International Journal of Scientific Research in Science and Technology
Print ISSN: 2395-6011 | Online ISSN: 2395-602X (www.ijsrst.com)
doi : https://doi.org/10.32628/IJSRST523103100

Brucellosis : Review on Routine and Molecular Technique for Diagnosis of Neglected Disease

Jinal Patel
Assistant Professor, School of Sciences, P P Savani University, Kosamba, Surat, Gujarat, India

ARTICLEINFO

Article History:

Accepted: 05 May 2023
Published: 30 May 2023

Publication Issue
Volume 10, Issue 3
May-June-2023

Page Number
525-530

ABSTRACT

Brucellosis is a zoonotic infection transmitted from animals to humans through the consumption of contaminated food, direct contact with an infected animal, or inhalation of aerosols. The latter method is extremely effective given the relatively low concentration of organisms required to induce infection in humans and has put this centuries-old disease back in the spotlight. Brucella is a facultative intracellular pathogen that has the ability to survive and multiply in phagocytes and cause abortion in cattle and ripple fever in humans. *Brucella spp.*, especially *B. Abortus*, *B. melitensis,* and *B. suis* pose a serious public health problem. *B. melitensis* is the most common cause of human brucellosis in India. Human brucellosis still poses several challenges for clinicians and scientists, including understanding the pathogenic mechanism, severity, and progression, and developing improved therapeutic regimens. Molecular studies have shed light on the pathogenesis of Brucella to develop new diagnostic tools that will be useful in developing countries where brucellosis is a common but often overlooked disease. This overview brings together all these questions, especially pathogenicity and new diagnostic tools.

Keywords: Brucellosis, virulence, zoonotic disease, serodiagnosis, molecular methods.

I. INTRODUCTION

Brucellosis is a global zoonotic disease with high human morbidity. According to the WHO, about 500,000 cases of this disease are registered worldwide every year [1,2]. Brucellosis remains an uncontrolled problem in regions with high endemicity, such as the Mediterranean, Middle East, Africa, Latin America, and parts of Asia [2,3]. The incidence of brucellosis in India was first detected at the start of the last century and has since been reported in almost all states [4]. Transmission occurs mainly through livestock, sheep, goats, pigs, and camels through direct contact with blood, placenta, fetuses, or uterine secretions or through consumption of contaminated raw animal products especially raw milk and soft cheese.

Copyright: © 2023, the author(s), publisher and licensee Technoscience Academy. This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial License, which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited

Brucellosis is the most common bacterial infection worldwide [5].

The disease is specifically manifested by fever of unknown origin with several clinical symptoms. Patients regularly suffer from severe focal complications such as spondyloarthritis, neurobrucellosis, or brucella endocarditis [6]. The clinical features and symptoms of brucellosis in humans overlap with many other infectious and non-infectious sickness [7], such as typhoid fever, rheumatic fever, spinal tuberculosis, pelvic inflammatory disease, cholecystitis, thrombophlebitis, autoimmune diseases and cancer [8-10]. The clinical picture is nonspecific and laboratory tests should confirm the diagnosis. The correct diagnosis of is important as the disease is characterized by treatment failure and relapses, a chronic course, and sometimes serious complications such as damage to the bones and joints [11].For the unknowing doctor, diagnosing of human brucellosis can be problematic. Asking the patient about animal contact and eating habits at this time can be helpful if brucellosis is suspected if the patient admits to owning or working with livestock and showing symptoms of brucellosis such as bursitis, infertility, or miscarriages in their animals or in patients mentioned has cravings for unpasteurized fresh dairy products [1].

An initial diagnosis of brucellosis can be made with some serological tests for antibodies to brucellosis, but the " gold standard" remains isolation and identification of the bacterium. However, the cultural investigation is dangerous and insensitive. Despite intensive efforts for more than a century to develop a definitive diagnostic technique for brucellosis, the diagnosis still relies on the combination of multiple tests to avoid false negative results [12].

