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Host-Pathogen Interactions, Diagnostics, and Control Measures for Brucellosis in Ruminants-A review

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ABSTRACT

Ruminants are usually endemic to brucellosis, a disease caused by *B. abortus*, *B. melitensis*, *B. suis* and *B. ovis*. *Brucella* can affect animals of any age, regardless of sex, and is typically linked to substantial morbidity, spontaneous abortion both in ruminants (last trimester), and humans. *Brucella* species form a closely related monophyletic cluster with DNA-to-DNA hybridization values that are close to 100%. The *Ochrobactrum* genus members are the nearby phylogenetic ancestors of *Brucella*, which share over 97% identity with the *Brucella* consensus sequence of the 16S rRNA gene. Lipopolysaccharide (LPS), Type IV secretion system (T4SS), Urease, Cytochrome oxidase and BvrR/BvrS system are important virulence factors which help bacteria to invade and cause infection in a host. Stamp's modified Ziehl-Neelsen (ZN) staining, immunohistochemistry, serological tests, and bacterial culture (gold standard) are the important tests for screening and diagnosis of brucellosis. Test and slaughter policies are not feasible to control brucellosis in developing nation such as India because cows are often used as a symbol of zeal and pride. Therefore, to reduce the burden of brucellosis in ruminants, attention must be focused on proper screening and segregation, vaccination, better cleanliness and hygiene, better management, increased farmer awareness, and more robust cows, in addition to the disease reporting system, such as diagnostic options and surveillance in farm animals.

HIGHLIGHTS:

- *Brucella* species can invade and persist in both phagocytic and non-phagocytic host cells.
- LPS, T4SS, Urease, Cytochrome oxidase and BvrR/BvrS system are important virulence factors.
- Test and slaughter, test and segregation, vaccination, increasing farmer awareness about the disease are important measures to control brucellosis.

Keywords: Brucellosis, diagnosis, pathogenesis, ruminants, virulence

Brucellosis is a zoonotic disease caused by gram-negative, facultative, intracellular bacteria of the genus *Brucella* that affects a variety of domestic and wildlife mammals (Moreno, 2014). Bacteria are short rods/coccobacilli measuring about 0.5 to 0.7 by 0.6 to 1.5 µm, nonmotile, non-spore-forming, and steady-growing belonging to the family "Brucellaceae". The bacteria are placed in the alpha-2 subclass of the Proteobacteria, along with the genera Mycoplana, Pseudochrobactrum, Paenochrobactrum, Daeguia, Crabtreella, and Ochrobactrum (Whatmore *et al.,* 2016). The genus *Brucella* includes the six known species *B. abortus*, *B. melitensis*, *B. suis*, *B. canis*, *B. ovis*, and *B. neotomae* as well as the two provisional marine species *B. pinnipedialis* and *B.* ceti; B. *microti* and *B. inopinata* have been newly proposed *Brucella* species isolated from the

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common vole and a breast implant respectively (Moreno, 2014; Pappas *et al.,* 2005).

Ruminants population are mostly susceptible to brucellosis which is typically caused by *B. abortus*, *B. melitensis*, *B. suis* and *B. ovis* (OIE, 2016). More than 300 million of the 1.4 billion cattle worldwide are thought to be infected with the pathogen (de Figueiredo *et al.,* 2015). Infection in adult female cattle is usually localized in the reproductive system and can cause placentitis, abortion (3rd trimester), premature delivery and results in economic loss to farmers. Most infected animals miscarry only once in their lifetime, but they can remain infected for life. Brucellosis in adult male cattle causes orchitis, epididymitis and can cause infertility in both sexes (Deka *et al.,* 2018). In some tropical nations, hygroma, which can develop in the joints of the legs, is a typical brucellosis symptom (OIE, 2016). The important risk factors associated with bovine brucellosis disease are age, sex, breed, history of abortion, retention of placenta, repeat breeding etc. (Fig. 1).

Brucellosis origin, zoonotic potential and challenges to diagnosis

Since the Roman Empire, brucellosis history and related clinical symptoms have been documented, and *Brucella* has always been important in terms of zoonotic transmission (Godfroid *et al.,* 2005). *Brucella*-like creatures resembling coconuts were discovered in carbonated cheese during the Roman Empire (Capasso, 2002). A significant development occurred in 1887 when Sir David Bruce identified the bacterium as *Micrococcus melitensis* from the spleen of a British soldier who had died in Malta from Mediterranean fever. In honor of Sir David Bruce, the name was eventually changed to *Brucella melitensis* (Godfroid *et al.,* 2005). *Brucella abortus*, which causes brucellosis (undulant fever) in humans and abortions in cattle, was discovered by Bang (Mantur and Amarnath, 2008).

