

AN OVERVIEW OF HUMAN BRUCELLOSIS: A NEGLECTED ZONOTIC DISEASE IN LIVESTOCK

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SUMMARY: Brucellosis is a neglected, zoonotic bacterial disease in humans with over 500, 000 new cases reported each year worldwide. *Brucella abortus*, *B. melitensis*, *B. suis* and *B. canis* have been detected in humans as causative agents of this zoonotic infection. The incidence of brucellosis varies greatly with several factors such as socio-economic status, association with livestock, occupation and food safety practices. Brucellosis is an endemic disease in Sri Lanka and only a few cases have been reported in humans in recent years. Human brucellosis is a common disease in the Mediterranean region, Middle East, Asia, South and Central America and Africa. The organism infects multiple hosts. Multiple routes of infection have been described for humans, the oral route being the most common. The common clinical signs in humans are undulant fever, fatigue, headache, chills and myalgia. Other signs and symptoms such as arthralgia, orchitis/epididymitis, acute renal failure, endocarditis, splenic abscess, spondylitis, arthritis, encephalitis hepatitis, osteoarticular manifestations, meningitis, and respiratory and cardiac complications also had been reported. Bacteriological culture, serology and nucleic acid amplification assays have been used as the basic diagnostic methods of brucellosis. The prevention strategy of human brucellosis recommended by the World Health Organization (WHO) mainly consists of creating public awareness, improving hygienic practices in livestock farms and slaughterhouses, promoting the consumption of pasteurized milk, consumption of cooked meat, adhering to hygienic practices during laboratory handling of the organism and proper handling of companion animals. The recommended prevention and control methods of brucellosis in animals include culling of infected animals, vaccination and restriction of the movement of serologically positive animals. In conclusion, further emphasis must be given to improving diagnostic testing and raising awareness of brucellosis and its zoonotic implications among humans.

INTRODUCTION

Brucellosis is a neglected, zoonotic bacterial infection in humans (Zheng *et al.*, 2018). The disease was first reported in the 1850s in Malta, in a patient that had acquired the infection through the consumption of infected goat milk (Deng *et al.*, 2019). In addition to the terms “Mediterranean fever” and “Malta fever” used for the disease, it is also known as “undulant fever” based on the frequent clinical outcome while the latest name “Brucellosis” is a tribute to Sir David Bruce, the military physician who discovered the causative agent *B. melitensis* (Zheng *et al.*, 2018). Multiple species of animals are infected and importantly,

over 300 million cattle were infected with *Brucella* species globally in 2018 (Deng *et al.*, 2019; Khurana *et al.*, 2021).

Bovine brucellosis is endemic both in the dry and wet zones of Sri Lanka and 5-10% of the cattle herds were reported to be serologically positive for brucellosis (Priyantha, 2011). In contrast, only isolated cases of caprine, ovine and swine brucellosis have been reported (Bandara and Mahipala, 2002; Priyantha, 2011). However, the prevalence of brucellosis in each livestock species is not known due to lack of recent local studies. In addition, calves are vaccinated against brucellosis with S 19 live attenuated vaccine in the endemic

areas (Priyantha, 2011) which may have had some impact on the prevalence. The socio-economic factors on the prevalence of brucellosis in livestock have been assessed in the dry zone and poverty has been identified as a key factor for spreading the disease (Kothalawala *et al.*, 2017). Human brucellosis may be misdiagnosed or under-reported in local medical practices mainly due to prevalence of several febrile infectious diseases with similar clinical presentation namely dengue, malaria, chikungunya, and viral fever (Rafaath *et al.*, 2021). In addition, the author believes that lack of specific diagnostic tests in rural settings, lack of awareness of the disease and lack of comprehensive history and medical records of the patients also lead to misdiagnosis of human brucellosis. Since brucellosis is an endemic disease in livestock, companion and wild animals, there is a high possibility of contracting the disease by humans employed in livestock and food industry. Therefore, the objective of this review is to summarise the information available on human brucellosis to improve the awareness of the disease among veterinarians, medical practitioners and scientist in Sri Lanka.

Human brucellosis is caused by several *Brucella* species. (**Table: 1**). The organism requires special growth conditions including 10% CO₂ (except S 19 vaccine strain) and incubation at 37°C for 72 hours (**Table: 2**). It is an intracellular organism, importantly a quick urease reactor that the urease positivity can be detected within 30 minutes. Since different brucella species show similar morphological and culture characteristics as described in Table: 1, expert knowledge is required to differentiate the species.

