**2. Review of Literature**

**2.1. General study**

**2.1.1. Etiology of Brucellosis**

Brucellae are small, non-motile, non-sporulating, non-toxigenic, non-fermenting, facultative, intracellular, gram-negative coccobacilli parasites that may, based on DNA homology, represent a single species (Moreno & Moriyon, 2002). Six species are importent for animal Brucellosis: *B. abortus*, *B. melitensis*, *B. suis*, *B. ovis*, *B. canis* and *B. neotomae* (Foster *et al.,* 2007). *B. abortus* usually causes brucellosis in cattle, bison and buffalo (Acha and Szyfres, 2003). *B. melitensis* is the most important species in sheep and goats, but *B. ovis* can also cause infertility in rams. *B. canis* causes disease almost exclusively in dogs. It can cause disease in humans, although this is rare even in countries where the infection is common in dogs (Carmichael, 1990). Some species of *Brucella* contain biovars. Seven biovars are recognized for *B. abortus* (1, 7-10, 12, 13), three for *B. melitensis* (1, 7, 8), and five for *B. suis*. The other species have not been differentiated into biovars, although variants exist (Corbel., 1997). *B. melitensis* biotypes-1 and 3 have been isolated from goats and sheep and cattle which is one of the most virulent species of *Brucella* and constitutes a hazard for humans (Jacques *et al.,* 2007). *Brucella melitensis* infection is emerging as an increasingly serious public health problem in some countries.

In humans, brucellosis can be caused by *B. abortus*, *B. melitensis*, *B. suis* biovars 1-4 and, rarely, *B. canis* or marine mammal *Brucella* (Acha and Szyfres, 2003; Brew *et al.,* 1999 and McDonald *et al.,* 2006). *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 (Acha and Szyfres, 2003; De Massis *et al.,* 2005).

One or more unnamed species of *Brucella* have been found in marine mammals. Formal names proposed for marine mammal isolates are *B. maris* for all strains, or *B. pinnipediae* for strains from pinnipeds (seals, sea lions and walruses) and *B. cetaceae* for isolates from cetaceans (whales, porpoises and dolphins) (Foster *et al.,* 2007).

**2.1.2. Epidemiology:**

Brucellosis is the most common zoonosis in the world. Although brucellosis and its transmission were discovered over 100 years ago but the disease remains a worldwide problem, especially in developing countries. Brucellosis is well controlled in most developed countries. *B. abortus* is found in cattle-raising regions except in Japan, Canada, some European countries, Australia, New Zealand and Israel, where it has been eradicated (Corbel, 1997). This organism has been reported from Africa and India, but it does not seem to be endemic in northern Europe, North America (except Mexico), Southeast Asia, Australia or New Zealand. *B. ovis* probably occurs in most sheep-raising regions of the world. It has been reported from Australia, New Zealand, North and South America, South Africa and many countries in Europe (Ashenafi *et al*., 2007). It is more common in countries that do not have good standardized and effective public health and domestic animal health programs. Areas currently listed as high risk are the Mediterranean basin (Portugal, Spain, Southern France, Italy, Greece, Turkey, and North Africa), South and Central America, Eastern Europe, Asia, Africa, the Caribbean, and the Middle East (Schutze *et al.,* 2000).

*Brucella* species also found in marine mammal populations in the North Atlantic Ocean, the Mediterranean Sea, the Arctic including the Barents Sea, the Atlantic and Pacific coasts of North America; the coasts of Peru, Australia, New Zealand and Hawaii; and in the Solomon Islands and the Antarctic (Corrente *et al*., 2010).