Brucellosis, an occupational direct anthropozoonosis, can be acquired from infected animals to humans through contact with their aborted fetuses and associated materials, ingestion of raw milk, or consumption of unpasteurized dairy products (OIE, 2016). *Brucella* species are considered as category B bioterrorism agent due to their ability to aerosolize and the minimal number of pathogenic organisms needed for infection. The estimated financial risk of such an attack is second only to anthrax and tularemia, with an infectious dose of 10 to 100 organisms (Ducrotoy *et al.,* 2016). The acute symptoms of brucellosis often manifest between two and four weeks after exposure; however, the disease can have a subtle beginning, with some cases not being detected for up to six months (Spickler, 2018). Weakness, fever, excessive sweating (especially at night), weight loss, generalized pain, testicular swelling, and burning urination from orchitis and urethritis are among the symptoms experienced by infected humans, with the most prevalent consequence being arthritis. Every year, around 500,000 cases of human brucellosis are reported worldwide (Ducrotoy *et al.,* 2016; Pal *et al.,* 2017). However, this alarming number can only be regarded as an approximate number considering many instances go unreported as a result of inappropriate diagnosis, inadequate surveillance, and incomplete reporting (WHO, 1997).

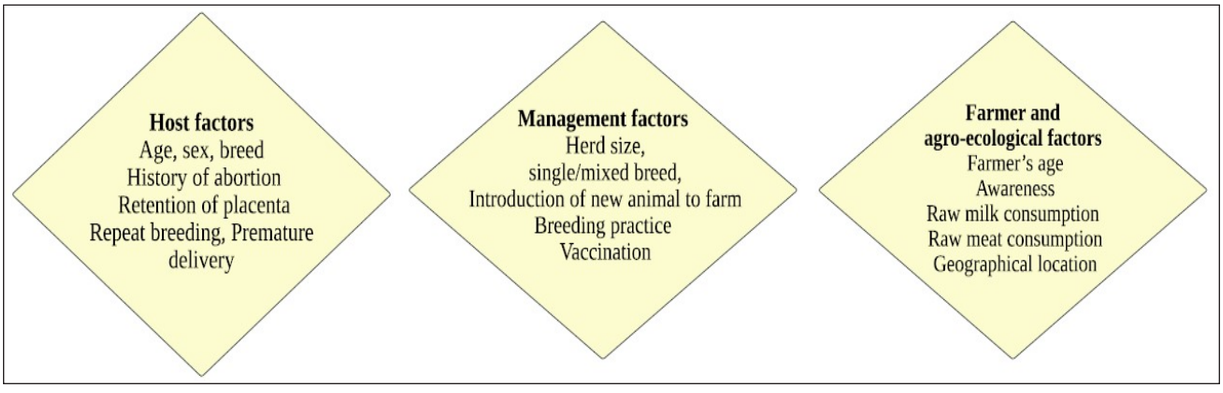

Fig. 1: Important risk factors of brucellosis in ruminants (Deka *et al.,* 2018)

Brucella species form a closely related monophyletic cluster with DNA-to-DNA hybridization values that are close to 100% (Al Dahouk *et al.,* 2010). The Ochrobactrum genus members are the nearby phylogenetic ancestors of *Brucella*, which share over 97% identity with the *Brucella* consensus sequence of the 16S rRNA gene. Species like *O. antrophi* and *O. intermedium* appear to be more related to the *Brucella* than to other species in their own genus (Velasco *et al.,* 1998; Gee *et al.,* 2004; Scholz *et al.,* 2008; Bohlin *et al.,* 2010). This noteworthy similarity has significant suggestions for both the accurate diagnosis of the infection and proper identification of *Brucella* (Yagupsky *et al.,* 2019).

Brucellosis is an endemic disease in many developing nations across the world, such as the Middle East, Asia, Africa, and South America, as well as in the United States, where disease foci remain because of the insistent infection in wildlife species (de Figueiredo *et al.,* 2015). This review of *Brucella* host-pathogen interactions, virulence factors, and diagnostics is intended to understand the disease pathophysiology and diagnosis and to provide the measures to control the disease worldwide.