Table 1. Natural hosts and zoonotic risk in different species of *Brucella* (Deng *et al.*, 2019; Khurana *et al.*, 2021., Zheng *et al.*, 2018).

Brucella Species	Natural host	Zoonotic risk
<i>B. melitensis</i> (biovar 1-3)	Goat, sheep	High
<i>B. abortus</i> (biovar1-6,7,9)	Cattle, buffaloes	High
<i>B. suis</i> (biovar1-5)	Pig, wild boar, hare, reindeer, caribou, rodent	High
<i>B. ovis</i>	Sheep	Not reported

<i>B. neotomae</i>	Desert rat	Low
<i>B. canis</i>	Dog	Low
<i>B. ceti</i>	Dolphin	Low
<i>B. pinnipedialis</i>	Seal	Low
<i>B. microti</i>	Wild voles	Not known
<i>B. papionis</i>	Baboon	Not known
<i>B. vulpis</i>	Red foxes	Not known

Table 2. Characteristic features of the *B. abortus* and *B. melitensis* (Deng *et al.*, 2019; Khurana *et al.*, 2021., Zheng *et al.*, 2018).

Size	0.5 - 0.7 µm x 0.6 - 1.5 µm
Shape	coccobacilli
Gram test	Gram Negative
Growth	Slow growing bacterium (72 hours at 37°C)
Special requirement	Required 10% CO ₂ for the growth
H ₂ S production	Only <i>B. abortus</i> is positive for H ₂ S production test (lead acetate paper method)
Urease Test	Positive for urease test
Motility	Non-motile
Catalase test	Positive
Oxidase test	Positive
Indole test	Negative

Epidemiology

Over 500, 000 new cases of human brucellosis are annually reported worldwide (Ramin and MacPherson, 2010). Although brucellosis is found globally, the high risk of infection was noticed in the Mediterranean region, the Middle East, Asia, South and Central America and Africa (Adetunji *et al.*, 2019). Relatively less incidence of human brucellosis is reported in developed countries in comparison with developing countries (Khurana *et al.*, 2021). The incidence of human brucellosis in China had been increased from 1.41 to 4.22 per 100,000 people between 2005- 2014 (Liang *et al.*, 2020). The incidence of brucellosis was 28-160 per 100, 000 person-years in Ethiopia (Tadesse, 2016). In addition, the annual incidence rates of human brucellosis reported from Arabic countries including Yemen (88.6 cases/100,000 person-years), Syria (40.6/100,000 person-years), and Iran (18.6/100,000 person-years) in 2014–2017 (Lai *et al.*, 2021). The incidence of human brucellosis was low in Europe; only 381 confirmed and cumulative cases were reported by 28 European Union (EU) countries in 2017 with an overall rate of 0.09

cases/100,000 person-years (Lai *et al.*, 2021). Compared to the other EU countries, the number of cases reported from Greece, Italy, Spain and Portugal were high (Lai *et al.*, 2021). The cases reported in Sweden were mostly due to the travelers arriving from the countries where brucellosis is endemic. In the USA, brucellosis is found commonly in immigrant families, especially in border states such as Texas (Serpa *et al.*, 2018). However, *B. abortus* has not been reported in Western and Northern Europe, Canada, Japan, Israel, Australia and New Zealand (Adetunji *et al.*, 2019).

B. melitensis has been identified as the main pathogen in high-risk regions such as the Middle East and Asia while *B. abortus* and *B. suis* commonly cause human brucellosis in USA (Adetunji *et al.*, 2019). Human brucellosis has been reported common in abattoir workers and in pregnant women in Cameroon (El-Koumi *et al.*, 2013). Handling of fetuses and uterine contents has been identified as a potential risk factor for brucellosis in abattoirs workers in Africa (Awah-Ndukum *et al.*, 2018). In addition, ingestion of raw milk is the main causes of human infection in children in the African continent (Awah-Ndukum *et al.*, 2018). Furthermore, human brucellosis was mostly associated with small ruminants such as goat and sheep than rest of the livestock species, and therefore consumption of unpasteurized goat milk was identified as the most common route of infection in humans in Southeast Asia (An *et al.*, 2021).

