CHAPTER - I

INTRODUCTION

Zoonotic infections are infections which are naturally transmitted between vertebrate animals and man, with or without an arthropod intermediate. Zoonotic infections represent an important group of occupationally-acquired infectious diseases in agricultural and meat industry workers. The different occupational groups exposed to animals include Veterinary practitioners, Livestock inspectors, Slaughter house workers, Butchers, Meat industry workers, Health care workers, laboratory workers and farmers, and they are at high risk of acquiring these infections than the general population. Among these, Veterinarians and butchers are occupationall high risk individuals, because they are directly exposed to contaminated body fluids and tissue materials of infected animals.

1.1 BRUCELLOSIS

Brucellosis is an important Zoonotic disease distributed all over the world. It is an uncontrolled serious re-emerging disease with greater public health importance. The disease has been known as Undulant fever, Bangs Disease, Gibralter fever, Mediterranean fever and Malta fever. The causative organism, Brucella species, was first isolated in 1887 by Sir David Bruce, who recovered the organism from splenic culture of British soldiers dying of Malta fever (Radolf,1994). In India it has been reported from many areas and is considered to be the most neglected disease.
Brucellosis became recognized as a Zoonotic infection of great economic importance and concern to the livestock industry in many part of the world. It is a zoonotic disease of bacterial origin, which gets accidentally transmitted to humans from cattle, sheep, goat, pigs and camels through direct contact with blood, placenta, fetus or uterine secretion, or through consumption of contaminated raw animal products like unpasteurized milk and soft cheese (Acha, 1986). In developed countries, some human infections are reported in individuals working with meat packing and dairy related occupations (Young, 1995).

1.2 HIGH RISK OCCUPATIONAL GROUPS

The people who are in close contact with animals, because of their profession can be included in high risk occupational group. Such commonly exposed groups include Veterinarians and slaughterhouse workers. In slaughterhouse workers the risk of exposure is more because contact with different infectious sources like carcasses and viscera of slaughtered animals and the aerosols present in the slaughtering area are acting as the causes of infection (Young, 1991).

1.3 TAXONOMY OF BRUCELLA

The major causative agent Brucella, is a small non motile aerobic facultative intracellular Gram negative coccobacilli belongs to α2 subdivision of the proteobacteria. Other members in this group include Ochrobactrum, Rhizobium, Rhodobacter, Agrobacterium, Bartonella and Rickettsia.

In 1985 Verger and colleagues used DNA-DNA Hybridisation techniques to investigate 51 Brucella strains representing all species and found that all of the isolates had greater than 96 ± 4%
homology with each other (Verger et al., 1985). The scientist proposed that all Brucellae belonged to a single species (Brucella melitensis) and that the recognized species should be regarded as biovars of Brucella melitensis i.e., B. melitensis biovars melitensis, abortus and suis. Multilocus enzyme electrophoretic analysis of Brucella strain also supported this monospecific genus proposal (Gandara et al., 2001).

1.3.1 Classification

Brucella can be classified into different species based on their CO₂ requirement, H₂S production, sensitivity to dyes such as basic fuchsin and thionin, agglutination by monospecific sera, phage lysis and oxidative metabolic tests with amino acids and carbohydrates. The two major species are Brucella melitensis and Brucella abortus infecting primarily goats or sheep, cattle and swine, respectively. The other important species is Brucella suis which mainly infect swine. All these three major species of Brucella are pathogenic to human beings.

Brucella melitensis is the most pathogenic species, while Brucella abortus and Brucella suis are of intermediate Pathogenicity. The incubation period is usually about 10- 30 days, but may sometimes be very prolonged.

1.3.2 Antigenic structure

The structure of the Brucella A and M antigens are well studied by application of high resolution Nuclear Magnetic Resonance techniques (Caroff et al., 1984 and Bundle et al., 1987). This approach was necessitated by the difficulty in isolating and identifying the monosaccharide of Brucella O-polysaccharide
antigens, 4-amino-4, 6-dideoxy-D-mannose, which existed in both A and M antigens as the N-formyl derivative.

There were two clearly identified Brucella polysaccharide antigens A and M, as typified by the strains *B. abortus* 1119-3 and *B. melitensis* 16M, respectively. Smooth strains such as *B. abortus* 45/20 and S19 also carried the o-polysaccharide, but in low amounts, and they had low molecular weights. This observation is consistent with the evidence that immunization with R-type strains can induce circulating anti-A-antigen polysaccharide antibodies (Alton et al., 1975).

