V. DISCUSSION

Tuberculosis is one of the most widespread infectious bacterial disease due to *Mycobacterium tuberculosis*, which is the leading cause of death due to a single infectious agent among adults in the world, but an unknown proportion of cases are due to *Mycobacterium bovis* and *M. africanum* has been prevalent since ancient times which is clinically indistinguishable (Buddle *et al.*, 2005 and Hein and Griebel, 2003). It was estimated that the proportion of human cases due to *M. bovis* accounted for 3.1% of all forms of tuberculosis and the epidemic of HIV infection in developing countries, particularly countries in which *M. bovis* infection is present in animals and the conditions favor zoonotic transmission, could make zoonotic tuberculosis a serious public health threat to persons at risk.

Of the total Asian cattle and buffalo populations, 94% of the cattle and more than 99% of the buffalo populations in Asia are either only partly controlled for bovine tuberculosis or not controlled at all. 94% of the human population lives in countries where cattle and buffaloes undergo no control or only limited control for bovine TB. Therefore, the global incidence of Tuberculosis is greatly underestimated. In 2005, 3.3 million cases were reported to the Global tuberculosis Programme of WHO, whereas a more likely number is 8.8 million. The total number of new cases will double by the year 2010, because of the HIV epidemic,
while demographic factors, such as population growth and changes in population structure, will largely account for the expected increase in TB incidence worldwide.

The disease has been reported to be existing since ages, even in Egyptian mummies. Although the causative organism has been identified by Robert Koch in 1882 itself, the disease is still a major problem. In the present situation all efforts are to be diverted in eradication of the disease, but in the absence of vaccines capable of stimulating strong CMI response, importance should be given for control measures. Disease control programmes based on regular tuberculin testing and removal of infected animals has been found to be successful in eradicating or markedly reducing bovine tuberculosis from cattle herds in many industrialized countries. These programmes are not affordable or acceptable in many parts of the world and it is particularly in these areas where bovine tuberculosis constitutes a public health risk. More than 94% of the world’s population lives in countries in which the control of bovine tuberculosis in cattle or buffaloes is limited or absent (Cousins and Roberts, 2001).

A vaccine against TB was developed by Albert Calmette and Camille Gu´erin in 1921, using a live attenuated strain of M. bovis, bacillus Calmette–Gu´erin (BCG). To date, some three billion people have been vaccinated with BCG worldwide. BCG’s efficacy is very variable,
ranging from 80% to 0% (Kaufmann, 2006 and Buddle et al., 2003b, c). Trails in cattle were initiated by Calmette and Gue’rin in the 1920s and followed up by many others (summarised by Francis, 1947; Skinner et al., 2003). Overall, BCG vaccination in cattle, induced some reduction in the severity of disease against experimental challenge, although it appeared to be ineffective in field trials as a vaccine against natural infection. One problem with the use of BCG in cattle is that vaccinated animals can react in the tuberculin skin test (Berggren, 1981). “Test and slaughter” control programmes introduced in many countries in the 1960s and 1970s achieved dramatic results and vaccination with BCG was not continued, principally as it was not compatible with these programmes.

India, with its population of over 1000 million, is estimated to account for nearly 30 per cent of the global tuberculosis burden. Tuberculosis continues to be a major health problem in India because of its high mortality and morbidity. This has prompted the search for new, improved tuberculosis vaccines. A heap of promising new approaches has been developed during the last two decades. Advances in gene and antigen identification, availability of genome sequences, a greater understanding of immune mechanisms possibly able to control mycobacterial disease, the development of adjuvants and delivery systems to stimulate T-cell immunity.
Over the past 10 years, a number of candidate vaccines have been tested in cattle with variable success. In recent years, only a few live attenuated mycobacterial vaccines other than BCG have been tested in cattle and two attenuated *M. bovis* strains induced protection against bovine tuberculosis in a situation when BCG vaccine was ineffective (Buddle *et al.*, 2002). DNA vaccines that have induced protection in small animal models have generally produced disappointing results when used alone in cattle (Wedlock *et al.*, 2003; Skinner *et al.*, 2003).

Major roadblocks to design of vaccines that protect against tuberculosis are the lack of surrogate markers of protection and lack of suitable animal model (Kaufmann, 2006 and Flynn, 2006). Understanding the mechanism involved in development of protective T-cell memory responses is an important component of tuberculosis vaccine enhancement efforts. Thus, there is currently a need to characterize T cell mediated immunity in additional animal model of tuberculosis. In the present study, we used the bovine model of tuberculosis to determine the ability of recombinant DNA and recombinant BCG expressing Rv3881c protein with conventional BCG to elicit cytolitic–effector T-cell response upon re-exposure to vaccine antigen *in vitro*. 
5.1 Single Intradermal test

In the present study, very low level of incidence was found using SID test in fifteen organized cattle herds in and around Bangalore. A total of 2668 animals from fifteen private and a government dairy farms were screened by SID test, of which 64 showed positive reaction and the overall percentage of incidence was 2.4% (Table.1). Overall incidence was ranging from 0 percent to 22 percent. Out of 1716 female animals screened, 56 showed (3.26%) positive reactions when compared to 3 animals (0.48%) out of 620 male animals screened (Table. 2). Irrespective of the sex, 5 animals (1.56%) below one year age group were positive out of 332 screened. Among different breeds (Table.1), out of 2106 Holstein crossbred animals screened, 59 were (2.81%) positive, whereas, out of 723 Jersey crossbred animals, only 5 were (0.69%) positive and out of 239 local breeds of animals namely Hallikar, Amrutmahal and others, none were (0%) positive. Thus, high prevalence of disease was noticed in HF crossbreds (Table.1 and 2).

