CHAPTER I

INTRODUCTION

The economy of India is dependent mainly on agriculture. Livestock production is a vital source of providing dietary protein for the rapidly growing human population and it is therefore, important to define strategies for controlling infectious diseases that are undermining the livestock industry. Approximately 70% of human population lives in villages and agriculture is the main occupation. India has 199.1 million cattle, 105 million buffaloes, 140 million goats and 71.6 million sheep, of which Punjab has 2.64 million cattle, 6.17 million buffaloes, 0.41 million goats and 0.44 million sheep. The estimates of milk, egg and wool production in 2003–2004 were 839.1 million kilograms, 3068 million numbers and 0.554 million kilograms, respectively (www.husbandrypunjab.org).

There are a number of constraints that impede the way for full exploitation of the genetic worth of these animals. Among such factors are the infectious diseases that undermine the dairy industry and prove disastrous wherever they get neglected. One such important disease that can have serious repercussion on the production and reproduction of dairy animals is brucellosis. Although brucellosis has devastating effect on economy of dairy industry, its control has been mainly ignored in our country due to low mortality rates in the face of other diseases that cause significant mortalities. Brucellosis is caused by Gram negative bacteria of the genus *Brucella*, which are facultative intracellular coccobacilli that belong to the α2-Proteobacteriacea family (Garrity 2001). Although there is evidence supporting the notion that the genus *Brucella* should be re-classified as a mono-specific genus with several biotypes (Verger *et al* 1985), division of the genus into six classical *Brucella*...
species is still widely used for historical and clinical reasons. These species are *Brucella melitensis*, *Brucella abortus*, *Brucella suis*, *Brucella ovis*, *Brucella canis*, and *Brucella neotomae* (Osterman and Moriyon 2006). *Brucella* spp., have been classified based on their strong affiliation to specific natural hosts (Boschiroli *et al* 2001). With the exception of *B. ovis* and *B. neotomae*, all other species are capable of infecting man (Hartigan 1997). In addition to the classical *Brucella* spp., the genus has recently been expanded to include marine isolates, which have been divided into two species, *Brucella ceti* and *Brucella pinnipedialis*, based on their preferential hosts i.e. cetaceans and pinnipeds, respectively (Foster *et al* 2007).

Brucellosis is worldwide in distribution and is more common in countries with poor animal and public health programmes (Capasso 2002). Though it has been eradicated in many developed countries in Europe, Australia, Canada, Israel, Japan and New Zealand (Geering *et al* 1995), yet it remains an uncontrolled problem in regions of high endemicity such as the Africa, Mediterranean, Middle East, parts of Asia and Latin America (Refai 2002). In India brucellosis was first recognised in 1942 and is now endemic throughout the country. The disease is reported in cattle, buffalo, sheep, goats, pigs, dogs and humans. *B. abortus* biotype-1 in cattle and buffaloes and *B. melitensis* biotype-1 in sheep, goats and man are the predominant infective biotypes (Renukaradhya *et al* 2002).

Brucellosis is a zoonotic disease transmitted to humans from infected animals. *Brucella* species continues to pose a human health risk globally despite strides in eradicating the disease from domestic animals. It is a systemic disease that can involve any organ or system of the body. However the most common manifestation is fever. Human brucellosis is usually manifested as an acute or subacute febrile illness which may persist and progress to a chronic form (Mantur *et
al 2007). It is a well characterized occupational disease in shepherds, abattoir workers, veterinarians, dairy industry professionals and laboratory personnel (Agasthya et al 2007).

Though infection may occur through the skin, conjunctiva or respiratory mucosa by inhalation (Crawford et al 1990), the most common route of infection in cattle is the gastrointestinal tract (Crawford et al 1990), from where infection spreads to local lymph nodes where *Brucella* replicates intracellularly in phagocytes (Anderson et al 1986). Invasion of lymphatic vessels is followed by bacteraemia leading to systemic infection, favouring colonisation of the gravid uterus, male genital organs, and mammary glands (Ko and Splitter 2003). The mammary gland is another target organ that is important in transmitting the infection through contaminated milk. *B. abortus* induces a multifocal interstitial mastitis with interstitial accumulation of macrophages and intra-acinar infiltration of neutrophils (Emminger and Schalm 1943), associated with moderate numbers of predominantly intracellular organisms.