Pathogenesis and Important Virulence Factors of *Brucella*

Brucella species can invade and persist in both phagocytic and non-phagocytic cells of the host. *Brucella* exhibits strong tissue tropism and proliferate within the vacuoles of dendritic cells (DCs), placental trophoblasts, and macrophages. However, *Brucella* can also proliferate within other cells types, such as murine fibroblast (NIH3T3) or epithelioid cell (HeLa) (Pizarro-Cerda *et al.,* 2000; Celli, 2006; de Figueiredo *et al.,* 2015; Xavier *et al.,* 2010). Chronic infection arises from the organism's capacity to survive in the host cells, where *Brucella* spreads through the lymphoreticular system to ultimately cause hepatic, cardiovascular, lymphoreticular, osteoarticular and neurologic disease (de Figueiredo *et al.,* 2015). Although the intracellular existence of *Brucella* in the host cells prevents it from host innates and adaptive immune responses and shields the organism from the effects of some antibiotics (Martirosyan and Gorvel, 2013). The disease is associated with detectable splenomegaly along with elevated lymphohistiocytic cells in the spleen as well as minor decline in the percentage of splenic CD4+ and CD8+ T cells, and significant increase in the percentage of splenic macrophages (de Figueiredo *et al.,* 2015).

Brucella strains penetrate the host cells through a zipper-like mechanism and advances through the layer of mucosal epithelial cells where the macrophages and dendritic cells (professional phagocytes) engulf the bacteria. In addition, bacteria can also persist within non-phagocytic cells for up to 72 hours after infection before breaking through the epithelial barrier and entering phagocytic cells (Gorvel and Moreno, 2002; de Figureido *et al.,* 2015). Non-opsonized *Brucella* organisms are internalized into macrophages via. lectin or fibronectin receptors whereas opsonized *Brucella* organisms are internalized through complement and Fc receptors. Opsonized bacteria are more likely to be eliminated within the macrophages than the non-opsonized bacteria. With in the mononuclear phagocytic cells, *Brucella* exist in a special vacuole called *Brucella*-containing vacuole (BCV), alter intracellular trafficking, and convert the vacuole into a replicative compartment called brucellosome. *Brucella* adapts quickly after invasion to the low nutritional availability of the microenvironment inside the BCV, according to experimental evidence (Kohler *et al.,* 2002). The bacteria with in the macrophages avoid the host immune response, proliferates and spread through the lymph nodes to other tissues using cellular tropism and then ultimately translocate to the ideal tissues in reproductive tract such as placental trophoblasts, fetal lung, male genitalia, skeletal tissues, reticuloendothelial system, and endothelium (de Figureido *et al.,* 2015; Kim, 2015; Carvalho *et al.,* 2008). Bacteria persuades acute or chronic infection of the reproductive tract and can results in severe reproductive diseases or abortion (He, 2012).

Important virulence factors which help bacteria to invade and cause infection in a host are Lipopolysaccharide (LPS), Type IV secretion system (T4SS), Urease, Cytochrome oxidase and BvrR/BvrS system (two component system).

LPS consists of lipid A, oligosaccharide core and O-antigen and is different and non-classical in *Brucella* as compared to other Gram-negative bacteria such as *E. coli*. Lipopolysaccharide from *Brucella* strains is not as much of toxic and active than the classical LPS isolated from *E. coli* (Cardosos *et al.,* 2006; Christopher *et al.,* 2010). *Brucella* strains can be either smooth or rough, expressing smooth LPS (S-LPS) or rough LPS (R-LPS) as main surface antigen. The structure of smooth LPS

consists of lipid A (contains aminoglycose, fatty acid and β-hydroxymiristic acid), core (covers mannose, glucose, quinovosamine) and O-chains (composed of 4-formamido-4,6-dideoxymannose). The structure of the R-LPS is similar to the S-LPS, except for O-chains, which are greatly reduced or absent (Corbel, 1997).

T4SS is a multiprotein complex that take part in the secretion of bacterial macromolecules (Cascales and Christie, 2003). This system, which is typified by the virB operon, which codes for 12 proteins (11860 bp), is highly similar to T4SSs found in *Rhizobia*, such as the phytopatogenic *Agrobacterium tumefaciens* (O'Callaghan *et al.,* 1999). With in the macrophages *Brucella* spp. resides in *Brucella*-containing vacuole (BCV); BCV interacts with the endoplasmic reticulum (ER) and results in the formation of specific *Brucella* -multiplication compartment (Kohler *et al.,* 2002). *Brucella* replicates intracellularly in these endoplasmic reticulum-associated compartments (becomes niche for *Brucella*) found in macrophages, epithelial cell lines, and placental trophoblasts and can cause chronic infection. The acquisition of endoplasmic reticulum membrane rests on a functional virB secretion system i.e. T4SS (Celli *et al.,* 2003).