1.4 VIRULENCE OF BRUCELLA SPECIES

There is no toxin production by Brucella species. The lipopolysaccharide of the Brucella cell wall is the major virulent factor. The LPS of *B. melitensis, B. abortus* and *B. suis* contains two major antigenic determinants called A for abortus and M for melitensis (Bundle et al., 1992). In addition to providing markers for Biovars determination, these molecules have role in the virulence of the organism.

1.5 RESISTANCE

Brucella is a heat labile organism and can be destroyed by heat at 60°C in 10 minutes and also by 1% phenol in 15 minutes. They are killed by pasteurisation. They may survive in soil and manure for several weeks. They remain viable for 10 days in refrigerated milk, one month in ice-cream, four months in butter and for varying periods in cheese depending on its pH. They may also survive for many weeks in meat. They are sensitive to direct sunlight and acid, and tend to die in butter milk. *Brucella*
*melitensis* may remain alive for six days in urine, six weeks in dust and ten weeks in water.

### 1.6 TYPES OF BRUCELLOSIS

Human infection can be of three forms – Acute, Chronic and latent or subclinical infections. Acute Brucellosis is mostly due to *Brucella melitensis*. It is also called as undulant fever. But only some case shows the undulant pattern. It is associated with prolonged bacteremia and irregular fever.

Chronic Brucellosis may be no bacteremic with low grade infection and periodic exacerbations. The symptoms are related to a state of hypersensitivity in the patients. Common clinical manifestations consist of lassitude and joint pain with minimal or no pyrexia. The illness will last for several years.

Latent infections are also called subclinical infections. In this stage there is no clinical manifestation of the disease so the diagnosis is purely dependant on the basis of serological evidences.

### 1.7 MODE OF TRANSMISSION

Human Brucellosis is acquired from animals, directly or indirectly. Almost all domestic species can be affected with Brucellosis except cats which are resistant to Brucella infection. It is mainly an infection of reticulo endothelial system. Organism from the infected animal enters into human through wounds, conjunctiva or by inhalation or ingestion of products from infected animals.
Brucella is transmitted to human by three principal routes: direct contact with infected animal tissue, ingestion of contaminated meat or dairy products, and inhalation of aerosolised organisms (Corbel, 1997).

1.8 PATHOGENESIS

Brucella species are facultative intracellular organisms and their disease spectrum is partially explained by the ability of the organism to evade host defense mechanisms by virtue of intracellular existence. Invaded bacteria are opsonised by antibodies and are phagocytosed by polymorphonuclear leucocytes. They survive there and are disseminated through lymphatic system to various organs where it produces localised infections (Lapaque et al., 2005). The organism is able to escape phagocytic killing through inhibiting the phagosome-lysosome fusion and reproducing inside macrophages (Young, 2005).

The organisms are carried into the lymph nodes and the blood stream and become sequestrated in various parts of the reticulo endothelial system like liver, spleen, and bone marrow (Memish, 2001). In these sites, the PMNs eventually degenerate and release the intracellular organisms. The bacteria in-turn is endocytosed by macrophages and monocytes. Multiplication continues within these cells and eventually the cells are killed, releasing the organisms.

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). Inside the phagocytic cells they survive, presumably by adenine
and guanine monophosphate mediated suppression or inactivation of the intracellular myeloperoxidase-peroxide defence mechanism and by the production of superoxide dismutase, which blocks formation of toxic oxygen radicals (Bricker et al., 1990).

The “Undulant” fever pattern seen in Brucellosis is associated with the periodic release of bacteria and their components from phagocytic cells. Release of bacteria into the peripheral circulation results in hematogenous seeding into other organs and tissues, thereby leading to the clinical manifestations of human Brucellosis.

1.9 CLINICAL MANIFESTATIONS

The symptoms are varied consisting of muscular and articular pain, asthmatic attacks, nocturnal drenching sweats, exhaustion, anorexia, constipation, nervous irritability and chills. The usual complications are articular, osseous, visceral or neurological systems. Symptoms can be highly variable, ranging from non specific flue like symptoms usually seen in acute form to undulant fever, arthritis, orchitis and epididymitis (Plummet et al., 1998). The symptoms usually start after 2-4 weeks of initial infection.