A number of socio-economic factors, and animal husbandry practices may contribute to the high incidence of brucellosis in developing countries compared to the developed countries (Awah-Ndukum *et al.*, 2018). The socio-economic factors associated with human brucellosis have been studied in limited localities in the world. Human brucellosis was reported to be common among agriculturalists (81.9%) and pastoralists (12.4%) (Li *et al.*, 2020). Occupational or domestic exposure to livestock is considered as the main risk factor for human brucellosis. In addition, socio-cultural practices such as consumption of raw blood and dairy products and slaughtering of animals within the homesteads in some communities are also risk factors identified for human brucellosis (Njeru *et al.*, 2016).

The individuals and families of livestock farmers, veterinarians, abattoir workers and livestock keepers are considered as the high-risk category of the infection (Njeru *et al.*, 2016). In addition, a seasonal variation has been shown to influence the incidence rate of human brucellosis and accordingly, most of the clinical cases were reported in summer period (Njeru *et al.*, 2016). The frequent animal grazing in summer has been identified as the reason for the high incidence of human brucellosis in livestock associated communities (Njeru *et al.*, 2016). Similarly, it has been shown that temperature, day length and evaporation contribute significantly to seasonal fluctuation of human brucellosis in China (Liu *et al.*, 2012).

Most of the clinical cases of human brucellosis were observed in the 25-59 years age category with an over-representation of males (male to female ratio 2.64:1) although the disease had been reported in all age groups in both males and females (Njeru *et al.*, 2016). Associated gender differences of clinical brucellosis had been observed in Saudi Arabia; the incidence of brucellosis was found high in men than in women while most of the clinical cases have been reported in summer (Alkahtani *et al.*, 2020). Further, incidence of clinical brucellosis in Saudi Arabia was shown to be high in individuals aged between 21-40 years (Alkahtani *et al.*, 2020). In Bangladesh, the prevalence of human brucellosis was reported to be 4.4% and prolonged history of livestock farming has been identified as the main reason for their vulnerability (Rahman *et al.*, 2012). In Sudan, over 23.3% of human patients who sought medical assistance for febrile illness were positive for brucellosis (Madut *et al.*, 2018). Interestingly, the incidence of clinical brucellosis in goat farmers was 60 times higher than in cattle farmers in Bangladesh (Rahman *et al.*, 2012). The sero-prevalence of human brucellosis varied from state to state in the Indian peninsula; it was as low as 1.83% in Nagpur (Ghugey *et al.*, 2021) while as high as 5.1% in Karnataka (Patil *et al.*, 2016).

When Sri Lankan scenario is concerned, brucellosis is an endemic disease in livestock and it has been reported mainly in cattle, buffaloes and goats while a few cases reported in sheep, pigs and wild animals since 1956 (Priyantha, 2011; Bandara and Mahipala, 2002; Kothalawala *et al.*, 2017, 2018). However, the reported incidence of brucellosis was low in humans although it is identified as a

significant disease in livestock in Sri Lanka. (Priyantha, 2011; Karunanayake *et al.*, 2019). The sero-prevalence of brucellosis in Sri Lanka was reported to be 8.4% (Karunanayake *et al.*, 2019). This study was conducted in four provinces including Central, North-Western, North-Central and Western Provinces of Sri Lanka with healthy individuals representing livestock farmers (n=818), veterinary staff (n=190), abattoir workers (n=137) and urban-dwellers without any contacts with livestock or livestock related workplaces (n=149). The same study highlighted that livestock farmers and people who are in close contact with livestock were at high risk of human brucellosis (Karunanayake *et al.*, 2019). A clinical case of human brucellosis had been reported in 2009 in Moneragala district of Sri Lanka (Udugama *et al.*, 2018). Furthermore, close contact with livestock had been identified as the main risk factor for human brucellosis in the reported cases in the country (Kothalawala *et al.*, 2017; Karunanayake *et al.*, 2019). Therefore, brucellosis is considered an important disease among farming communities in Sri Lanka and other developing countries (Kothalawala *et al.*, 2017, 2018; Deng *et al.*, 2019; Khurana *et al.*, 2021).