Urease, a metalloenzyme, breaks down urea into carbonic acid and ammonium, and results in an increase in pH. This characteristic of urease enables *Brucella* to survive in acid environment and may protect *Brucella* during passage through the digestive tract (stomach), when the bacteria access their host through the oral route (Seleem *et al.,* 2008; Bandara *et al.,* 2007). *Brucella* has two non-identical urease operons i.e. ure-1 and ure-2 in two separate genomes separated by 1Mb of DNA. These operons encode structural genes (ureA, ureB, ureC) and accessory genes (ureD, ureE, ureF, ureG) (Bandara *et al.,* 2007). All *Brucella* bacteria, with the exception of *B. ovis*, produce urease (Sangari *et al.,* 2007).

Cytochrome oxidase is an enzyme that helps *Brucella* survival in the oxygen-poor environment of macrophages. *Brucella* has two high oxygen-affinity oxidases i.e. cytochrome cbb3-type and cytochrome bd (ubiquinol oxidases) encoded by two operons in the genome. Cytochrome cbb3 oxidase is expressed in vitro and facilitates the colonization of anoxic tissues (maximal action is achieved in microaerobiosis) whereas, Cytochrome bd oxidase is expressed during intracellular multiplication and facilitates adaptation to the replicative niche (Loiser-Meyer *et al.,* 2005).

Brucella genome has two open reading frames (ORF) i.e. BvrR [encodes BvrR proteins (237 amino acid)] and BvrS [encodes BvrS proteins (601 amino acid), located in cell membrane] that controls intracellular replication of *Brucella* (Sola-Landa *et al.,* 1998; Martinez-Nunez *et al.,* 2010). BvrR/BvrS system regulates multiple genes expression (Viadas *et al.,* 2010) and mutants of BvrR/BvrS show structural changes in LPS, but O-chains remains unchanged. These mutants persist extracellularly and do not infect the cell because they are unable to activate GTPase (Cdc42) prior entry into cell (Guzman-Verri *et al.,* 2001). BvrR/BvrS is also in responsibility for limited lysosome fusion and intracellular trafficking (Lopez-Goni *et al.,* 2002). It has been demonstrated that BvrR/BvrS system controls virB expression through positive stimulation of vjbR transcription (Glowacka *et al.,* 2018).

DIAGNOSTICS

Stamp's modified Ziehl-Neelsen staining

Microscopic analysis of smears made from secretions, affected tissues, and exudates, utilizing modified Ziehl-Neelsen (Stamp) staining, may facilitate in a tentative diagnosis. *Brucella* are not truly acid-fast but resistant to decolorization by weak acids, and finally stain red. Bacteria appear as coccobacilli or short rods, usually arranged singly but sometimes in pairs or small groups. Some organisms can mimic *Brucella*, including *Coxiella burnetii* and *Chlamydia abortus* (Spickler, 2018). Tilak *et al.* (2016) carried out a study to detect *Brucella* colonies in blood culture bottles of humans using Gram staining and Stamp's modified cold Ziehl-Neelsen (ZN) staining using 0.5% acetic acid as decolorizer. All the analysed blood culture fluids showed small Gram-negative coccobacilli on gram staining and pink coccobacilli on ZN staining respectively.

Immunohistochemistry

Immunohistochemistry in formalin-fixed, paraffin-embedded tissues is a useful tool for the diagnosis of brucellosis. Ilhan and Yener (2008) carried out an immunohistochemical study in Turkey to identify

Brucella melitensis antigens in sheep abortions that occurred naturally. Sections of the lung, liver, kidney, and spleen of each fetus were stained with immunoperoxidase. *Brucella melitensis* antigens were identified in 33 out of 110 fetuses (30%). Among the 33 positive cases of fetuses, *Brucella* antigens were discovered in the lung (22.7%), liver (19%), spleen (11.8%), and kidney (5.4%). Microscopic investigations revealed that the primary locations of *Brucella* antigens were in the cytoplasm of lung neutrophils and macrophages, as well as in the cytoplasm of macrophages in portal infiltrates and Kupffer cells in the liver.

