Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
Accessible at: www.pvj.com.pk

REVIEW ARTICLE

Bovine Brucellosis: Old and New Concepts with Pakistan Perspective

Muhammad Abubakar*, Mehwish Mansoor and Muhammad Javed Arshed

National Veterinary Laboratory, Park Road, Islamabad, Pakistan
*Corresponding author: mabnvl@gmail.com

ARTICLE HISTORY

Received: May 02, 2011
Revised: September 01, 2011
Accepted: October 16, 2011

Key words:
Bovine
Brucella abortus
Occurrence
Pakistan Perspective

ABSTRACT

Brucellosis is considered to be one of the most widespread zoonoses in the world. According to OIE, it is the second most important zoonotic disease in the world after rabies. The disease affects cattle, swine, sheep, goats, camels and dogs. It may also infect other ruminants and marine mammals. The disease is manifested by late term abortions, weak calves, still births, infertility and characteristic lesions are primarily placentitis, epididymitis and orchitis. The organism is excreted in uterine discharges and milk. The disease is economically important, is one of the most devastating transboundary animal diseases and also a major trade barrier. Although not yet reported, some species of *Brucella* (e.g., *B. abortus*) are zoonotic and could be used as bioweapons. Brucellosis has a considerable impact on animal and human health, as well as wide socio-economic impacts, especially in countries in which rural income relies largely on livestock breeding and dairy products. Considering the poor health infrastructure and manpower in rural areas, the focus should be on preventive measures coupled with strengthening the curative health care services for early diagnosis and treatment. The incidence of brucellosis is increasing particularly in large dairy herds in Pakistan. Several studies have been conducted using sero-diagnostic techniques to determine the prevalence of brucellosis in different provinces, districts and livestock farms in government and private sector.

To Cite This Article: Abubakar M, M Mansoor and MJ Arshed, 2012. Bovine brucellosis: old and new concepts with Pakistan perspective. Pak Vet J, 32(2): 147-155.

INTRODUCTION

Brucellosis is considered by the Food and Agriculture Organisation (FAO), the World Health Organisation (WHO) and the Office International des Epizooties (OIE) as one of the most widespread zoonoses in the world (Schelling *et al.*, 2003). According to OIE, it is the second most important zoonotic disease in the world after rabies. The disease affects cattle, swine, sheep, goats, camels and dogs. It may also infect other ruminants and marine mammals. Synonyms of Brucellosis include: undulant fever, Malta fever, Mediterranean fever, enzootic abortion, epizootic abortion, contagious abortion, and Bang's disease. It is an important zoonotic disease and causes significant reproductive losses in sexually mature animals (Forbes and Tessaro, 1996; Wadood *et al.*, 2009). The disease is manifested by late term abortions, weak calves, still births, infertility and characterized mainly by placentitis, epididymitis and orchitis, with excretion of the organisms in uterine discharges and milk (England *et al.*, 2004).

It also causes morbidity and considerable loss of productivity (Pappas, 2006). The disease is important from economic point of view; it is one of the most devastating trans-boundary animal diseases and also a major barrier for trade (Gul and Khan, 2007).

Brucellosis was first recognized as a disease affecting human-beings on the island of Malta in the 19th and early 20th centuries. It represents a cause of health problems in a herd. In addition to its direct effects on animals, brucellosis causes economic losses through abortions, stillbirths or the death of young stock. The disease can also have a blow on exports and have negative impact on the efforts to improve breeding. Brucellosis has a considerable impact on animal and human health, as well as wide socio-economic impacts, especially in countries in which rural income relies largely on livestock breeding and dairy products (Maadi *et al*., 2011). The economic importance of livestock goes beyond direct food production. Skins, fibers, manure (fertilizer or fuel), draught power, and capital are also livestock benefits. Livestock provides a lifeline for a large proportion of 95% of the world's rural population that lives in the developing world and cultivates 64% of the world's arable land (Hoffmann, 1999; Wadood *et al*., 2009).

Considering the poor health infrastructure and manpower in rural areas, the focus should be on preventive measure together with strengthening the curative health care services for early diagnosis and treatment. Measures against brucellosis should aim at the control and, if possible, the eradication of the agent in the animal reservoir. As the disease often goes undetected the identification of infected herds and animals is of prime importance. Studies by Aulakh *et al.* (2008) showed that brucellosis is widespread in cattle and buffaloes and the only alternative to control and eradicate the disease is a statutory mass vaccination of livestock.

Zoonotic importance: In humans, brucellosis can be caused by *B. abortus*, *B. melitensis*, *B. suis* biovars 1-4 and, rarely, *B. canis*. From public health view point, brucellosis is considered to be an occupational disease that mainly affects farm labor, slaughter-house workers, butchers, veterinarians (Yagupsky and Baron, 2005). Transmission typically occurs through contact with infected animals, materials with skin abrasions, inhalation of aerosols or ingestion of contaminated or unpasteurized dairy and food products (Young, 1998; Christopher *et al.*, 2010).

Worldwide prevalence of brucellosis in human population has been studied and reviewed. The Mediterranean Basin, south and Central America, Eastern Europe, Asia, Africa, the Caribbean and the Middle East are considered as high-risk countries. In the Eastern Mediterranean Region, the incidence of disease ranges from 1 per 100,000 to 20 per 100,000 populations. Brucellosis is endemic in Saudi Arabia, where the national sero-prevalence is 15% (Memish, 2001).

Mukhtar and Kokab (2008) showed that brucellosis is also a public health problem in Pakistan by conducting a sero-prevalence study of brucellosis in abattoir workers of Lahore. Symptoms in human brucellosis can be highly variable, ranging from non–specific, flu-like symptoms (acute form) to undulant fever which may progress to a more chronic form and can also produce serious complications affecting the musculoskeletal, cardiovascular, and central nervous systems, other problems like arthritis, orchitis and epididymitis. It also gives rise to a chronic granulomatous infection, causing clinical morbidity that requires combined prolonged antibiotic treatment (Baba *et al.*, 2001; Grillo *et al.*, 2006).

Human incidence of brucellosis can only be controlled by decreasing the incidence of disease in animals, especially livestock species. It is a serious public health challenge having socio-economic problems and an unaccounted financial burden which needs joint efforts, promotion of inter-sectoral action, regional and international cooperation, as well as technical and financial support.

ETIOLOGY

Worldwide, six species of the genus *Brucella* have been recognized. The genus *Brucella* contains a group of very closely related bacteria. The first member of the group, *B. melitensis*, affects primarily sheep and goats, the second member of the group, *B. abortus*, affects primarily cattle while the other members include *B. suis*, *B. ovis*, *B. neotomae and B. canis* (Corbel, 1998). Cross transmission of brucellosis can occur between cattle, swine, sheep, goats and other species including dogs, horses, bison, rein deer and camels (FAO, 2003).

Brucella is small, non-motile, anerobic, Gram-negative coccobacilli. The cells are short and slender; the axis is straight; the ends are rounded; the sides may be parallel or convex outwards. In length they vary from about 0.5 - 0.7 µm, in breadth vary from 0.5 - 1.5 µm, occurring singly, in pairs or short chains (Leslie *et al.*, 1998).

