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Brucellosis in India: Its pathogenesis, clinical manifestations, and diagnostic procedures

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Abstract

Brucella epidemiology has a great impact on the economy of livestock production farms across different states of India. This leads to heavy economic losses for the farms and food production industries. The Brucella family has multiple biovars in each strain, which affects cattle, buffalo, goats, sheep, equine, swine, and canine species to marine and wildlife. With widespread zoonosis within different species, the bacteria pose a great threat to public health in humans, causing undulant fever, remitting fever, Maltese fever, and Gibraltar fever. Prevalence of *Brucella abortus* and *Brucella melitensis* among other strains have the most severe impacts on animal health. While many countries have reached milestone success in control of its epidemiology, when sick cows cannot be treated or culled due to religious beliefs, India's struggle with brucellosis begins. The main objective of this review is to highlight the bacterium *Brucella* with an overview of brucellosis in various livestock animals.

1. Introduction

Brucellosis is a bacterial disease with significant economic, public health, and veterinary importance. The Food and Agriculture Organization and the World Health Organization define it as one of the most prevalent zoonotic diseases that can variably affect the productivity of animals. The disease is prevalent in most countries across the world and has been categorised under OIE List B diseases. The *Brucella* family affects a wide species of animals, from goats, sheep, cattle, buffalo, pigs, dogs, horses, camels, and bison. Brucellosis in India is a common but often neglected disease. Serious infections of *Brucella* can have life threatening effects on humans as well as animals. In India, brucellosis in livestock is responsible for an estimated loss of US $3.4 billion per year, out of which cattle and buffaloes account for 95.6% of total losses as a result of abortions, temporary infertility, and sterility in animals. The average loss is US$ 18.2 per buffalo, followed by 6.8 per cattle, 0.7 per sheep, 0.6 per pig, and 0.5 per goat (Singh et al. 2015). *Brucella* comprises ten host-specific species of *B. abortus* (cattle), *B. melitensis* (sheep and goat), *B. suis* (pig), *B. canis* (canids), *B. ceti* (cetaceans), *B. microti* (Microtusarvalis), *B. neotomae* (Neotomal epida), *B. ovis* (sheep), *B. pinipedialis* (pinnipeds), and *B. inopinata* (human) (Whatmore 2009; Minharro et al. 2013; Foster et al. 2007; Scholz et al. 2008; Scholz et al. 2010). Of these, *B. cetaceae* and *B. pinnipediae* have been isolated from marine organisms. Human brucellosis is often regarded as undulant fever, remitting fever, Maltese fever, and Gibraltar fever.

2. Colonial morphology

The disease is caused by the genus *Brucella*, which is a coccobacillary bacterium measuring 0.6-1.5 µ long and 0.6- 0.7 µ in length. The organism grows well over 5-10% blood agar within 3-5 days. The colonies grow on serum with pinpoint, smooth, glistening, translucent appearing colonies that tend to become opaque with ageing. They usually exist singly, but less frequently in small groups of pairs. The organisms are non-motile, facultative intracellular pathogens, and appear pleomorphic in older cultures. The bacteria are susceptible to long exposure to sunlight, acidic pH, and pasteurization at 60

ºC.

3. Biochemical tests

The bacteria is catalase, oxidase, and urease positive, which reduce nitrate. Strains of *B. ovis* and *B. neotomae* are oxidase negative, while *B. ovis* is urease negative. The organisms are capnophilic and require 5-10% CO_2 tension. The IMViC pattern is (-,-,-,-) and the culture produces H_2S gas.

4. Transmission

In India, over 80% of the population lives in close proximity to livestock, causing more human-animal interaction and a high degree of zoonosis of diseases. Seroprevalence suggests a transmutability of 0.9%-19% which is especially higher in veterinarians and farmers (Hemashettar and Patil 1991). Transmission of *Brucella* take place among animals with contact to the abortion material, retained placenta, and to a lesser extent through glandular orchitis and accessory sex glands in males. Feed contaminated with conjunctival inoculation, skin scrapings, ingesta, mucous, nasal and eye discharge is the primary source of infection for animals in a herd. Community pasture lands serve a larger risk of the infection. Transmission of the bacteria from dam to infant is frequently observed through the route of milk. The condition can be especially aggravated when calves are pooled together for manual feeding through buckets. Zoonotic transmission in humans is also observed through the same route as *B. abortus* can remain viable in damp environment, milk, and water for up to four months.

