosis of human brucellosis in Kuwait. These studies showed that the use of nested primers gave increased sensitivity and higher specificity providing a better molecular diagnostic approach for human brucellosis. Costa *et al.,* (2013) evaluate species-specific nested PCR based on a previously described (Xavier *et al.,* 2010) species-specific PCR assay for detection of *Brucella ovis* in semen and urine samples of experimentally infected rams. The results showed that performance of the species-specific nested PCR was significantly more sensitive as compare to genus-specific PCR. Izadi *et al.,* (2014) evaluated and compared the performance of *bcsp* 31 gene based nested and semi nested PCR in detecting the *Brucella* spp. in dairy products. They concluded that nested PCR has higher sensitivity and accuracy as compared to semi nested PCR.

Multiplex PCR

Multiplex-PCR is a widely used molecular biology technique in which amplification of multiple targets can be achieved in a single tube using multiple primers and a temperature-mediated DNA polymerase in a thermal cycler. This technique has advantage over uniplex PCR in terms of considerable savings in time, less expense on reagents, less contamination in making reaction mixture and detection of multiple pathogens at once (Elnifro *et al.,* 2000).

The first multiplex PCR for identification of different species of *Brucella* was published in 1994, since then, numerous multiplex PCRs have been described for identification of *Brucella* at the species level and partly at the biovar level using different primer combinations. Bricker and Halling, (1994) described a multiplex AMOS PCR for identification of *Brucella abortus, B. melitensis, B. ovis,* and *B. suis* at the species level by using five primers. This multiplex PCR was also able to detect biovars 1, 2, and 4 of *B. abortus*; all 3 biovars of *B. melitensis*; biovar 1 of *B. suis* and biovar 1 of *B. ovis* but was unable to differentiate other *Brucella* species (such as *B. canis, B. neotomae, B. pinnipedialis, and B. ceti*) and individual biovars within a species like *B. abortus* biovars 3, 5, 6, 7, and 9 and *B. suis* biovars 2, 3, 4, and 5. Over a period of time many scientists have tried to improve this assay by incorporation of additional strain-specific primers (Bricker and Halling, 1995; Ewalt and Bricker, 2000; Bricker *et al.,* 2003; Ocampo-Sosa *et al.,* 2005) and also tried validation of this assay on a large number of reference strains as well as field strains (Kamal *et al.,* 2013 and Orzil *et al.,* 2016). Ewalt and Bricker, (2002) developed a multiplex *Brucella abortus* species-specific polymerase chain reaction (BaSS-PCR) for identification as well as differentiation of *Brucella abortus* field and vaccine strains. Due to reports of misdiagnosis by this assay in different laboratory Bricker and co-workers described an improved multiplex BaSS-PCR that can specifically recognize field strains of *B. abortus* biovars 1, 2 and 4 and can distinguish the aforementioned strains from vaccine strains, other *Brucella* species and unrelated bacteria that might give cross reactions (Bricker *et al.,* 2003). Ferrao-Beck *et al.,* (2006) developed a multiplex PCR assay based on sequence variation in omp2b gene for analysis of polymorphism in *Brucella suis*. Though, this assay successfully

differentiate the biovars 1, 2 and 3 of *B. suis* in reference strains but was found unable to differentiate *B. suis* biovar 1 from biovars 2 and 3 in the field strains isolated from animals.

Garcia-Yoldi *et al.,* (2006) developed a multiplex PCR assay (Bruce-ladder) to identify all *Brucella* spp. including 6 terrestrial, marine species and the vaccine strains S19, RB51, and Rev. 1. There after several multiplex PCR assays (Lopez-Goni *et al.,* 2008; Kang *et al.,* 2011; Kumar *et al.,* 2011 and Mirnejad *et al.,* 2013) were developed by researchers with slight modifications and inclusion of newly designed species specific primers in Bruce-ladder PCR assay with an aim of identification of all *Brucella* spp., different biovars and vaccine strains in a single PCR test. The results of these assays concluded that Bruce-ladder PCR assay has advantage over AMOS PCR in terms of identification and differentiation of all *Brucella* species and the vaccine strains in the same test with lesser time requirement to perform the assay, minimal sample preparation and minimal contamination (Lopez-Goni *et al.,* 2008). Schmoock *et al.,* (2011) developed a multiplex PCR based microarray assay to detect and differentiate *Brucella* spp. The gene targets included genus-specific sequences in bcsp31, perA, cgs, and omp2b, as well as chromosomal regions showing species-specific hybridization patterns. This newly developed *Brucella* array tube assay was found to be an easy-to-handle molecular test for high-throughput and parallel analysis which allows fast response in brucellosis outbreaks.

Several authors have also published the multiplex assay showing high sensitivity and specificity of the assay related to *Brucella* by taking either *Brucella* genus or *Brucella* species as one of the member of multiplex assay (Saunders *et al.,* 2007 and Moustacas *et al.,* 2013).

Although, several researchers have successfully developed and validated the multiplex PCR assays for diagnosis of animal and human brucellosis but, development and validation of such multiplex assay requires laborious optimization (Cha and Thilly, 1993 and Brownie *et al.,* 1997) and there are always chances of formation of primer-dimers, nonspecific amplification and template contamination during the PCR reaction.

Real-time PCR

Real-Time PCR, also known as quantitative PCR (qPCR), is a variant of PCR. In contrast to conventional PCR, it monitors the amplification and detection of the targeted DNA molecule during the progression of reaction that is in "real time". Real-time detection of PCR products is made possible by adding a fluorescent molecule in the reaction that reports an increase in the amount of DNA with a proportional increase in fluorescent signal. Detection of amplified products in real-time PCR can be done mainly by two common chemistries: (i) intercalating of non-specific fluorescent dyes with any double-stranded DNA (e.g. - Sybr Green dye), and (ii) DNA probes (e.g. - TaqMan Probe) consisting of sequence-specific oligonucleotides and labelled with a fluorescent reporter that gives fluorescence only after hybridization with complementary sequence of target DNA.

