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Brucellosis in Livestock: Epidemiology, Pathogenesis, and One Health Control Strategies

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Key Points

- Brucellosis is an important zoonotic disease affecting cattle, sheep, goats, pigs and humans, with major reproductive and economic consequences in livestock.
- *Brucella* is primarily transmitted through reproductive discharges, aborted materials, milk, semen and contaminated environments, creating significant risks for farmers, veterinarians, abattoir workers and other exposed groups. Pasted markdown
- Effective control requires vaccination, biosecurity, movement control, surveillance, safe handling of infectious materials and a coordinated One Health approach involving animal, human and environmental health sectors. Pasted markdown

Abstract

Brucellosis is a significant zoonotic disease throughout the world, caused by intracellular gram-negative bacteria of the genus *Brucella*, which results in substantial reproductive losses in livestock and serious public health implications in humans. Brucellosis affects a wide range of livestock species including cattle, buffalo, sheep, goats and pigs. *Brucella abortus*, *B. melitensis*, and *B. suis* are the most epidemiologically important species of *Brucella*. Brucellosis is endemic in various livestock production systems in India with a large variation in seroprevalence between states and species. Transmission to man is mainly by ingestion of unpasteurised dairy products and by occupational exposure to infected animals and birth products. Human brucellosis is an acute febrile syndrome that may evolve with severe focal complications of the osteoarticular, cardiovascular and neurological systems requiring prolonged combination of antimicrobial therapy. Effective control will require an integrated

One Health approach involving targeted vaccination, biosecurity, surveillance, movement controls, and continued community education.

Keywords: Brucellosis; *Brucella*; Zoonoses; One Health

Introduction

Brucellosis is a major bacterial zoonosis of global importance caused by Gram-negative, facultative intracellular coccobacilli of the genus *Brucella* [1]. The disease involves infections in a variety of animal species and man. The principal causative species are *Brucella abortus*, *B. melitensis*, *B. ovis*, and *B. suis*, each with a certain degree of host specificity that determines its epidemiological and clinical profile [2]. *B. Abortus* remains the most frequent pathogen in cattle, but *B. melitensis* is the main pathogen in sheep and goats and is known to be the most virulent species in humans with frequent reports from the Middle East, the Mediterranean basin, Central Asia and Latin America [3]. *B. melitensis* and *B. abortus* can infect other species, particularly camels, while B. *ovis* has a very restricted host range, affecting only sheep of all ages and no recognised zoonotic potential [4]. *B. suis* (biovars 1, 2 and 3) is the main cause of swine brucellosis, however, biovar 2 is mainly associated with European wild boar (*Sus scrofa*) populations, where it has endemically established, creating a persistent sylvatic reservoir affecting the biosecurity of domestic swine [5], with a limited host range and geographic distribution. *Brucella* resides in macrophages and dendritic cells in a specialised intracellular niche, the *Brucella*-containing vacuole (BCV), actively preventing phagosome-lysosome fusion and exploiting the endoplasmic reticulum pathway to form a niche for replication [6]. It escapes the immune system by synthesising an atypical lipopolysaccharide (LPS) with significantly lower TLR-4 stimulatory activity, and a Type IV Secretion System (T4SS/VirB) that translocate effector proteins to manipulate host intracellular signalling, autophagy and programmed cell death pathways [7]. The disease in animals is largely manifested by reproductive failure such as abortion, infertility, retained placenta, orchitis and epididymitis [8]. Systemic shedding of the organism through uterine discharges, milk, urine, and semen facilitates both intra-herd transmission and zoonotic spillover [9]. For definitive diagnosis, isolation of *Brucella* organism from clinical specimens like abortion material, udder secretions, and post mortem tissue samples is required. Due to the zoonotic risks of *B. abortus*, *B. melitensis,* and *B. suis* to humans, especially those occupationally exposed such as veterinarians, abattoir workers and laboratory staff, all potentially contaminated tissues, bacterial cultures and laboratory materials must be handled according to appropriate biosafety containment protocols [5].