Brucellosis is a significant and increasing veterinary and public health problem in India. In India 80% of the population live in approximately 575000 villages and thousands of small towns; have close contact with domestic/ wild animal population owing to their occupation. *B. melitensis*, and *B. abortus. B. melitensis* is the most virulent and common strain for man and it causes severe and prolonged disease with a risk of disability. Bovine brucellosis is widespread in India and increase in recent times, due to increased trade and rapid movement of livestock (Renukaradhya *et al.,* 2002). In Bangladesh, Brucellosis is found in Bagerhat, Bogra, Gaibangha, Mymensingh and Sirajgonj districts (Rahman *et al*., 2011). The overall seroprevalence of brucellosis in Bangladesh is 5% while in Mymensingh (2%), Tangail (16.66%), Pabna (11.52%), Faridpur (2.92%), Bogra (2%) (Rahman *et* *al.,*1982). Brucellosis in goat and sheep is recently detected in Mymensingh and Bogra districts of Bangladesh (Rahman and Rahman., 2011).

**2.1.3. Transmission**

The transmission of *Brucella* infection in a region depends upon several factors like food habits, methods of processing milk and milk products, unpasteurized dairy products, social customs, husbandry practices, climatic conditions, socioeconomic status, and environment hygiene. Environmental sanitation is particularly important in the context of air borne transmission. Brucellosis is almost invariably transmitted to man from infected domestic animals. However, it has been documented beyond doubt, the possibility of human to human transmission of *Brucella* infection. Animal products such as milk and meat products also play an important role in the disease transmission. Dairy products prepared from unpasteurized milk such as soft cheeses, yoghurts, and ice-creams may contain high concentration of the bacteria and consumption of these is an important cause of brucellosis. Camel milk is also considered to be the important source of the infection in Middle East countries and Mongolia. Bacterial load in animal muscle tissues is low, but consumption of undercooked traditional delicacies such as liver has been implicated in human infection. Some particular food habits, such as eating aborted fetuses seen in Ecuador, may be implicated in causing human brucellosis. Crushing the umbilical cord of newborn lambs and kids with the teeth is another risky habit. Consuming fresh goat’s milk combined with herbal extracts to obtain relief from chronic ailments is reported to be one more risky habit. Skinning stillborn lambs and kids and aborted fetuses, which may be heavily contaminated with *Brucella* spp., also presents a high risk of brucellosis. Contamination of skin wounds may be a problem for persons working in slaughterhouses or meat packing plants or for veterinarians.

Hunters may be infected through skin wounds or by accidentally ingesting the bacteria after deer, elk, moose, or wild pigs that they have killed. Inhalation is often responsible for a significant percentage of cases in abattoir employees. In addition, laboratory acquired *Brucella* infection due to accidental ingestion, inhalation and mucosal or skin contact is a major health hazard for the laboratory workers handling the cultures of the virulent or attenuated strains. The disease has been recognized as one of the common laboratory- transmitted infections and has been reported to occur in clinical, research, and production laboratories. The presence of brucellosis in wild animals, with a potential for continuous transfer to domestic animals and from them to humans is another epidemiological issue. Males are affected more commonly than females which may be due to risk of occupational exposure. Although human brucellosis affects all age groups, it is said to be rare in childhood. However, in areas, where *B. melitensis* is endemic, pediatric cases are seen (Mantur *et al.,* 2004). Free grazing and movement with frequent mixing of flocks of sheep and goats also contribute to the wide distribution of brucellosis in these animals have reported a severe outbreak of brucellosis in an organized dairy farm. The much higher seroprevalence rate has been also noted in specific risk groups such as abattoir workers.

Those with a professional risk of acquiring infection include livestock producers, abattoir workers, shepherds, farmers, veterinarians, and laboratory personnel. Brucellosis is common in rural areas because farmers live in close contact with their animals.

**2.1.4. Clinical Sign**

In cattle, *B. abortus* causes abortion (usually occur during the second half of gestation), stillbirth and weak calves, the placenta may be retained and lactation may be decreased. Epididymitis, seminal vesiculitis, orchitis and testicular abscesses are sometimes seen in bulls. Infertility occurs occasionally in both sexes, due to metritis or orchitis/epididymitis (Acha and Szyfres, 2003). Hygromas, particularly on the leg joints, are a common symptom in some tropical countries. Arthritis can develop after long-term infections. Systemic signs do not usually occur in uncomplicated infections, and deaths are rare except in the fetus or newborn. Infections in non-pregnant females are usually asymptomatic. The brucellae localizes in the supra-mammary lymph nodes and mammary glands of 80% of the infected animals (Hamdy and Amin, 2002). Most infected cows abort only once although the placenta will be heavily infected at subsequent apparently normal calving (Morgan, 1969).