Many researchers have developed real-time PCR assays for detection and differentiation of *Brucella* spp. based on genus specific and species specific genes. In this series of development of assays, firstly Redkar *et al.,* (2001) developed a fluorescence resonance energy transfer (FRET) based real time PCR

assays to specifically identify *B. abortus* biovars, *B. melitensis*, and biovar 1 of *B. suis*. In this study they designed and used a common IS711 based genus specific forward primer while reverse primers and hybridization probes were species-specific. Newby *et al.,* (2003) evaluated SYBR green I, 5'-exonuclease and hybridization probes for real time detection of *B. abortus* targeting alkB gene and the IS711element and reported comparable sensitivity for all three assays, however, hybridization probe assay shows highest specificity. Probert *et al.,* (2004) redesigned the primer and probe using the similar gene targets that were described by Redkar *et al.,* (2001) and developed a TaqMan based multiplex real time PCR assay for detection of *Brucella* spp., *B. abortus*, and *B. melitensis*. Bogdanovich *et al.,* (2004) developed and validated a 5′- hydrolysis probe based real time PCR assay targeting the perosamine synthetase gene (per) along with internal amplification control (IAC) for direct verification of suspected *Brucella* colonies on agar plates. In this study, *Brucella* specific primers were used as described previously (Lubek *et al.,* 2003) while, three different TaqMan probes (6-carboxyfluorescein [FAM] labeled) that is: Bruc1, in close proximity to the 3′ end of the forward primer; Bruc2, in the middle of the amplified fragment; and Bruc3, within a few nucleotides of the 3′ end of the reverse primer were designed. Queipo-Ortuno *et al.,* (2005) developed a SYBR Green I based Light Cycler real-time PCR (LC-PCR) assay targeting bcsp31 gene for detection of *Brucella* DNA in serum samples and compared it with PCR-ELISA assay, conventional PCR assay and dot-blot hybridisation. The results showed that analytical sensitivity of the LC-PCR assay was higher than those of conventional PCR procedures, followed by dot-blot hybridisation and PCR-ELISA. In another study, Kattar *et al.,* (2007) developed three different real-time PCR assays for diagnosis of human brucellosis at genus level using three different target genes i.e. 16S-23S ITS, omp25 and omp31 for primer and probe designing. They evaluated these assays on whole blood and paraffin-embedded tissues of humans and found that 16S-23S ITS primer and probe were most sensitive and could be used for the diagnosis of human brucellosis in the clinical samples.

Fretin *et al.,* (2008) describes four single real time PCR assays based on single nucleotide polymorphism (SNP) signatures (*pts*P-1677, *pyr*H816–817, *rpo*B-244 and *dna*K-1005) for the rapid identification and biovar characterization of *B. suis*. The present assay was evaluated on 137 field strains of worldwide origin and the results showed that allelic profiles were unique and globally consistent for each *B. suis* biovar, however, some *B. suis* biovar 3 field strains matched the allelic profile of *B. suis* biovar 1. Hinic *et al.,* (2008) describe a novel PCR assay using seven individual reactions for the rapid detection of the *Brucella* genus, and the differentiation between six recognized *Brucella* species in both conventional and real time format. The primer and TaqMan probes in this study were designed from: BMEII0466 gene for *B. melitensis*, BruAb2_0168 gene for *B. abortus*, BR0952 gene for *B. suis*, BOV_A0504 gene for *B. ovis*, BMEII0635–0636 genes for *B. canis* and BMEII0986–0988 genes for *B. neotomae*. In continuation, Hinic *et al.,* (2009) validated this novel real time PCR assay for the detection of *Brucella* spp. in blood and tissue samples from naturally infected wild boars. The authors also compared the performance real time PCR assay with the results of bacterial isolation and three different serological tests for detection of brucellosis: RBT, i-ELISA and c-ELISA and found real-time PCR assay as high sensitive and appealing assay for diagnosis of Brucellosis. Bounaadja *et al.,* (2009) developed and evaluated the

performance of three real time PCR assays similar to Kattar *et al.,* (2007) but the target genes for designing of primer and TaqMan probe were different i.e. IS711, bcsp31 and per genes. The results of this study showed that the IS711-based assay was the most sensitive method to detect *Brucella* at genus level.

Winchell *et al.,* (2010) developed a rapid SYBR Green based real-time PCR assay identification and differentiation of *Brucella* and described a new technique i.e. High-Resolution Melt Analysis (HRM) for analysis of real time PCR results. The same HRM analysis was further used by Piranfar *et al.,* (2015) for development of a multiplex real time PCR assay for detection and differentiation of *Brucella abortus* and *Brucella melitensis*. Hansel *et al.,* (2015) developed a novel real time PCR assay for identification of *Brucella suis* biovars 1-4 in clinical samples. The primers and TaqMan probe in the present assay were designed from BS1330_II0657 locus encoded on chromosome 2 of *B. suis* biovars 1. The authors claimed this assay as a novel method that can detect all practically relevant *B. suis* bv 1–4based on single qPCR probe. Kim *et al.,* (2015) developed a new real-time PCR assay to distinguish *B. abortus* from other *Brucella* species by using a hybprobe designed from a specific SNP on fbaA gene. The present real-time PCR showed greater sensitivity than that of conventional PCR and previously described TaqMan probe based real-time PCR assays which make it a valuable tool for differentiating *B. abortus* infection with rapidity and accuracy. Nan *et al.,* (2016) developed a rapid cycleave PCR assay for differentiating the vaccine strain *Brucella abortus* A19 from field strains. This study was designed on SNP (C_{587} –T_{587}) in BAbS19_I07270 (arginyl–transfer RNA–protein transferase) locus. The primer and probe were designed based on this SNP. Kaden *et al.,* (2017) described a novel real time PCR assay for specific detection of *Brucella melitensis* by designing a new primer and TaqMan probe from acetyl-CoA acetyltransferase gene having two base pair deletion which makes this assay as highly specific for *B. melitensis*.