BRUCELLA ORGANISM

Brucella is a genus of bacteria in the family *Brucellaceae*, class *Alphaproteobacteria*, and order *Rhizobiales*, closely related to *Agrobacterium*, *Bartonella*, and *Ochrobactrum* [6]. It is an obligate aerobic but capnophilic Gram-negative, nonmotile, non-spore-forming, non-encapsulated coccobacillus (0.5-0.7 × 0.6-1.5 µm) [10]. Several species particularly *B. abortus* require supplemental CO_2 for primary isolation [11]. The organism is catalase positive, oxidase variable and has a urease enzyme which is functional and has differential activity among species as a major biochemical identification marker. The classical taxonomy recognises six main nomenspecies including *B. abortus*, *B. melitensis*, *B. suis, B. neotomae*, *B. ovis,* and *B. canis* are further subdivided into biovars according to cultural characteristics, CO_2 requirement, H_2S production, urease activity, dye sensitivity, and monospecific serum agglutination [12]. Discovery of new isolates has increased the genus to 12 accepted species including *B. ceti* and *B. pinnipedialis* from sea mammals, *B. microti* from foxes and voles, *B. inopinata* from a human breast-implant infection, *B. papionis* from a still-born baboon, and *B. vulpis* from red foxes [13]. The *Brucella* genome consists of two circular chromosomes, a larger (~2.1 Mb) encoding essential metabolic functions and a smaller (~1.1–1.2 Mb) harbouring virulence-associated genes. Although the average nucleotide identity across species is >90%, classical polyphasic taxonomy is still the standard recommended by the WOAH for epidemiological and diagnostic purposes [14]. The LPS of the cell envelope possesses an atypical lipid A component, which leads to a significantly lower TLR-4 stimulatory activity and is a major mechanism of innate immune evasion [7]. The O-polysaccharide chain distinguishes the smooth strains (*B. abortus*, *B. melitensis*, *B. suis*) from the naturally rough strains (*B. ovis*, *B. canis*) with important implications for serology and vaccine design [15]. Outer membrane proteins (Omp2a, Omp2b, Omp16, Omp19 and the immunodominant Bp26) are critically important for adherence to host cells and immune modulation and are being actively investigated as subunit vaccine candidates [16].

BRUCELLOSIS IN CATTLE

In most cases brucellosis in cattle is caused by biovars of *Brucella abortus*, but in regions where cattle are kept with small ruminants, *B. melitensis* may also be implicated as an etiologic agent, a cross-species transmission dynamic of increasing epidemiological concern in mixed farming systems throughout the Middle East, North Africa and Central Asia. *B. suis* can sporadically cause bovine infection, especially in regions where feral swine populations constitute a known sylvatic reservoir [17]. Brucellosis is widespread worldwide, but a number of countries, mainly in Western Europe, North America and Australasia have successfully achieved and maintained official freedom from both *B. abortus* and *B. melitensis* through continued eradication programs combining test-and-slaughter policies with systematic

vaccination. Infection in young animals and non-pregnant females is usually asymptomatic, but latent infections can develop, which can be reactivated in conditions of immunosuppression or pregnancy [5]. When infected with *B. abortion* or *B. melitensis*, placentitis develops in pregnant females with a histopathological picture of necrotising cotyledonitis, intercotyledonary thickening, and prominent vasculitis. Abortion occurs often between the fifth and ninth month of gestation. The pathogenesis of abortion is attributed to the tropism of *Brucella* for trophoblastic cells, the bacteria preferentially colonise the trophoblasts of the placenta, using erythritol, a four-carbon polyol found in high concentrations in foetal bovine tissues, as a preferred carbon source that stimulates bacterial growth in the uterus. There is significant shedding of the organism through the placenta, foetal fluids and vaginal secretions, with bacterial loads in aborted material as high as 10^{10} organisms per gram of placental tissue posing an extreme risk of environmental contamination and transmission [5]. Mammary tissue and regional lymph nodes are commonly infected and the organism is shed continuously in milk and colostrum [1]. Colostrum from infected dams is a very important source of neonatal infection since calves ingesting *Brucella* contaminated colostrum may develop latent infections which become clinically apparent at sexual maturity or at first pregnancy. Infected bulls may develop orchitis and epididymitis with the *Brucella* organisms recoverable from seminal fluid and capable of venereal transmission [17]. The latter route of infection is of particular concern in artificial insemination programs where semen quality testing is not sufficient to exclude infected donors. Moreover, the organism can be transmitted via urine, a relevant route in densely stocked housing systems [18]. Hygromas, a fluctuant fluid-filled swellings, of the carpal and tarsal joints, are a common clinical presentation in some tropical regions and may be the sole clinical indicator of infection in some cases; hygroma fluid has been shown to contain viable *Brucella* organisms in high concentrations [5].