II. LABORATORY DIAGNOSIS

Serological tests

Rose Bengal test: The Rose Bengal Plate agglutination test (RBT) is a rapid test that was originally developed for screening in veterinary medicine but is now widely used to diagnose brucellosis in humans [13-15]. The high sensitivity, ease, and speed of use, as well as its low cost, have made it very popular in hospital emergency departments for the diagnosis of febrile syndromes [16]. In this test, 30, mL of serum is applied to a glossy white plate and mixed with an equal volume of RBT antigen using a toothpick, then at room temperature for 8 minutes shaken, instead of the 4 minutes recommended for animal brucellosis and visible aggregation and/or the appearance of the typical border is counted as a positive test [7]. Positive sera are tested further as follows. Eight drops of ml of saline were dropped onto the plate and the first was mixed with ml of an equal volume of normal positive serum (half dilution of serum). Then transfer 30 ml of this first dilution into the second drop using a micropipette and mix to obtain a 1/4 dilution. From to 1/128, dilutions from 1/8 to 1/128 are obtained for successive transfers and mixes, taking care to rinse the pipette tip between transfers. Finally, drops are tested with an equal volume (30 mL) of RBT reagent such that the final dilutions are between 1/4 and 1/256 [17, 18].

Serum Agglutination Test: The serum agglutination test (SAT) is performed by mixing serial dilutions of serum, typically 1:20 to 1:360, with Brucella antigen in tubes. After overnight incubation, agglutination is read with the naked eye or binoculars. As a guideline, agglutination at titers of 1:160, or greater is considered diagnostic as long as the patient shows signs and symptoms of the disease. In endemic areas, the diagnostic cut-off of must be set to at least a higher titer value 1:320 to ensure sufficiently high specificity, since many asymptomatic individuals have a titer of at the lower cut-off of 1:160 [19]. Sometimes SAT is prepared in the presence of the reducing agents 2-mercaptoethanol (2-ME) or dithiothreitol (DTT). These reducing agents destroy the

agglutinating activity of immunoglobulin M (IgM) and leave IgG intact [20].

Enzyme Immunoassay: The enzyme immunoassay ELISA is recognized as a sensitive and rapid method for diagnosing brucellosis. The detection of specific immunoglobulins with a single, simple, and rapid test is the main advantage of the ELISA test [21,22,7]. The advantages of ELISA in diagnosing brucellosis in endemic areas, it may be useful as a screening test in areas with a low incidence of the disease [23]. Blood samples will be collected from the patients and controls and assayed for Brucella IgM and IgG antibodies by ELISA using commercially available ELISA kits according to the standard procedure. According the study by Memish et al., the sensitivity of SAT to the diagnosis of brucellosis was similar to the combination of IgM and IgG ELISA tests [24]. In another study, Ciffici et al. found a sensitivity of 94.3%, 97.1%, and 71.4% each for SAT, IgG ELISA, and IgM ELISA [25]. Hasibi et al. evaluated the accuracy of the ELISA for diagnosing human brucellosis and determined the optimal cut-off value for ELISA results. The IgG ELISA was found to be more reliable than the IgM ELISA in diagnosing human brucellosis. Using cutoffs of 10 IU/mL and 50 IU/mL gave the highest sensitivity of (92°).9%) and the highest specificity (100%) for IgG ELISA [26].