Serological tests

The rose bengal plate test (RBPT), standard tube agglutination test (STAT), complement fixation test (CFT), milk ring test (MRT), enzyme-linked immune sorbent assay (ELISA), rapid slide agglutination test (RSAT), agar gel immunodiffusion (AGID), counter immunoelectrophorsis (CIE), and immunochromatographic test (ICT) are among the serological tests that can be used to identify antibodies against smooth or rough strains of the *Brucella* organism (Sharma *et al.,* 2023). It has been suggested to use a battery of serological tests for the diagnosis of brucellosis due to the varying sensitivities/specificities of individual tests (O.I.E 2016). Buffered *Brucella* agglutination tests (BBATs) [which includes rose bengal plate test (RBPT) and the buffered plate agglutination test (BPAT)], Enzyme Linked Immuno-Sorbent Assay (ELISA) and Fluorescence polarization assay (FPA), are considered as appropriate screening tests for the control of brucellosis at the national or local level. For international trade purposes, the serum agglutination test (SAT) is generally considered unsatisfactory. The complement fixation test (CFT) is more robust and specific than the SAT, with a standardized unitage system, although it can be affected by anti-complementary activity. Certain enzyme-linked immunosorbent assays (ELISAs) and the fluorescence polarization assay (FPA) have diagnostic performance characteristics that are on the same level with or better than the CFT, and because they are more easily performed and less complicated, their use may be recommended (OIE, 2022). Prevalence with several serological tests used for the screening and diagnosis of *Brucella* infection in ruminants and their comparative effacies are as follows:

Legesse *et al.* (2023) conducted a study to assess the occurrence of brucellosis in sheep, goats, and cattle as well as to compare the efficacy of the Rose Bengal Plate Test (RBPT), Complement Fixation Test (CFT), and Indirect-Enzyme-Linked Immunosorbent Assay (I-ELISA). A total of 2317 sera samples were tested and found 189 were positive for RBPT, 191 for I-ELISA, and 48 for CFT. Using indirect ELISA as a reference test, sheep and goats showed 100% sensitivity to RBPT, while cattle showed 74% sensitivity. The RBPT specificity values for sheep, goats, and cattle were 98.69%, 99.28%, and 100%, respectively. Sheep had a CFT sensitivity of 5.56%, goats of 10.48%, and cattle of 52.94%. Sheep, goats, and cattle had 100% specificity on CFT. A cross-sectional study was carried out by Sharma *et al.* (2023) to determine the prevalence of brucellosis in stray cattle populations kept in cow shelters of Punjab, India. A total of 587 blood samples were collected and tested using Rose Bengal plate test (RBPT), standard tube agglutination test (STAT) and Indirect Enzyme Linked Immunosorbent Assay (I-ELISA) and found 62 (10.56%), 63 (10.73%) and 68 (11.58%) animals were seropositive using RBPT, STAT and I-ELISA respectively.

Koto and Boru (2023) conducted a cross-sectional study to determine seroprevalence of brucellosis in sheep and goats using serological tests RBPT and Indirect ELISA. Serum samples from 238 sheep and goats that appeared healthy and had not received vaccinations were taken. The results indicated that 8 (3.7%) and 18 (7.56%) animals were found to be positive by Indirect ELISA and RBPT, respectively. The RBPT prevalence was higher (7.7%) than indirect ELISA (3.7%). This might be the result of cross-reactions between *Brucella* and other bacteria that have similar epitopes. Deka *et al.* (2021) conducted a cross-sectional investigation using I-ELISA in the Indian states of Bihar and Assam with a total of 740 bovine serum samples and found sero-positivity of brucellosis was 15.9% and 0.3% in Assam and Bihar respectively. Shome *et al.* (2019) carried out a study to determine the seroprevalence of brucellosis in 15 states of India using protein G indirect ELISA. A total of 12,054 samples of bovine serum (9236 cattle and 2818 buffalo) were used, and the results revealed true prevalence of 8.3% and 3.6% in cattle and buffalo, respectively. Isloor *et al.* (1998) determined serological investigation of brucellosis in cattle and buffalo using RBPT and STAT with total of 30,437 bovine sera samples

(23,284 cattle and 7,153 buffalo) in 23 Indian states. The results revealed an overall sero-prevalence of 1.9% in the cattle population and 1.8% in the buffalo population.