BRUCELLOSIS IN SHEEP AND GOATS

Brucellosis in sheep and goats is primarily caused by *Brucella melitensis* which is considered to be the most pathogenic and zoonotically important *Brucella* species worldwide with sporadic cases associated with *B. abortus* and *B. suis* also were also reported [3]. *B. melitensis* infection in small ruminants leads to significant reproductive losses like late term abortion, stillbirth, weak neonates, decreased milk yield, and infertility, all of which have significant economic implications to pastoral and agropastoral communities in endemic areas. A systematic review and meta-analysis estimated the global pooled prevalence of *B. melitensis* in small ruminants was 2.46% in goats and 8.07% in sheep [19]. The main routes of transmission are via the placenta, foetal fluids, and vaginal discharges of infected ewes and does during and for several months after abortion or parturition. The organism can also be

isolated from a range of tissues including cervical and mesenteric lymph nodes, spleen, uterus, epididymis, and testes. The bacterial shedding is also high in mammary secretions and seminal fluid [5]. *B. ovis*, causing genital disease such as epididymitis, orchi-epididymitis, placentitis, abortion and infertility in sheep. The high perinatal lamb mortality is also associated with ovine brucellosis. Contrary to *B. melitensis*, *B. ovis* is a naturally rough species lacking the O-polysaccharide chain of smooth LPS [20]. This makes standard smooth-LPS based serological tests (Rose Bengal Plate Test, Complement Fixation Test) unreliable for the detection of *B. ovis* and necessitates species-specific diagnostic tests such as the agar gel immunodiffusion (AGID) test using *B. ovis* antigen or ELISA with rough LPS or outer membrane protein antigens. *B. ovis* is usually shed in the semen of infected rams and venereal transmission through contact with ewes is the dominant route of infection. However, ram to ram transmission through the oro-nasal route occurs with considerable frequency and is epidemiologically significant within intensively managed flocks [21]. Infected ewes may also shed the organism in vaginal secretions and milk, rendering ewe to ram and lactating ewe to lamb transmission as important routes. Cattle, goats and deer have been shown to be susceptible to infection with *B. ovis* in an experimental study [22]. Transmission of *B. ovis* from rams to red deer stags have been reported under natural condition when red deer stags are kept in prolonged contact with infected rams [23], highlighting the significance of managing the wildlife interface in brucellosis control programs under the One Health concept.

BRUCELLOSIS IN SWINE

Brucella suis (biovars 1,2,3) are the primary cause of porcine brucellosis, but sporadic infections by *B. abortion* and *B. melitensis* is a rare occurrence when there is close interspecies contact [24]. Among the five recognised biovars of *B. suis*, biovar 1 is the most common and virulent type and has been reported extensively in domestic and feral pig populations in the southern United States, Australia, and Oceania. It has also been responsible for occupationally acquired human infections in hunters, abattoir workers, and processors who handle infected animal material. Biovar 2 is less virulent for humans but is well established in wild boar (*Sus scrofa*) populations in Europe and constitutes a persistent sylvatic reservoir with important implications for domestic pig biosecurity throughout Central and Eastern Europe. Biovar 3 is mainly distributed in the America and Asia, while biovars 4 and 5 are associated with reindeer/caribou and small rodents, respectively, and have limited significance in commercial swine production. A 2023 systematic review estimated the pooled global prevalence of porcine brucellosis in swine at 2.1%, with substantially higher prevalence in feral pigs (15.0%) than in domestic pigs (1.1%), highlighting the epidemiological importance of wild suids and the wildlife-livestock interface in porcine brucellosis [25]. The pathogen is primarily transmitted

