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An overview of Bovine brucellosis: A neglected endemic zoonotic disease

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Abstract
Globally over the last two decades emergence of many zoonotic diseases has observed and India also experienced recently with COVID-19, Nipah, Bird flu, Pandemic H1N1 influenza etc. Brucellosis is one of the world's neglected zoonotic diseases, which is caused by the genus *Brucella*. Major factors responsible for the spread of the disease are lack of resources, poor management and lack of awareness. In case of cattle, abortion during last trimester is a predominant sign. Besides this high temperature and decreased production is also observed, while in human's brucellosis is characterized by undulant fever, Malta fever, general malaise, and arthritis. Isolation and identification of pathogen remain the gold standard test, which needs experience. *Brucella* abortus, strain 19 and RB51 are the most widely used vaccine strains to protect against *Brucella* infection and related abortions in cattle. Moreover, it is very important to note that no vaccine is available either for bovines or human beings which is highly protective, safe and effective. One Health approach can aid in control of brucellosis, both in animals and humans. In this review, several aspects of brucellosis, diagnosis, treatment, prevention and control are reviewed.

Keywords: Brucellosis, zoonoses, diagnosis, control programme, prevention

Introduction
Brucellosis is a contagious, chronic infectious disease of animals and humans in different regions of worldwide (Pappas *et al.* 2006 and Franc *et al.* 2018) [59, 26]. After following the effective preventive measures and vaccine strategies brucellosis in livestock and transmission of infection to the human population has been significantly decreased, in some parts of the world, it remains an uncontrolled problem in regions of high endemicity such as the Latin America, Middle East, Mediterranean, Africa, and parts of Asia (Corbel, 1997 and Refai, 2002) [15, 63]. The World Health Organization (WHO) has selected brucellosis as one of the most serious "ignored zoonoses" in the world and each year, it is estimated that there are approximately 500,000 new cases of humans infected with this disease (Franc *et al.* 2018 and Khan and Zahoor, 2018) [26, 37]. Due to its ease of aerosol transmission WHO designated *Brucella* as a hazard group III pathogen (Yuguda *et al.* 2019) [80].
J. A. Marston, an assistant surgeon, for the first time described brucellosis as Mediterranean gastric remittent fever in 1861 from his base in Malta in the 19th century (Marston, 1861) [48]. Sir David Bruce discovered the cause of the disease in 1887 and reported numerous small coccal organisms from autopsy samples taken from the spleens, livers, and kidneys of infected British soldiers in Malta. He isolated and identified the organism and named it as *Micrococcus melitensis* (Bruce, 1887) [11]. Later, it was renamed as *Brucella melitensis* in his honor by Meyer and Shaw (Tazerart, 2022) [73]. Epidemiological evidence shows that in India brucellosis is present in different species of farm animals such as cattle, sheep, goats, buffalo, yaks, camel, horses, pigs and wild life (Renukaradhya *et al.* 2002) [64]. Disease in animals referred as Bang's disease, enzootic abortion, and contagious abortion whereas in humans Malta fever, Mediterranean fever, intermittent fever, and undulant fever (Franc *et al.* 2018) [26]. *Brucella spp.* are Gram-negative coccobacilli, characterized by being non-capsulated, non-motile, non-spore forming pathogen, (Dahouk *et al.* 2013) [4] and facultative intracellular with small size of of 0.6–1.5 µm in length and 0.5–0.7 µm in diameter that categorized in the family of *Brucella*ceae. The family *Brucella*ceae comprises the genus *Brucella* and six further genera, including *Ochrobactrum*, *Daeguia*, *Crabtreella*, *Mycoplana*, *Pseudochrobactrum*, and *Paenochrobactrum* which are phylogenetically members of the order *Rhizobiales* within the class Alphaproteobacteria (Leclercq *et al.* 2020) [41]. The genus *Brucella* currently comprises twelve species that infect different wildlife and domestic animal species (Whatmore *et al.* 2016) [78]. Among these, according to their pathogenicity, six *Brucella* species have been

categorized and preferred hosts as *Brucella abortus* (cattle), *B. melitensis* (goats and sheep), *B. ovis* (rams), *B. canis* (dogs), *B. suis* (pigs), and *B. neotomae* (Common voles, desert wood rat). *B. melitensis*, *B. suis* and *B. abortus* are the most important pathogenic species in man (Wareth *et al.* 2014 and Kaynak *et al.* 2016) [36, 77]. Two new *Brucella* spp., *B. ceti* (dolphins, porpoises and whales), and *B. pinnipedialis* (walruses and seals) have been recently reported from marine mammal hosts according to their pathogenicity and preferred hosts (Cvetnić *et al.* 2016) [17].

