ZOONOTIC IMPORTANCE OF BRUCELLOSIS

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INTRODUCTION

Worldwide, brucellosis remains a major source of disease in domesticated animals and humans. Among the common infectious diseases causing contagious abortions, infertility and herd economic losses in terms of milk and calf crop in bovine in India, Brucellosis is being predominantly considered as foremost one. Although reported incidence and prevalence of the disease vary widely from country to country, bovine brucellosis caused mainly by *B. abortus* is still the most widespread form.

Besides cattle, buffaloes and one humped and two humped camel and horses; studies indicate that feral animals such as swine, deer, fox, hare and rodents etc. are also susceptible to *Brucella* infection.

In humans, ovine/caprine brucellosis caused by *B. melitensis* is by far the most important clinically apparent disease. The disease has an added importance in countries like India, where conditions are conducive to widespread human infection on account of unhygienic conditions and poverty.

OETIOLOGY

Six major species of brucella are presently known, they are namely, *Brucella melitensis, B. suis, B. abortus, B. canis, B. neotomae and B. ovis*. Of these *Brucella melitensis, Brucella suis, Brucella abortus and B. canis* have public health implications. *Br. melitensis* occurs more frequently than the other types in the general population and it is the most pathogenic and invasive species of *Brucella*, followed in order, by *Br. suis and Br. abortus*. 
Bovine brucellosis is usually caused by *Br. abortus*, less frequently by *B. melitensis*, and rarely by *B. suis*. Infection is widespread globally. Several countries in Northern and Central Europe, Canada, Japan, Australia and New Zealand are believed to be free from the agent.

Brucella are gram negative, coco bacillary and intracellular bacterial organisms that can survive within host cells causing a chronic infectious disease that may persist throughout the life of an animal. Besides other biochemical properties, the presence of Erythritol stimulates and enhances their growth. The organisms settle in the cells that are capable of providing the nutrient erythritol, hence their site of predilection is cells of the genital tracts of animals. Erythritol has been found to be present in the placenta of cattle, sheep, goats, swine and dogs, as well as in the seminal vesicles and testes of the male of these animals. Brucella organisms are transported into the lymphatic system and may replicate there locally; they also may replicate in the kidney, liver, spleen, breast tissue, or joints, causing both localized and systemic infection.

**TRANSMISSION**

Majority of the susceptible animals can acquire infection through conjunctiva or skin, broken or unbroken or by ingestion. Air borne transmission of the disease is also reported.

Transmission of *B. abortus* is very likely to occur via the oral route because cattle tend to lick aborted fetuses and the genital discharge of an aborting cow. Exposure to Brucella organisms is also likely to occur in utero or when calves born to healthy dams are fed on colostrum or milk from infected dams.

It has been established that brucellosis in bulls does not always result in infertility, although semen quality may be affected. Bulls that remain fertile and functionally active will shed Brucella organisms with the semen during the acute phase of the disease. Shedding, however, may cease or become intermittent. In contrast to artificial insemination, bulls used in natural service may fail to spread the infection, as the infected semen is not deposited in the uterus.
While indirect exposure to Brucella organisms could be mediated by wildlife, birds and waterways (contaminated with urine, uterine discharge, or slurry from aborting cattle) it seems that only dogs carry pieces of placenta or aborted fetuses from one place to another causing direct exposure.

Contamination of a cowshed or pasture takes place when infected cattle abort or have a full-term parturition. Although it is generally accepted that *B. abortus* is not excreted for any considerable time before abortion occurs, excretion in the vaginal discharge of infected cattle may occur as early as 39 days after exposure. A massive excretion of Brucella starts after abortion and may continue for 15 days. Once the fetal membranes are expelled the uterine discharge diminishes and the number of Brucella organisms excreted decreases rapidly. Although the infectious material from the genital tract usually clears after 2-3 months, some infected cattle become carriers of Brucella and excrete it intermittently for many years.
Brucella are sensitive to direct sunlight, disinfectant and pasteurization. In dry conditions they survive only if embedded in protein. Brucella can survive in tap water for several months at 4-8°C, 2.5 years at 0°C, and several years in frozen tissues or medium. Brucella can also survive up to 60 days in damp soil, and up to 144 days at 20°C and 40% relative humidity.