With the increase in artificial insemination, transmission of the disease is less frequent in cattle. However, venereal transmission through semen causes a significant number of infections in cattle, sheep, goats, horses, and pigs. This is especially true in caprine, ovine, and canine species where sharing of male breeding stock is practiced or where male animals are allowed to directly run with large number of females. Human to animal route of transmission is very rare. Other factors that are involved in the contamination of herds include newly added infected animals, unclean milking machines, transhumance of summer grazing lands, licking between animals, etc. Pasteurization of milk can significantly help avoid such infections.

5. Pathogenesis

Somatic antigens of *Brucella* are of major value in differentiating the biotypes in Brucella (Alton et al. 1975). The bacteria has two antigenic determinants: 'A' dominant from *B. abortus,* and 'M' dominant from *B. melitensis* (Wilson et al. 1932). These antigens are present on the LPS complex (Omp25). *The B. abortus* ratio for antigen A to M is 20:1 while the same ratio for B. melitensis is 1:20. The ratio is intermediate for *B. suis*. The three *Brucella* species of main concern in India - *B. abortus*, *B. melitensis*, and *B. suis*, have an endemic prevalence in different states of the country (Chauhan et al. 2000). *B. abortus* predominantly affects cattle and buffalo and can take a toll on the economic condition of a dairy farm. In India, *B. abortus* biotypes (type-1, 2, 4, 6, and 9) have been isolated from cattle, buffalo, sheep, goats, and pigs, while *B. melitensis* biotypes (type-1 and 3) have been isolated from goats, sheep, and cattle. Biotyping is usually done using biochemical assays, i.e., urease, catalase, and hydrogen sulfide production, and macroscopic or molecular observations. While *B. abortus*, *B. suis*, and *B. canis*, causing undulant fever, are rarely found infectious, *B. melitensis* is prominent in humans and causes Maltese or Malta fever. Symptoms caused by the disease include flu-like symptoms, sweating, constipation, headaches, myalgia, sexual impotence, nervousness, loss of appetite, chills, and severe lethargy (Koshi et al. 1971; Mousa et al. 1987). Diseases caused by the biotypes of these two variants are indistinguishable and may be equally severe. The prevalence of Brucellosis in the livestock population of a country can be directly related to its presence in the human population.

Pathogenesis of *Brucella*

Entry of bacteria in the animal through various routes

Entry of bacteria into regional lymph nodes and penetrance into enteral epithelial cells (Ackermann et al. 1988)

Infection of lymphatics causes bacteraemia

Localization of bacteria in reproductive organs, spleen, liver, mammary glands, foetus, placenta, joints, and lymph nodes

Entry of bacteria in cells with affinity to erythritol containing tissue like chorio–allantoic trophoblast

Abortion

Phagocytosis by body cells. Bacteria survive inside macrophages and prevent the fusion of lysosomes with phagosomes

Bacteria are protected from lysosomes and multiply within phagosomes

Antibodies are thus ineffective; macrophages rupture and bacteria continue to multiply

Susceptibility to *Brucella* is usually lower in calves where

the organism is usually shed through faces. Mucosal membranes, conjunctivae, and intact skin are routes of infection in adult cattle. The organism enters the lymphatic circulation and multiplies in the thoracic ducts, whereafter it passes to different parenchymatic tissues. The bacterium has adapted to survive in phagocytic cells to cause granulomatous lesions in parenchymatous and reticulo-endothelial tissue. *Brucella* shows a strong affinity for erythritol, a saccharide alcohol present in gravid uterus and placenta and thus, is most infectious to genital organs (Halling et al. 2005). In adult infected females, organisms are shed through colostrum and milk and may persist contaminating for several months to two years. According to Preez et al. (2015), the majority of infected cows continue to be chronically infected, which increases the risk of direct spread.