*B. melitensis* mainly causes abortions, stillbirths and the birth of weak offspring (Lilenbaum *et al.,* 2007). Acute orchitis and epididymitis can occur in males, and may result in infertility. Arthritis is seen occasionally in both sexes (Acha and Szyfres, 2003). *B. ovis* affects sheep that can cause epididymitis, orchitis and impaired fertility in rams (Acha and Szyfres, 2003). Abortions, placentitis and perinatal mortality can be seen in ewes.

**2.1.5. Treatment**

Treatment failure and relapse rates are high due to intracellular localization of *Brucella* organism (Seleem *et al.,* 2008). There is no practical treatment for infected cattle or pigs, but long-term antibiotic treatment is sometimes successful in infected dogs and human. Antibiotic treatment has also been used successfully in some valuable rams, but it is usually not economically feasible. In regions where the prevalence is high, the only way of controlling and eradicating this Disease is by vaccination of all susceptible hosts and elimination of infected animals (Briones *et al.,* 2001). No vaccines are made for dogs and human (Henk and Kadri, 2005). Some forms of localized disease, such as endocarditis, may require surgery.

**2.1.6. Prevention and control**

Nationwide eradication programs for *B. abortus*, *B. melitensis* and *B. suis* include quarantines of infected herds, vaccination, test-and-slaughter and/or depopulation techniques, cleaning and disinfection of infected farms, and various forms of surveillance and trace backs.

Herd additions should come from brucellosis-free areas or accredited herds. Domesticated animals should always be kept from contact with wild animal reservoirs. Commercial *B. abortus* and *B. melitensis* vaccines are available for cattle, sheep and goats. The most commonly used vaccines against bovine brucellosis are *B. abortus* strain 19 and the recently USDA approved strain RB51 (Moriyon *et al.,* 2004). 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 (Adone *et al.,* 2008).

*B. abortus, B. melitensis* and *B. suis* can be eradicated from a herd by test-and-removal procedures, or by depopulation (Briones *et al.,* 2001). Good management can reduce the incidence of infection in an infected herd. Transmission is reduced by immediate disposal of the placenta, contaminated bedding and other infectious material, followed by thorough cleaning and disinfection. The prevalence of *B. ovis* can be decreased by examining rams before the breeding season and culling rams with palpable abnormalities (Corbel *et al.,* 1997).

**2.2. Review study or diagnosis of Brucellosis**

Buffered Brucella antigen tests like Milk ring test, serological test like Rose Bengal Plate test (RBT), slow agglutination Test (SAT), Tube agglutination Test (TAT), mercaptoethanol test and/or Enzyme-linked immunosorbent assays ( ELISA) (indirect, competitive, Avidin-Biotin), Complement fixation test, Interferon gamma test, Fluorescence polarisation assay are generally used to detection of brucellosis in livestock. Staining methods, culture (basal media, selective media),identification and typing, Nucleic acid recognition methods (Bruce-ladder multiplex PCR), Identification of vaccine strains are also used for detection the organism. Other tests like brucellin skin test, native hapten and cytosol protein-based tests are sometime prefer to detect the *Brucella* in animal(OIE, 2010**).**

**2.2.1. Microscopic examination**

Microscopic examination of smears stained with the Stamp's modification of the Ziehl-Neelsen method can be used for a presumptive diagnosis (Joshi *et al.,* 2005). *Brucell*a species are not truly acid-fast, but they are resistant to decolorization by weak acids, and stain red against a blue background. Brucellae are coccobacilli or short rods, usually arranged singly but sometimes in pairs or small groups (Dahouk *et al.,* 2003).