1.10 DIAGNOSIS OF BRUCELLOSIS

Diagnosis can be established by laboratory methods such as serology and blood culturing. The diagnosis of Brucellosis can be challenging and is frequently delayed or missed because the clinical picture may mimic other infectious or non infectious conditions (Araj, 1999 and Yagupsky, 1999).

The diagnosis can be based on cultural isolation, serological tests and in recent years by biotechnological techniques. Cultural
isolation is time consuming, cumbersome and require establishment of advanced laboratories and trained personnel. Diagnosis can be established by laboratory methods such as serology and blood cultures. Prolonged incubation periods, specialised growth media and subcultures are required for the isolation of these fastidious slow growing bacteria. However cultures are not always positive when other tests are positive (Romero et al., 1995).

In India at present serological methods are mainly used for diagnosis of Brucellosis. Many serological tests, both conventional and novel methods are being used for the diagnosis of human Brucellosis, such as agglutination test, Indirect Immunofluorescence, Rose Bengal Plate Test, Standard tube agglutination test and ELISA. The most commonly used test are the serum agglutination test, Coombs anti Brucella test and Rose Bengal test (Orduna et al., 2000).

Several sources of errors have to be guarded before doing the tests. The serum often contains blocking or non agglutinating antibodies. For overcoming the prozone phenomenon several dilutions of the samples should be tested. A positive agglutination test may be produced by cholera, tularemia or yersinia infection or immunization. Cholera induced agglutinins may be differentiated by the agglutinin absorption test or by treating the serum with 2-mercaptoethanol. The agglutinin titres are expressed in international units using standard reference serum for comparison.

1.11 IMMUNITY IN BRUCELLOSIS

In Brucellosis both IgM and IgG antibodies appear in 7-10 days after onset of clinical infection. As the disease progresses, IgM antibodies decline, while the IgG antibodies persist or increase
in titre. In chronic infections, IgM may often be absent and only IgG can be demonstrated. The agglutination test is usually positive in acute infection but may be only weakly positive or even negative in chronic Brucellosis. So a negative agglutination test may not exclude the possibility of Brucellosis.

1.12 TREATMENT

The treatment of human Brucellosis is a controversial area because of the spectrum of the disease, the possibility of chronic infection, and the development of complications (Radolf, 1994). Successful treatment requires prolonged antimicrobial therapy usually with combination of agents, and in some cases surgical intervention is also indicated. The administration of agents that penetrate and have activity within phagocytic cells are pre-requisites for therapy, since Brucellae are facultative intracellular pathogens.

Human infection can be treated with oral tetracycline for six weeks in combination with intramuscular streptomycin, or gentamycin daily for 2-3 weeks can be recommended for Brucellosis (Young, 1995). Doxycycline and Rifampin administered orally for at least 6 weeks is also recommended for treatment. The usual regimen is a combination of doxycycline for 45 days with streptomycin intramuscularly daily for the first two weeks in adults, and in children cotrimoxazole along with rifampicin or Gentamycin.

1.13 PROPHYLAXIS
The infection can be prevented by reducing exposure to infected animals and by eliminating infected herds. An attenuated live vaccine can be given to individual with occupational risk of exposure. Prompt and accurate diagnosis helps the public health personnel in reducing the morbidity and also by preventing transmission through education and prevention programmes. All these measures are aimed at farm and meat industry workers, health workers and other high-risk occupational groups.

1.14 AIMS AND OBJECTIVES

The present study is undertaken to determine the current status of Seroprevalence of Brucella specific antibodies among person in high risk occupational group with the following aims and objectives -

- To identify the prevalence of Brucellosis among occupationally exposed and high-risk individuals

- To prove the risk of transmission of Brucellosis to Butchers and Veterinarians to find out the most suitable and sensitive technique for the diagnosis.

- To find out the effectiveness of Conventional and Newer techniques for the diagnosis of Brucellosis.

- To detect the most susceptible individuals among the most high risk occupational groups.

- To determine the factors predisposing these infections.

1.15 HYPOTHESIS:
1. Veterinary practitioners are at high risk in acquiring brucellosis, compared to other occupational groups like slaughter house workers, livestock inspectors and farmers.

2. Rose Bengal Plate Test (RBPT) is a rapid screening test for detecting brucellosis