via ingestion of contaminated feed containing birth fluids, abortive material and uterine secretions. The eating of aborted foetuses and placental tissue, as part of the pig's natural scavenging behaviour, promotes fecal-oral transmission within and between herds. Sexual transmission is another important route, with shedding of *B. suis* in semen is a well-documented risk to artificial insemination programs. Environmental persistence also facilitates transmission, as *B. suis* can survive in moist soil, slurry, and organic material for weeks to months under temperate conditions, especially if there is no direct sunlight or desiccation. After the first bacteraemia, *B. suis* preferentially colonises the reproductive system of both sexes by using erythritol as a preferential carbon source that stimulates intracellular proliferation. Pregnant females are attacked by the organism at the placenta and foetus, causing necrotising placentitis and foetal bacteraemia. In males it invades the testes, prostate, epididymides, seminal vesicles and bulbo-urethral glands. Lesions progress sequentially from hyperplasia to abscess formation to eventual sclerosis and atrophy in chronic infection [26]. Abortion is the hallmark clinical sign of infected females, which occurs at any time during pregnancy but is most common between days 50-110 of gestation. In chronically infected herds, the primary sign is infertility, which is more common than abortion, and is frequently manifested as repeat breeding, irregular return to oestrus and reduced litter sizes, which may be confused with nutritional or management deficiencies in the absence of targeted serological surveillance. Polyarthritis of several joints with accompanying swelling and lameness may occur in both sexes, and in severe cases may be complicated by posterior paralysis or spondylitis from vertebral osteomyelitis.

HUMAN BRUCELLOSIS

Human brucellosis is a systemic zoonosis of major public health importance worldwide, caused mainly by *Brucella melitensis*, *B. abortus,* and *B. suis*. A recent evidence-based global assessment estimated that approximately 1.6-2.1 million new human cases occur annually, with the greatest burden occurring in resource-limited regions of Asia and Africa and important endemic foci also present in the Mediterranean, middle East, Central Asia, sub-Saharan Africa, and Latin America [27]. Transmission occurs via three main routes ingestion of unpasteurised dairy products and undercooked meat, inhalation of infectious aerosols, and conjunctival exposure, with the aerosol infectious dose estimated to be only 10-100 organisms [28]. Occupationally exposed groups including veterinarians, abattoir workers, animal handlers and slaughter house personnel, are at increased risk of *Brucella* infection as compare to general population. On entry, *Brucella* organisms are phagocytosed by mononuclear phagocytes and distributed haematogenously to the reticulo-endothelial system. Intracellular survival of *Brucella* within the *Brucella*-containing vacuole (BCV) results in persistent infection. The

acute clinical syndrome, classically known as undulant or Malta fever, is characterised by undulating fever, drenching night sweats, profound malaise, arthralgia, myalgia, and hepatosplenomegaly. The presentation is notoriously non-specific and mimics typhoid, tuberculosis and leishmaniasis, contributing significantly to diagnostic delay. The most consistent focal complication is osteoarticular brucellosis, frequencies vary widely among studies, manifesting as sacroiliitis, spondylitis, and peripheral arthritis with risk of vertebral destruction and permanent neurological sequelae. The neuro-brucellosis is an uncommon but potentially severe complication, with reported frequencies vary widely among different studies, and includes meningitis, meningo-encephalitis, and myelitis. Brucellar endocarditis is less common but has the highest mortality and most often involves the aortic valve. The adverse pregnancy outcomes for gestational brucellosis includes spontaneous abortion and intrauterine foetal death. The combination of antimicrobial therapy remains the cornerstone of treatment. The standard therapeutic regimen includes doxycycline with rifampicin for six weeks or with streptomycin for two to three weeks. However, intracellular persistence of *Brucella,* relapse following treatment, and emerging antimicrobial resistance underscore the need for improved therapeutic strategies [3].