Unlike most bacteria, *Brucella* species are facultative intracellular pathogens (Jarvis *et al.*, 2002) and can usually be found in the reticulo-endothelial and reproductive systems. They grow rather slowly on ordinary nutrient media while their growth is improved by serum or blood. The ability of *Brucella* to replicate and persist in host cells is directly associated with its capacity to cause persistent disease and to circumvent innate and adaptive immunity (Fichi, 2003). The presence of rough or smooth lipo-polysaccharide correlates with the virulence of the disease and smooth are generally more virulent. *Brucella* species and their different biotypes are currently distinguished by differential tests based on sero-typing, phage typing, dye sensitivity, CO_2 requirement, H_2S production, and metabolic properties.

TRANSMISSION

B. abortus is transmitted by contact with the placenta, fetus, fetal and vaginal fluids from infected animals. Animals are infectious after either abortion or full-term parturition. *B. abortus* may also be found in the milk, semen, feces and hygroma fluids. Shedding in milk can be prolonged or lifelong or may be intermittent (Bercovich, 1998). Few infected cattle become chronic carriers. Infection usually occurs by ingestion and through mucous membranes, but *B. abortus* can be transmitted through broken skin. Although the mammary gland is usually colonized during the course of an infection, it can also be infected by direct contact, with subsequent shedding of the organisms in the milk (Stableforth, 1959).

Disease is spread through contamination of placental material and vaginal discharges of aborting animal (Woodhead and Aitken, 1889). *B. abortus* can also be spread through fomites. Reservoirs of infection have been reported in a wide range of domestic animals, birds and carnivores such as dogs. The transmission of brucellosis by ticks, fleas or mosquitoes from an infected herd to a non-infected herd has never been proven (OIE, 2009).

Brucella can survive for longer periods in conditions of high humidity, low temperatures, no sunlight and in soil; and can remain viable for several months in water, aborted fetuses, and manure under appropriate conditions. However, the importance of their environmental persistence in manure and soil in regards to transmission is unclear as direct contact with infectious material appears to be most important for lateral transmission (McEwen and Paterson, 1939). In previously unexposed and unvaccinated cattle, *B. abortus* spreads rapidly and abortion storms are common. The most significant feature of bovine brucellosis epidemiology is the shedding of large numbers of organisms during the 10 days after abortion or calving of infected cows and the consequent contamination of the environment. The movement of

infected cattle into a herd can result in transfer of the disease when cattle ingest the bacteria from aborted fetuses, placenta, and discharges from cows that have aborted or contaminated pasture or water (Park *et al.*, 2005).

GEOGRAPHICAL DISTRIBUTION

Brucellosis is the most common zoonosis in the world, accounting more than 500,000 cases in animals and humans alike, annually (Pappas *et al.*, 2006). Though its distribution is worldwide; yet brucellosis is more common in countries with poorly standardized animal and public health programs. Advances in control and eradication practices have led to complete eradication from many developed countries like USA, Israel, Canada, Japan & New Zealand, however it remains an uncontrolled problem in highly endemic areas such as Africa, Middle East, Asia and Latin America (Refai, 2000).

Geographically brucellosis has been reported in Asia, Africa, South and Central America, the Mediterranean Basin, Sahara (McDermott and Arimi, 2002) and the Caribbean and these are the regions where cattle raising are mostly preferred. Infected or exposed animals have also been found along the Atlantic and Pacific coasts of North America; the coasts of Peru, Australia, New Zealand and Hawaii (OIE, 2009).

Incidence of brucellosis is reported to be the highest in bovines and prevalence range of 0.85-23.3% has been reported from a wide range of countries. In camels, brucellosis has been reported from Arabian and African countries (0.0-17.20%) (Refai, 2000). Brucellosis is wide-spread in African countries, although with varying prevalence (Thimm and Wundt, 1976).

The worldwide distribution of brucellosis has been reviewed by Memish and Balkhy (2004). They observed that in Central American countries, bovines are the most affected hosts with herd infection rates ranging from 10-25%. In Mexico, brucellosis is one of the most serious bacterial diseases in livestock and humans alike, even after the development of control strategies at national level. Brucellosis has been a well-known disease in Latin American countries with prevalence rates of 10-25%. The Netherlands and England were considered to be free of bovine brucellosis by the turn of the century (Godfroid and Kashbohrer, 2002).

Brucellosis-positive herds were still reported in France, Ireland and Italy, but the incidence has been declining (Godfroid *et al.*, 2002). 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 (Taleski *et al.*, 2002). While bovine, caprine, ovine and porcine brucellosis exist in most sub-Saharan African countries, the true prevalence is either poorly reported or completely unknown (McDermott and Arimi, 2002). High incidence of brucellosis has also been reported from Sub continent particularly India and Pakistan (Park *et al.*, 2005).

OCCURRENCE IN PAKISTAN

Although the exact incidence of bovine brucellosis in Pakistan is unknown but it has been reported to vary from 3.25 to 4.4% in different areas of Pakistan (Naeem *et al.*, 1990). The incidence of brucellosis in Pakistan is increasing particularly in large dairy herds. Several studies have been conducted using sero-diagnostic techniques to determine the prevalence of brucellosis in different provinces, districts and livestock farms in government and private sector. A very limited review literature is present about the prevalence of brucellosis at national level.

In a cross sectional study conducted by Abubakar *et al.* (2010) to determine sero-prevalence of *B. abortus* in Punjab at village level, it was found to be 5.06% in cattle as compared to 7.74% in buffaloes. They further confirmed that the incidence of brucellosis increased with age after testing the sera of animals from different age groups utilizing ELISA as confirmatory diagnostic tool. Shafee *et al.* (2011) found the overall prevalence of Brucellosis in Quetta to be 3 and 8.5% in cattle and buffaloes using MRT and i-ELISA, respectively.

To assess the current situation of brucellosis at various government and private farms in Kohat, serological survey in cattle & sheep/goats was conducted by Hamidullah *et al.* (2009). In their study, 17.58% cattle and 32.5% sheep/goats were found sero-positive. Earlier, Qureshi and Masood (1988) reported 14.2% brucellosis in cattle at livestock farms. Ahmed and Munir (1995a) reported the prevalence of brucellosis in different livestock species in Pakistan to be 5.78, 9.33, 4, 5.05 and 5.56% in horses, dogs, poultry, buffaloes and cattle, respectively.

Relationship of disease with livestock production systems: In Ethopia, Gebretsadik *et al.* (2007) conducted sero-epidemiological investigation of bovine brucellosis in the extensive cattle production system. Herd-level sero-prevalence in the transhumant management system which was found to be 80% was significantly higher than prevalence in the sedentary system. Similar observations are made by several researchers form other countries (Kagumba and Nandokha, 1978; Maiga *et al.*, 1996).

Higher sero-prevelance rate in extensive cattle production system could be attributed mainly to the large herd size and movement of herds. According to one finding, large herd size enhances the exposure potential through increased contact within the herd and with other infected herds, common feeding and watering points and relatively poor management, thus promoting transmission of disease (Hellmann *et al.*, 1984; Omer *et al.*, 2010).

