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Brucellosis: An Overview and Current Scenario

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***Abstract* – Brucellosis is an infectious disease having zoonotic importance. There are nine brucella species currently identified. Brucellosis is affecting many animal species. Clinical signs exhibited in different species are variable. Various molecular and serological methods have been developed for the prompt and precise diagnosis of the disease. Current global and Indian scenario shows that brucellosis is still prevalent in many countries causing a high threat to animals as well as human beings**

***Keywords* – Brucellosis, Zoonotic, Serological.**

I. INTRODUCTION

Brucellosis is an important zoonotic disease prevalent in many parts of the world. J. A. Martson described brucellosis, as "Mediterranean gastric remittent fever", in 1861 [1]. Sir David Bruce isolated the organism from the spleen and he called the organism as *Micrococcus melitensis* [2] and later it was renamed *Brucella melitensis*. The genus *Brucella* consists of many species: *B. melitensis* (goat, sheep, and camel), *B.abortus* (cow), *B.suis* (swine, reindeer, caribou, rodent), and *B. canis* (dog). Brucellosis causes infertility, delayed heat, interrupted lactation, loss of calves, wool, meat and milk production. The disease is very relevant because of its zoonotic transmission which is mainly by contact with animals especially the placenta and fetus during delivery and use of raw animal products (Corbel, 1997). The abattoir workers, farm labourers and veterinarians are at great risk because brucellosis causes a serious illness in human beings. The disease causes mainly abortion in animals and women and permanent infertility in males. The prevention and control programmes are still in its infancy so that the disease remains as a big threat to human and animals. This article emphasizes the disease in detail and current scenario.

II. BRUCELLOSIS: AN OVERVIEW

Etiology

Brucella spp. are facultative intracellular gram-negative cocco-bacilli, non-spore-forming and non-capsulated. Nine Brucella species are currently identified, seven of them that affect terrestrial animals are: *B. abortus, B. melitensis, B. suis, B. ovis, B. canis, B. neotomae*, and *B. microti* [4] and two that affect marine mammals are: *B. ceti* and *B. pinnipedialis* [5]. The first three species are called classical Brucella and within these species, seven biovars are recognized for *B. abortus*, three for *B. melitensis* and five for *B. suis*. The remaining species have not been differentiated into biovars.

Each species has an average genome size of approximately 3.29 Mb and consists of two circular chromosomes, Chromosome I, is approximately on average 2.11 Mb and Chromosome II is approximately 1.18 Mb. The G + C content of all Brucella genomes is 57.2% for Chromosome I and 57.3% for Chromosome II [6]. Organism has no classic virulence genes encoding capsules, plasmids, pili or exotoxins [7]

Clinical Signs

The characteristic clinical sign following infection is abortion [8]. The main causative organism of cattle is *B. abortus*, but it can be infected by *B. suis* and more commonly by *B. melitensis*. *B. melitensis* and *B. suis* can be transmitted by cow's milk and cause a serious public health threat [8]. Abortion (premature or full term birth of dead or weak calves) is usually in the second half of gestation with retention of placenta and metritis [8]. There is an estimated 25% reduction in milk production in infected cows. Organism localizes in the supra-mammary lymph nodes and mammary glands of 80% of the infected animals and secrete the pathogen in milk during their entire life time [8]. Most infected cows abort only once although the placenta will be heavily infected at subsequent apparently normal calvings [9].

The main etiologic agent of brucellosis in goats is *B. melitensis*, but it can also get infected with *B. abortus* [10]. As in cattle, brucellosis in goats is characterized by late abortion, stillbirths, decreased fertility and low milk production [10]. Sheep brucellosis can be divided into classical brucellosis and ram epididymitis. Ram epididymitis is caused by non-zoonotic agent *B. ovis*, while classical brucellosis is caused by *B. melitensis* and constitutes a major public health threat equal to goat brucellosis [8]. In pigs *B. suis* abortion, orchitis, lameness, hind limb paralysis, or spondylitis; are the main clinical symptoms [11].