The *B. suis* has five biovars (type 1 to 5). *B. suis* biotype 1 has been consecutively reported in various piggeries in Southern India, causing decreased litter size and increased abortions (Shome et al. 2018).

The *B. melitensis* was reported in ovine, caprine, and cattle species followed by its highest zoonosis in human compared to other strains. There are three biovars (type 1 to 3). *B. melitensis* biovars-1, 2, and 3 were consecutively reported in the countryside of Delhi and Haryana (Sen and Sharma 1975).

6. Clinical signs

6.1 Cattle

The disease is almost always caused by *B. abortus* and comes with an incubation period of 30-60 days. After the causation of bacteraemia, the infection localizes to the placenta or foetus. In nonpregnant animals, the infection localizes to the udder, causing interstitial mastitis. In males, the localized infection causes glandular orchitis and inflammation of epididymis. An abortion in the last trimester is a cardinal sign of infection.

6.2 Equine

B. abortus and *B. suis* are the most common infectants. The cardinal signs of this infection in equines include fistulous withers or poll evil. The condition is followed by inflammation of the supraspinous bursa. Equine brucellosis is distinguished by the bursal sac filling with viscous fluid which eventually ruptures.

6.3 Ovine and caprine

The infection is caused by *B. melitensis* and *B. ovis* and affects pregnancy in the last trimester. The organism causes heavy losses on farms, with the death of young ones as a result of sporadic abortion. In goats, articular and periarticular hygromas may cause articular gout. Complications include orchitis in males, fertility abnormalities, and abortions. Microscopic lesions in the ductus deferens of rams include hyperplasia, perivascular oedema, and lymphocyte infiltration (Rahaley et al. 1984).

6.4 Swine

Cardinal signs of infection by *B. suis* include prolonged bacteraemia, abortion in early and late gestation (2nd and 3rd month), lameness, posterior paralysis, spondylitis, metritis, and smaller litter size (Megid 2010). The organism particularly infects the cotyledons, causing necrosis. Microscopic granulomatous lesions are characteristic in swine. Findings show a greater prevalence in finishing pigs compared to other ages due to active involvement in breeding activities (Ruiz-Fons et al. 2007).

6.5 Canines

Infection is caused by *B. canis* with an incubation period of 6-21 days, resulting in abortion during the last trimester or on the 50th day. Abortion is followed by yellow-brown to dark-brown discharges that persist for up to 6 weeks. Microscopic lesions are characterized by hyperplasia and plasmacytosis of lymph nodes, followed by hyalinization of glomeruli. In males, orchitis and testicular atrophy resulting in decreased spermatogenesis are the hallmarks of brucellosis. The ultimate result is improper functionality and infertility.

6.6 Marine mammals

The infection is caused by *B. cetaceae* in marine mammals like dolphins and whales. The organism is both a systemic and a secondary invader, resulting in meningoencephalitis, abortion, placentitis, and debilitation in dolphins.

6.7 Wild animals

The organism has similar clinical signs in wild animals like crows, vultures, bears, coyotes, and moose. Brucellosis is also prevalent in the gene pool of wild deer and buffaloes in India and the US.

7. Diagnostic procedures

Bovine brucellosis is endemic to all states of India and appears to increase during summer season. Epidemiological investigation of brucellosis relies greatly upon seroprevalence studies. Animals with a history of reproductive failure and abortion are typically screened for brucellosis using qualitative tests such as the Rose Bengal Plate Test (RBPT), and quantitative tests such as the serum tube agglutination test (SAT) and the Coombs test. Generalised tests such as the Whey Agglutination Test and enzyme-linked immunosorbent assay (ELISA) can also be used for diagnosis. In RBPT, the *Brucella* is stained with Rose Bengal dye. 0.3 ml of antigen and 0.3 ml of antibody are mixed together. Agglutination after 4 minutes is indicative of a positive result. In SAT, a serum sample is mixed with *Brucella* antigen without dye. Development of 50% agglutination at 1:40 and above dilutions is positive, while 1:20 and below 1:40 are considered suspicious. These days, advanced tests like the Brucella microagglutination test (BMAT), a modified version of the tube serum agglutination test (SAT), are preferred for their relevance. A brucellin PPD

test can be employed to test unvaccinated herd animals for *brucella* upon the occurrence of positive serological reactors.