Moreover, it was observed that cattle herds in sedentary system are small in size and sedentary with little possibility of contact with other infected herds, thus, there was less risk of acquiring the disease. Several studies in this regard have also been conducted in Pakistan. It was shown that the incidence of disease is higher in animals kept at organized farms rather than small holdings (Ahmad *et al.*, 1990; Ahmad *et al.*, 1994**;** Ahmed and Munir 1995b; Lodhi *et al.*, 1995). The reason being increased herd densities and lack of proper management facilities at farm level.

Risk Factors for Brucellosis: There are so many factors that can affect the pervasiveness of brucellosis in various species of livestock. Prevalence of brucellosis can vary

according to climatic conditions, geography, species, sex and age (Gul and Khan, 2007). Brucellosis occurs in sexually mature animals, the bacteria localizing mainly in the reproductive tract especially in pregnant animals; there is also evidence that mammary gland may be even more favored for localization than the reproductive tract (Anonymous, 2007).

Age-wise prevalence has also been studied by Abubakar *et al.* (2010) who showed that the incidence of brucellosis increased with age, and the incidence is high in sexually mature animals. Similar results were presented by Aulakh *et al.* (2008) who studied age-wise prevalence of brucellosis in cattle in Punjab (India). It has been reported by different workers that brucellosis is highly prevalent in mature females and males are less affected (Hussein *et al.,* 2005). However, there are controversies regarding this statement.

In general, brucellosis can be found in any season of the year. The epidemic peak occurs from February to July and is closely related to the months associated with delivery and abortion in animals (Shang *et al.,* 2002). In humans, prevalence of the disease is high (39.5%) in summer season (Salari *et al.,* 2003).

DIAGNOSIS OF BRUCELLOSIS

The development of a definitive diagnostic test for brucellosis remains an elusive target. Ever since the development of the first serologic test for brucellosis by Bruce more than a century ago; a definitive diagnostic technique has been actively pursued. In a herd, the most important tool for correct diagnosis of disease without laboratory aid is on the basis of the most obvious clinical sign i.e., persistent late-term abortion rates of >5% in the herd (Martin- Mureno *et al.,* 1983). However, other causes of abortion should also be considered and the disease should be differentially diagnosed from other diseases like trichomoniasis, vibriosis, leptospirosis, listeriosis, infectious bovine rhinotracheitis and various mycoses on the basis of clinical signs, history and the most important serological analysis. As signs and symptoms of brucellosis are unspecific, culture and serology are necessary for diagnosis (Colmenero *et al.,* 1996). Some general laboratory findings might suggest the diagnosis e.g., leukopenia and relative lymphocytosis (Martin-Moreno *et al.,* 1983; Schussler *et al.,* 1997). Liver enzymes are also found to be elevated in many cases.

Serological tests are relatively easy to perform and provide a practical advantage in detecting the prevalence of *Brucella* infection. Classically, direct diagnosis is performed by cultivation in artificial media, with posterior identification of the isolates by its morphology and growth characteristics of the colonies, however; disadvantages of these procedures are the high costs, time necessary for growth and identification of the isolates, apart from high risk for personnel (Fekete *et al.,* 1992).

The criterion standard test for diagnosis of brucellosis is the isolation of the organism from the blood or tissues (e.g., bone marrow, liver aspiration).The sensitivity of blood cultures is usually between 40-50%. Any fluid can be cultured (e.g., synovial, pleural, cerebrospinal), but the yield is usually low. Evaluation of cerebrospinal fluid reveals a mild-to-modest lymphocytic pleocytosis in 88-98%. Protein levels are elevated in conjunction with normal glucose levels (Gotuzzo *et al.,* 1986).

Accurate diagnosis of brucellosis requires bacteriological isolation and detection of the pathogen in the laboratory, which is impractical for regular screening of large populations (Lulu *et al.,* 1988; Yagupsky, 1994). Serological tests can be nonspecific owing to cross-reaction or sub-sensitive or high immunity reactions, depending on sub-clinical or endemic prevalence of the disease (Ariza *et al.,* 1992; Weynants *et al.,* 1996; Godfroid *et al.,* 2002). However, accurate diagnosis of brucellosis has some constraints.

In field conditions, it is quite difficult to differentiate between the antibody titers of vaccinated and infected animal and there is not even a single test which is able to do so. Thus, sera are usually screened with any simple test of high sensitivity and then positive results are confirmed with a more elaborate test of high specificity. For this purpose, some indirect (Alonso *et al.,* 1988) and competitive (Asarta, 1989) enzyme-linked immuno-sorbent assays, the complement fixation test and gel precipitation tests (Alonso *et al.,*1988) have been proposed or used as confirmatory tests.

Screening tests: *Brucella* Milk Ring Test can be for screening the herd and to indicate level of infection in a herd. The test can be applied to monitor the dairy herds at regular intervals. Although relatively cheap and easy to perform, this test does not give accurate results. There are a high percentage of false positive results.

Standard tests: Standard tests for the diagnosis of brucellosis are Rose Bengal Precipitation Test (RBPT), Serum Agglutination Test (SAT) and Complement Fixation Test (Memish and Balkhy, 2004). Rose Bengal Precipitation Test and Serum Agglutination Test are quantitative measurements of antibodies and are affected by many factors. RBPT which was officially introduced in Britain in 1970 is rapid, simple and sensitive but it has moderate specificity (Falade, 1983). Thus, 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 ELISA. However, the negative predictive value of RBPT is high as it excludes active brucellosis with a high degree of certainty (Gul and Khan, 2007). A test based prevalence study of brucellosis in Pakistan using RBPT by Omer *et al.* (2000) showed the incidence of 35.90% in cattle, 33.3% in sheep, 16.70% in goats and 3.10% in camels.

A sero-surveillance study conducted by Lodhi *et al.* (1995), for Faisalabad and surrounding areas revealed that sero-positive percentage obtained through RBPT and SAT was 12.6 and 2.4%, respectively. They suggested further studies to recommend more accurate and standard protocol for diagnosis. In another study, by Nasir *et al.* (2004), sero-prevalence of Brucellosis at government and private farms in Punjab was confirmed using RBPT and SAT. Results of two sero-diagnostic tests indicated that RBPT detected higher percentage of sero-positive animals as compared to SAT.

Bacteriological Identification: The absolute diagnosis of brucellosis requires isolation of the bacterium from blood

or tissue samples. The sensitivity of blood culture varies, depending on individual laboratory practices and how actively the obtaining of cultures is pursued. The percentage of cases with positive cultures ranges from 15 to 70% (Memish *et al.,* 2000).

A variety of samples can be collected for culture and microscopic examination. Milk samples and vaginal swabs are particularly useful for diagnosis in live cattle. In addition, *B. abortus* can often be isolated from the secretions of non-lactating udders. This organism can also be cultured from aborted fetuses (stomach contents, spleen and lung) or the placenta. The spleen, mammary and genital lymph nodes, udder and late pregnant or early post-parturient uterus are the most reliable samples to collect at necropsy. *B. abortus* can also be cultured from semen, the testis or epididymis, and arthritis or hygroma fluids. Serum samples and milk samples can be collected for serology (OIE, 2009).

Brucella can be microscopically examined through modified Ziehl-Neelsen staining method which is not a definitive test. *Brucella* species are not truly acid-fast, but they are resistant to decolorization by weak acids, and stain red against a blue background (Mitchell and Humphreys, 1931). Other organisms such as *Chlamydophila abortus* and *Coxiella burnetii* can resemble *Brucella* (OIE, 2009).