Apart from the above discussed novel real time PCR assays, a lot of work has been done so far on the validation aspect of these real time PCR assays in different countries (Al Dahouk *et al.,* 2007; Queipo-Ortuno *et al.,* 2008; Amoroso *et al.,* 2011; Doosti and Dehkordi, 2011; Kumar *et al.,* 2015; Mukherjee *et al.,* 2015; Awwad *et al.,* 2016; Kumar *et al.,* 2017; Saini *et al.,* 2017 and Patel *et al.,* 2018). All these studies have found real time PCR as fast, sensitive and reliable tool for early detection of causative organism in the biological samples so that control and eradication programmes can be adopted as early as possible to minimise the losses to animal husbandry sector.

Apart from these, many researchers either have used *Brucella* with other bacteria or different spp. of *Brucella* for the development and validation of multiplex real time PCR assays for simultaneous detection of more than two causative agents in a single reaction (Probert *et al.,* 2004; Kumar *et al.,* 2011; Selim *et al.,* 2014 and Tutar *et al.,* 2018).

Queipo-Ortuno *et al.,* (2009) developed a fluorescent hybridization probe based multiplex real time PCR assay for rapid detection and differential diagnosis of extra pulmonary tuberculosis from brucellosis by targeting bcsp31gene for *Brucella* genus and senX3-regX3 gene for *Mycobacterium tuberculosis* for designing of primers and probe. Sanjuan-Jimenez *et al.,* (2013) developed and compared different SYBR Green based multiplex real time PCR strategies for the simultaneous differential

diagnosis between extrapulmonary tuberculosis and focal complications of brucellosis in human clinical cases. In this study, three different primer pair combinations (senX3-regX3+ IS711, senX3-regX3+ bcsp31 and IS6110+ IS711) for simultaneous detection of *Mycobacterium tuberculosis* complex and *Brucella* spp. were evaluated and compared in single tube multiplex real time PCR format and the results showed that senX3-regX3+ IS711 pair was 100% specific in detection of the above discussed targets in clinical samples

Loop-mediated isothermal amplification (LAMP)

Loop mediated isothermal amplification (LAMP) is a single tube that amplifies DNA with high specificity, efficiency and rapidity under isothermal conditions (Notomi *et al.*, 2000). In contrast to the PCR technology in which the reaction is carried out with a series of alternating temperature steps or cycles, isothermal amplification is carried out at a constant temperature of 60–65 °C, and does not require a thermal cycler. This method employs a DNA polymerase with high strand displacement activity as well as replication activity and a set of four specially designed primers that recognize a total of six distinct sequences on the target DNA (Notomi *et al.*, 2000). Detection of amplification product can be determined via photometry for turbidity caused by an increasing quantity of magnesium pyrophosphate precipitate in solution as a by product of amplification (Mori *et al.*, 2001). After the discovery of LAMP assay by Notomi *et al.*, 2000 a lot of work have been done on developing LAMP assays (Ohtsuki *et al.*, 2008; Lin *et al.*, 2011; Pan *et al.*, 2011; Song *et al.*, 2012; Perez-Sancho *et al.*, 2013; Chen *et al.*, 2013; Soleimani *et al.*, 2013; Karthik *et al.*, 2014; Marcos *et al.*, 2015; Prusty *et al.*, 2016a, Prusty *et al.*, 2016b and Amenov *et al.*, 2017) for identification of *Brucella* spp. in infected animals as well as humans. The target genes for development of these different assays were similar to those which were used in PCR assays. Therefore, likewise PCR these assays targeted bscp-31 (Ohtsuki *et al.*, 2008; Marcos *et al.*, 2015 and Prusty *et al.*, 2016a), omp 25 (Lin *et al.*, 2011; Pan *et al.*, 2011; Song *et al.*, 2012; Chen *et al.*, 2013; Soleimani *et al.*, 2013 and Prusty *et al.*, 2016b) and IS711 (Perez-Sancho *et al.*, 2013 and Amenov *et al.*, 2017) for identification of *Brucella* at the genus-specific level while BruAb_0168 gene was used (Karthik *et al.*, 2014 and Kang *et al.*, 2015) for identification of *Brucella abortus*. These assays were found to be highly sensitive, specific and easy to perform and can be used in field conditions for detection of *Brucella* at genus level as well as at species level in clinical samples.

Other newly developed assay

Sergueev *et al.*, (2017) developed a highly sensitive and specific bacteriophage (*Brucella*phages- such as Tb, S708, Fz, Wb or Bk) based assay for detection of *Brucella abortus* in liquid cultures, blood and potentially in other key biological samples. The method allowed reliable detection of single *B. abortus* cells in simulated blood samples within 72 h and identification of higher concentrations of bacteria in shorter time (24-48 h).

Pal *et al.*, (2017) developed a new test for detection of *Brucella* in biological samples using based on hybridization of gold nanoparticles (AuNP) with pathogen specific DNA sequence which, on detection yield a visual colour change. In this study, thiol modified probe specific to bcsp31 was designed which codes for outer membrane protein of *Brucella*. The results showed that the AuNP-oligo probe can be used for the simple, rapid and "point-of-care" visual

detection of *Brucella* with high sensitivity and specificity from a broad range of bovine samples, including semen, milk and urine.