Brucella can survive 30 days in urine, 75 days in aborted foetuses and more than 200 days in uterine exudate. In bedding contaminated with infected faecal material Brucella will be destroyed at 56°C-61°C within 4.5 hours. However, there are conflicting reports as to its survival in liquid manure. It has been found that Brucella can survive in faeces, slurry, or liquid manure 85-103 days in the winter, 120-210 days in spring, 30-180 days in summer, and 50-120 days in autumn. Although *B. abortus* is relatively resistant and may survive for a considerable time, the environment is not considered to be an important source of infection.
Age, sex, stage of pregnancy and natural resistance to Brucella may influence the progression of infection. Heifers born to infected dams usually test seronegative for Brucella for a long period. Pregnant females are more likely to become infected than non-pregnant cattle or males. This is because a gravid uterus sustains growth of the organism. Furthermore, the course and incidence of the disease is also influenced by natural resistance to infection with Brucella.

**CLINICAL SYMPTOMS**

The disease is usually asymptomatic in nonpregnant females. Following infection with *B. abortus* or *B. melitensis*, pregnant adult females develop a necrotic placentitis and ulcerative endometritis, usually resulting in abortion between the second and ninth month of pregnancy or sometimes giving birth to the nonviable fetus. Young calves removed from the source of infection usually throw it off but may excrete the organisms in the faeces for a variable period. The disease may run an acute course with abortion “storm” when introduced into a clean and unprotected herd. Brucella organism may cause abortion from two to ten months stage of gestation in buffaloes. Brucella being the self limiting infection maximum number of females may abort when are infected once and fetus may be carried to full term in subsequent pregnancies, although, in few cases second or even third abortion may occur in the same buffalo. After abortion and subsequent apparently normal calvings, large number of organisms are excreted in the placenta, vaginal discharge and milk, this excretion may persist for some weeks to years in exceptional cases. Thus in the infected females abortion, premature births, decreased milk yield, delayed conception and temporary or permanent infertility is a common sequel of the disease.

In acute infections, the organism is present in most major body lymph nodes. Infected adult male cattle may develop orchitis and epididymitis, which may be a cause of infertility in both sexes; such affected males are also considered to be potential spreaders of the disease when used for Artificial Insemination.
Hygromas, usually involving leg joints, are a common manifestation of brucellosis in some tropical countries and may be the only obvious indicator of infection; the hygroma fluid is often infected with Brucella.

**DIAGNOSTIC TECHNIQUES**

All abortions in cattle should be treated as suspected brucellosis and should be investigated. The clinical picture is not pathognomonic, although the herd history may be helpful. Unequivocal diagnosis of Brucella infections can be made only by the isolation and identification of Brucella, but in situations where bacteriological examination is not practicable, diagnosis must be based on serological methods. There is no single test by which a bacterium can be identified as Brucella. A combination of growth characteristics, serological and bacteriological methods is usually needed.

In areas with high prevalence of brucellosis, testing individual serum, plasma or milk samples is a highly sensitive diagnostic tool that is recommended by the OIE.

It should be stressed that the serum agglutination test (SAT) is generally regarded as being unsatisfactory for the purposes of international trade. The complement fixation test (CFT) is diagnostically more specific than the SAT, and also has a standardized system of unitage. The diagnostic performance characteristics of some enzyme-linked immunosorbent assays (ELISAs) like I-ELISA, C-ELISA and the fluorescence polarisation assay (FPA) are comparable with or better than that of the CFT, and as they are technically simpler to perform and more robust, their use may be preferred.

For the control of brucellosis at the national or local level, the buffered Brucella antigen tests (BBATs), i.e. the rose bengal test (RBT) and the buffered plate agglutination test (BPAT), as well as the ELISA and the FPA, are suitable screening tests. Positive reactions should be retested using a suitable confirmatory strategy.

In an eradication programme the RBT can be used as a screening test. As with the serum I-ELISA numerous variations of the milk
I-ELISA are in use. Serum I-ELISA can be performed with ELISA kit which is readily available from ADMAS Bangalore (India). With this kit around forty serum samples can be tested at a time, costing Rs.25 to 40/ per sample. In lactating animals, the MRT can be used for screening herds for brucellosis. In large herds (>100 lactating cows), the sensitivity of the test becomes less reliable. False-positive reactions may occur in cattle vaccinated less than 4 months prior to testing, in samples containing abnormal milk (such as colostrum) or in cases of mastitis.