A definitive diagnosis can be made if *B. abortus* is cultured from the animal. However, it is stated after evaluation of bacteriological culture techniques that the sensitivity of the B. *abortus* culturing is low (Navarro *et al.,* 2004). In addition, the culture technique is time-consuming and presents a great threat of infection for the laboratory personnel, as *Brucella* species are class III pathogens.

Serological Diagnosis: Serological diagnosis of brucellosis is used widely in most of the countries as criteria for control and eradication of disease. Conventionally, several techniques are used for the detection of *Brucella* antibodies. Each one of the technique detects different antibody isotypes, to determine an animal seropositive to brucellosis (Nielsen *et al.,* 1996). Although the serological tests have higher sensitivities as compared to culture techniques, but their specificities are generally low (Al-Attas *et al.,* 2000).

Different studies and trials have been conducted throughout the world for evaluation of *Brucella* diagnostic techniques. In a study, ELISA was compared with other serological techniques and was found to be more sensitive and specific. It is also confirmed from the findings that the standard tests like RBPT and SAT have low specificity because these tests detect only the antibodies to the LPS (lipopolysaccharide) antigen of *B. abortus* (Al-Attas *et al.,* 2000), which is similar to that of other Gram negative bacteria like Salmonella, *E. coli*, *Yersinia enterocolitica*, *Vibrio cholerae* etc. This antibody cross reactivity contributes towards low specificity of these tests.

Enzyme linked immuno-sorbent assay (ELISA): The protocol for Indirect ELISA for the detection of *Brucella* antibodies in milk & serum has been described by Limet *et al*. (1998). The introduction of indirect immuno-enzymatic techniques in serological diagnosis has allowed the achievement of higher sensitivity and specificity levels than most commonly used conventional techniques (Neilsen *et al.,* 1996). Indirect enzyme-linked immuno-sorbent assays (ELISAs) typically use cytoplasmic proteins as antigens. ELISA measures class M, G, and A immunoglobulins, which allows for a better interpretation of the clinical situation and overcomes some of the shortcomings of the serum agglutination test. A comparison with the serum agglutination test yields higher sensitivity and specificity (Almuneef and Memish, 2003). At present, application of the ELISA technique is considered a better test in early detection of infection than classical diagnostic tests like complement fixation, agglutination and precipitation (Rojas and Alonso, 1995). These ELISA assays have also been approved by International Office of Epizootics (OIE, 2009).

In certain studies conducted worldwide and even in Pakistan for the comparison of standard diagnostic tests and other serological techniques, it was concluded that ELISA assays are more accurate than tests like SAT and MRT, revealing high percentages of sero-positive samples. In this regard, Shafee (2007) confirmed the prevalence of Brucellosis in Quetta city using indirect ELISA assays. The overall prevalence was found to be 3% and 8.5% in cattle using MRT and i-ELISA, respectively. Indirect enzyme linked immunosorbent assays (I-ELISAs) have been used in various countries for sero-diagnosis of brucellosis in cattle and other animals (Romero *et al.,* 1995; Dajer *et al.,* 1998; Omer *et al.,* 2001) however, none of the diagnostic test has been standardized in buffaloes (Guarino *et al.,* 2001).

In contrast to above study, Munir *et al.* (2008) developed Immuno-capture ELISA assay using lipopolysaccaride (LPS), reported high sensitivity values and approved this test for the screening of buffalo herds. Results of study conducted by Hussain *et al.* (2008) to determine seroprevalence of brucellosis in cattle, buffalo and human population in Pakistan showed that RBPT and ELISA can be used efficiently for mass screening of *Brucella* antibodies in both animals and humans but ELISA is more sensitive and reliable. Moreover, the efficiency of ELISA has been evaluated for diagnosis of brucellosis in other species as well. El-Razik *et al*. (2007) have suggested its efficiency as a screening and confirmatory diagnostic test in goats and sheep.

Polymerase Chain Reaction (PCR): Molecular studies have now highlighted the pathogenesis of *Brucella*, for the development of newer diagnostic tools that will be useful in developing countries where brucellosis is a common disease. PCR testing for *Brucella*e is a recent advance with promising potential. It would allow for rapid and accurate diagnosis of brucellosis. PCR was first developed in the early 1990s and recently it has been used routinely for more accurate and specific diagnosis of brucellosis and other infectious agents (Asif *et al.,* 2009).

Two major genetic targets are the *Brucella* gene BCSP31 and the 16S-23S rRNA operon (Debeaumont *et al.,* 2005; Navarro *et al.,* 2006). The 16S-23S rRNA operon has been shown in studies to be more reliable in terms of sensitivity but is not yet widely used in clinical practice and needs more standardization. Possible applications would include evaluating cases of relapse and monitoring response to therapy. Other promising tests include real-time PCR, and

PCR-ELISA, but the clinical role for these tests remains to be defined (Mitka *et al.*, 2007).

Asif *et al.* (2009) demonstrated that PCR is the most authenticated test for diagnosis of brucellosis. They presented the first ever report of molecular characterization of *B. abortus* BSCP31 gene from Pakistan. Their study revealed that SAT, RBPT and other standard tests should be only used for screening the herds but not for confirmatory diagnosis in individual animals. The sero-positive SAT samples should be subjected to PCR.

In Pakistan, a study was conducted by Akhtar *et al.* (2010) which was aimed at comparing the efficacy of conventional diagnostic methods and evaluation of PCR for the diagnosis of bovine brucellosis. The efficacy for RBPT and MRT was calculated in terms of specificity and sensitivity in cattle and buffaloes. In the continuation of this study polymerase chain reaction (PCR) was evaluated for its diagnostic efficacy of quick *B. abortus* isolation from same samples. The antigenic detection of *Brucella* using PCR gave more positive results than conventional RBPT and MRT. Therefore, the combination of both conventional tests along with serum PCR can be recommended. Moreover, in our circumstances PCR cannot be used as initial screening tests for large herds because of high cost as compared to other two tests, unchecked quality control measures, sample contamination and time consumption.

Diagnostic Plans in Pakistan: In Pakistan, veterinarians mostly rely on the above described conventional serological tests due to the lack of more specific diagnostic facilities and economic constraints. The most widely performed tests at government livestock laboratories in Pakistan are Rose Bengal Precipitation Test and Serum Agglutination Test (Gul and Khan, 2007; Asif *et al.*, 2009).

PREVENTION AND CONTROL

Compatible relationships of *Brucella* species with the hosts including variable incubation periods, long survival time in both extracellular and intracellular environments, asymptomatic carrier stages and resistance to treatment are the major problems. These and animal husbandry factors such as nomadism, co-mingling, and increasing population sizes assure difficulties in control of disease (Rahman *et al.*, 2006). Brucellosis control programs based on various strategies, including vaccination and/or test-and-slaughter of infected animals, has been successful in controlling the disease in animals in several countries. Brucellosis can be prevented in humans by controlling, or better, eliminating the disease in the animal population, avoiding consumption of raw milk, raw milk products and adopting hygienic practices. Proper heat treatment of milk or milk products is important for effective prevention of brucellosis in humans. Moreover, brucellosis must be included in public health education, and public awareness programs, particularly in the rural areas of Pakistan and efforts should be directed towards preventive measures but not curative services.