Molecular techniques: Polymerase chain reaction (PCR) assays can be used for the amplification and detection of Brucella DNA in pure cultures and clinical specimens. PCR-based assays direct detection of Brucella DNA in patients with brucellosis is difficult due to the low number of bacteria in clinical samples and the inhibitory effect of matrix components [27]. The QIAamp™ DNA Mini Kit (Qiagen Inc., Valencia, CA, USA) and the UltraClean™ DNA BloodSpin Kit (MO BIO Laboratories Inc., Carlsbad, CA, USA) are among the seversal commercial kits that have been used successfully Extraction of Brucella DNA from whole blood, serum and tissue samples were used [28]. brucellosis by amplification of a genomic target sequence from a *Brucella spp*. Studies have shown that the standard PCR method is more sensitive than microbiological methods not only in diagnosing the infection but also in the early detection of recurrences [29–31]. Badura et al. compared the sensitivity of 3-pair primers amplifying three different fragments, including the BCSP 31 gene (B4/B5), the B. abortus 16S rRNA sequence (F4/R2), and the omp2 gene (JPF/ JPR). that the sensitivity of primer pair B4/B5, primer pair JPF/JPR and primer pair F4/R2 was 98%, 88.4%, and 53%, respectively. and 1% [32]. Navarro et al. [33] also compared PCR methods using these three primer pairs as described in above. Their results also showed that the three primers tested showed a difference in sensitivity in the presence of human genomic DNA.

Real-time PCR

Real-time PCR is a valuable technique for quantifying nucleic acids in single blood samples. It is highly reproducible, fast, sensitive, and specific [34]. Queipo-Ortuño et al. [27] reported that the sensitivity of the SYBR Green I Light Cycler-based real-time PCR analysis with serum samples was 93.3%, which is more than 90%, and 65% with PCR ELISA with whole blood culture blood samples and blood cultures using a panel of seven primer sets, Winchell et al. They developed a real-time PCR method to analyze members of Brucella isolates and concluded that it has the potential to detect new species [31]. Cattar et al. [35] developed three real-time PCRs for the diagnosis of human brucellosis at the genus level using the hybridization probes and the primers of 16S-23S ITS, omp25, and omp31. Their results showed that the real-time PCR reaction using the ITS 16S-23S primers and their probes was the most sensitive, indicating their potential for diagnosing human brucellosis in the clinical laboratory. Nested and Semi-Nested PCR Nested PCR means that two different PCR primer pairs are used for a single locus [36]. In semi-nested PCR, there are two different PCR primer pairs, but

the second primer pair has an identical primer to the first pair [37]. Nested-PCR and semi-nested-PCR tests are currently being developed to identify Brucella in human blood samples, and later to study their clinical practice for diagnosing brucellosis in humans. Lin et al. described a nested PCR for the laboratory diagnosis of human brucellosis [38].

III.CONCLUSION

The diagnosis of brucellosis remains a difficult task. According to various study a combination of two or more tests reduces the number of misdiagnoses. The combination of the Rose Bengal positive test and the Coombs or Brucellacapt test is a good diagnostic criteria with 80% specificity and 100% sensitivity among serological tests. The LC blood culture technique is a culture technique and productive highly sensitive and gives faster results than the traditional cultivation technique, but it must be performed with all precautions. Regardless of disease stage, standard PCR and other techniques are more sensitive than blood cultures and more specific than serological tests. New molecular diagnostic techniques such as the -LAMP method can be preferred over other molecular methods because of the simplicity, low cost (compared to PCR), sensitivity, and specificity of and can be a useful tool for the rapid detection of *Brucella spp.* in epidemiological studies and resource constraints in developing countries. In addition, the MLVA-16 high-throughput genotyping technique is useful for tracing the source of Brucella infection, especially when processing a large number of samples in a short period of time.

IV. REFERENCES

[1]. Young EJ. 1995. An overview of human brucellosis. Clin Infect Dis 21: 283-289.

[2]. Corbel MJ. 1997. Brucellosis: an overview. Emerg Infect Dis 3: 213-221.

[3]. Refai M. 2002. Incidence and control of brucellosis in the Near East region. Vet Microbiol 90: 81-110.

[4]. Renukaradhya GJ, Isloor S, Rajasekhar M. 2002. Epidemiology, zoonotic aspects, vaccination and control/eradication of brucellosis in India. Vet Microbiol 90: 183-195.

[5]. Yagupsky P, Baron EJ. 2005. Laboratory exposures to brucellae and implications for bioterrorism. Emerg Infect Dis 11: 1180-1185.

[6]. Colmenero JD, Reguera JM, Martos F, Sánchez-De-Mora D, Delgado M, et al. 1996. Complications associated with Brucella melitensis infection: a study of 530 cases. Medicine (Baltimore) 75: 195-211.