Brucella is a multi-host bacterium, and the organism has been isolated in a range of host including cattle, buffalo, goat, sheep, pig, dog, camel and other wild animals (Table 1). Humans are the incidental hosts of brucellosis, and the bacterium can be transmitted via various routes, including oral, conjunctival, respiratory, cutaneous, placental, blood and through bone marrow transplantation (Adetunji *et al.*, 2019). Direct exposure to contaminated animal products such as consumption of unpasteurized milk, exposure to genital secretions and aborted fetuses, inhalation of infectious aerosols and accidental vaccine inoculations are the main route of transmission of brucellosis in humans (Al Anazi *et al.*, 2019). In addition, human to human transmission has also been reported in isolated cases in which the disease was transmitted between humans through breastfeeding, sexual activity and blood transfusions (Adetunji *et al.*, 2019). Four species of *Brucella* are considered pathogenic to humans including *B. melitensis*, *B. suis*, *B. abortus* and *B. canis* (Adetunji *et al.*, 2019). Among these species, severe clinical infection is usually caused by *B. melitensis* in humans (Adetunji *et al.*, 2019). Although human to human transmission had been reported and human is considered an accidental

host or the dead-end host for the bacterium (Zheng *et al.*, 2018).

Clinical disease

The high recovery rate is the common and positive observation of clinical brucellosis in humans, as over 61% of patients responded to the antimicrobial therapy (Shi *et al.*, 2018). However, the duration of treatment was reported to be as long as 30-50 days (Shi *et al.*, 2018). It is important to realise that the clinical signs of human brucellosis are highly nonspecific and may include undulant fever, fatigue, headache, chills, myalgia and arthralgia. According to a study from China, fatigue (67%), fever (64%), arthralgia (63%) and sweating (54%) were the most common clinical signs reported in the patients diagnosed with human brucellosis (Shi *et al.*, 2018). Based on another study carried out in China by Zheng *et al.*, (2018), fever (87%), fatigue (63%), arthralgia (62%) and myalgia (56%) were identified as the most common clinical signs. Another more recent study from China by Jiang *et al.*, (2019) reported that fever (93.3%), joint pain (69.8%), sweating (45.2%), fatigue (38.6%) and splenomegaly (34.0%) as the main clinical signs of human brucellosis. Joint pain, low backache, fatigue and night sweating were reported as signs of childhood brucellosis in a study conducted in India (Dutta *et al.*, 2018). Other less frequently reported clinical signs of human brucellosis include orchitis/epididymitis, acute renal failure, endocarditis, splenic abscess, spondylitis, arthritis, encephalitis, hepatitis, meningitis and respiratory and cardiac complications (Njeru *et al.*, 2016). Although clinical signs vary among patients due to several host factors including age, duration of the infection, immunological status, and concurrent metabolic disorders of the host, it has been identified that fever, arthralgia, myalgia, back pain, enlarged lymph nodes, splenomegaly, hepatomegaly, skin rashes, pharyngitis and hematological and respiratory complications were more common in children with brucellosis than in adults (Bukhari, 2018). In addition, chills, headaches and weight loss were observed commonly in children with chronic infection of clinical brucellosis than others (Bukhari, 2018). Furthermore, a higher rate of abortion and intrauterine fetal deaths were reported in serologically positive pregnant women compared to pregnant women without brucellosis (Patra *et al.*, 2018).

In south India, acute brucellosis infection is common than chronic or sub-acute infection (Patra *et al.*, 2018). Fever is the main indicator of acute infection while back pain is the main indicator of chronic brucellosis in human (Patra *et al.*, 2018). Bone or joint pain remains as one of the severe complications of human brucellosis described in literature (Shi *et al.*, 2018). The age, presence of back pain and joint tenderness had been identified as the main risk factors for unfavorable prognosis in human brucellosis (Shi *et al.*, 2018). Arthritis, especially in large joints such as the knee and hip joints were reported as a common clinical finding in human brucellosis (Shi *et al.*, 2018; Ibrahim and Al-Shahrani, 2021). In addition to knee and hip joints, arthritis has been reported in ankles, shoulders, elbows, wrists and sternoclavicular joints in patients with brucellosis (Shi *et al.*, 2018; Ibrahim and Al-Shahrani, 2021). Sacroiliitis is also reported as a complication of brucellosis in humans (Slobodin *et al.*, 2016). Importantly, arthritis may present with monoarticular, oligoarticular or polyarticular distributions while brucella-induced spondylitis at the disco-vertebral junction has been reported at the lumbar region, especially in L4 and L5, and thoracic and cervical regions (Adetunji *et al.*, 2019). Furthermore, brucella-induced osteomyelitis has been reported to occur secondary to inflammation and necrosis of bone tissues (Adetunji *et al.*, 2019). Osteomyelitis caused by brucellosis often presents as a motor neuronal weakness with a high rate of therapeutic failure (Adetunji *et al.*, 2019; Ibrahim and Al-Shahrani, 2021). Therefore, about 27% of human brucellosis cases end up with osteoarticular lesions (Adetunji *et al.*, 2019). Further, spinal brucellosis is also often associated with therapeutic failure against antimicrobial treatments in humans and therefore early diagnosis is required to decrease morbidity and mortality (Tali *et al.*, 2015).