**2.2.2. Culture**

Culture from the blood of a patient provides definite proof of brucellosis (Dahouk *et al.,* 2003). *Brucella* species can be recovered from numerous tissues and secretions, particularly fetal membranes, vaginal secretions, milk (or udder secretions in non-lactating cow), semen, arthritis or hygroma fluids, and the stomach contents, spleen and lung from aborted fetuses (Radostits *et al.,* 2000). *Brucella* spp. can be isolated on a variety of plain media, or selective media such as Farrell's medium or Thayer-Martin’s modified medium. Enrichment techniques can also be used. Colony morphology varies with the species. Colonies of smooth forms (*B. abortus*, *B. suis, B. melitensis* and marine mammal *Brucella*) are round with smooth margins (Poester *et al.,* 2010).

**2.2.3. Nucleic acid recognition methods**

Polymerase chain reaction (PCR) techniques are also available for most species. rRNA sequencing has defined the phylogenetic relationship of *Brucella.* This organism is detected by a PCR that is otherwise specific for *Brucella* (Costa *et al.,* 1996)*.*

**2.2.4. Serological test**

Serological tests are not completely specific and cannot always distinguish reactions because most serological tests rely on the unique antigenic properties of lipopolysaccharides (LPS) that are shared among the *Brucella* species (Munoz *et al.,* 2005). Serological tests commonly used to test individual cattle or herds include the buffered *Brucella* antigen tests (rose Bengal test and buffered plate agglutination test), complement fixation, indirect or competitive enzyme-linked immunosorbent assays (ELISAs) and the fluorescence polarization assay. The classical Rose Bengal test (RB) is often used as a rapid screening test (Ruiz-Mesa *et al.,* 2005). Supplemental tests such as complement fixation or rivanol precipitation are often used to clarify the results from plate or card agglutination tests. ELISAs or the *Brucella* milk ring test (BRT) can be used to screen herds by detecting antibodies in milk.

**2.2.5. Other tests**

**2.2.5.1. Brucellin skin test:** An alternative immunological test is the brucellin skin test, which can be used for screening unvaccinated herds. The brucellin skin test has a very high specificity, such that serologically negative unvaccinated animals that are positive reactors to the brucellin test should be regarded as infected animals (Saergerman *et al*., 1999). Also, results of this test may aid the interpretation of serological reactions thought to be FPSR due to infection with cross reacting bacteria, especially in brucellosis-free areas (Massis *et al*., 2005).

**2.2.5.2. Serum agglutination test (SAT):** While not recognized as a prescribed or alternative test, the SAT has been used with success for many years in surveillance and control programmes for bovine brucellosis. Its specificity is significantly improved with the addition of EDTA to the antigen (Lord *et al*., 1989).

**2.2.5.3. Native hapten and cytosol protein-based tests:** Native hapten tests are highly specific in S19 vaccination contexts, and have been used successfully in an eradication programme in combination with the RBT as a screening test (Asarta *et al*., 1989).

**2.2.5.4. Interferon gamma test:** In general, the interferon gamma test involves stimulation of lymphocytes in whole blood with a suitable antigen, in this case, Brucellin has been shown to work well and then measuring the resulting gamma interferon production by a capture ELISA (Weynants *et al.,* 1998).

**2.2.6. Hematological diagnosis**

Normal hematological parameters of exotic cow was demonstrated by Research Animal Resources (RAR), University of Minnesota that is Hb: 8-15 gm/dl,PCV: 24-48%, TLC: 4-12 Thousand/μl, DLC (Neutrophil: 20-40, Lymphocyte: 40-70, Monocyte: 1-6, Eosinophil: 0-4, Basophil: 0-2), MCV: 40-60 fl, MCH: 11-17 pg, MCHC: 30-36 g/dl (RAR, 2011).

In human, fetal systemic infection with multifocal liver and lung nodules caused by *B. abortus* where hemoglobin was 11.4 gm/dl and WBC 2290/mm and neutrophil 69.9% (Tiller *et al.,* 2010).

Disseminated intravascular coagulation in case of bovine brucellosis changes hematocrit (21%) and hemoglobin (7 gm/dl) (Dogan, 2010).