This study concludes as in developing countries like India there are always chances of re-emergence and outbreaks of brucellosis in the animal population. Therefore, for implementation of control and eradication programmes of brucellosis, in these countries, requires rapid and early detection of *Brucella* genus at the species and at the biovar level in the outbreaks so that losses to the animal husbandry can be minimised. Although, gold standard for diagnosis of brucellosis remains isolation of *Brucella* spp. from biological samples, PCR-based methods are more useful and practical in implementing the control and eradication strategies for brucellosis in developing countries. PCR-based assays allow rapid and more-sensitive identification of the causative organisms in biological samples, compared with traditional techniques. However, PCR protocols lack standardization and most of the new assays that have been developed for identification and typing of *Brucella* spp. still need validation for use with clinical samples. Also, during development, standardization, validation and implementation of an assay for routine laboratory testing of brucellosis, false-positive results due to specimen contamination or amplicon carryover, standard positive controls, negative controls and internal control, also require strict attention.

References

Al Dahouk, S., Nockler, K., Scholz, H.C., Pfeffer, M., Neubauer, H. and Tomaso, H. (2007). Evaluation of genus-specific and species-specific real-time PCR assays for the identification of *Brucella* spp. *Clin. Chem. Lab. Med.* 45(11): 1464-1470.

Al Nakkas, A..F, Wright, S.G., Mustafa, A.S. and Wilson, S. (2002). Single-tube nested PCR for the diagnosis of human brucellosis in Kuwait. *Ann. Trop. Med. Parasitol*. 96(4): 397-403.

Al-Nakkas, A., Mustafa, A.S. and Wright, S.G. (2005). Large-scale evaluation of a single-tube nested PCR for the laboratory diagnosis of human brucellosis in Kuwait. *J. Med. Microbiol*. 54(8): 727-730.

Amenov, A.A., Kalendar, R.N., Abeldenov, S.K., Musakhmetov, A.S., Li, P.K., Kiribayeva, A.K., Khassenov, B.B. (2017). Development and application of rapid xtreme chain reaction and loop-mediated isothermal amplification assays for the detection of leukaemia and brucellosis of cattle. *Eurasian J. Appl. Biotechnol*. 3: 49-55.

Amoroso, M.G., Salzano, C., Cioffi, B., Napoletano, M., Garofalo, F., Guarino, A. and Fusco G. (2011). Validation of a real-time PCR assay for fast and sensitive quantification of *Brucella* spp. in water buffalo milk. *Food Control* 22: 1466-1470.

Awwad, E., Farraj, M., Essaw, T., Sabri, I., Adwan, K., Rumi, I., Manasra, A., Bărăităreanu, S., Gurău, M.R. and Daneş, D. (2016). Detection of *Brucella* genome by real time PCR from the milk of small ruminants in the West Bank, Palestine. *Bullet. UASVM Vet. Med.* 73(1): 89-94.

Baddour, M.M, and Alkhalifa, D.H. (2008). Evaluation of three polymerase chain reaction techniques for detection of *Brucella* DNA in peripheral human blood. *Canadian J. Microbiol.* 54(5): 352-357.

Baily, G.G., Krahn, J.B., Drasar, B.S. and Stoker, N.G. (1992). Detection of *Brucella melitensis* and *Brucella abortus* by DNA amplification. *J.*

Trop. Med. Hyg. 95: 271-275.

Bartlett, J.M.S. and Stirling, D. (2003). A short history of the Polymerase Chain Reaction. In: Methods in Molecular Biology (2nd edn.). pp. 3-6.

Bogdanovich, T., Skurnik, M., Lubeck, P.S., Ahrens, P. and Hoorfar, J. (2004). Validated 5' nuclease PCR assay for rapid identification of the genus *Brucella*. *J. Clin. Microbiol*. 42: 2261-2263.

Bounaadja, L., Albert, D., Chénais, B., Hénault, S., Zygmunt, M.S., Poliak, S. and Garin-Bastuji, B. (2009). Real-time PCR for identification of *Brucella* spp.: a comparative study of IS711, bcsp31 and per target genes. *Vet Microbiol*.137(1-2): 156-164.

Bricker, B.J. and Halling, S.M. (1994). Differentiation of *Brucella abortus* bv. 1, 2, and 4, *Brucella melitensis*, *Brucella ovis*, and *Brucella suis* bv. 1 by PCR. *J. Clin. Microbiol*. 32: 2660-2666.

Bricker, B.J. and Halling, S.M. (1995). Enhancement of the *Brucella* AMOS PCR assay for differentiation of *Brucella abortus* vaccine strains S19 and RB51. *J. Clin. Microbiol.* 33: 1640-1642.

Bricker, B.J., Ewalt, D.R., MacMillan, A.P., Foster, G. and Brew, S. (2000). Molecular characterization of *Brucella* strains isolated from marine mammals. *J. Clin. Microbiol*. 38:1258-1262.

Bricker, B.J., Ewalt, D.R., Olsen, S.C. and Jensen, A.E. (2003). Evaluation of the *Brucella abortus* species-specific polymerase chain reaction assay, an improved version of the *Brucella* AMOS polymerase chain reaction assay for cattle. *J. Vet. Diagn. Invest*. 15: 374-378.

Brownie, J., Shawcross, S., Theaker, J., Whitcombe, D., Ferrie, R., Newton, C. and Little, S. (1997). The elimination of primer-dimer accumulation in PCR. *Nucleic Acids Res.* 25: 3235-3241.

Carr, J., Williams, D.G. and Hayden, R.T. (2010). Molecular detection of multiple respiratory viruses. In: Techniques and applications for the clinical laboratory. pp. 289-300.

Cha, R.S. and Thilly, W.G. (1993). Specificity, efficiency, and fidelity of PCR. *PCR Methods Appl*. 3: 18-29.

Chen, S., Li, X., Li, J. and Atwill E.R. (2013). Rapid detection of *Brucella* spp. using Loop-Mediated Isothermal Amplification (LAMP). *Methods Mol Biol*.1039: 99-108.

Cloeckaert, A., Verger, J.M., Grayon, M., Paquet, J.Y., Garin-Bastuji, B., Foster, G. and Godfroid, J. (2001). Classification of *Brucella* spp. isolated from marine mammals by DNA polymorphism at the omp2 locus. *Microbes Infect*. 3: 729-738.