Outbreaks of bovine brucellosis are associated with abortion during the last trimester of gestation, production of weak newborn calves and infertility in cows and bulls (Enright et al 1984). Importantly, the outcome of infection in cattle is dependent on age, reproductive and immunological status, natural resistance, route of infection, infectious challenge, and virulence of the infective strain (Nicoletti, 1980). It is also a major impediment for the trade. Death may occur as a result of acute metritis, followed by retained fetal membranes (Radostits et al 2000). *Brucella abortus* has a strong tropism to the uterus during the last trimester of gestation, which is thought to be due to high concentrations of erythritol and steroid hormones. Erythritol favours bacterial survival since it can be metabolised by *B. abortus* as a
source of carbon and energy (Samartino and Enright 1996). Erythrophagocytic trophoblastic cells located at the base of chorionic villi of ruminants (Santos et al 1996) are considered the primary site of invasion of fetal placental tissues, from where \textit{B. abortus} disseminates to intercotyledonary trophoblasts (Anderson et al 1986). \textit{Brucella} multiplication induces infiltration of inflammatory cells, trophoblastic necrosis, vasculitis, and ulceration of the allantochorion. Consequently, fetal maternal metabolic exchanges are compromised resulting in abortion (Anderson et al 1986).

Laboratory diagnostics for veterinary pathogens have traditionally relied on methods of detecting the pathogen by culture or antibodies, using a variety of techniques such as neutralisation, enzyme-linked immunosorbent assay, agar gel immunodiffusion and complement fixation. In the past fifteen years, veterinary diagnosticians have incorporated new molecular techniques such as the polymerase chain reaction and Western blot, and improved older techniques through the use of recombinant antigens, monoclonal antibodies and synthetic peptides. The clinical diagnosis of brucellosis is complicated by variable incubation period and absence of apparent clinical signs, except abortion. While culture and isolation of \textit{Brucella} spp. is regarded as the “gold standard” test for laboratory diagnosis of brucellosis, its sensitivity is low because the \textit{Brucellae} are fastidious micro-organisms that can easily be overgrown by other contaminating bacteria. More importantly, the procedure is associated with high risk of infection to laboratory personnel (Alton et al 1988). Therefore, serological tests are often relied upon for the diagnosis of brucellosis. Although several serological tests have been developed and used for the laboratory diagnosis of brucellosis, they all lack qualities of an “ideal test” and no single test is appropriate in all epidemiological situations due to problems of
sensitivity and/or specificity. The buffered *Brucella*-antigen tests; the Rose Bengal test (RBT) and buffered plate agglutination test are very sensitive, and often used in many eradication programmes, but positive reactors require further investigations with other tests (OIE 2008). The complement fixation test (CFT) is used in many countries as a confirmatory test due to its higher specificity since it detects IgG1 antibodies, but may give rise to positive reactions in *B. abortus* S19 vaccinated cattle (Nielsen, 2002). Indirect or competitive ELISA and fluorescent polarisation assay are also employed as confirmatory tests (Nielsen, 2002). An alternative to isolation is the use of PCR-based methods for detecting *Brucella* genomic DNA (Leal-Klevezas *et al* 1995 and Bricker, 2002).

The approach to control, prevention or eradication of brucellosis in a region or country will depend on many factors, such as the level of infection in the herds or flocks, type of husbandry, economic resources, public health impacts and potential international trade implications. In a country like India innovative approaches need to be worked out to overcome the basic problems of ban on cow slaughter, distress sale of animals following the positive serological diagnosis of brucellosis and absence of a disease control strategy for the prevention and control of the disease. Keeping this in view the present study was undertaken with the following objectives:

1. Evaluation of different serological tests for diagnosis of brucellosis in cattle and buffaloes and seromonitoring of *Brucella* positive animals.
2. Molecular diagnosis of brucellosis in affected animals by PCR.
3. Identification of the risk factors associated with occurrence of brucellosis in cattle and buffaloes and impact of managerial practices on its control.