Hematological changes were also reported in human patients with brucellosis and thrombocytopenia, leukocytosis, anaemia and pancytopenia were the most common abnormalities reported in adults (El-Koumi *et al.*, 2013; Zheng *et al.*, 2018). In children, anemia (43%), leukopenia (38%), leukocytosis (20%) and pancytopenia (18%) were the most commonly reported hematological picture (El-Koumi *et al.*, 2013). However, thrombocytopenia was less commonly reported in children affected with brucellosis than adults (Serpa *et al.*, 2018) and it is important to

note that the mean platelet volume and neutrophils to lymphocytes ratio have been used as markers of childhood arthritis resulted from brucellosis (Bozdemir *et al.*, 2017). In addition, Shi *et al.*, (2018) reported that high erythrocyte sedimentation rate (69%), increased C-reactive protein (39%), high alanine aminotransferase (ALT) (33%) and high aspartate aminotransferase (AST) (20%) have been the most common laboratory findings reported in patients with acute brucellosis.

It is evident that brucella infects multiple organs in humans and therefore, various clinical presentations of human brucellosis have been reported. Further, the clinical presentation of brucellosis is shown to be different between adults and children. Skin rashes, respiratory and cardiac complications and orchitis/epididymitis were more prevalent in children with brucellosis (Zheng *et al.*, 2018). In addition, hepato-splenomegaly was reported in more than 60% of the children with brucellosis (Serpa *et al.*, 2018). Importantly, over 90% of the patients with brucellosis from Bosnia and Herzegovina and Europe recovered after antimicrobial therapy. However, the recovery rate was as low as 7.32% in patients with the relapsing of disease after a prolonged interval (Ahmetagić *et al.*, 2015). The commonly reported complications of brucellosis were hepatitis, followed by osteoarthritis, respiratory diseases, cardiovascular diseases, central nervous system dysfunction, hemophagocytic syndrome and orchitis/epididymitis (Zheng *et al.*, 2018). The rate of misdiagnosis of human brucellosis is reported to be high in non-pastoral areas (Zheng *et al.*, 2018) (Zheng *et al.*, 2018).

Although significant reproductive-related clinical signs were reported in animals infected with brucellosis, less pronounced obstetric abnormalities were reported in humans (Bosilkovski *et al.*, 2020). However, the reproductive related effects of brucellosis were shown to be significant in brucella infected pregnant women than in healthy pregnant women (Bosilkovski *et al.*, 2020). Seroprevalence of brucellosis in pregnant women varied from 1.5-12.2%; the cumulative incidence of brucellosis cases per 1000 delivered obstetrical discharges in endemic regions was 0.42–3.3. Abortions (2.5-54.5%), intrauterine fetal death (0-20.6%) and preterm deliveries (1.2-28.6%) were reported as obstetrics outcomes of brucellosis observed in infected pregnant women (Bosilkovski *et al.*,

2020). It was realised that the hospital staff or delivery team are at risk of acquiring the infection by handling fetal membranes and fluids of infected mothers or babies (Bosilkovski *et al.*, 2020). Congenital or neonatal brucellosis may cause low birth weight, delays in development or even premature infant death and thus, early treatment for brucellosis has been shown to reduce the frequency of adverse outcomes (Bosilkovski *et al.*, 2020).

Brucella organism has been isolated from human placenta, aborted fetuses and preterm stillbirths. In addition, the organisms have been isolated in blood, urine and other tissues of the female reproductive tract. Miscarriage due to brucellosis was found to be common in the first and the second trimester of pregnancy in women (Arenas-Gamboa *et al.*, 2016). The rates of preterm delivery, intrauterine fetal death and poor fetal growth were significantly higher in Israeli-Arab localities with a high incidence of brucellosis compared to localities where the disease was not reported (Bosilkovski *et al.*, 2020). Maternal bacteraemia, disseminated intravascular coagulation, placentitis and acute febrile reaction are the main causes of abortion and stillbirths in infected pregnant women (Arenas-Gamboa *et al.*, 2016). Although brucellosis is a multi-systemic disease in humans, a minimum kidney involvement has been reported and rarely a rapidly progressive glomerulonephritis had been reported (Shebli *et al.*, 2021). However, cellulitis has been reported as uncommon clinical sing of brucellosis in young men (Subramaniam and Ali, 2019).