The principal cause for human brucellosis worldwide is *B. melitensis*. *B. melitensis* type 1 predominating in India (Mantur *et al.* 2006) [45] and Spain (Colmenero *et al.* 1996) [16], type 2 in North Western Greece and type 3 in Turkey (Bodur *et al*. 2003) [10] which accounts for up to 90% of all brucellosis cases. Apart from well-known endemic regions, in many areas of worldwide brucellosis considered as a neglected disease leading to serious health and economic concern for the livestock populations by affecting animals such as cattle, buffalo, camel, sheep and goat (Singh *et al.* 2015, Lokamar *et al.* 2020 and Lai *et al.* 2021) [39, 42, 72]. Infertility, fetal death, late-gestation abortion, missed reproductive cycles, birth of weak offspring with low birth weight, decreased cattle productivity, loss in market value of animals, lost draught power, and increased veterinary costs in farms are the primary consequences of this disease (Dereje *et al.* 2018 and El-Diasty *et al.* 2021) [18, 21]. In many underdeveloped countries brucellosis poses a serious public health risk due to the rise in incidences of morbidity in both humans and animals. Humans infected with the disease either by coming into direct contact with sick animals or by consuming contaminated milk and milk products (Dione *et al.* 2022) [19].

Diagnosis

Clinical diagnosis of Brucellosis is made based upon epidemiological patterns, history of exposure, clinical signs and laboratory tests. World Health Organization (WHO) notified that around millions of cases were reported every year whereas the actual rate of incidence is 10-25 times more than the stated number of cases. Lack of specific guidelines for brucellosis diagnosis cases is one of the reason to this condition. During the first week of illness in acute form of disease, immune response is mainly comprised of IgM antibodies, as the time passes the IgG levels hikes in the secondary immune response, such variation in serological pattern of disease suggests seven possible clinical subtypes of the brucellosis modulating the epidemiological scenery of this disease (Avijgan *et al*. 2019 and Singh *et al.* 2021) [7, 72].

Isolation and identification of Pathogen

Isolation of bacterial pathogens is a confirmatory diagnosis but usually takes two weeks (Radostits *et al.* 2000) [61]. Blood agar base or Columbia agar solid media used for isolation of *Brucella*. Serum–dextrose agar (SDA) or glycerol dextrose agar are other satisfactory media to observe colonial morphology (Alton *et al.* 1988) [5]. Isolation of *Brucella* from milk, blood and other body fluids, can be done by Castaneda's medium, which prevents interference in Bio-typing when grown in broth (Mantur *et al.* 2018) [46]. *B. melitensis* does not require serum and carbon dioxide for growth whereas *B. abortus* requires. Farrell's selective medium avoids growth of contaminants and such media is used for isolating the bacteria from milk samples.

Brucella abortus biovar 3 was isolated from milk sample of dairy cattle, organs of aborted fetus, fetal membranes and placenta. The primary isolation of organism was done by using selective serum dextrose agar medium along with Farrell's medium, then grams staining and were identified by phase contrast microscopy (Mathew *et al.* 2015) [49]. Similarly, *Brucella* species were isolated from seropositive cattle having history of abortion. vaginal swabs (8.69%) and placental cotyledon (11.1%) contains *B. abortus* where as from aborted foetal abomasal contents and milk of animal no isolate was detected (Geresu *et al.* 2016) [27]. Modified Agrifood. Research and Technology Center of Aragon (CITA) medium (mCITA) was better for selective isolation of *Brucella spp.* compared to Farrell's medium (FM) (Ledwaba *et al.* 2020) [40].