**COLLECTION OF MATERIAL**

For the diagnosis of animal brucellosis by cultural examination, the choice of samples usually depends on the clinical signs observed. The most valuable samples include aborted fetuses (stomach contents, spleen and lung), fetal membranes, vaginal secretions (swabs), milk, semen and arthritis or hygroma fluids. From animal carcasses, the preferred tissues for culture are those of the reticulo-endothelial system (i.e. head, mammary and genital lymph nodes and spleen), the late pregnant or early post-parturient uterus, and the udder. Growth normally appears after 2–3 days, but cultures should not be discarded as negative until 8–10 days have elapsed.

**Tissues**: Samples are removed aseptically with sterile instruments and macerated using a tissue grinder with a small amount of sterile phosphate buffered saline (PBS).

**Vaginal discharge**: A vaginal swab can be taken by using tampon technique after abortion or parturition. It is an excellent source for the recovery of Brucella and far less risky for the personnel than abortion material.

**Milk**: Samples of milk must be collected cleanly after washing and drying the whole udder and disinfecting the teats. It is essential that samples should contain milk from all quarters, and 10–20 ml of milk should be taken from each teat. The first streams are discarded and the sample is milked directly into a sterile vessel.
**Serum**: Paired sera samples may be collected, first soon after abortion and second after three weeks after abortion. Serum samples may be stored at -20°C.

**Dairy products**: Dairy products, such as cheeses etc. need to be carefully homogenized before culture, after they have been ground in a tissue grinder.

All samples should be cooled immediately after they are taken, and transported to the laboratory in the most rapid way. On arrival at the laboratory, milk and tissue samples should be frozen if they are not to be cultured immediately. Use of laboratory animals should be avoided unless absolutely necessary.

Brucella organisms are among the most dangerous bacteria with which to work in terms of the risk of producing laboratory-acquired infections. For transporting Brucella cultures, the caps of the bottles or canisters should be screwed tightly down and sealed with PVC tapes. Bottles should be wrapped in absorbent paper or cotton wool, sealed in polyethylene bags and packed into a rigid container in accordance with the requirements of the International Air Transport Association (IATA) for shipping dangerous goods. As *Brucella* cultures are infectious agents, they are designated UN2814 and a Declaration of Dangerous Goods must be completed. The requirements for submitting samples from suspected cases of brucellosis are similar and the IATA regulations should be reviewed before sending samples. Other international and national guidelines should also be followed.

**DISEASE CONTROL AND ERADICATION**

Bovine brucellosis may be controlled with an effective vaccination programme or eradicated using a test and slaughter programme. However, the combination of these two programmes has been proved better with considerable success in several countries where eradication of the disease has been achieved.
Under existing socio-economic conditions in India, test and elimination by slaughter method can not be practiced. Therefore methods like:

1} sanitary control or herd management,
2} test and segregation of reactors,
3} treatment of infected animals to control level of infection
4} test and elimination of reactors {wherever possible},
5} gradual building up of an immune herd by

Vaccination with eradication as an ultimate aim might be used successfully either alone or in conjunction with each other. However legislation is needed to effectively control and eradicate brucellosis.

It is also recognized that in absence of maintenance of high level of environmental sanitation taking all steps to reduce intensity of exposure, the attempt to eradicate the disease will prove to be futile.

Treatment:

The drugs like Oxytetracyline @ 5mg/kg body weight intravenously in combination with Strptomycin @ 11mg/kg body weight intramuscularly per day for five consecutive days may be used to control the level of infection. This combination treatment may be repeated as per the requirement. However it is essential to remember that from public health point of view prolonged treatment of infected domestic animals with a high dosage of antibiotics can not be undertaken due to the appearance of antibiotics in the human food chain which interferes with the production of milk products. Moreover, as Brucella are facultative intracellular bacteria, relapses after treatment usually occur. Therefore, efforts are directed at prevention or eradication of brucellosis.

Vaccination:

Usually, whenever more than 10% abortions take place in a particular herd calf hood vaccination is advised. Vaccination of calves with B. abortus strain 19 increases resistance to infection, but protection
may not be complete and some vaccinated cattle may become infected. Also some vaccinated cattle may develop antibodies that interfere with diagnostic test results. In some countries strain RB 51 vaccine from an attenuated rough B. abortus strain has largely replaced strain 19 vaccines because it does not cause production of antibodies that interfere with serological tests. The vaccine is also safe for use in cattle and effectively prevents brucellosis and abortions that are caused by infection with B. abortus. But the RB 51 vaccine can cause infection in calves if administered to pregnant cattle. Even humans can be at risk for infection with RB 51 if they have contact with aborted infected tissue, placenta, blood or amniotic fluid without wearing gloves, masks or eye protection. Another potential risk for health hazard is vaccine administration by unintentional needle stick injuries. For brucellosis vaccines, there have been cases of suspected or confirmed adverse effects in humans reported.