BRUCELLOSIS: INDIAN PERSPECTIVE

Brucellosis is one of the most economically and epidemiologically important zoonotic diseases in India. However, its impact remains under-recognized relative to its likely burden, particularly because of under-diagnosis, underreporting, and limited systematic surveillance. The disease affects a wide variety of livestock species in the country including cattle, buffalo, sheep, goats, pigs and camels and poses a major constraint on livestock productivity, reproductive efficiency and rural livelihoods in one of the world's largest livestock-holding nations. According to 20th livestock census, India has one of the largest population of cattle and largest buffalo population, making it a major reservoir of bovine livestock. Buffaloes in the world, providing a substantial population of susceptible bovine hosts relevant to the epidemiology of brucellosis. The reported prevalence of brucellosis in India is widely variable depending on species, region, production system and diagnostic tool used. Serological surveys conducted across India have demonstrated substantial variation in bovine brucellosis seroprevalence among states and between herd and animal level estimates. A recent meta-analysis estimated the prevalence of brucellosis at 16.6% (95% CI:13.0-21.1%) in cattle and 14.2% (95% CI: 8.9-21.8%) in buffaloes in India during 1990-2019 [29].

Caprine and ovine brucellosis are primarily due to *B. melitensis* has been reported in several states of India. A serological and molecular survey conducted in six districts of Rajasthan between July 2020 and February 2022 reported cumulative seroprevalence of 0.58%

in goats and 2.43% in sheep based on combined RBPT and indirect ELISA results, although prevalence varied according to the diagnostic method used [30]. In 2022, a sero-epidemiological survey in Rajasthan (one of the states with the highest small ruminant population in India) showed seroprevalence rates of 8.9% and 6.4% in goats and sheep, respectively. Multivariable logistic regression analysis identified abortions, shared grazing and introduction of new animals as significant risk factors. The co-circulation of *B. abortus* in cattle and *B. melitensis* in small ruminants pose high risk of interspecies transmission within sharing grazing systems and mixed farming households. Molecular epidemiological studies using whole genome sequencing (WGS) have provided evidence for more common cross-species spillover events than previously recognized in India's agropastoral landscapes [31-33].

B. suis has been reported to cause swine brucellosis from several regions especially in the states where backyard and semi-intensive pig keeping is prevalent like Assam, Nagaland, Meghalaya and other north-eastern states and parts of Uttar Pradesh [34]. The real burden of porcine brucellosis in India is still not well characterised because of limited targeted surveillance of swine populations. Human brucellosis in India is also underreported, as most cases remain undiagnosed due to its non-specific febrile presentation, limited clinical awareness among physicians and lack of confirmatory diagnostic facilities outside tertiary care centres. Hospital based seroprevalence studies in India have detected *Brucella* antibodies in 17.5% of patient presenting with acute undifferentiated illness [35]. Seroprevalence studies among occupationally exposed individuals in India have reported *Brucella* seropositivity of up to 9.46% (using RBPT), with veterinarians, abattoir workers, dairy farmers, and livestock handlers indentified as disproportionately high-risk groups, among whom awareness and clinical recognition of brucellosis remain critically poor [36].

DIAGNOSIS OF BRUCELLOSIS

The intracellular nature of the pathogen, non-specific clinical manifestations, and the substantial cross-reactivity of many serological tests with other bacterial antigens, make the accurate and timely diagnosis of brucellosis in both animals and humans a critical and consistently challenging aspect of disease surveillance and clinical management. Bacteriological culture remains the gold standard, providing clear evidence of active infection and permitting biovar characterisation and antimicrobial susceptibility testing. Suitable samples are placental cotyledons, aborted foetal tissues, vaginal swabs, milk, semen and lymph node aspirates in animals and blood or bone marrow in humans, the latter being 15-20% more sensitive than peripheral blood culture in subacute and chronic cases. The sensitivity of detection has been significantly improved by the BACTEC automated blood culture system with mean time-to-positivity of 3.5-5 days. Importantly, all *Brucella* culture work must be

performed under Biosafety Level 3 (BSL-3) conditions, which is a biosafety requirement that severely limits culture-based diagnosis in resource-limited endemic settings.