Cultural and serological techniques were compared for brucellosis diagnosis in 248 cattle from those, 80.9% animals were detected positive in at least any one serological test while 45.2% showed positivity in all five serological tests such as microserum agglutination test, indirect enzyme linked immune sorbent assay (iELISA), competitive enzyme linked immune sorbent assay (cELISA), complement fixation test (CFT) and fluorescence polarization assay. Overall analysis suggested that along with serological tests, bacterial culture methods should be encouraged and performed for confirmation of brucellosis (O'Grady *et al.* 2014) [53].

Serological tests

Serological tests are important for monitoring of the disease, surveillance, control and eradication programmes worldwide. Several serological tests such as Rose Bengal plate test (RBPT), standard tube agglutination test (SAT), immune capture agglutination, CFT, milk ring test, Coombs test, ELISA and lateral flow assay (LFA) are frequently employed to diagnose brucellosis (Lucero *et al.* 2003) [43]. At the point of sample collection RBPT and LFA can be performed; such that the time required for diagnosis reduced (Ezama *et al.* 2018) [22].

Rose Bengal Plate Test (RBPT)

The RBPT sensitivity is very high, but has less specificity (Barroso *et al.* 2002) [8]. From Southern Ethiopia 384 serum samples of cattle used for the detection of *Brucella* specific antibodies using RBPT, overall seroprevalence, 4% was reported. Abortion and retained foetal membrane (RFM) are found significantly associated with seropositivity (Yilma, 2016) [75]. RBPT is having better relative sensitivity and specificity in comparison with that of SAT and CFT for human samples (Teng *et al.* 2017) [74].

Complement fixation test (CFT)

The CFT is considered as better serological test for control and surveillance programmes of brucellosis. CFT is a very specific test that can detect IgM and IgG1 antibodies. The CFT quantitatively measure more of the IgG1 type antibodies than the IgM type antibodies, as the inactivation process results in partial destruction of IgM antibodies (Buchanan and Faber, 1980) [13].

Standard tube agglutination test (SAT)

The SAT quantifies IgM and IgG, the quantity of specific IgG is measured by 2-mercaptoethanol (2ME) treatment of serum sample. IgG antibodies are important for detection of active brucellosis and is an excellent indicator of active brucellosis,

thus a rapid decline in the titre of IgG antibodies indicates successful treatment. Persistence of SAT antibodies in some successfully treated patients indicate over diagnosis of human brucellosis which results in wrong treatment (Memish and Almuneef 2002; Mantur *et al.* 2006) [44, 45].

Brucellin test

This test is especially useful as a confirmatory test in unvaccinated animals and as per OIE it was an alternative test (OIE, 2009) [54]. It measures delayed type hypersensitivity reaction. This test is more specific than common serological assays (Pouillot *et al*. 1997) [57]. Disadvantage with this test its sensitivity is low which makes it a good test for herd but not for individual certification. Other rapid tests are preferred, as it takes long time and effort.

Enzyme-linked immunosorbent assay (ELISA)

As per a study ELISA is a suitable alternative to culturing techniques to detect *Brucella* antigen, having 100% sensitivity and 99.2% specificity (AlShamahy and Wright, 1998) [6]. Wang *et al.* (2015) developed a highly advanced version of a monoclonal antibody-based cELISA against LPS for the diagnosis of Bovine brucellosis, which revealed higher specificity than the commercially available cELISAs and RBPT (Ahmed *et al*. 2011; Kirit *et al.* 2017)[1, 38, 76]. Praud *et al.* 2016) [58] evaluated three commercially available cELISA kits and fluorescence polarization assay (FPA) for Bovine brucellosis diagnosis and compared these with RBPT, CFT, indirect ELISA and FPA [58]. The most sensitive tests were found as FPA, competitive ELISA and RBPT. CFT, SAT and RBPT were found to be highly specific. However, these three cELISA kits could not be recommended as a single screening test because of low specificity.

