

Review Article

**Epidemiology of Bovine Brucellosis-a Review of Literature**

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**Abstract**

Brucellosis is mainly caused by *Brucella abortus* in bovines which results in great effect on economy, reduced milk production, abortions in last trimester, long calving interval. In Pakistan incidence is increases day by day due to unawareness. Brucellosis is also a greater Zoonotic risk for human being, especially for veterinarians. It is diagnosed by different tests e.g. Milk Ring Test (MRT), Rose Bengal Plate Test (RBPT), Serum Agglutination Test (SAT) and ELISA. *Brucella* is also considered a strong bioterrorist. Brucellosis is controlled by medication and vaccination. RB51 vaccine is used. Now a day's DNA vaccines are used. Brucellosis eradication program is needed in Pakistan with the help of government, international organizations like OIE, FAO to prevent the spreading of the disease to neighboring countries.

**Key Words:** Bovine, Brucellosis, Epidemiology

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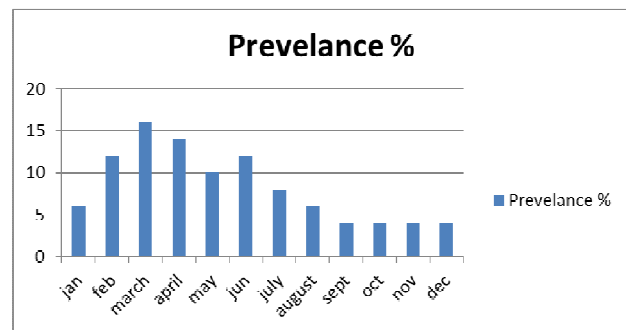
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**Introduction**

Brucellosis is named after Sir David Bruce, who in 1886 isolated the causative agent from a soldier in Malta where the disease caused considerable morbidity and mortality among British military personnel. During the 19th century, brucellosis was thus known as Malta or Mediterranean fever [1]. Brucellosis infection is caused by species of the bacterial genus *Brucella* [2, 3]. These are non-motile, facultative and intracellular coccobacilli bacteria. They act as facultative intracellular parasites [4]. There are six different species of *Brucella*, whereby *Brucella abortus* is the predominant species infecting cattle [2]. Apart from cattle, goats, sheep, pigs, buffaloes, camels, reindeer and, less frequently, other mammals are affected by brucellosis [1]. It is characterized by abortion, with excretion of the organisms in uterine discharge and in milk. Major economic losses result from abortion, loss of calves, and reduced milk yield in females and infertility in males [5]. It is a zoonotic infection and a serious threat to public health. The *Brucella* may enter the body through digestive tract, lungs or mucosal layers and intact skin. Then it may spread through blood and the lymphatic system to any other organ where it infects the tissues and causes localized infection [6]. Although, exact incidence of the Brucellosis in bovines in Pakistan is not known but it has been reported to vary from 3.25 to 4.4 percent in different areas of Pakistan [7]. Brucellosis is one of the world's major zoonotic problems. Though, it has been eradicated in many developed countries in Europe, Australia, Canada, Israel, Japan and New Zealand [8].

**Distribution**

The *Brucella* species are cosmopolitan in distribution and outbreaks are periodically occurs throughout the year. The epidemic peak occurs from February to July (Fig. 1) then it suppress. The peak epidemics are associated with higher mortalities and abortion in animals [9].



**Fig. 1** Prevalence of Brucellosis in China from 1950 to 1999 with reference to month.

**Economic losses**

Brucellosis contributes in major economic losses just because of lower calving rate due to temporary infertility or abortion, resulting in a decreased milk production, increased replacement costs as well as lowered sale value of infected cows [20]. General economic losses, however, go far beyond the financial losses suffered by cattle producers alone. Not only cattle but also other species might be affected including human beings [21]. The Major economic losses are categorized as following

- 1- Losses due to abortion in the affected animal population
- 2- Diminished milk production, *Brucella* mastitis and contamination of milk.

- 3- Culling and condemnation of infected animals due to breeding failure.
- 4- Endangering animal export trade of a nation.
- 5- Human Brucellosis causing reduced work capacity through sickness.
- 6- Government costs on research and eradication schemes.
- 7- Losses of financial investments.

### Diagnosis/Sero-surveillance

Testing of livestock for brucellosis is done by culture and serology or by testing milk samples [22]. The main serological test used for diagnosis of brucellosis is the Rose Bengal Plate Test (RBPT), which has very high (>99%) sensitivity but low specificity [4]. As a result, the positive predictive value of this test is low and a positive result is required to be confirmed by some other more specific test like serum agglutination test (SAT) and ELISA. However, the negative predictive value of RBPT is high as it excludes active brucellosis with a high degree of certainty. The sensitivity and specificity of the SAT test are 95.6 and 100.0%, respectively, while that specificity of the ELISA is 45.6% [23]. Milk ring test is based on agglutination of antibodies present in the milk sample with an antigen employed [24].

### Treatment

The broad spectrum antibiotics are used against the infection by different bacterial strains and choice of the antibiotics depends on the type and severity of infection among antibiotics .