Diagnosis and laboratory tests

As the clinical signs of brucellosis in humans are non-specific, laboratory confirmation is required for accurate diagnosis. Conventional bacterial isolation and identification, serological tests and nucleic acid amplification assay are used as laboratory diagnostic aids for human brucellosis (Deng *et al.*, 2019). The average incubation period of human brucellosis is 2 to 4 weeks. However, short incubation periods such as 5 days and long incubation periods such as 6 months also have been reported in literature (Deng *et al.*, 2019). Although conventional methods are used to detect human brucellosis, isolation of the organism from blood cultures is a challenging task due to persistent low-grade bacteraemia of brucellosis in humans (Yagupsky *et al.*, 2019). The low-grade

bacteraemia that leads to reduced circulating bacterial load in the peripheral circulation is shown to cause low isolation rate of human brucellosis (Pappas and Papadimitriou, 2007). As the infection progresses, the organism is cleared from the blood and localized in macrophages (Yagupsky *et al.*, 2019). The organism re-enters the blood stream intermittently making isolation of the bacterium by blood culture further difficult (Yagupsky *et al.*, 2019). The literature on blood culturing for brucella is not consistent and high variability of success rate has been described. The isolation of the organism by blood culture is most possible at the early stage of the disease (Shemesh and Yagupsky, 2011). However, over 70% success in isolation of bacterium by blood culture was reported from Southern India (Patra *et al.*, 2018). Haemolysed blood cultures and recently introduced automated systems have shown a high rate of detection than conventional methods (Pappas and Papadimitriou, 2007). In addition, bone marrow aspirates have shown a high rate of isolation of the organism than conventional blood cultures (Yagupsky *et al.*, 2019). Furthermore, the brucella organism can be isolated from other body fluids and tissue samples including genital exudates, bone tissue, synovial fluid and cerebrospinal fluid (Yagupsky *et al.*, 2019).

Both specific and nonspecific media are used for laboratory isolation and identification of the organism from clinical samples. Further, conventional, and quantitative PCR techniques are being used to confirm the isolate as brucella species. Importantly, two circular chromosomes (2.1 and 1.2 Mb) are found in brucella, and GC content is 57.2% (Yagupsky *et al.*, 2019). Several target genes have been used to detect the brucella species as *omp2*, *omp31*, *omp28*, *16rRNA*, *IS711*, and *bcs31*. (Horvat *et al.*, 2011; Yagupsky *et al.*, 2019; Kumar, 2011). Assays are optimized to detect brucella species using blood, serum and other tissue fluids (Yagupsky *et al.*, 2019). In addition, MALDI TOF MS and Vitek MS are also used for molecular characterization of brucella species. Although, fast, accurate and reproducible diagnosis of brucellosis is achieved by MALDI TOF MS, misidentification of *B. melitensis* as *Ochrobactrum anthropic* has been reported (Lista *et al.*, 2011; Yagupsky *et al.*, 2019). The detection of the organism in blood culture is recommended at the early stage of the disease, especially with nonspecific febrile illness (Yagupsky *et al.*, 2019).

However, testing multiple samples during prolonged incubation periods (maximum 4 weeks) are always recommended (Yagupsky *et al.*, 2019). Although serological diagnosis is not 100% accurate for the diagnosis of brucellosis in humans, it is considered an indispensable diagnostic tool in human diagnostic laboratories.

The type of antibodies produced against the smooth lipopolysaccharides of the brucella bacterium vary according to the duration of infection and the order is, serum concentration of IgM in the first week, IgG1 in the second week, and IgG2 and IgA in the latter part of the infection (Al Dahouk and Nöckler, 2011). Rose Bengal plate test (RBPT) and ELISA are also used in human diagnostic laboratories. RBPT, standard agglutination test (SAT), micro agglutination test, mercapto-ethanol test, Coombs antiglobulin agglutination test, Brucella Coomb's gel test CFT and immune-capture agglutination test are used as serological laboratory tests (Al Dahouk and Nöckler, 2011; Yagupsky *et al.*, 2019). In addition, fluorescent resonance energy transfer (FRET), fluorescent polarization immunoassay (FPA), fluorescence in situ hybridization (FISH) test, dipstick assays and lateral flow assays (LFA) have been optimized as the new diagnostic tests (Yagupsky *et al.*, 2019). Currently, some diagnostic tests such as intradermal skin tests are not used as diagnostic aids for human brucellosis. (Yagupsky *et al.*, 2019).