[7]. Araj GF. 1999. Human brucellosis: a classical infectious disease with persistent diagnostic challenges. Clin Lab Sci 12: 207-212.

[8]. Lulu AR, Araj GF, Khateeb MI, Mustafa MY, Yusuf AR, et al. 1988. Human brucellosis in Kuwait: a prospective study of 400 cases. Q J Med 66: 39-54.

[9]. Madkour MM. 2001. Epidemiologic aspects: Madkour's brucellosis. Springer, New York, USA.

[10]. Young EJ. 1989. Brucellosis: clinical and laboratory aspects, Corbel MJ (edt), CRC Press Inc, Florida, USA.

[11]. Al Dahouk S, Tomaso H, Nöckler K, Neubauer H, Frangoulidis D (2003) Laboratory-based diagnosis of brucellosis--a review of the literature. Part II: serological tests for brucellosis. Clin Lab 49: 577-589.

[12]. Poiester FP, Nielsen K, Samartino LE, Yu WL. 2010. Diagnosis of Brucellosis. The Open Veterinary Science Journal 4: 46-60.

[13]. Morgan WJ, MacKinnon DJ, Lawson JR, Cullen GA .1969. The rose bengal plate agglutination test in the diagnosis of brucellosis. Vet Rec 85: 636-641.

[14]. Oomen LJ, Waghela S. 1974. The rose Bengal plate test in human brucellosis.Trop Geogr Med 26: 300-302.

[15]. Díaz R, Maravi-Poma E, Fernández JL, García-Merlo S, Rivero-Puente A. 1982. [Brucellosis: study of 222 cases. IV. Diagnosis of human brucellosis].Rev Clin Esp 166: 107-110.

[16]. Ruiz-Mesa JD, Sánchez-Gonzalez J, Reguera JM, Martín L, Lopez-Palmero S, et al. 2004. Rose Bengal test: diagnostic yield and use for the rapid diagnosis of human brucellosis in emergency departments in endemic areas. Clinical Microbiology and Infection 11: 221-225.

[17]. Smits HL, Abdoel TH, Solera J, Clavijo E, Diaz R. 2003. Immunochromatographic Brucella-specific immunoglobulin M and G lateral flow assays for rapid serodiagnosis of human brucellosis. Clinical and Diagnostic Laboratory Immunology 10: 1141-1146.

[18]. Díaz R, Ariza J, Alberola I, Casanova A, Rubio MF. 2006 Secondary serological response of patients with chronic hepatosplenic suppurative brucellosis. Clinical Vaccine Immunology 13: 1190-1196.

[19]. Martín Moreno S, Guinea Esquerdo L, Carrero González P, Visedo Orden R, García Carbajosa S, et al. 1992. Diagnosis of brucellosis in an endemic area. Evaluation of routine diagnostic tests. Medicina Clinica (Barcelona) 98: 481-485.

[20]. Al Dahouk S, Tomaso H, Nöckler K, Neubauer H, Frangoulidis D. 2003. Laboratory-based diagnosis of brucellosis--a review of the literature. Part II: serological tests for brucellosis. Clin Lab 49: 577-589.

[21]. Osoba AO, Balkhy H, Memish Z, Khan MY, Al-Thagafi A, et al. 2001. Diagnostic value of Brucella ELISA IgG and IgM in bacteremic and non-bacteremic patients with brucellosis. J Chemother 13 Suppl 1: 54-59.

[22]. Pappas G, Akritidis N, Bosilkovski M, Tsianos E. 2005. Brucellosis. N Engl J Med 352: 2325-2336.

[23]. Karplus R, Ramlawi A, Banai M, Maayan S. 2007 The use of ELISA in a seroprevalence study of Brucella antibodies in West Bank Palestinian women of childbearing age. Int J Infect Dis 11: 367-368.