Costa, L.F., Nozaki, C.N., Lira, N.S.C., Antunes, J.M.A.P., Xavier, M.N., Costa, E.A., Paixão, T.A., Santos, R.L. and Megid, J. (2013). Species-specific nested PCR as a diagnostic tool for *Brucella ovis* infection in rams. *Arq. Bras. Med. Vet. Zootec*. 65(1): 55-60.

Cutler, S.J., Whatmore, A.M. and Commander, N.J. (2005). Brucellosis – New aspect of an old disease. *J. Appl. Microbial*. 98(6): 1270-1281.

Doosti, A. and Dehkordi, G.P. (2011). Application of real-time PCR for identification and differentiation of *Brucella abortus* and *Brucella melitensis* in cattle. *Bulgarian J. Vet. Med.* 14(2): 109-115.

Elnifro, E.M., Ashshi, A.M., Cooper, R.J. and Klapper, PE. (2000). Multiplex PCR: optimization and application in diagnostic virology. *Clin. Microbiol. Rev.* 13(4): 559-570.

Ewalt, D.R. and Bricker, B.J. (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. *J. Clin. Microbiol*. 38: 3085-3086.

Ewalt, D.R. and Bricker, B.J. (2002). Identification and differentiation of *Brucella abortus* field and vaccine strains by BaSS-PCR. In: Methods in molecular biology series: PCR detection of microbial pathogens. Sachse, K., Frey, J. (edt.), pp. 97-108. Humana Press Inc., Totowa, NJ.

Fekete, A., Bantle, J.A., Halling, S.M. and Sanborn, M.R. (1990). Preliminary development of a diagnostic test for *Brucella* using polymerase chain reaction. *J. Appl. Bacteriol*. 69: 216-227.

Fekete, A., Bantle, J.A., Halling, S.M. and Stich, R.W. (1992). Amplification fragment length polymorphism in *Brucella* strains by use of polymerase chain reaction with arbitrary primers. *J. Bacteriol*. 174: 7778- 7783.

Ferrao-Beck, L., Cardoso, R., Munoz, P.M., de Miguel, M.J., Albert, D., Ferreira, A.C., Marín, C.M., Thiébaud, M., Jacques, I., Grayon, M., Zygmunt, M.S., Garin-Bastuji, B., Blasco, J.M. and Sá, M.I. (2006). Development of a multiplex PCR assay for polymorphism analysis of *Brucella suis* biovars causing brucellosis in swine. *Vet Microbiol*. 115: 269-277.

Fretin, D., Whatmore, A.M., Dahouk, S.A., Neubauer, H., Garin-Bastuji, B., Albert, D., Van Hessche, M., Menart, M., Godfroid, J., Walravens, K. and Wattiau, P. (2008). *Brucella suis* identification and biovar typing by real-time PCR. Vet. Microbiol. doi:10.1016/j.vetmic.2008.04.003.

Garcia-Yoldi, D., Marin, C.M., de Miguel, M.J., Munoz, P.M., Vizmanos, J.L., 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. *Clin. Chem*. 52: 779-781.

Hänsel, C., Mertens, K., Elschner,M.C. and Melzer, F. (2015). Novel real-time PCR detection assay for *Brucella suis*. *Vet. Rec. Open* 2: e000084. doi:10.1136/vetreco-2014-000084.

Henault, S., Calvez, D., Thiébaud, M., Boulière, M. and Garin-Bastuji, B. (2000). Validation of a nested-PCR based on the IS6501/711 sequence for the detection of *Brucella* in animal samples. In: Proceedings of the Brucellosis 200 International Research Conference 9I including the 53rd Brucelllosis Reserach Conference, Nimes, France. Paris: INRA.

Hinić, V., Brodard, I., Thomann, A., Cvetnić, Ž., Makaya, P.V., Frey, J. and Abril, C. (2008). Novel identification and differentiation of *Brucella melitensis*, *B. abortus*, *B. suis*, *B. ovis*, *B. canis*, and *B. neotomae* suitable for both conventional and real-time PCR systems. *J. Microbiol. Methods* 75: 375-378

Hinić, V., Brodard, I., Thomann, A., Holub, M., Miserez, R. and Abril, C. (2009). IS*711*-based real-time PCR assay as a tool for detection of *Brucella* spp. in wild boars and comparison with bacterial isolation and serology. *BMC Vet. Res.* 5: 22-29.

Imaoka, K., Kimura, M., Suzuki, M., Kamiyama, T. and Yamada A. (2007). Simultaneous detection of the genus *Brucella* by combinatorial PCR. *Jpn. J. Infect. Dis*. 60: 137-139.

Izadi, A., Moslemi, E., Tabatabaei, P.A.S. and Kheiri, M.H. (2014). *Brucella* spp. detection in dairy products using nested and hemi nested PCR

techniques. *Ann. Biol. Res*. 5(1): 124-131.

Kaden, R., Ferrari, S., Alm, E. and Wahab, T. (2017). A novel real-time PCR assay for specific detection of *Brucella melitensis*. *BMC Infect. Dis.*17: 230-235.

Kamal, I.H., Gashgari, B.A., Moselhy, S.S., Kumosani, T.A. and Abulnaja, K.O. (2013). Two-stage PCR assay for detection of human brucellosis in endemic areas. *BMC Infect. Dis.* 13: 145-149.

Kang, S., Her, M., Kim, J.W., Kim, J.Y., Ko, K.Y., Ha, Y.M. and Jung, S.C. (2011). Advanced multiplex PCR assay for differentiation of *Brucella* Species. *Appl. Environ. Microbiol.* 77(18): 6726-6728.