Polymerase chain reaction (PCR) assay

PCR is a quick diagnostic method, which may be applied even on samples of poor quality. This could be useful for epidemiological analysis as well as for molecular characterization. A number of sequences were recognized as targets for genus-specific PCR assays for confirmation of *Brucella* species, *viz*., omp2 and bcsp31,16S rRNA and the 16S-23S region (Habtamu *et al.* 2013) [33]. A more sensitive and specific unique repeat sequence PCR (URS-PCR) has also been validated for confirmatory diagnosis of *B. abortus* and *B. melitensis* (Alamian *et al.* 2017) [2].

Novel techniques and modifications

Several field level tests, *viz*., Lateral flow assay (LFA) and latex agglutination found easy to use and quick. It has been found that sensitivity as well as specificity of the LFA for positive cases is more than 95% (Mizanbayeva *et al.* 2009 and Marei *et al.* 2011) [50, 47]. Researchers were showing interest of using circulating microRNAs (miRNAs) as clinical biomarkers (Ghai and Wang, 2016) [28]. Infection with *B. melitensis* can modulate the *in vitro* expression of miRNAs impacting the immunological responses in host body (Rong *et al.* 2017) [65]. Loop-mediated isothermal amplification (LAMP) of DNA as well as real-time PCR have been proved as sensitive, quick and specific diagnostics for *B. abortus* and other *Brucella spp.* directly from clinical specimens (Patra *et al.* 2019) [56]. Real-time recombinase polymerase amplification (RPA) was developed targeting the bcsp31 gene and 94% sensitivity was found (Qin *et al.* 2019) [60]. Next generation sequencing of cerebrospinal fluid would be used for fast diagnosis of human neurobrucellosis, which enables early treatment and better prognosis (Fan *et al.* 2018) [23]. Rapid vertical flow technology using lipopolysaccharide of *Brucella* spp. was used for detection of anti-*Brucella* antibodies, this developed assay had an accuracy of 98% and hence can be used for early diagnosis of brucellosis at field level (Shi *et al.* 2020) [70].

Treatment

Due to intracellular survival of *Brucella* and its adaptability in the macrophages, antibiotic treatment of brucellosis in domestic animals is unsuccessful (Farid *et al.* 1961 and Seleem *et al.* 2008) [24, 69]. Relapse of infection is very common in man and success rate of treatment is low. Combination of drugs should be selected wisely for *brucellosis* treatment in man, to prevent the side effects and emergence of resistance (Villate and Casallas, 2020) [79]. Either ciprofloxacin and/or ceftriaxone as single drug for treatment of brucellosis cases by researchers but results were not promising (Doganay and Aygen, 1992) [20]. Combination therapies are preferred over monotherapy to reduce the chances of disease relapses (Feiz *et al.* 1973 and Ranjbar *et al.* 2020) [25, 62]. Another regimen is use of doxycycline in dose of 100 mg twice daily orally along with 600–900 mg (15 mg/kg BW) of rifampin once a day for 6 weeks by oral route, amikacin two times a day for a week can also be included in the regimen to formulate triple drug therapy (Villate and Casallas, 2020) [79]. Moreover, tauroursodeoxycholic acid or ginseng saponin fraction A is also been reported to inhibit intracellular replication of *Brucella*.

Singh and co-workers, 2015 described effective management treatment of Bovine brucellosis as it is very important in infected dairy cattle herd [71]. Researchers had reported a novel and successful immunotherapy for treatment of Bovine brucellosis in cows by using RB51 phage lysates (as RL) and S19 (as SL). The cocktail of these two phage lysates (RL and SL) were injected subcutaneously in 2 mL-dose and even after 3 month-period of immunization by phage cocktail, blood samples were found negative for presence of *Brucella*. Among these two phage lysates, RL projected stronger cell-mediated immune response while SL stimulated higher level of humoral immune response. Results are promising to encourage the use of bacteriophage lysates in treatment of Bovine brucellosis (Saxena and Raj, 2018) [68].