Diagnostic procedures like Milk Ring Test (MRT) or Abortus Bang's Ring (ABT) are successfully equipped for testing of Brucellosis in large herds. These tests are based on the detection of antibodies in milk against antigens. Upon a positive test result, all animals are individually tested for Brucella. The formation of a white ring over a pink stained inactive brucella infected solution occurs upon a positive finding. Microscopic examination of chorionic foetal stomach content and uterine discharge using Koster's or Macchiavello staining method confirms the gram-negative *Brucella* organisms. The partially Ziehl-Nelson negative bacteria are resistant to decolourization by weak acids and stain red when Stamp's modification of the ZN method is applied. *Brucella* organisms stain red against a blue background in this staining. A fluorochrome or peroxidase-labelled antibody conjugate based on the technique can also be used. Bacterial examination over Albimi agar reveals small, round, convex, and translucent colonies with smooth glistening surfaces.

The Compliment Fixation Test (CFT) and Fluorescence Polarisation Assay are other widely accepted confirmatory tests. These tests require an advanced laboratory and highly trained staff to be carried out. The Fluorescence Polarisation Assay is an easy antibody-antigen interaction determination technique employed for international livestock trade (Falzon et al. 2019). There is no serological test available for the diagnosis of *B. canis*.

8. Resistance and treatment course

Brucella organisms can be easily killed by disinfection with 1% phenol in 15 minutes and pasteurization at 60 °C for 10 minutes (Gulbaz and Kamber 2016). The organism can remain viable in refrigerated milk for up to 10 days, up to one month in ice-cream and four months in butter and meat. Brucellosis (especially in bovidies caused by *B. abortus*) is difficult to treat. An antibiotic course of streptomycin, tetracycline, chloramphenicol, gentamicin or a combination of doxycycline and rifampin prophylactically for 6-8 weeks can prove effective (Glynn and Lynn 2008). Combinations of two or more drugs are preferred since the bacteria incubates within the cell. The rate of relapse of the disease can reach up to 15% within the first six months after treatment. Longer courses of antibiotics may help avoid osteomyelitis, meningitis, and relapse of infection. The gold standard regimen for adult animals includes an intramuscular injection of streptomycin 1 g for 14 days and doxycycline 100 mg twice daily for 45-50 days through oral route.

In India, the epidemiological factors of infection, such as animal populations, remain untraced after diagnosis. Upon diagnosis, the priority becomes treatment of patients with drug courses, causing a setback to prevention and control measures (Corbel 2006; Hull and Schumaker 2018).

Live attenuated vaccine of cotton smooth S19 *B. abortus* isolated from milk is administrated to female calves upto 5 months. Administration to male calves is avoided to prevent its localisation in testes. Smooth strains of *Brucella* produce a very high titre of antibodies against the O-polysaccharide (McGiven et al. 2015). Inactivated/ killed vaccine of S45/50 rough bacterin is less preferred vaccine as it does not show immunological response toward smooth form. The vaccine has thus been discontinued. The RB15 vaccine is a natural, stable, rough stain that provides good protection against abortion storm. The vaccine needs to be administered to calf from 4-12 months. It does not provide a serological response, thus not interfering with the Brucellosis surveillance program while protecting against brucellosis-related complications (Moriyon et al. 2004). It is therefore the mandatory and only official vaccine in many countries. As these vaccines need to be administered in calf stage, they are also referred to as calfhood vaccines. Vaccines for ovines and caprines targeted against *B. melitensis* and *B. ovis* include the Live Rev1 vaccine and the inactive H38 vaccine. At present, there are no vaccines for swine infected by *B. suis*. Swine have been administered and experimented with RB51 vaccines, but the results have appeared to be mixed and unreliable (Lord et al. 1998; Edmonds et al. 2001).