The World Health Organization (WHO) has long been involved in brucellosis surveillance and control including research and development of vaccines to prevent animal brucellosis (Munir *et al*., 2010). Efforts are directed at detection and prevention because no practical treatment is available. WHO has been implementing regional control programs in Middle East and Latin American countries with collaboration of OIE and FAO.

Brucellosis is a neglected disease in Pakistan, where few studies have been carried out to estimate its prevalence. A full description of the epidemiology of the disease is needed for planning interventional strategies for its prevention and control. Mukhtar and Kokab (2008) provided the following guidelines which should be considered for control of brucellosis:

- Proper diagnosis
- Scheduled vaccination programs for young animals
- Screening of herds, livestock markets, abattoirs & subsequent removal of diseased
- Awareness among the farmers, livestock & public health authorities

Strategies for Control: Like all other bacterial diseases, brucellosis is highly infectious and contagious disease with rapid intra and inter-herd spreading potential (Ahmad, 2005). Thus, a single control strategy could not be recommended. However, several countries have been declared brucellosis free because of continuous efforts and implementation of strategic control measures for eradication.

There are three kinds of control measures:

1. Reducing or eliminating the source or reservoir of infection by quarantine, destruction of reservoir, early detection of disease and environmental control (Ahmad, 2005). Quarantine is usually imposed on animals entering a country or establishment so that any disease they may be carrying or incubating can be identified. In this way, *Brucella* infections have been eliminated from the United States.
2. Breaking the connection between the source of the infection and susceptible animals by general cleaning and sanitation measures.
3. Reducing the number of susceptible population by immunization. This concept is called herd immunity. Mass immunization as a preventive technique has the advantage of allowing the freedom of movement to resistant animals, unlike environmental control, in which the animal is confined to the controlled area.

The countries which are qualified as brucellosis free are those where all the cattle herds are serologically negative for the disease and none of the animals have been found positive for the past five years (WHO). The system for control is decided by the country concerned. However, tactics such as on farm quarantine, movement restrictions and biosecurity are used, at some stage in at least all the eradication programs. Surveillance, either passive or active, has been an underlying feature of most programs led by many countries, abattoirs being the major source of data. New Zealand is free from brucellosis and the methods used for eradication exemplify a range of disease control strategies such as stamping out affected herds, compulsory treatment, vaccination, and test and removal (Davidson, 2002). Similar strategies for control have been reported by America. In Egypt, two approaches

are used, one is to test the animals and then slaughter the infected ones having positive serologic tests; while the other approach is vaccination of the animal population (Fathey and Moghney, 2004).

Vaccination: Vaccination as the sole means of brucellosis control has been proven to be effective. Reduction in the number of positive animals in a herd is directly related to the percentage of vaccinated animals. However, when proceeding from a control to an eradication program, a test and slaughter program is necessary. Modified live vaccines are available against *Brucella* spps. *B. abortus* S19, RB51 and *B. melitensis* Rev.1 are proven effective vaccines against *B. abortus* in cattle and against *B. melitensis* and *B. ovis* in sheep and goats, respectively (Elberg, 1996). Despite the availability, these vaccines have several drawbacks, including residual virulence for animals and humans (Gamboa *et al.,* 2009).

Choice of Ideal Brucellosis vaccine: Live vaccines have proved superior to inactivated products for the prevention of brucellosis (Nicoletti, 1990). They are effective, inexpensive, and immunity is more persistent. The ideal live vaccine should not produce disease in vaccinated animals; it should prevent infection in both sexes at any age, it should not stimulate persistent antibodies interfering with accurate sero-diagnosis (it should give very few false positive results), it should be biologically stable, free of reversion to virulence *in vitro* and *in vivo and* non-pathogenic for humans (Adams, 1990). The ideal live vaccine should also contain specific genetic or phenotypic markers that would make it easy to differentiate from field isolates.

S19 vaccine: *B. abortus* "strain 19" or S19 (here after, S19) is a spontaneously attenuated strain discovered by Dr. John Buck in 1923 (Graves, 1943). Live, attenuated strain S19 had been used worldwide since the early 1930s as an effective vaccine to prevent brucellosis in cattle, until it was replaced by RB51 in 1990s. *Brucella* Strain 19 maintains its smooth appearance derived from the presence of the extracellular lipopolysaccharide (LPS) (Mukherjee *et al.,* 2005). Caporale *et al.* (2010) studied to evaluate the efficacy of RB51 in water buffalo compared to the *B. abortus* S19 vaccine (S19). A statistical significanct difference was found when evaluation was performed to assess the immunogenicity values obtained in buffalo vaccinated with S19, compared to those obtained in buffalo vaccinated with the RB51 vaccine and in the unvaccinated control group.

RB51 vaccine: *B.rucella abortus* strain RB51 vaccine has been developed in United States and tested for its efficacy and safety. This mutant strain of *B. abortus* does not produce cross-reacting antibodies in vaccinated cattle that are detected in the routine surveillance tests. It means that cattle vaccinated with RB51 remain negative on the brucellosis surveillance tests and do not give false positive results (Edmondson and Breitmeyer, 1996). This is because *Brucella* strain RB51 is rough as it lacks the lipopolysaccharide O chain, this feature gives it an advantage because it does not induce the antibodies that are detected by official diagnostic tests, resulting in the differentiation of vaccinated from infected animals (Herrera *et al.,* 2010).

At present, over 5 million calves have been vaccinated subcutaneously with the recommended dose of 1-3.4 x 10^{10} organisms without deleterious effects. Unpublished observations regarding protective efficacy suggest that immunization should start with animals not younger than 4 months (OIE, 2004). Pregnant cattle can be safely vaccinated without the induction of abortion or placentitis (Young, 1998).

Both vaccines have the disadvantages of causing abortion in a proportion of pregnant animals, and of being pathogenic for humans. Several approaches have been followed to overcome the main problem encountered in animal vaccination with live attenuated smooth *Brucella* strains, i.e. inability to distinguish vaccinated animals from infected animals by the current standard serological tests (Fensterbank *et al.,* 1986).

SITUATION IN PAKISTAN

As stated earlier, brucellosis is a neglected disease and no official policy for brucellosis eradication exists in Pakistan. Therefore, no mandatory measures have been adopted to curtail the spread of the disease in government and private herds (Akhtar *et al.,* 1990). Veterinary Services, economic conditions and methods of farming in the country, suggest that the appropriate method for the control of brucellosis is immuno-prophylaxis, although vaccines against brucellosis are not manufactured in the country (Akhtar *et al.,*1990; Afzal *et al.,* 2000).

The most popular vaccine for brucellosis in large ruminants is *Brucella abortus* strain 19 (Afzal *et al.,* 2000). Owing to the high prices of cattle and buffalo, the test-and-slaughter method is not a pragmatic approach to the eradication of bovine brucellosis in Pakistan. Testing, isolation and separate management of reactors is the only viable option to limit the spread of brucellosis in official and large private herds. However, the impact of such a policy in Pakistan has yet to be demonstrated.