Brucella specimens and culture

Aborted fetuses, the placenta, vaginal swabs, milk, semen, lymph nodes, and affected tissues can all harbor *Brucella*. Blood can be an appropriate medium for culture in *B. canis* infected dogs, because of prolonged bacteremia (Spickler, 2018). Globally, approximately 2% of human cases of brucellosis are caused by the laboratory-acquired infections of *Brucella* (Measureur *et al.,* 2018). The bacterium can enter the host through a variety of entry points such as the respiratory mucosa, conjunctivae, gastrointestinal tract, and abraded skin. A human infection can be established with remarkably few viable organisms (10–100 cells); bacteria can survive for weeks or even months on inanimate surfaces (Doganay and Aygen, 2003; Yagupsky and Baron, 2005). *Brucella* can live for 120–210 days in the spring, 30-180 days in the summer, 50–120 days in autumn, and 85–103 days in the winter in faeces, slurry, or liquid manure (Kerimov, 1983). For up to 50 days following infection, bacteria were cultivated from the feces of wolves experimentally infected with *B. abortus* biovar 1 (Tessaro and Forbes, 2004). The prolonged survival of *Brucella* species in manure, slurry, and faeces could pose a health risk to humans and animals.

Brucella in a laboratory can be isolated through a variety of commercially available dehydrated basal media are available, such as tryptose (or trypticase) soy agar (TSA) and *Brucella* medium base. Strains like *B. abortus* biovar 2 requires the addition of 2-5% bovine or equine serum to basal media to grow (Alton *et al.,* 1988). *B. abortus* and *B. ovis* requires 5–10% CO_2 for their growth but the growth of *B. melitensis* or *B. suis* is not dependent this but such a CO_2 enriched-atmosphere is ideal for the culture of all *Brucella* (OIE, 2022). Castaneda, a biphasic, non-selective medium is suggested for culture of *Brucella* from blood and other body fluids or milk where enrichment is needed. The medium is used because, in broth medium, *Brucella* have a tendency to dissociate, making it difficult to use conventional bacteriological techniques for biotyping (OIE, 2022). Media such as serum–dextrose agar (SDA) or glycerol–dextrose agar, can also be used for isolation (Alton *et al.,* 1988). SDA is usually favored medium for observation of colonial morphology (OIE, 2022). Barua *et al.* (2016) conducted a study for isolation of *Brucella melitensis* using castenda medium from patients suspected for human brucellosis in India. A total 102 blood samples were collected and of these, 18 isolates recovered on blood culture using castenda medium. The biochemical, PCR, PCR-RFLP and 16s rRNA sequencing discovered that all isolates were of *B. melitensis* and matched precisely with reference strain *B. melitensis* 16M.

Modified Farrell's medium containing polymyxin B sulphate (5 mg), bacitracin (25 mg), natamycin (50 mg), nalidixic acid (5 mg), nystatin (100mg) and vancomycin (20 mg), is the most commonly used selective medium for the isolation of *Brucella* (Stack *et al.,* 2002). Farrell's medium in conjugation with Thayer–Martin's modified medium has been considered best strategy for primary isolation of *Brucella* from veterinary field samples (Alton *et al.,*1988). Morales-Estrada *et al.* (2016) in Mexico used Farrel's medium containing antibiotics to culture *Brucella* from cow and goat manure in serologically positive animals. A total of 20 cow fecal samples (seropositive on card agglutination test and rivanol test) and 10 goat fecal samples (seropositive on card agglutination test and complement fixation test) were collected directly from anal openings, and bacterial isolation was performed on *Brucella* selective agar plates containing antibiotics and further identified by using BCSP31 PCR and species identification by using Bruce-ladder multiplex PCR. *B. melitensis*, *B. abortus*, and *B. suis* were found in cow manure, and *B. abortus* was found in goat manure.

De Miguel *et al.* (2011) formulated a selective and translucent culture medium (CITA) for selective isolation of *Brucella* that contains blood agar as a base supplemented with 5% sterile calf serum and antibiotics such as vancomycin (20 mg/liter), colistin methane sulfonate (7.5 mg/liter), nitrofurantoin (10 mg/liter), nystatin (100,000 International Units/liter), and amphotericin B (4 mg/liter). de Nardi Junoir *et al.* (2015) conducted a study to compare *Brucella* agar, CITA, and Farrell's media for selective isolation of *Brucella* abortus from the semen of bovine bulls in Brazil. A total of 335 semen samples were collected and concurrently subjected to microbiological culture in *Brucella* agar, Farrell media, and CITA media. The *B. abortus* B19 strain was isolated from 5 (1.49%) out of 335 semen samples using the three selective media. However, Farrell's medium was thought to be the best

selective medium for microbiological diagnosis because it allowed the isolation of samples without bacterial, commensal, or fungal contamination of plates.