Karthik, K. Rathore, R., Thomas, P., Arun, T.R., Viswas, K.N., Agarwal, R.K., Manjunathachar, H.V. and Dhama, K. (2014) Loop-mediated isothermal amplification (LAMP) test for specific and rapid detection of *Brucella abortus* in cattle, Vet. Q. 34(4): 174-179.

Kattar, M.M., Zalloua, P.A., Araj, G.F., Samaha-Kfoury, J., Shbaklo, H., Kanj, S.S., Khalife, S. and Deeb, M. (2007). Development and evaluation of real-time polymerase chain reaction assays on whole blood and paraffin-embedded tissues for rapid diagnosis of human brucellosis. *Diagn. Microbiol. Infect. Dis*. 59: 23-32.

Keid, L.B., Soares, R.M., Vasconcellos, S.A., Chiebao, D.P., Salgado, V.R., Megid, J. and Richtzenhain, L.J. (2007). A polymerase chain reaction for detection of *Brucella canis* in vaginal swabs of naturally infected bitches. *Theriogenology* 68: 1260-1270.

Kim, J.Y., Kang, S., Lee, J.J., Lee, K., Sung, S.R., Jung, S. C., Park, Y.H., Yoo, H.S. and Her, M. (2015). Differential diagnosis of *Brucella abortus* by real time PCR based on a Single-Nucleotide Polymorphisms. *Clin. Microbiol*. 4: 5-10.

Kumar, S., Tuteja, U., Kumari, S., Singh, D.K., Kumar, A. and Kumar, O. (2011). Rapid multiplex PCR assay for the simultaneous detection of the *Brucella* genus, *B. abortus*, *B. melitensis* and *B. suis*. *J. Microbiol. Biotechnol*. 21(1): 89-92.

Kumar, V., Kumar, A., Debnath, A., Batra, K., Bansal, N., Gupta, A.K., Tiwari, A. and Maan, S. (2015). Development of real time PCR assay for diagnosis of *Brucella* spp. *Haryana Vet.* 54(2): 99-102.

Kumar, V., Maan, S., Kumar, A., Batra, K., Chaudhary, D., Dalal, A., Gupta, A.K., Bansal, N., Sheoran, N. and Maan, N.S. (2017). Real time PCR assay for differentiation of *Brucella abortus* and *Brucella melitensis. Indian J. Anim. Res.* doi:10.18805/ijar.v0iOF.8464.

Leal-Klevezas, D.S., Martinez-Vazquez, I.O., Lopez-Merino, A. and Martinez-Soriano, J.P. (1995). Single-step PCR for detection of *Brucella* spp. from blood and milk of infected animals. *J. Clin. Microbiol*. 33: 3087-3090.

Leal-Klevezas, D.S., Martinez-Vazquez, I.O., Garcia-Cantu, J., Lopez- Merino, A. and Martinez-Soriano,J.P. (2000). Use of polymerase chain reaction to detect *Brucella abortus* biovar 1 in infected goats. *Vet. Microbiol*. 75: 91-97.

Lin, G.Z., Zheng, F.Y., Zhou, J.Z., Gong, X.W., Wang, G.H., Cao, X.A. and Qiu, C.Q. (2011). Loop-mediated isothermal amplification assay targeting the omp25 gene for rapid detection of *Brucella* spp. *Mol Cell Probes*. 25: 126-129.

Lopez-Goni, I., Garcia-Yoldi, D., Marin, C.M., de Miguel, M.J., Munoz, P.M.,

Blasco, J.M., Jacques, I., Grayon, M., Cloeckaert, A., Ferreira, A.C., Cardoso, R., Correa de Sa, M.I., Walravens, K., Albert, D. and Garin-Bastuji, B. (2008). Evaluation of a multiplex PCR assay (Bruceladder) for molecular typing of all *Brucella* species, including the vaccine strains. *J. Clin. Microbiol*. 46: 3484-3487.

Lübeck, P.S., Skurnik, M., Ahrens, M.P. and Hoorfar, J. (2003). A multiplex PCR-detection assay for *Yersinia enterocolitica* serotype O:9 and *Brucella* spp. based on the perosamine synthetase gene. In: Skurnik, M., Bengoechea, J.A. and Granfors, K. (edt). The genus of *Yersinia*. pp. 451-453, Kluwer Plenum, New York.

Marcos, D.T., Gioffré, A.K., Cucchi, M.E.C., Caimi, K.C., Ruybal, P., Zumárraga, M.J. and Cravero, S.L. (2015). LAMP technology: Rapid identification of *Brucella* and *Mycobacterium avium* subsp. *Paratuberculosis. Braz. J. Microbiol*. 46(2): 619-626.

Mirnejad, R., Mohamadi, M., Piranfar, V., Mortazavi, S.M., and Kachuei, R. (2013). A duplex PCR for rapid and simultaneous detection of *Brucella* spp. in human blood samples. *Asian Pac. J. Trop. Med.* 6(6): 453-456.

Mori, Y., Nagamine, K., Tomita, N. and Notomi, T. (2001). "Detection of loop-mediated isothermal amplification reaction by turbidity derived from magnesium pyrophosphate formation". *Biochem. Biophys. Res. Commun*. 289(1): 150-154.

Moustacas, V.S. , Silva, T.M.A., Costa, L.F., Xavier, M.N., Carvalho Jr, C.A., Costa, É.A., Paixão, T.A. and Santos, R.L. (2013). Species-specific multiplex PCR for the diagnosis of *Brucella ovis*, *Actinobacillus seminis*, and *Histophilus somni* infection in rams. *BMC Vet. Res*. 9: 51-58.

Mukherjee, F., Nagmani, K., Surendra, K.S.N.L., Subramanian, B.M., Bahekar, V.S., Prasad, A., Rana, S.K., Muthappa, P.N., Sharma, G.K. and Srinivasan, V.A. (2015). Optimization and validation of a diagnostic real-time PCR for bovine brucellosis. *Adv. Anim. Vet. Sci.* 3(11): 577-587.