9. Conclusion

Brucellosis is a zoonotic disease of great importance to livestock farmers across India. It levies unbearable economic losses on marginal farmers across the country. The zoonotic nature of the causing bacteria then poses a threat to the general public's health. Despite all of its losses, the disease is often neglected. The National Animal Disease Control Programme aims at controlling Brucellosis through continuous testing and awareness programs. Intervention strategies are expected to tackle the spread of *Brucella*. Unfortunately, the lack of adequate infrastructure, favourable policies, and awareness has significantly contributed to the spread of the disease, which elevates in numbers during summers. The outcome of these intervening strategies is thus variable and inconsistent. The positive aspects that thus remain are the increasing literacy of the nation and the advancement in testing technology, which allows rapid and accurate diagnosis. The development of a new generation of vaccines has significantly contributed to the success of controlling the disease.

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References

Ackermann MR, Cheville NF, Deyoe BL. (1988). Bovine ileal dome lymphoepithelial cell: endocytosis and transport of *B. abortus* strain 19. Veterinary Pathology 25: 28-35.

Alton GG, Jones LM, Pietz DE. (1975). Laboratory Techniques in Brucellosis. 2nd ed. World Health Organization (WHO) Monograph Series No. 55. WHO, Geneva, Switzerland.

Chauhan HC, Chandel BS, Shah NM. (2000). Seroprevalence of brucellosis in buffaloes in Gujarat. Indian Veterinary Journal 77: 1105-1106.

Corbel MJ. (2006). Brucellosis in humans and animals. Produced by the World Health Organization in collaboration with the Food and Agriculture Organization of the United Nations and World Organization for Animal Health.

Edmonds MD, Samartino LE, Hoyt PG, Hagius SD, Walker JV, Enright FM, Schurig GG, Elzer P. (2001). Oral vaccination of sexually mature pigs with Brucella abortus vaccine strain RB51. American Journal of Veterinary Research 62: 1328-1331.

Falzon LC, Traore S, Kallo V, Assamoi JB, Bonfoh B, Schelling E. (2019). Evaluation of the fluorescence polarization assay as a rapid on-spot test for ruminant brucellosis in Cote d'Ivoire. Frontiers in Veterinary Science 10(6): 287.

Foster G, Osterman BS, Godfroid J, Jacques I, Cloeckaert A. (2007). *Brucella ceti* sp. nov. and *Brucella pinnipedialis* sp. nov. for *Brucella* strains with cetaceans and seals as their preferred hosts. International Journal of Systemic and Evolutionary Microbiology 57: 2688-2693.

Glynn MK, Lynn TV. (2008). Brucellosis. Journal of American Veterinary Medicine Association 233(6): 900-908.

Gulbaz G, Kamber U. (2016). The detection of Brucella bacteria with PCR and bacteriological method in raw milk and some of the dairy products which are consumed in Kars. Bulletin of University of Agricultural Science and Veterinary Medicine Cluj-Napoca Veterinary Medicine 73: 127-132.

Halling SM, Peterson-Burch BD, Bricker BJ, Zuerner RL, Qing Z, Li LL, Kapur V, Alt DP, Olsen SC. (2005). Completion of the genome sequence of Brucella abortus and comparison to the highly similar genomes of *Brucella melitensis* and *Brucella suis*. Journal of Bacteriology 187(8): 2715-2726.

Hemashettar BM, Patil CS. (1991). Brucellosis among practicing veterinarians. Indian Journal of Medical Microbiology 9: 45-47.

Hull NC, Schumaker BA. (2018). Comparisons of brucellosis between human and veterinary medicine. Infection Ecology and Epidemiology 8(1): 1500846. https://doi.org/10.1080/20008686.2018.1500846

Koshi G, Eapen M, Singh G. (1971). Brucellosis- an oft forgotten clinical entity. Indian Journal of Medical Science 25(5): 324-328.

Lord VR, Cherwonogrodzky JW, Schurig GG, Lord RD, Marcano MJ, Melendez GE. (1998). Venezuelan field trials of vaccines against brucellosis in swine. American Journal Veterinary Research 59: 546-551.