Camels can be infected by *B. abortus* and *B. melitensis*. Milk from infected camels is a major source of infection [12]. The main etiologic agent for dog brucellosis is *B. canis*, but sporadic cases of brucellosis in dogs caused by *B. abortus*, *B. suis* and *B. melitensis* have been reported [8]. Dogs infected with *B. canis* show reproductive related conditions similar to cattle or non reproductive tract related conditions like ocular, musculoskeletal, or dermatologic lesions [8,13]

Zoonotic aspects and human brucellosis

Five brucella species can infect humans and the most pathogenic and invasive species for human is *B. melitensis*, followed by *B. suis*, *B. abortus* and *B. canis* [8]. The zoonotic nature of the marine brucellae (*B. ceti*) has been documented [14]. The incubation period of of human brucellosis normally is 1–3 weeks. *B. melitensis* is associated with acute infection, but the infections with other species are usually subacute and prolonged [8]. Most

common symptoms of brucellosis include undulant fever, night sweats with peculiar odor, chills and weakness. Common symptoms also include malaise, insomnia, anorexia, headache, arthralgia, constipation, sexual impotence, nervousness and depression [8]. Human brucellosis is also known for complications and involvement of internal organs and its symptoms can be very diverse depending on the site of infection and include encephalitis, meningitis, spondylitis, arthritis, endocarditis, orchitis, and prostatitis [8]. Spontaneous abortions, mostly in the first and second trimesters of pregnancy, are seen in pregnant women infected with brucella [15]. Rarely endocarditis is also associated with *B. melitensis* infection. It accounts for at least 80% of deaths due to brucellosis [16]. Lack of appropriate therapy during the acute phase may result in localization of brucella in various tissues and organs and lead to subacute or chronic disease that is very hard to treat [17]. Symptoms and signs of brucellosis usually referred as fever of unknown origin can be confused with other diseases including enteric fever, malaria, rheumatic fever, tuberculosis, cholecystitis, thrombophlebitis, fungal infection, autoimmune disease and tumors [8].

There is high possibility of zoonotic transmission in endemic places via contact with infected animals or consumption of their products, mostly milk and milk products. Some specific occupational groups including farm workers, veterinarians, ranchers, and meat-packing employees are considered at higher risk [18]. *B. abortus* and *B. suis* infections usually affect occupational groups, while *B. melitensis* infections occur more frequently than the other brucella species in the general population [8]. Consumption of raw sheep or goat milk containing *B. melitensis* is an important source of human brucellosis worldwide and has caused several outbreaks. The prevalence of human brucellosis in some countries is seasonal, reaching a peak usually after kidding and lambing [19].

Diagnosis

The diagnosis can be made tentatively from the clinical signs. Diagnostic tools include isolation and identification of *Brucella* from clinical samples, detection of antigen, genome and antibodies. Main source of antigen in cows is placental fluid at the time of calving. Culture of organism provides definite proof of brucellosis but there can be false negative results. Antigen detection by ELISA is also a useful tool for the diagnosis. Polymerase Chain reaction has also been developed for diagnosis of brucellosis. Nucleotide sequencing may help in exploring the various strains and study the phylogeny.

Antibody detection is also a valuable tool for diagnosis. Antibodies usually begin to appear in the blood at the end of the first week of the disease, IgM appearing first followed by IgG [8]. Rose Bengal Plate Test (RBPT) and Serum Agglutination Test (SAT). Agglutinations tests, however, are not useful for diagnosis of infection caused by *B. canis.*

Serum agglutination test is referred as the standard tube Brucella agglutination test. 2 mercaptoethanol (2ME) and complement fixation tests (CFTs) are used for chronic brucellosis, where active infection continues even though agglutination titers return to low levels [8]. Milk ring test is used detecting antibodies in the pooled sample.

Other useful tests for diagnosis of human brucellosis are counter immunoelectrophoresis (CIEP), Coomb's test, immunocapture agglutination test, latex agglutination, the indirect enzyme-linked immunosorbent assay (ELISA) [8,20].