Prevention and control

Globally there is an increase in trade of animal products that is responsible for spread of various disease causing organism. Animal products transportation should be done as per general principles and procedures provided in the International Zoo-Sanitary Code of the OIE along with those of prevalent practices in a locality. Along with various testing procedures for animals, quarantine measures specified in this code should followed (OIE, 2016) [55]. Test-and-slaughter policy was adopted by most South East Asian countries to eradicate the animal brucellosis (Zamri-Saad and Kamarudin, 2016) [81]. Brucellosis-free herd should be selected for semen introduction in the farm as it acts as important risk factor for spread of the pathogen (Cardenas *et al.* 2019) [14]. It was concluded that there is a need of nation and worldwide comprehensive surveillance program for planning, control and eradication policies to decrease the transmission of brucellosis from animals to human beings (Ryu *et al.* 2019) [66]. Animal brucellosis is controlled by identifying infected animals,

prevention of pathogen to spread from infected animals and herds to noninfected herds, removal of reservoirs of *Brucella* infection. (Gwida *et al.* 2010) [32]. Nepomuceno and co-workers (2018) developed an individual based-mathematical model to show dynamics of Bovine brucellosis in Brazil and concluded that to eradicate the disease, approaches like isolation of infected animals and reduction of the size of population are essential [52]. Pasteurization of milk was another protective mechanism. Vaccination of cattle is recommended to control Bovine brucellosis in enzootic areas with high prevalence rates.

Vaccination

Vaccination is the best way to prevent, control and eradication of brucellosis in endemic areas (Briones *et al.* 2001) [12]. The most common *Brucella spp., viz*., strain 19, RB51 and Rev1 are widely used as vaccine strains to protect against *Brucella* infection. Further studies were needed to know their use in other susceptible animals and requires the development of novel effective vaccines in near future (Jezi *et al.* 2019) [35]. The most common and very efficient vaccines being used against Bovine brucellosis were, *B. abortus* strains 19 and RB51. *B. abortus* S19 was a result of natural attenuation lacking 720-bp region in the erythritol catabolic genes (Sangari *et al.* 1994 and Gheibi *et al.* 2018) [29]. The strain RB51 vaccine does not interfere with serodiagnostic results unlike strain 19 vaccine (Moriyon *et al.* 2004) [51]. *B. melitensis* strain Rev1 is the best vaccine for the prevention of brucellosis in goats and sheep (Benkirane *et al.* 2014) [9]. Omp16, Adk, SecB, etc., were various recombinant proteins studied for their potential to be utilized as vaccine against brucellosis (Alizadeh *et al*. 2019 and Huy *et al*. 2020) [3, 34]. Recently a combined subunit vaccine of BP26, Omp25 and L7/L12 antigens was found to exhibit better protection against challenge than single antigen but lesser protection when compared to *B. abortus* S19 (Gupta *et al.* 2019) [31]. Besides conventional vaccines, new DNA vaccines and multivalent fusion DNA vaccine have also been developed as preventive measures. Multivalent DNA vaccines significantly induced high level of humoral immune response in terms of increased IgM, IgG, IgG2a, and enhanced cell-mediated immune response evidenced as high IFN-c and lymphoproliferative response of splenocytes (Gomez *et al.* 2017) [30].

Conclusion

Brucellosis is the most prevalent animal and zoonotic diseases with worldwide occurrence. It is an endemic disease in India. The prevalence of this disease due to numerous hygienic, social, economic, cultural and political factors. Prophylaxis programs of brucellosis depends on early, accurate and precise diagnosis of the disease. Diagnosis of this disease is by history, symptoms of disease, bacteriological isolation and identification, serological tests, and various molecular tests including PCR-based assays. However, all these tests have some strengths and limitations.

In disease prevalent areas, nation-wide comprehensive monitoring, surveillance programs with adequate funding in different geographical areas in all the countries should be conducted in order to assess the magnitude of the disease. Prevention, control and eradication strategies having collaboration of various departments should also be in force. Registration and proper identification of animals, excellent veterinary/medical services and adequate compensation are essentially required. Proper liaison between the medical and veterinary professionals and raising awareness about occupational risk hazards could aid in decreasing the incidence of brucellosis.

Zoonotic diseases are always a threat to human beings because animals, humans and environment are always dependent on each other and we don't know how many challenges like SARS-Cov-2, Pandemic Influenza etc. will confront humans in future. Therefore, it is necessary to develop a good communication, co-operation between medical, veterinary, wildlife sciences and ecologists including sharing knowledge and laboratory facilities which will add an immense strength to control diseases and their emergence, re-emergence in future.

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