McGiven J, Howells L, Duncombe L, Stack J, Ganesh NV, Guiard J, Bundle DR. (2015). Improved sero-diagnosis of bovine brucellosis by novel synthetic oligosaccharide antigens representing the capping M epitope elements of Brucella O-polysaccharide. Journal of Clinical Microbiology 53(4): 1204-1210.

Megid J. (2010). Clinical manifestations of brucellosis in domestic animals and humans. Open Veterinary Science Journal 4: 119-126.

Minharro S, Mol JP, Dorneles EMS, Barbosa RP, Neubauer H, Melzer F, Poester FP, Dasso MG, Pinheiro ES, Soares Filho PM, Santos RL, Heinemann MB, Lage AP. (2013). Biotyping and genotyping (MLVA16) of *Brucella abortus* isolated from cattle in Brazil, 1977 to 2008. PLoS One 8: 12. https://doi.org/10.1371%2Fjournal.pone.0081152

Moriyon I, Grillo MJ, Monreal D, Gonzalez D, Marin C, Lopez-Goni I, Mainar-Jaime RC, Moreno E, Blasco JM. (2004). Rough vaccines in animal brucellosis: structural and genetic basis and present status. Veterinary Research 35(1): 1-38.

Mousa AR, Muhtaseb SA, Almudallal DS, Khodeir SM, Marafie AA. (1987). Osteo-articular complications of brucellosis: a study of 169 cases. Reviews of Infectious Diseases 9(3): 531-543.

Preez JHD, Malan F. (2015). Brucellosis in cattle. Farmer's weekly. https://www.farmersweekly.co.za/animals/cattle/brucellosis-in-cattle/

Rahaley RS, Dennis SM. (1984). Histopathology of experimental brucellosis in rams following vaccination with *Brucella ovis*. Australian Veterinary Journal 61(11): 353-356.

Ruiz-Fons F, Vidal D, Hofle U, Vicente J, Gortazar C. (2007). Aujeszky's disease virus infection patterns in European wild boar. Veterinary Microbiology 120: 241-250.

Scholz HC, Hubalek Z, Sedlacek I, Vergnaud G, Tomaso H, Al Dahouk S, Melzer F, Kampfer P, Neubauer H, Cloeckaert A, Maquart M, Zygmunt MS, Whatmore AM, Falsen E, Bahn P, Göllner C, Pfeffer M, Huber B, Busse HJ, Nockler K. (2008). *Brucella microti* sp. nov., isolated from the common vole Microtus arvalis. International Journal of Systemic and Evolutionary Microbiology 58: 375–382.

Scholz HC, Nockler K, Gollner C, Bahn P, Vergnaud G, Tomaso H, Al Dahouk S, Kampfer P, Cloeckaert A, Maquart M, Zygmunt MS, Whatmore AM, Pfeffer M, Huber B, Busse HJ, De BK. (2010). *Brucella inopinata* sp. nov., isolated from a breast implant infection. International Journal of Systemic and Evolutionary Microbiology 60: 801-808.

Sen GP, Sharma GL. (1975). Speciation of seventy-eight Indian strains of Brucella: An epidemiological study. Indian Journal of Animal Science 45: 537-542.

Shome R, Kalleshamurthy T, Natesan K, Jayaprakash KR, Byraredd K, Mohandoss N, Sahay S, Shome BR, Hiremath J, Rahman H, Barbuddhe SB. (2018). Serological and molecular analysis for brucellosis in selected swine herds from Southern India. Journal of Infection and Public Health 10: 10-13.

Singh BB, Dhand N, Gill JPS. (2015). Economic losses occurring due to brucellosis in Indian livestock populations. Preventive Veterinary Medicine 119(34): 211-215.

Whatmore AM. (2009). Current understanding of the genetic diversity of Brucella, an expanding genus of zoonotic pathogens. Infection, Genetics and Evolution 9(6): 1168-1184.

Wilson GS, Miles AA. (1932). The serological differentiation of smooth strains of Brucella group. British Journal of Experimental Pathology 13: 1-13.

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