REFERENCES

Abubakar M, MJ Arshed, M Hussain, Ehtisham-ul-Haq and Q Ali, 2010. Serological evidence of *Brucella abortus* prevalence in Punjab province, Pakistan-a cross-sectional study. Transbound Emerg Dis, 57: 443-447.

Adams LG, 1990. Development of live *Brucella* vaccines. In: Advances in brucellosis Research. (Adams LG ed), Texas A&M University Press, pp: 250-276.

Afzal M, M Ashraf and M Jahangir, 2000. Immune Response of buffaloes to vaccination with *Brucella abortus* strain 19. Rev Sci Tech Off Int Epiz, 19: 867-870.

Ahmad K, 2005. Control of animal diseases caused by bacteria: Principles and approaches, Pak Vet J, 25: 200-202.

Ahmad R, S Javed and M Latif, 1990. An investigation on the prevalence & treatment of brucellosis in buffaloes and cows. Pak Vet J, 10: 107-109.

Ahmad R, MA Munir and M Latif, 1994. Production systems and brucellosis in buffaloes. Pak J Agric Sci, 31: 341-344.

Ahmed R and MA Munir, 1995a. Epidemiological investigations of brucellosis in horses, dogs, cats and poultry. Pak Vet J, 15: 85-88.

Ahmed R and MA Munir, 1995b. Epidemiological investigations of brucellosis in Pakistan. Pak Vet J, 15: 169-172.

Akhtar S, M Afzal, S Ali and MI Khan, 1990. Effects of reactor retention on the spread of brucellosis in Jersey cattle and buffalo herds. Rev Sci Tech Off Int Epiz, 9: 1179-1185.

Akhtar R, ZI Chaudhry, AR Shakoori, M Ahmad and A Aslam, 2010. Comparative efficacy of conventional diagnostic methods and evaluation of polymerase chain reaction for the diagnosis of bovine brucellosis. Vet World, 3: 53-56.

Al-Attas RA, M Alkhalifa and AR Alqurashi, 2000. Evaluation of PCR, culture and serology for the diagnosis of acute human brucellosis. Ann Saudi Med, 20: 224-228.

Almuneef M and ZA Memish, 2003. Prevalence of *Brucella* antibodies after acute brucellosis. J Chemother, 15: 148-153.

Alonso U, BI Moriyon, R Diaz and JM Blasco, 1988. Enzyme-linked immunosorbent assay with *Brucella* native hapten polysaccharide and smooth lipopolysaccharide. J Clin Microbiol, 26: 2642-2646.

Anonymous, 2007. Brucellosis background. American Veterinary Medical Association. www.avma.org/public_health/brucellosis_bgnd.asp Accessed on February 20, 2007

Ariza J, T Pellicer, R Pallares, A Foz and F Gudiol, 1992. Specific antibody profile in human brucellosis. Clin Infect Dis, 14: 131–140

Asarta A, 1989. Erradicacion de la brucellosis en el ganado vacuno de Navarra. In: Sociedad Espaola de Microbiologia, Actas del XII Congreso Nacional de Microbiologia, Pamplona, Spain, pp: 371-375.

Asif M, AR Awan, ME Babar, A Ali, S Firyal and QM Khan, 2009. Development of genetic marker for molecular detection of *Brucella abortus*. Pak J Zool Suppl Ser, 9: 267-271.

Aulakh HK, PK Patil, S Sharma, H Kumar, V Mahajan and KS Sandhu, 2008. A Study on the Epidemiology of bovine brucellosis in Punjab (India) using milk-ELISA. Acta Vet Brno, 77: 393–399.

Baba MM, SE Sarkindared and F Brisibe, 2001. Serological evidence of brucellosis among predisposed patients with pyrexia of unknown origin in the north eastern Nigeria. Cent Eur J Pub Health, 9: 158–161.

Bercovich Z, 1998. Maintenance of *Brucella abortus*-free herds: a review with emphasis on the epidemiology and the problems in diagnosing brucellosis in areas of low prevalence. Vet Quart, 20: 81-88.

Caporale V, B Bonfini, E Di Giannatale, A Di Provvido, S Forcella, A Giovannini, M. Tittarelli and M Scacchia, 2010. Efficacy of *Brucella abortus* vaccine strain RB51 compared to the reference vaccine *Brucella abortus* strain 19 in water buffalo. Vet Ital, 46: 13-19.

Christopher S, BL Umapathy and KL Ravikumar, 2010. Brucellosis: review on the recent trends in pathogenicity and laboratory diagnosis. J Lab Physicians, 2: 55-60.

Colmenero JD, JM Reguera, F Martos, D Sánchez-De-Mora, M Delgado, M Causse , A Martín-Farfán and C Juárez, 1996. Complications associated with *Brucella melitensis* infection: a study of 530 cases. Medicine (Baltimore), 75: 195-211.

Corbel MJ, 1998. *Brucella*. J Syst Bacteriol, 2: 842-844.

Dajer AA, RE Gutierrez and VD Zapato, 1998. Use of the ELISA and rivanol agglutination tests for the diagnosis of bovine brucellosis in Yucatan, Mexico. Vet Mexico J, 29: 167-171

Davidson RM, 2002. Control and eradication of animal diseases in New Zealand. New Zealand Vet J, 50: 6-12.

Debeaumont C, PA Falconnet and M Maurin, 2005. Real-time PCR for detection of *Brucella* spp. DNA in human serum samples. Eur J Clin Microbiol Infect Dis, 24: 842-845.

Edmondson A and RE Breitmeyer, 1996. UCD Vet Views, California Cattleman, April 1996. University of California, Davis. http://www.vetmed.ucdavis.edu/vetext/INF-BE_cca/INF-BE_cca96/INF-BE_cca9604.html

Elberg S, 1996. Rev.1 *Brucella melitensis* vaccine. Part III: Veterinary Bulletin, 66**:** 1193-1200

El-Razik KA, YA Ghazi and EM Salama, 2007. Monitoring of *Brucella* reactor does following milk examination using different techniques. Pak J Biol Sci, 10: 240-244.

England T, L Kelly, R D Jones, A MacMillan and M Wooldridge, 2004. A simulation model of brucellosis spread in British cattle under several testing regimes. Prev Vet Med, 63: 63-73.

FAO 2003. Guidelines for coordinated human and animal brucellosis surveillance. FAO Animal Produc Heal Paper, 156: 3-4.

Falade S, 1983. Some observations n the use of Rose Bengal plate and tube agglutination in caprine brucellosis. Trop Vet, 1: 49-53.

Fathey AR and A Moghney, 2004. A preliminary study on brucellosis on camels at Behira province. Assuit Univ Bull Environ Res, 7: 39-43.

Fekete A, JA Bantle and SM Halling, 1992. Detection of *Brucella* by polymerase chain reaction in bovine fetal and maternal tissues. J Vet Diagn Invest, 4: 79–83.

Fensterbank R, 1986. Brucellosis in cattle, sheep and goats: diagnosis, control and vaccination. Rev Sci Tech Off Int Epiz, 5: 605-618.

Fichi TA, 2003. Intracellular survival of *Brucella*: defining the link with persistence. Vet Microbiol, 92: 213-223.