Prevention and control

Brucella infections in animals have an important economic impact especially in developing countries as they cause abortion in the pregnant animals, reduce milk production and cause infertility. In regions with high prevalence of the disease, the only way of controlling and eradicating this zoonosis is by vaccination of all susceptible hosts and elimination of infected animals [21]. The most commonly used vaccines against bovine brucellosis are *B. abortus* strain 19 and the recently USDA approved strain RB51; the latter unlike strain 19 does not interfere with serological diagnoses [22]. The use of *B. abortus* strain 19 vaccine leads to the production of antibodies whose persistence depends mainly on the age of the animals at the time of vaccination. Based on test and slaughter coupled with control by vaccination, for a policy of eradication to be successful, there must be rigid control of the age at which strain 19 vaccination is allowed [23]. *B. melitensis* strain Rev1 although highly infectious to human, is considered as the best vaccine available for the control of ovine and caprine brucellosis, especially when administrated at the standard dose by the conjunctival route. However, the Rev1 vaccine shows a considerable degree of virulence and induces abortions when administered during pregnancy. Also, the antibody response to vaccination cannot be differentiated from the one observed after field infection, which impedes control programs. Attempts have been made to develop new live attenuated rough *B. melitensis* vaccines, which are devoid of the O-side chain. Those vaccines await further evaluation in field experiments [24]. Vaccination alone will not eradicate Brucella as the immunity produced by Brucella vaccines are not absolute and can be circumvented by increasing the level of infection. It is obvious, therefore, that a policy of vaccination is more likely to succeed if combined with good measures of husbandry [9]. Live human vaccines *B. abortus* strain 19-BA and strain 104M are being used only in the former Soviet Union and China, respectively [8].

III. BRUCELLOSIS: GLOBAL SCENARIO

Brucellosis is the most common zoonosis in the world, accounting for the annual occurrence of more than 500,000 cases [25]. In Central America, bovines are the main natural hosts for *Brucella* [26]. The prevalence of bovine brucellosis is reported to be between 4- 8%, with higher prevalence in dairy herds which causes an economic loss of US$ 25 million per year. El Salvador is the the country with less bovine brucellosis (close to 1% prevalence), while Guatemala and Costa Rica has highest prevalence rate. The incidence of the disease is high where

the laboratory facilities for diagnosis and control are limited in most of the countries of Central America except Costa Rica and Panama. In a serological survey performed in Costa Rica [27], a prevalence of 45% was found in a high risk population of 384 individuals. *Brucella abortus* biotype 1 and 2 were identified as most common biotypes in CA. There are many reports of *B. suis* in swine and humans in all CA countries, *B. melitensis* in ovines and humans in Guatemala and *B. canis* in dogs in Costa Rica. *B. melitensis* is absent in ovine and caprine population in El Salvador and Costa Rica. Restricted surveys have failed to reveal antibodies against Brucella in wild mammals. Disease prevalence among humans in the countries of Central America still remain inconspicuous, even though the demands of the Ministry of Health to report all cases of brucellosis. It is reported that high prevalence of brucellosis among animals in the country, where consumption of unpasturized dairy products is common. Plate agglutination and rose Bengal are commonly used as screening tests in rural areas. Rivanol and 2-mercaptoethanol tests are commonly used confirmatory assays. Complement fixation and competitive ELISA are commonly used for trading and exportation purposes [12]. Human infections are primarily diagnosed by plate agglutination with antigen from human febrile illness [27]. Other tests, such as RID, PCR and specialized immunoenzymatic assays or immunodiagnosis of tissue samples are performed in research laboratories [28].

Brucellosis control Programmes in CA for the control of brucellosis are based on calf vaccination and elimination of the reactors. *B. abortus* S19 was the official vaccine which was introduced in 1990. *B. abortus* RB51 is the currently used vaccine CA countries. Adult vaccinations and revaccination are commonly unregulated practices among farmers, mainly in areas of high prevalence. Recent studies in areas of high prevalence revealed significantly higher rates of abortion and infection in RB51 than in S19 vaccinated herds in previous years [26]. Rev 1 vaccine has been used sporadically in ovine and caprine herds in Guatemala.