Mullis, K.B., Henry, A.E., Norman, A., Glenn, T.H., Randal, K.S. and Stephen, J.S. (1986). Process for amplifying, detecting, and/or-cloning nucleic acid sequences. U.S. Patent 4,683,195.

Nan, W., Tan, P., Wang, Y., Xu, Z., Mao, K., Peng, D., Chen, Y. (2014). Duplex PCR for differentiation of the vaccine strain *Brucella suis* S2 and *B. suis* biovar 1 from other strains of *Brucella* spp. *Vet. J.* 201(3): 427-428.

Nan, W., Zhang, Y., Tan, P., Xu, Z., Chen, Y., Mao, K. and Chen, Y. (2016). A rapid cycleave PCR method for distinguishing the vaccine strain *Brucella abortus* A19 in China. *J. Vet. Diagn. Invest.* 28(3): 214-218.

Nashwa, M., Helmy, H., Zaki, M. and Sami, S.A. (2007). Identification and differentiation of *Brucella melitensis* Rev.1 vaccine and *B. melitensis* biovar 3 fields isolates in Egypt by serological and PCR–RFLP techniques. *J Appl Sci Res* 3: 841-847.

Navarro, E., Escribano, J., Fernandez, J.A. and Solera, J. (2002). Comparison of three different PCR methods for detection of *Brucella* spp. in human blood samples. *FEMS Immunol. Med. Microbiol*. 34: 147-151.

Newby, D.T., Hadfield, T.L. and Roberto, F.F. (2003). Real-time PCR detection of *Brucella abortus*: a comparative study of SYBR green I, 5‟-exonuclease, and hybridization probe assays. *Appl. Environ. Microbiol.* 69: 4753-4759.

Notomi, T., Okayama, H., Masubuchi, H., Yonekawa, T., Watanabe, K., Amino, N. and Hase, T. (2000). Loop-mediated isothermal amplification of DNA. *Nucleic Acids Res*. 28(12): 63.

OIE, (2009). Terrestrial Animal Health Code, brucellosis (World Organisation for Animal Health).

Ocampo-Sosa, A.A., Agüero-Balbin, J., Garcia-Lobo, J.M. (2005). Development of a new PCR assay to identify *Brucella abortus* biovars 5, 6 and 9 and the new subgroup 3b of biovar 3. *Vet Microbiol*. 110: 41-51.

Ohtsuki, R., Kawamoto, K., Kato, Y., Shah, M.M., Ezaki, T. and Makino, S.I. (2008). Rapid detection of *Brucella* spp. by the loop-mediated isothermal amplification method. *J. Appl. Microbiol*. 104:1815-1823.

Orzil, L.L., Preis I.S., Almeida, I.G., Souza, P.G., Filho, P.M.S., Jacinto, F.B. and Fonseca A.A. Jr. (2016). Validation of the multiplex PCR for identification of *Brucella* spp. *Ciência Rural, Santa Maria* 46(5): 847-852.

Pal, D., Boby, N., Kumar, S., Kaur, G., Ali, S.A., Reboud, J., Shrivastava, S., Gupta, P.K., Cooper, J.M. and Chaudhuri, P. (2017). Visual detection of *Brucella* in bovine biological samples using DNA-activated gold nanoparticles. *PLoS ONE* 12(7): e0180919.

Pan, W., Wang, J.Y., Shen, H., Zhao, M., Ju, C., Dong, X., Yi, L. and Chen, J.D. (2011). Development and application of the novel visual loop-mediated isothermal amplification of Omp25 sequence for rapid detection of *Brucella* sp. *J. Anim. Vet. Adv*. 10: 2120-2126.

Patel, K.B. Chauhan, H.C., Patel, S.S., B Patel, K., Shrimali, M.D. and Chandel, B.S. (2018). Molecular detection of *Brucella abortus* using bscp31 and IS711 gene based PCR assay in cattle and buffalo. *Buffalo Bulletin* 37(1): 71-80.

Perez-Sancho, M., Garcıa-Seco, T., Arrogante, L., Garcıa, N., Martınez, I., Diez-Guerrier, A., Perales, A., Goyache, J., Domınguez, L. and Alvarez, J. (2013). Development and evaluation of an IS711-based loop mediated isothermal amplification method (LAMP) for detection of *Brucella* spp. on clinical samples. *Res. Vet. Sci*. 95: 489-494.

Piranfar, V., Sharif, M., Hashemi, M., Vahdati, A.R. and Mirnejad, R. (2015). Detection and discrimination of two *Brucella* species by multiplex real-time PCR and high-resolution melt analysis curve from human blood and comparison of results using RFLP. *Iran J. Basic Med. Sci.* 18: 909-914.

Probert, W.S., Schrader, K.N., Khuong, N.Y., Bystrom, S.L. and Graves, M.H. (2004). Real-time multiplex PCR assay for detection of *Brucella* spp., *B. abortus*, and *B. melitensis*. *J. Clin. Microbiol*. 42: 1290-1293.

Prusty, B.R., Chaudhuri, P., Chaturvedi, V.K., Saini, M., Mishra, B.P. and Gupta, P.K. (2016a). Visual detection of *Brucella* spp. in spiked bovine semen using loop-mediated isothermal amplification (LAMP) assay. *Indian J. Microbiol*. 56: 142-147.

Prusty, B.R., Tabassum, R., Chaudhuri, P., Chaturvedi, V.K., Saini, M., Mishra, B.P. and Gupta, P.K. (2016b). A closed tube loop-mediated isothermal amplification assay for identification of *Brucella* Species in Bull Semen. *Proc. Natl. Acad. Sci., India, Sect. B Biol. Sci.* 1-7.

Queipo-Ortuño, M.I., Colmenero, J.D., Baeza, G. and Morata, P. (2005). Comparison between lightcycler 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. *Clin. Infect. Dis*. 40: 260-264.