Forbes LB and SV Tessaro, 1996. Infection of cattle with *Brucella abortus* biovar 1 isolated from a bison in Wood Buffalo National Park. Can Vet J, 37: 415-419.

Gamboa AM, TA Fitch, MM Kahl-McDonagh, G Gomez and AC Rice-Ficht, 2009. The *Brucella abortus* S19 DeltavjbR live vaccine candidate is safer than S19 and confers protection against wild-type challenge in BALB/c mice when delivered in sustained-release vehicles. Infect Immun, 77: 877-884.

Gebretsadik B, K Belihu and Y Asfaw, 2007. Sero-epidemiological investigation of bovine brucellosis in the extensive cattle production system of Tigray Region of Ethiopia. Int J Appl Res Vet Med, 5: 65-71.

Graves RR, 1943. The Story of John M. Buck's and Matilda's contribution to the cattle industry. J Amer Vet Med Ass 102: 193–195.

Grillo MJ, MJ DeMiguel, PM Munoz, CM Marin, J Ariza and JM Blasco, 2006. Efficacy of several antiniotic combinations against *Brucella melitensis* Rev1 experimental infection in BALB/c mice. J Antimicrob Chemother, 58: 622–626.

Godfroid J, C Saegerman, V Wellemans, K Walravens, J Letesson, A Tibor, A McMillan, S Spencer and M Sanna, 2002. How to substantiate eradication of bovine brucellosis when aspecific serological reactions occur in the course of brucellosis testing. Vet Microbiol 90: 461–477.

Godfroid J and A Kasbohrer, 2002. Brucellosis in the European Union and Norway at the turn of the twenty-first century. Vet Microbiol, 90: 135–145.

Gotuzzo E, C Carrillo, J Guerra and L Llosa, 1986. An evaluation of diagnostic methods for brucellosis--the value of bone marrow culture. J Infect Dis, 153: 122-125.

Guarino A, G Fusco, L Serpe, P Gallo, DI Matteo, G Urbani, M Tittarelli, M D Ventura and R Condoleo, 2001. Indirect ELISA for the diagnosis of brucellosis in water buffaloes (*Bubalus bubalis*) in Italy. Vet Rec, 149: 88-90.

Gul ST and A Khan, 2007. Epidemiology and epizootology of brucellosis: A review. Pak Vet J, 27: 145-151.

Hamidullah M, R Khan and I Khan, 2009. Seroprevalence of brucellosis in animals in district kohat NWFP and comparison of two serological tests. Pak J Sci, 61: 242-243.

Hellmann E, C Staak and M Baumann, 1984. Bovine brucellosis among two different cattle populations in Bahr el Ghazal Province of Southern Sudan. Tropenmed Parasitol, 35: 123-126.

Herrera L, F Suárez-Güemes, B Arellano Reynoso, E G Palomares-Resendiz, R Hernández-Castro and E Díaz Aparicio, 2010. Experiences in Mexico of the vaccination in bovines and goats, with *Brucella abortus* RB51. Current research, technology and education topics in applied microbiology and microbial biotechnology, A. Mendez vilas (Ed), pp: 694-699.

Hoffmann D, 1999. Asian Livestock to the Year 2000 and beyond. Working Paper series 1/2, 1-44.

Hussain I, MI Arshad, MS Mahmood and M Akhtar, 2008. Seroprevalence of Brucellosis in Human, Cattle, and Buffalo Populations in Pakistan. Turk J Vet Anim Sci, 32: 315-318.

Hussein AA, AS Sayed and MA El Feki, 2005. Seroepidemiological study on human brucellosis in Assiut Governorate. Egypt J Immumol, 12: 49-56.

Jarvis BW, TH Harris, N Qureshi and GA Splitter, 2002. Rough lipopolysaccharide from *Brucella abortus* and *Escherichia coli* differentially activates the same mitogen-activated protein kinase signaling pathways for tumor necrosis factor alpha in RAW 264.7 macrophage-like cells. Infect Immun, 70: 7165-7168.

Kagumba M and E Nandokha, 1978. A survey of the prevalence of bovine brucellosis in east Africa. Bull Anim Health Prod Afr, 26: 224-229.

Leslie, C, A Balows and M Sussman, 1998. Microbiology and microbial infections. Syst Bacteriol, 2**:** 829-830.

Limet JN, P Kerkhofs, R Wijffels and P Dekeyser, 1988. Diagnostic serologique de la brucellose bovine par ELISA. Ann Med Vet, 132: 565-575.

Lodhi LA, H Jamil, ZI Qureshi and I Ahmad, 1995. Sero-prevalence of brucellosis in buffaloes in & around Faisalabad. Pak Vet J, 15: 127-128.

Lulu, A R, GF Araz, MI Khatib, MY Mustafa, AR Yusuf and FF Fenech, 1988. Human brucellosis in Kuwait: a prospective study of 400 cases. Q J Med, 66: 39-54.

Maadi H, M Moharamnejad and M Haghi, 2011. Prevalence of brucellosis in cattle in Urmia, Iran. Pak Vet J, 31: 81-82.

Martin-Moreno S, O Soto-Guzman and J Bernaldo-de- Quiros, 1983. Pancytopenia due to hemophagocytosis in patients with brucellosis: a report of four cases. J Infect Dis, 147: 445-449.

Maiga S, MD Traore, M Niang and I Toure, 1996. Sero-epidemiological investigation of bovine Brucellosis in the dairying belt of Bamako, Mali. Proceedings of 18th International Conference, Bamako. January, 1996.

McDermott JJ and SM Arimi, 2002. Brucellosis in sub-Saharan Africa: epidemiology, control and impact. Vet Microbiol, 90: 111–134.

McEwen AD and PFW Paterson, 1939. An estimate of a suitable infective dose of *Brucella abortus* for immunization tests on cattle. J Comp Pathol, 52: 116-128.

Memish Z, MW Mahmood, S Al Mahmoud, M Al Shaalan and MY Khan, 2000. *Brucella* bacteraemia: clinical and laboratory observations in 160 patients. J Infect, 40: 59-63.

Memish Z, 2001. Brucellosis Control in Saudi Arabia: Prospects and Challenges. J Chemother, 13: 11–17.

Memish ZA and HH Balkhy, 2004. Brucellosis and International Travel. J Travel Med, 11: 49–55.

Mitchell CA and FA Humphreys, 1931. Studies in *Brucella melitensis* (abortus) infection of cattle. Cornell Vet, 21: 57-67.

Mitka S, C Anetakis and E Souliou, 2007. Evaluation of different PCR assays for early detection of acute and relapsing brucellosis in humans in comparison with conventional methods. J Clin Microbiol, 45: 1211-1218.

Mukherjee F, J Jain, MJ Grillo, J M Blasco and M Nair, 2005. Evaluation of *Brucella abortus* S19 vaccine strains by bacteriological tests, molecular analysis of *ery* loci and virulence in BALB/c mice. Biologicals, 33: 153–160.

Mukhtar F and F Kokab, 2008. *Brucella* serology in abattoir workers. J Ayub Med Coll Abbottabad, 20: 57-61.

Munir R, ST Rehman, R Kausar, S M S Naqvi and U Farooq, 2008. Indirect Enzyme Linked Immunosorbent Assay for diagnosis of brucellosis in buffaloes. Acta Vet Brno, 77: 401–406.