### Vaccination

The use of live attenuated rough strain *B. abortus* RB51 for vaccine production is common for decades. It is produced from a rifampicin-resistant mutant of *B. abortus* strain 2308 and denominated *B. abortus* strain RB51 [26]. ‘‘R’’ standing for ‘‘rough’’ and ‘‘B’’ for *Brucella*. The 51 does not stand for the number of passages which were necessary to select strain RB51, it refers to an internal laboratory nomenclature used at the time it was derived. Strain RB51 is attenuated as indicated by studies carried out in mice, guinea pigs, goats and cattle, from all of which it is cleared in a relatively short time. Furthermore, it has no, or highly reduced characteristics [26-28]. When used in single vaccination protocols its protective effect in cattle is similar to that induced by strain 19 [29, 30]. Current experiments underway in the field under both high and low brucellosis prevalence indicate that immunity

induced by strain RB51 (at least 1 year after vaccination) is similar to or better than that induced by strain 19 [31]. The strain can be isolated using a selective medium [32] and identified by molecular typing [33].

### DNA Vaccines

The basic premise of DNA vaccines involves the introduction of gene(s) encoding protein antigens responsible for stimulating a protective immune response [34]. The gene(s) is on a plasmid vector that has the ability to replicate in prokaryotes without expressing the protein but it has the ability to replicate and express the protective antigen in the immunized eukaryote. These DNA vaccines are useful for targeting the *in vivo* expressed gene(s) of intracellular pathogens like *Brucella*, even though they can also be used for the expression of toxins and protective antigens from other organism [35]. In the field of *Brucella* vaccines, there have been a limited number of studies utilizing this DNA technology. The majority of the effort has been in the area of small animal models. It remains to be shown that the method will be transferable to farm animals. Several investigators have shown that DNA-based vaccines induce long-lived humoral and cellular immune response against a variety of antigens from bacteria, virus and parasites [35]. This has not been true in the case of *Brucella* antigens as seen from the limited numbers of publications. Once an animal is injected with the DNA vaccines, the cells harboring the genes of interest could continue to express antigens for extended periods of time, thereby inducing a strong immune response conferring long lived immunity associated with memory cells [36] using DNA vaccines encoding the *bacterio ferritin or p39* (a putative periplasmic binding protein) of *Brucella* that were able to induce typical Th1- dominated immune response in BALB/c mice as demonstrated by the presence of interferon-gamma (INFg) and immunoglobulin G isotype. They also demonstrated that only the p39 gene (injected intramuscularly) was able to induce a moderate level of protection against wild type *B. abortus* 544 challenges. They also demonstrated that just purified protein p39 adjuvanted with CpG oligodeoxynucleotide (ODN) was able to induce a significant level of protection against a *Brucella* challenge. Recently, [37] have shown that *B. abortus lumazine synthase (BLS) gene in a pCDNA3-based* vaccine was able to induce a significant level of antigen specific immune response in BALB/c mice. It induced a humoral response with IgG2a as the dominant isotype as well as INFg which is basically a Th1 response. They also demonstrated that repeated vaccination induces a

significant level of protection against a *B. abortus* 544 challenge in a BALB/c mouse model. The immune response to individual antigens, including those of *Brucella* spp is also [38, 39], governed by the genetic background of an individual.

### Zoonotic risk and genetic diversity

Members of the genus *Brucella* represent some of the world's major zoonotic pathogens responsible for enormous economic losses and considerable human morbidity [40,41]. There is considerable circumstantial evidence of the significance of brucellosis over human history [42]. As biosafety level, some *Brucella* are also considered bioterrorist threats [43-45] reflecting previous weaponisation [46]. The fact that the organism is highly infectious, can be readily aerosolized and outbreaks might be difficult to detect due to non-specific symptoms associated with infection. *Brucella* species are characterized by extremely high levels of nucleotide similarity but vary widely in host tropisms, microbial and disease phenotypes and pathogenicity. For many years molecular studies and the development of molecular typing tools were hampered by this lack of diversity. However, gradual progress was made in identifying useful markers and tools [47-49]. Human infections present in various forms with the most common symptoms being fever, malaise, sweats and lymphadenopathy but may also lead to the development of severe complications such as endocarditis, meningoencephalitis, arthritis, spondylitis, orchitis and psychological disturbance [50].

Measuring genetic diversity within a population is of particular importance for bacterial pathogens as it can reflect differences in virulence, antibiotic susceptibility and other phenotypes important for treatment and control of disease and knowledge of the population structure can shed light on the epidemiology, evolution and emergence of pathogenic organisms. There have been very few classical bacterial population genetic studies applied to *Brucella* to date. Such studies were carried out using multilocus enzyme electrophoresis (MLEE) the gold standard approach for studying the population genetics and global molecular epidemiology of bacteria [51]. MLEE indexes diversity by assessing electrophoretic variants of enzymes. Application of this approach to *Brucella* simply confirmed the apparent lack of genetic diversity with 99 *Brucella* isolates found to correspond to only six electrophoretic types with some of these containing multiple species [46]. The first *Brucella* genomes became available in 2002 when the publication of the *B. melitensis* 16 M genome was

followed a few months later by *B. suis* 1330 [52, 53]. In light of the extreme homogeneity between the classical *Brucella* species early attempts to identify useful epidemiological markers and to understand the phylogenetics and inter-species relationships of the group advanced only slowly. However, it is now clear that the classical taxonomy based on host specificity and phenotype and which predates molecular characterization represents an astonishingly accurate picture of genetic relationships. While it has been debated for many years whether the degree of differentiation merits species status it is now becoming apparent that *Brucella* species are reproductively isolated and (with the exception of *B. suis/canis*) represent monophyletic lineages separated by long branch lengths [54, 55].

### Conclusion

Brucellosis is mainly caused by *Brucella abortus* in bovines which results in great effect on economy, reduced milk production, abortions in last trimester, long calving interval. Therefore, there is dire need of better control strategies in developing countries like Pakistan including novel strategies like protein and DNA vaccination.

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