Queipo-Ortuno, M.I., Colmenero, J.D., Bravo, M.J., Garcıa-Ordonez, M.A. and Morata, P. (2008). Usefulness of a quantitative real-time PCR assay using serum samples to discriminate between inactive, serologically positive and active human brucellosis. *Clin. Microbiol. Infect*. 14: 1128–1134.

Queipo-Ortuno, M.I., Colmenero, J.D., Bermudez, P., Bravo, M.J. and Morata, P. (2009). Rapid differential diagnosis between extrapulmonary tuberculosis and focal complications of brucellosis using a multiplex real-time PCR assay. *PLoS One* 4(2): e4526.

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

Rijpens, N.P., Jannes, G., Van Asbroeck, M., Rossau, R. and Herman, L.M. (1996). Direct detection of *Brucella* spp. in raw milk by PCR and reverse hybridization with 16S-23S rRNA spacer probes. *Appl. Environ .Microbiol*. 62: 1683-1688.

Romero, C., Gamazo, C., Pardo, M. and Lopez-Goni, I. (1995). Specific detection of *Brucella* DNA by PCR. *J Clin Microbiol*. 33: 615-617.

Saini, S., Gupta, V.K., Gururaj, K., Singh, D.D., Pawaiya, R.V.S., Gangwar, N.K., Mishra, A.K., Dwivedi, D., Andani, D., Kumar, A. and Goswami, T.K. (2017). Comparative diagnostic evaluation of OMP31 gene based TaqMan® real-time PCR assay with visual LAMP assay and indirect ELISA for caprine brucellosis. *Trop. Anim. Health Prod.* doi: 10.1007/s11250-017- 1323-7.

Sangari, F.J. and Aguero, J. (1994). Identification of *Brucella abortus* B19 vaccine strain by the detection of DNA polymorphism at the ery locus. *Vaccine* 12: 435-438.

Sanjuan-Jimenez, R., Morata, P., Bermudez, P., Bravo, M.J. and Colmenero, J.D. (2013). Comparative clinical study of different multiplex real time PCR strategies for the simultaneous differential diagnosis between extrapulmonary tuberculosis and focal complications of brucellosis. *PLoS Negl. Trop. Dis.* 7(12): e2593.

Saunders, V.F., Reddacliff, L.A., Berg, T., Hornitzky, M. (2007). Multiplex PCR for the detection of *Brucella ovis*, *Actinobacillus seminis* and *Histophilus somni* in ram semen. *Aust Vet J.* 85: 72-77

Schmoock, G., Ehricht, R., Melzer, F., Elschner, M., Tomaso, H., Neubauer, H. and Al Dahouk, S. (2011). Development of a diagnostic multiplex polymerase chain reaction microarray assay to detect and differentiate *Brucella* spp. *Diagn. Microbiol. Infect. Dis.* 71: 341–353.

Seah, C.L., Chow, V.T. and Chan, Y.C. (1995). Semi-nested PCR using NS3 primers for the detection and typing of dengue viruses in clinical serum specimens. *Clin. Diagn. Virol*. 4(2): 113-120.

Selim, A.M., Elhaig, M.M. and Gaede, W. (2014). Development of multiplex real-time PCR assay for the detection of *Brucella* spp., *Leptospira* spp. and *Campylobacter foetus*. *Vet. Italiana* 50(4): 269-275.

Sergueev, K.V., Filippov, A.A. and Nikolich, M.P. (2017). Highly sensitive

bacteriophage-based detection of *Brucella abortus* in mixed culture and spiked blood. *Viruses* 9: 144-160.

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

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

Tutar, E., Akinci, K.S. and Akyol, I. (2018). Development and application of a new multiplex real-time PCR assay for simultaneous identification of *Brucella melitensis*, *Cronobacter sakazakii* and *Listeria monocytogenes* in raw milk and cheese. *Int. J. Dairy Tech.* doi.org/10.1111/1471-0307.12500.

Vemulapalli, R., McQuiston, J.R., Schurig, G.G., Sriranganathan, N., Halling, S.M. and Boyle, S,M. (1999). Identification of an IS711 element interrupting the wboA gene of *Brucella abortus* vaccine strain RB51 and a PCR assay to distinguish strain RB51 from other *Brucella* species and strains. *Clin. Diagn. Lab. Immunol*. 6: 760-764.

Verger, J.M., Grimont, F., Grimont, P.A.D. and Grayon, M. (1985). *Brucella*, a monospecific genus as shown by deoxyribonucleic acid hybridization. *Int. J. Syst. Bacteriol*. 35: 292-295.

Vizcaino, N., Caro-Hernandez, P., Cloeckaert. A. and Fernandez-Lago, L. (2004). DNA polymorphism in the omp25/omp31 family of *Brucella* spp.: identification of a 1.7-kb inversion in *Brucella cetaceae* and of a 15.1-kb genomic island, absent from *Brucella ovis*, related to the synthesis of smooth lipopolysaccharide. Microbes Infect. 6: 821-834.

Winchell, J.M., Wolff, B.J., Tiller, R., Bowen, M.D. and Hoffmaster, A.R. (2010). Rapid identification and discrimination of *Brucella* isolates by use of real-time PCR and high-resolution melt analysis. *J. Clin. Microbiol.* 48(3): 697-702.

Xavier, M.N., Silva, T.M.A., Costa, E.A., Paixao, T.A., Moustacas, V.S., Carvalho Jr, C.A., Sant-Ann, F.M., Robles, C.A., Gouveia, A.M.G., Lage, A.P., Tsolis, R.M. and Santos, R.L. (2010). Development and evaluation of a species-specific PCR assay for the detection of *Brucella ovis* infection in rams. *Vet. Microbiol*. 145: 158-164.

Yu, W.L. and Nielsen, K. (2010). Review of detection of *Brucella* spp. by polymerase chain reaction. Croat Med J. 51(4): 306-13.

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