Munir R, M Afzal, M Hussain, SMS Naqvi and A Khanum, 2010. Outer membrane proteins of *B. abortus* vaccinal and field strains and their immune response in buffaloes. Pak Vet J, 30: 110-114.

Naeem, K, S Akhtar and N Ullah, 1990. The serological survey of bovine brucellosis in Rawalpindi, Islamabad District. Pak Vet J, 10: 154-156.

Nasir A, Z Parveen, MA Shah and M Rashid, 2004. Sero-prevalence of brucellosis I animals at government and livestock farms in Punjab. Pak Vet J, 24: 144.

Navarro E, M A Casao and M A Solrea, 2004. Diagnosis of human brucellosis using PCR. Expert Rev Mol Diagn, 4: 115-123.

Navarro E, JC Segura and MJ Castano, 2006. Use of real-time quantitative polymerase chain reaction to monitor the evolution of *Brucella melitensis* DNA load during therapy and post-therapy follow-up in patients with brucellosis. Clin Infect Dis, 42: 1266-1273.

Nicoletti P, 1990. Vaccination against *Brucella*. Adv Biotechnol Processes, 13: 147-168.

Nielsen KH, L Kelly, D Gall, SJ Balsevicius, P Nicoletti and W Kelly, 1996. Comparison of enzyme immunoassays for the diagnosis of bovine brucellosis. Prev Vet Med, 26: 17-32.

OIE, 2004. In OIE manual of the diagnostic tests and vaccines for terrestrial animals, mammals, birds and bees. 5th Ed, OIE, Paris, pp: 409-443.

OIE, Terrestrial Animal Health Code Brucellosis, http://www.oie.int/ (2009), Accessed on 2 March, 2010.

Omer MK, G Holstan, E Skjerve, Z Woldehiwet and APG MacMillian, 2000. Prevalence of antibodies to *Brucella* species in cattle, sheep, goats, horses and camels in the State of Eritrea, influence of husbandry system. Epidemiol Infect, 125: 447- 453.

Omer MK, E Skjerve, AP Macmillan and Z Woldehiwet, 2001. Comparison of three serological tests in the diagnosis of *Brucella* infection in unvaccinated cattle in Eritrea. Prev Vet Med, 48: 215-222.

Omer MM, MT Mussa, MR Bakhiet and L Perrett, 2010. Brucellosis in camels, cattle and humans: associations and evaluation of serological tests used for diagnosis of the disease in certain nomadic localities in Sudan. Rev Sci Tech, 29: 663-669.

Park MY, Lee CS, Choi YS, Park SJ, Lee JS and Lee HB, 2005. A sporadic outbreak of human brucellosis in Korea. J Korean Med Sci, 20: 941-946.

Pappas G, P Papadimitriou, N Akritidis, L Christou and E VTsianos, 2006. The new global map of human brucellosis. Lancet Infect Dis, 6: 91–99.

Qureshi MA and SJ Masood, 1988**.** Latest trend of brucellosis in livestock at livestock experiment stations in the Punjab. Pak J Vet Res, 1: 39-44.

Rahman MS, MJ Uddin, J Park, J Chae, MB Rahman and MA Islam, 2006. A Short history of Brucellosis: special emphasis in Bangladesh. Bangladesh J Vet Med, 4: 1–6.

Refai M, 2000. Control of brucellosis in animals in Egypt. In: Proc. 2nd Intern. Symp. cum- Workshop of the Germany–Egypt-Region Inter-Alumni-Net (GEAR), St. Catherine, Germany.

Rojas X and O Alonso, 1995. ELISAs for the diagnosis and epidemiology of *Brucella abortus* infection in cattle in Chile. Arch Med Vet, 27: 45-50.

Romero C, M Pardo, MJ Grillo, R Diaz, JM Blasco, GI Lopez and IL Goni, 1995. Evaluation of PCR and indirect enzyme linked immuno-sorbent assay on milk samples for diagnosis of brucellosis in diary cattle. J Clin Microbiol, 33: 3198-3200.

Salari MH, MB Khalili and GR Hassanpour, 2003. Selected epidemiological features of human brucellosis in Yazd, Islamic Republic of Iran: 1993-1998. East Mediterr Health J, 9: 1054-1060.

Schelling E, C Diguimbaye, S Daoud, J Nicolet, P Boerlin, M Tanner and J Zinsstag, 2003. Brucellosis and Q-fever sero-prevalence of nomadic pastoralists and their livestock in Chad. Prev Vet Med, 61: 279 – 293.

Schussler JM, AZ Fenves and WL Sutker, 1997. Intermittent fever and pancytopenia in a young Mexican man. South Med J, 90: 1037-1039.

Shafee M, 2007. Seroprevalence of bovine brucellosis in Quetta. MPhil thesis, University of Veterinary and Animal Sciences, Lahore, Pakistan.

Shafee M, R Masood, AS Ali, M Ahmad and A Razzaq, 2011. Prevalence of bovine brucellosis in organized dairy farms, using milk ELISA, in Quetta city, Balochistan. Vet Med Int, Article ID 358950.

Shang DX, Y Donglou and Y Jiming, 2002. Epidemiology and control of brucellosis in China. Vet Microbiol, 90: 165–182.

Stableforth AW, 1959. Brucellosis, infectious diseases of animals: diseases due to bacteria. Rev Sci Tech Off Int Epiz, 1: 153-159.

Taleski V, L Zerva and T Kantradjiev, 2002. An overview of the epidemiology and epizootology of Brucellosis in selected countries of Central and Southeast Europe. Vet Microbiol, 90: 147–155.

Thimm B and W Wundt, 1976. The epidemiological situation of brucellosis in Africa. Devel Biol Standard, 31: 201-217.

Weynants V, A Tibor, PA Denoel, C Saegerma, J Godfroid, P Thiange and JJ Letesson, 1996. Infection of cattle with *Yersinia enterocolitica* O: 9 a cause of the false positive serological reactions in bovine brucellosis diagnostic tests. Vet Microbiol, 48: 101–112.

WHO, Report of the WHO Working Group Meeting on Brucellosis Control & Research, Geneva, 2-4 June 1992. World Health Organization, 1992, Geneva (unpublished document WHO/CDS/ VPH/92.109)

WHO Expert Committee on Biological Standardization: forty-seventh report. WHO technical report series 878, World Health Organization, Geneva, 1998.

Wadood F, M Ahmad, A Khan, ST Gul and N Rehman, 2009. Seroprevalence of brucellosis in horses in and around Faisalabad. Pak Vet J, 29: 196-198.

Woodhead GS and AP Aitken, 1889. Epizootic abortion: second report of a committee appointed by the Highland and Agricultural Society of Scotland. J Comp Pathol, 2: 97-105.

Yagupsky P, 1994. Detection of *Brucella melitensis* by BACTEC NR660 blood culture system. J Clin Microbiol, 32: 1899–1901.

Yagupsky P and EJ Baron, 2005. Laboratory exposures to *Brucella*e and implications for bioterrorism. J Emerg Infect Dis, 11: 1180–1185.

Young EJ, 1983. Human brucellosis. Rev Infect Dis, 5: 821-842.