It is known that existing vaccine strains of Brucella, such as B. abortus strains 19 and RB51, and B. melitensis strain REV1, can both protect against the Brucella species from which they were derived and cross protect against infection by other species, such as B. abortus, B. melitensis, B. ovis, B. suis, B. canis and B. neotomae.

ZOONOSIS

It is known that out of the major six species of Brucella, *Br. melitensis, Br. suis, Br. abortus* and *B. canis* have public health implications. Although many countries have eradicated *Brucella abortus* from cattle, in some areas *Brucella melitensis* has emerged as a cause of infection in this species as well as in sheep and goats. Despite vaccination campaigns with the Rev 1 strain, *B. melitensis* remains the principal cause of human brucellosis. *Brucella suis* is also emerging as an agent of infection in cattle, thus extending its opportunities to infect humans.

Brucellosis known as Malta fever or undulant fever are synonyms for brucellosis in man. Symptoms of acute brucellosis caused by *Brucella*
*abortus* are 'flu-like' and highly nonspecific. Chronic brucellosis is an insidious disease with vague symptoms that might be confused with other diseases affecting various organ systems. The commonly reported symptoms are fever, fatigue, malaise, chills, sweats, headaches, myalgia, arthralgia, and weight loss. The acute form of human brucellosis is characterized by an undulating fever, in addition to the signs and symptoms mentioned. The chronic form can also produce serious complications affecting the musculo-skeletal, cardiovascular, and central nervous systems. Lack of appropriate therapy during the acute phase may result in localization of bacteria in various tissues and lead to subacute or chronic disease that can have serious clinical manifestations.

Humans usually acquire brucellosis by oral, respiratory, or conjunctival routes, but ingestion of dairy products constitutes the main risk to the general public. Brucellosis is also recognized as an occupational hazard for farmers, veterinarians, and workers in the meat industry within areas with enzootic *B. abortus*. Farmers and workers in the meat industry may contract brucellosis percutaneous, conjunctival or by nasal mucous membrane infection. Veterinarians may become infected when handling aborted fetuses or apparently healthy calves born to infected cows and by performing gynaecological and obstetric manipulations, or rectal examination of infected cattle, as well as through inadvertent exposure to *B. abortus* strain 19 vaccine. Airborne transmission of bacteria to humans has also been documented in clinical laboratories and abattoirs.

Brucellosis is one of the most easily acquired laboratory infections, and strict safety precautions should be observed when handling cultures and heavily infected samples. Protective clothing and careful handling of infected animals can reduce occupation-related brucellosis, and avoiding unpasteurized dairy products should prevent infection in the general population. Because cattle and small ruminants are the major source of human infection, programmes to eradicate human brucellosis have been largely aimed at these animal species.
The appropriate antibiotic therapy for human brucellosis has been studied to some degree. Various drugs like Doxycycline, Rifampin, Streptomycin, corticosteroids etc. have been tried alone or in combinations in simple infection and chronic infection cases with higher success rates, however relapses have been reported in certain cases.

**Precautions:**

Veterinarians, farmers and workers can avoid occupation related Brucellosis by knowing the importance of personal hygiene and specific measures that are to be taken routinely to protect themselves against zoonotic agent like Brucellosis.

Protective clothing be used while handling every case. It may not be taken away from the site. They should be changed whenever they become soiled. The soiled clothings should not be taken home. Gloves, masks, head and foot covers, gowns or other body covers may be used whenever necessary. Injuries, animal bites, animal scratches, and cuts sustained should be viewed very seriously, while handling the cases. Hand washing is a crucial safety measure for safeguarding personnel while giving services. Disposable gloves are useful to prevent disease transmission between animals and humans. They are also useful to limit exposure to contact allergens. They should be discarded and disposed off properly after use. Although the proper use of disposable gloves provide an effective means of preventing hand contamination, hands can easily become contaminated during the removal of contaminated gloves. Person should avoid hand contact with their eyes, mouth, or other body surfaces with contaminated gloves or hands. Hands should be washed with soap and water whenever they touch contaminated or potentially contaminated surfaces, liquids, or body fluids. Hands should be routinely washed before eating, drinking, applying cosmetics, before touching contact lenses, and before leaving the place. Protective eye ware like goggles or other devices that completely shield the eyes should be used. Smoking, eating, drinking, applying cosmetics, installing contact lenses and similar procedures should be avoided while working.