The most common diagnostic method used at the level of field and laboratory is serology. The Rose Bengal Plate Test (RBPT) is the standard rapid screening test, but its specificity is compromised by cross-reactions with *Yersinia enterocolitica* O:9 and related organisms [37]. The Standard Tube Agglutination Test (SAT) is used as a confirmatory serological test for human brucellosis at a diagnostic titre of ≥ 1:160, while the 2-mercaptoethanol (2-ME) modification test run in parallel with SAT, destroy disulfide bonds to inactive IgM so that residual agglutination reflects IgG isotype activity, making it useful for follow-up monitoring and early detection of treatment failure rather than primary confirmation in suspected chronic cases [38]. The complement fixation test (CFT) has a higher specificity and is the reference test for certification for international trade [39]. Competitive ELISA (cELISA) and indirect ELISA (iELISA) formats offer enhanced sensitivity and are being increasingly utilised in national surveillance programs, while Bulk Tank Milk ELISA (BTM-ELISA) is a cost-effective non-invasive herd-level screening tool for dairy systems [40]. Fluorescence polymerization assay (FPA) has a sensitivity and specificity comparable to the complement fixation test (CFT). Molecular diagnostics (PCR) brought a revolution in brucellosis diagnosis with high sensitivity, specificity and rapid turnaround time independent of the viability of organisms. Multiplex PCR panels are of considerable value to outbreak investigations as they can identify species and biovars at the same time [5].

BRUCELLOSIS MANAGEMENT

Effective brucellosis management requires a comprehensive and multi-pronged approach including vaccination, biosecurity, movement control, surveillance, and community education with prevention at the animal reservoir level as the foundational pillar in the absence of a licensed human vaccine. Vaccination is the most effective intervention, S19 and RB51 are used in cattle and buffalo, with RB51 providing the crucial benefit of DIVA compatibility, whereas Rev1 remains the global standard for small ruminants, although its residual human pathogenicity necessitates strict biosafety handling. In India, test-and-slaughter which is the ultimate eradication strategy successfully implemented in Western Europe and North America, is severely impeded by significant socioeconomic and logistic challenges. Targeted test-and-cull in high-prevalence commercial dairy herds is a pragmatic intermediate approach. Some of the biosecurity measures are safe disposal of aborted materials, disinfection of premises, isolation of clinically affected animals and strict use of PPE by occupationally exposed personnel. Identification of unregulated cattle markets and cross-state buffalo trade corridors as the highest risk settings for disease dissemination in India. However, pre-movement testing

and quarantine of newly introduced animals remain critically under-utilized in movement control strategies. Surveillance combining bulk tank milk ELISA, RBPT, CFT, FPA and PCR at herd, abattoir and wildlife interface levels is critical in control and prevention of brucellosis. Finally, focused farmer education, veterinary training and inclusion of brucellosis awareness in the existing agricultural extension services and krishi vigyan kendras provide scalable institutionally sustainable approaches for progressive brucellosis control and long-term eradication in India.

CONCLUSION

Brucellosis is a major zoonotic disease globally and continues to be a cause of heavy economic losses and serious public health consequences. Heterogeneous seroprevalence, weak surveillance and inconsistent vaccination coverage are the major challenges for effective control of brucellosis in India. Prevention depends on vaccination of cattle and buffalo with S19 or RB51, small ruminants with Rev1, and strong biosecurity, movement controls and targeted surveillance. In humans, prolonged combination of antimicrobial therapy remains the standard treatment approach. However, the emergence of antimicrobial resistance and the absence of a licensed human vaccine highlight the need for new treatments. Recent advances in whole genome sequencing, DIVA compatible diagnostics and point of care molecular platforms provide promising tools to improve control of brucellosis in both livestock and humans. Coordinated One Health approach integrating veterinary, medical, wildlife and environmental health, along with community education, occupational health protection and equitable compensation is needed to effectively and sustainably control brucellosis.

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