In Mexico, brucellosis is main threat to the animals and human. Annually 5,000 human cases were reported in early 1900. Later, Secretariat of Agriculture had implemented a national campaininig to eradicate brucellosis and and the incidence was reduced to 2000 per year. The disease is present in all states of mexico, whereas highest prevalence among goats was found in the states of Chihuahua, Hidalgo, and Guanajuato. The common route of zoonotic transmission is by the consumption of unpasteurized cow and goat dairy products

In most countries of Latin America, brucellosis has become very popular in humans and animals, with *B. abortus* being the most common agent. In Argentina, human brucellosis is more common among the rural population, and is mainly linked to the consumption of fresh and unpasteurized goat cheese. The estimated disease prevalence among cattle in Argentina ranges between 10% and 13%, whereas for caprine brucellosis, it ranges between 20% and 25% [29]. In Brazil, most of the reported human cases are found in abattoir workers and meat processors [29]. There is no data regarding the prevalence of brucellosis in animals. In Venezuela prevalence among cattle and buffalo was found to average 10%. Control aqnd eradication programmes had been started in Venezuela [30].

The Netherlands and England are free from bovine brucellosis [31]. The incidence of brucellosis is declining in France, Ireland and Italy [32]. In the countries of central and south-eastern Europe, namely Greece, Macedonia, Yugoslavia and Bulgaria, sheep and goats remain a major reservoir of the disease, while cows are less important hosts. In Croatia, brucellosis has also been found in pigs. In most of these countries human disease goes largely unreported, and the true prevalence rates are unknown [31].

In sub-Saharan Africa, the prevalence of brucellosis among animals, mainly cattle, sheep, goats and pigs, is unknown. Since the economic status of most of these countries is poor, disease control has been very difficult, making chronic infection and infertility common place among the herds. Carcasses and abattoir products provide a continuous supply of the organism to maintain the infectious cycle among animals and humans. Outbreaks of bovine brucellosis in animals have occurred in most sub-Saharan African countries; however, no data are available from Benin, Burundi, Cape Verde, Congo, Equatorial Guinea, Rwanda, or Sierra Leone. In South Africa, more than 300 outbreaks took place each year from 1996 to 2000, with over 5,000 cases reported per year in humans. Most countries of West, East and Central Africa also had outbreaks, but the numbers of cases among animals and humans are less well defined [33].

IV. Brucellosis in India

In India brucellosis is prevalent among cattle, sheep, goats, dogs and pigs. Bovine brucellosis is present in almost all parts of India, and seems to be increasing among livestock. The disease was first recognized in India in 1942 and now it becomes endemic [34]. *B. abortus* biotype 1 and *B. melitensis* biotype 1 is prevalent in sheep, goat and man. Bovine brucellosis is endemic in all states of India and the incidence rate is increasing in recent times. Marketing of animals through local cattle yards and cattle fairs is main channel for the spread of brucellosis. As India has reached its highest milk production record, the movement of dairy animals is more extensive which contributes largely to the spread of brucellosis [34]. There was a high prevalence of brucellosis in milch goats of Bikaner district of Rajasthan (11.45%) [34] and serological survey of brucellosis by RBPT and STAT revealed that a prevalence of 1.9% in cattle and 1.8 % in buffaloes [35]. The study conducted by PD ADMAS indicated that 5% of cattle and 3% buffaloes were infected with brucellosis. Serological study of *B. melitensis* with RBPT, STAT and ELISA reveals incidence of 13.85%, 9.96% and 20.35% in Tamil Nadu State [36]. Prevalence rate for brucellosis was 8.58% in cattle, 8.85% in goat and 7.08% in sheep from the states of Rajasthan and Bihar. It is reported that there was a prevalence of 27% in western

states, 3% in eastern states, 8 % in northern states and 5 % in southern states. There are reports of incidence of brucellosis in veterinary and animal handlers [37].

V. Conclusion

Brucellosis is a highly infectious disease affecting animals as well as human beings. Many serological and molecular methods have been developed for the precise diagnosis of the disease. Vaccination of healthy animals, test and culling of positive animals are the main control measures implemented for the prevention and spread of the disease. Recent reports indicate that there is high prevalence of the disease in India and many other countries. So it is the high time to scientifically respond to the disease by implementing proper control strategies for the complete eradication of the disease.

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