[24]. Memish ZA, Almuneef M, Mah MW, Qassem LA, Osoba AO. 2002 Comparison of the Brucella Standard Agglutination Test with the ELISA IgG and IgM in patients with Brucella bacteremia. Diagnosis of Microbiol Infectious Diseases 44: 129-132.

[25]. Ciftçi C, Oztürk F, Oztekin A, Karaoğlan H, Saba R, et al. 2005. Comparison of the serological tests used for the laboratory diagnosis of brucellosis]. Mikrobiyol Bul 39: 291-299.

[26]. Hasibi M, Jafari S, Mortazavi H, Asadollahi M, Esmaeeli Djavid G. 2013. Determination of the accuracy and optimal cut-off point for ELISA test in diagnosis of human brucellosis in Iran. Acta Med Iran 51: 687-692.

[27]. Queipo-Ortuño MI, De Dios Colmenero J, Macias M, Bravo MJ, Morata P. 2008. Preparation of bacterial DNA template by boiling and effect of immunoglobulin G as an inhibitor in real-time PCR for serum samples from patients with brucellosis. Clin Vaccine Immunol 15: 293-296.

[28]. Al Dahouk S, Sprague LD, Neubauer H. 2013. New developments in the diagnostic procedures for zoonotic brucellosis in humans. Rev Sci Tech 32: 177-188.

[29]. Briones-Lara E, Palacios-Saucedo Gdel C, Martínez-Vázquez IO, Morales-Loredo A, Bilbao-Chávez Ldel P. 2007. Response to the treatment of brucellosis among children. Evaluation with Huddleson reaction and PCR. Revista Médica del Instituto Mexicano del Seguro Social 45: 615-622.

[30]. Elfaki MG, Al-Hokail AA, Nakeeb SM, Al-Rabiah FA. 2005. Evaluation of culture, tube agglutination, and PCR methods for the

diagnosis of brucellosis in humans. Med Sci Monit 11: MT69-74.

[31]. Morata P, Queipo-Ortuño MI, Reguera JM, García-Ordoñez MA, Pichardo C, et al. 1999. Posttreatment follow-Up of brucellosis by PCR assay. J Clin Microbiol 37: 4163-4166.

[32]. Baddour MM, Alkhalifa DH. 2008. Evaluation of three polymerase chain reaction techniques for detection of Brucella DNA in peripheral human blood. Can J Microbiol 54: 352-357.

[33]. Navarro E, Escribano J, Fernández J, 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.

[34]. Wang Y, Wang Z, Zhang Y, Bai L, Zhao Y, et al. 2014. Polymerase chain reaction-based assays for the diagnosis of human brucellosis. Ann Clin Microbiol Antimicrob 13: 31.

[35]. 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. J Clin Microbiol 48: 697-702.

[36]. Kattar MM, Zalloua PA, Araj GF, Samaha-Kfoury J, Shbaklo H, et al. 2007. Development and evaluation of real-time polymerase chain reaction assays on whole blood and paraffin-embedded tissues for rapid diagnosis of human brucellosis. Diagnostic Microbiology and Infectious Disease 59: 23-32.

[37]. Seah CL, Chow VT, Chan YC. 1995. Semi-nested PCR using NS3 primers for the detection and typing of dengue viruses in clinical serum specimens. Clin Diagn Virol 4: 113-120.

[38]. Lin GZ, Zheng FY, Zhou JZ, Gong XW, Wang GH, et al. 2011. Loop-mediated isothermal amplification assay targeting the omp25 gene for rapid detection of Brucella spp. Molecular and Cellular Probes (MCP) 25: 126-129.

Cite this article as :

Jinal Patel, "Brucellosis : Review on Routine and Molecular Technique for Diagnosis of Neglected Disease", International Journal of Scientific Research in Science and Technology (IJSRST), Online ISSN : 2395-602X, Print ISSN : 2395-6011, Volume 10 Issue 3, pp. 525-530, May-June 2023. Available at doi : https://doi.org/10.32628/IJSRST523103100

Journal URL : https://ijsrst.com/IJSRST523103100