Measures to Control Bovine Brucellosis

The traditional method of immunization, testing, quarantine, and slaughter with compensation programmes is less effective or practical in low and middle-income countries. More specific control strategies might be more beneficial (Mantur and Amarnath, 2008). But due to the introduction of vaccines like *B. abortus* strain 19 in cattle and *B. melitensis* strain Rev1 in goats and sheep, brucellosis in animals has been eradicated or nearly eradicated in many countries. There is already an established cattle brucellosis control programme in India. For the purpose of producing semen, the Government of India requires brucellosis-free populations, and all breeding bulls from artificial insemination facilities must undergo routine brucellosis testing (Renukaradhya *et al.,* 2002). The introduction of certified brucellosis-free herd's semen into the farm should be promoted as it serves as a significant risk factor (Cardenas *et al.,* 2019).

Today's increased traffic in animal products around the globe also contributes to the spread of certain diseases. One of them, brucellosis, is spread through consumption of raw milk, meat, and other unpasteurized dairy products. When transporting and trading livestock and animal products locally, regionally, nationally, and internationally, numerous rules and regulations must be carefully obeyed (OIE, 2016).

The majority of South East Asian nations typically employs the test and slaughter strategy to eradicate animal brucellosis (Zamri-Saad *et al.,* 2016). Because the cow is widely used as a synonym for religious fervour and pride, test and slaughter policies are significantly more difficult in India. Only a small number of Indian states, including Kerala and other northeastern regions, have legalized the slaughter of cattle (Renukaradhya *et al.,* 2002). Although a test and slaughter policy could significantly lower brucellosis incidence and prevalence, the disease could not be completely eliminated due to a number of factors, including the incompetence to contain or regulate animal movement, struggle to trace brucellosis infected animals, the purchase of animals without first testing them for brucellosis, and farmers' lack of awareness of and interest in the disease (Gwida *et al.,* 2010). Furthermore, it would result in substantial financial losses for poor farmers (Renukaradhya *et al.,* 2002). Therefore, the focus should be on effective surveillance mechanisms to identify infected animals, segregate them into separate sheds, vaccinate animals and prevent the spread of infection from infected herds to non-infected herds, remove *Brucella* infection reservoirs, and take preventive measures to prevent the disease from being introduced into a herd again (Gwida *et al.,* 2010).

Another strategy for brucellosis management is testing and segregation of diseased animals. A study done in Uttar Pradesh, India found that by routinely testing all animals and isolating seropositive animals at a different facility from the main farm, the incidence rate was reduced from 12.4% to 1.2%. Better housing, sanitary disposal of aborted materials, and calf vaccination have all been undertaken in addition to this (Kollannur *et al.,* 2007).

Vaccination in female calves and removing infected animals from endemic areas are the best ways to prevent, control, and eradicate brucellosis. In a study conducted by Beauvias *et al.* (2016), it was found that following vaccination of sheep and cattle, it takes 3.5 years to completely eliminate the disease from mixed cattle and sheep species farms endemic with *B. melitensis* according to the Susceptible-Exposed-Infectious-Recovered-Susceptible (SEIRS) model. The elimination period increased to 16.8 years when vaccine was only restricted to sheep. Vaccinating cattle in endemic locations is, therefore, absolutely necessary. The *Brucella abortus* strain 19, *Brucella abortus* RB51, and *Brucella melitensis* Rev1, are frequently used as vaccine strains to prevent *Brucella* infection and associated abortions in animals (Dadar *et al.,* 2019).

The alleged health benefits of raw milk products and the rural population's consumption of them in brucellosis-endemic regions have been a significant contributor to the increased incidence of human brucellosis, which urgently requires accurate preventive strategies that regularly molecularly detect and monitor the disease in livestock, their likely pathogen sources, and implementation of hygiene precautions during the processing of dairy products (Ganter, 2015). Additionally, there must be an increase in awareness among the populace that eating raw meat, milk and milk products puts them at risk of contracting a number of infections (Waring, 2005).

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