Blood pictures are also useful to diagnose brucellosis in humans. (Alumasa *et al.*, 2021). The most common hematological abnormalities reported in patients with human brucellosis include leukocytosis (24.1%), anemia (23.9%), thrombocytopenia (15.8%) and pancytopenia (13.2%) (Alumasa *et al.*, 2021). Febrile antigen brucella agglutination test (FABAT) and rose bengal plate test (RBPT) are the most frequently used diagnostic aids for brucellosis in developing countries (Alumasa *et al.*, 2021). The author believes that inadequate laboratory facilities, lack of awareness about human brucellosis among practitioners and farmers and non-specific nature of the clinical signs may cause underdiagnosis of human brucellosis in Sri Lanka, especially in livestock farmers.

Prevention, control, and treatment

The OIE control strategy consists of epidemic-sero surveillance, culling of positive animals and vaccination of livestock in the areas where eradication of bovine brucellosis is cost-effective. (<https://www.woah.org/en/disease/brucellosis>).

Similarly, according to the WHO, the elimination of infected animals by culling or vaccination of livestock has been recommended for controlling brucellosis. In addition, raising awareness about the disease, promotion of food-safety measures, occupational hygiene and laboratory safety have been highlighted as an important part of the control strategy in human brucellosis by WHO (<https://www.who.int/news-room/fact-sheets/detail/brucellosis>). The prevention strategy of human brucellosis consists of improving awareness of the disease, minimum handling of meat and milk products of infected animals, consumption of pasteurized milk, consumption of cooked meat and adhering to hygienic laboratory practices in the handling brucella (Deng *et al.*, 2019; Khurana *et al.*, 2021., Zheng *et al.*, 2018). The prevention and control of brucellosis in livestock species recommended by the OIE is considered as the most important method to control human brucellosis in endemic countries. The methods of prevention and control of brucellosis in livestock includes improving hygienic practices in livestock farms and slaughterhouses, prevention and control of brucellosis in animals by frequent screening, culling of infected animals, vaccination and movement restriction of the serologically positive animals. Further, raising awareness about the disease among the high-risk communities such as slaughterhouse workers, veterinarians, animal handlers and farming communities also helps reducing human brucellosis. In addition to livestock, proper handling of companion animals also help preventing the spread of the disease (Deng *et al.*, 2019; Khurana *et al.*, 2021., Zheng *et al.*, 2018). Furthermore, systematic surveillance of the disease in live animals and throughout the food chain is highly recommended (Deng *et al.*, 2019; Khurana *et al.*, 2021., Zheng *et al.*, 2018). According to the WHO recommendations, brucellosis in humans is treated with 100mg of doxycycline and rifampicin (15mg/kg/day) twice daily for 45 days. In addition, streptomycin and gentamicin are also used by the clinicians to treat human brucellosis (Deng *et al.*, 2019; Zheng *et al.*, 2018). Further, sulfamethoxazole-trimethoprim

with aminoglycosides or rifampicin are also used to treat brucellosis in children (Alavi and Alavi, 2013). A combination of rifampicin and trimethoprim-sulfamethoxazole is used for 6 weeks in most commonly used therapeutic regimes (Solera *et al.*, 1997).

CONCLUSION

Although human brucellosis is endemic in Sri Lanka, it is an underdiagnosed disease in the country, mostly due to the insufficient diagnostic facilities available at regional diagnostic laboratories. Nonspecific nature of clinical signs and concurrent presence of several other common diseases with similar clinical presentation also may have contributed to the low detection level of human brucellosis. Raising awareness and upgrading of diagnostic facilities are the key to improve diagnosis of human brucellosis in Sri Lanka. Improving hygienic practices, good management practices, improving biosecurity and vaccination of livestock may also help reducing the number of cases of human brucellosis. In addition, maintenance of hygienic practices and quality standards of food and milk processing would also reduce the incidence of brucellosis in humans.

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