I. INTRODUCTION

Tuberculosis is one of the most common chronic infectious and debilitating diseases of man and animals. It is still a major public health disease of economic importance in many countries of the world, although, emerged about 35,000 years ago. As per the WHO report (2005), about 40 % or 1/3rd of the world’s human population is infected with tuberculosis, of which 10% develops disease and 3 millions are dying every year. It is estimated that approximately 25% of the avoidable adult human deaths in the developing world are from tuberculosis.

Although the human disease is primarily caused by *Mycobacterium tuberculosis* and *Mycobacterium bovis* which is a primary causative agent of bovine tuberculosis is equally important in human disease. Similarly, bovine tuberculosis is a chronic contagious respiratory disease caused primarily by *M.bovis* and infections due to *M.tuberculosis*, is also common. Both *M.bovis* and *M.tuberculosis* are genetically and antigenically very similar and cause identical clinical disease in both man and bovines. There is considerable and continuing public health significance of *M.bovis* infection in humans and animals and the disease has emerged as a major zoonotic problem in many countries. The bacterium can be discharged through saliva, milk and other discharges of infected animals. Young animals and humans can contract the disease
either by drinking raw milk from infected dams or due to close proximity with the infected animal.

India ranks first as the largest producer of milk in the world with more than 13% of the total world output and 57% of Asia’s total production. India possesses enormously large bovine population comprising more than 200 million cattle and 80 million buffaloes. In India, bovine tuberculosis incidence is high and is found to be more common in milch animals than draught animals. Such animals form a nucleus for spread of disease to other animals and human beings. The incidence of disease is not only higher in the developing nations, but in the absence of any national control and eradication program, is increasing in most of the countries worldwide particularly in the Asian, African and Latin American countries.

The development of a cattle vaccine would be one of the best option for long-term control of Tuberculosis. Unfortunately, an effective vaccine is not currently available for bovines. Vaccination strategy, in conjunction with a more accurate diagnosis system and a well defined control programme, would be the most effective way to control the disease.

The immune response to tuberculosis is often described as bidirectional, as it protects the host against disease on one side, also assists the pathogen by causing the tissue damage required for effective
transmission on the other side. There is a need for greater precision in our understanding of the relationship between virulence and protective immunity in tuberculosis. In the early years of twentieth century, Calmette and Guerein developed tuberculosis vaccine by attenuating an initially virulent *M. bovis*. The Calmette-Guerin bacilli (BCG) vaccine remains one of the world’s most widely used vaccines even today. Delivered after birth, BCG reduces the childhood tuberculosis by around 0 - 70%, but has little or no effect on the predominant adult pulmonary disease responsible for the current global emergency.

The BCG vaccine protects against severe forms of childhood tuberculosis, but unfortunately does not lead to eradication of disease and protective activity of the vaccine weakens during adolescence. With 3 million deaths from Tuberculosis every year, there is a pressing need for improved vaccines. Gene deletion is an important part of the attenuation process that led to the development of recombinant-BCG vaccine, with loss of a group of nine genes, referred to as region of deletion (RD-1).

The current BCG vaccine contains modified *M. bovis*, which lacks approximately 130 genes originally found in wild variety and reintroduction of selected genes was known to increase immunogenicity of the BCG vaccine without reverting it to a pathogen. New genes or antigens using bacillary sub-units or pure DNA can be added to vaccines or an entirely new vaccine can be made, with an ultimate aim is to
improve BCG or preferably to make a vaccine that can be given soon after birth and produce lifelong protection. The naked DNA construct could stimulate CD4+ & CD8+ cells with proven high immunogenicity in small-rodents.

The recombinant DNA vaccine used in the present study was developed in the Department of Microbiology and Cell Biology, Indian Institute of Science, Bangalore consisting of a gene fragment designated as Rv3881c from \textit{M. tuberculosis} coding for a 49 KDa protein. It was found to be a strong Th1 cell stimulator in guinea pigs and showed protective potential against challenge with virulent \textit{M. tuberculosis}. However, no such trials have been done in case of bovines. Because bovines act as source of infection to human beings, it is essential to know the immunogenic potential of this recombinant vaccine with respect to T-lymphocyte sub sets required for protection against the disease in bovines. This approach could help in identifying the importance of Rv3881c protein in eliciting immune responses that usually correlates with protection.

Keeping this in view, the present study was taken up to evaluate the immune response to recombinant DNA and recombinant BCG vaccine, expressing Rv3881c protein in comparison with the conventional BCG vaccine in cattle with the following objectives.
1. Epidemiological study of tuberculosis in some of the organized farms in and around Bangalore

2. Study of the quantitative response of IFN-γ by ELISA in immunized animals

3. Study of CD4 and CD8 T cells response by Flow cytometry in vaccinated animals

4. Study of the quantitative response of IL-4 in bovines to the vaccines by Capture cytokine ELISA