The SID test is considered to be a reliable test in the cattle and therefore used as a prerequisite for the testing of disease-free buffaloes, but it has a lower specificity than the IFN-γ test. Two other problems associated with the intradermal tuberculin test, are the interval of testing and the cost factor. The animals have to be immobilized twice therefore making the test expensive and the test can only be done once every 3–4
months. The sensitivity of the intradermal tuberculin test using bovine PPD in calves however, compared well with that of the IFN-γ assay. More important however, is the fact that the IFN-γ assay can be run at very short intervals which is not possible with the intradermal tuberculin test.

Various workers have reported the incidence of tuberculosis in bovines based on tuberculin test in different parts of the country (Taneja, 1955; Basu et al., 1966; Lall et al., 1969; Guha and Sarkar 1970; Nagaraja et al., 1973; Kulshreshta et al., 1980; Habibi 1986; Prakash 1995 and Ashwathanarayana, 1997). Lall et al., 1969 reported that the incidence of tuberculosis in organized farms was 1.93% among 4249 cattle and 6.39% among 1292 buffaloes based on the results of tuberculin test. Guha and Sarkar 1970 studied 224 cattle including 45 clinically suspected cases in Calcutta and reported that 64(28.5%) animals had generalized tuberculosis at necropsy. In an organized cattle herd of 224 animals in Karnataka, a high incidence of 31.42% of tuberculin reactors was recorded by Nagaraja et al., 1973. Bovine PPD tuberculin was found considerably more specific (Francis et al., 1978) than the human PPD tuberculin in detecting tuberculous animals.

In the present study, the response to tuberculin skin test with B-PPD was lower in local cattle (B.tarus indicus), when compared to Jersy cross bred cattle and Holstein cross cattle. At the same time, the response to skin test is also lower or nil in males than to calves aged
below two years of age and females. The high reaction was noticed in milch animals than other animals. Similar results were also reported by Ashwatanarayan, 1997 and Isloor et al., 2008. A likely explanation could be that a higher proportion of Holstein cattle in India suffer from advanced disease. Since the test-and-slaughter-based control method is not applied in India, the disease could progress longer with a greater proportion of animals reaching a more severe disease status.

Similarly, Isloor, et al., 2008 reports 9% of incidence in the similar type of herds. It can be speculated that animals with dormant infections fail to respond to PPD stimulation or that repeated testing of animals with PPD increase the number of animals failing to respond (Thoen and Bloom, 1995). However, there is also the possibility of a false positive reaction, especially in the animal with a negative M. bovis culture result. Francis, et al., (1978) reported on the sensitivity and specificity of the various tuberculin tests using bovine PPD and other tuberculins. They concluded that the intradermal tuberculin test on the side of the neck delivered the highest possible sensitivity while the caudal tail fold test showed the highest possible specificity. However, they found the comparative intradermal tuberculin test to be less sensitive and specific and therefore suggested that only single tests should be used for surveys. A different observation applied to the cattle when injected with bovine and avian PPDs.
The comparative intradermal tuberculin test using bovine and avian PPD was more successful in detecting true bovine reactors than when bovine PPD was used alone (Bengis, Skukuza 2001). Due to the presence of other mycobacterial species in the environment, most cattle show some reaction to the intradermal PPD injections. It is therefore important to differentiate between true bovine reactors and animals that react to the avian tuberculin as well. However, the intradermal tuberculin test was still confusing at times due to false positive as well as false negative reactions. Menzies and Neill (2000) stated that *M. bovis* can only survive in the environment for a few weeks at most and that the mycobacterium is very rarely isolated from soil and pasture samples. Cattle-to-cattle transmission through naturally contaminated pasture also failed to cause disease. It can therefore be concluded that direct contact of an infected animal with a healthy animal is needed for the disease to be transmitted, hence the term nose-to-nose disease.

Taneja (1955) reported high incidence in Haryana cattle in Punjab and observed the age group of one to three years showing an incidence of less than eight percent, where as four years and older age group had an incidence of more than 45%. Nandy, (1958) found 17% positive reactors in Calcutta. Basu et al., (1966) reported high incidence of tuberculosis amoung milch cattle. Similarly, Ameni et al., (2006). Reported that, the level of IFN-γ and intradermal tuberculin test to the mycobacterial antigens (PPD-B) was significantly lower in Aris cattle, a Zebu *B.tarus*
indicus breed, than in Holstein cattle (B.tarus tarus) kept under the same husbandry conditions. The difference IFN-γ and tuberculin skin test in Zebu cattle maintained under identical conditions could be due to the different BoLA alleles in the two breeds affecting the recognition of mycobacterial antigens (Vordermeier. Et al., 2002).

In addition to breed, cattle husbandry was found to be an important factor affecting the intensity and distribution of the pathology of bovine TB, as well as the strength of the antigen specific skin reaction. As it was recorded in present study in few farms (private farm) where animals shown considerably higher reaction skin tests were found. The severity of bovine tuberculosis was significantly greater in cattle kept indoors at a higher population density than in those kept in a pasture. Similar findings were recorded by Ameni et al., (2006) and Radostitis. Et al., (1994). Housing predisposes cattle to tuberculosis, the closer animals are packed together, the greater the chance that tuberculosis will be transmitted. Apart from physical factors like close contact facilitating the transmission of ineffective aerosols between animals, it is also possible that stress caused by overcrowding or nutritional differences between housed and pastured animals contributed to the sever disease found in housed Holstein cattle.
5.2 IFN-γ assay

It is established that the dominant immune response to mycobacterial infections in cattle is cellular rather than humoral in nature (Thorns and Morris, 1983). Until recently there have been no practicable alternative cellular assays to the SI D test. Wood et al., (1990) described the interferon gamma (IFN-γ) assay, as a simple and rapid in vitro cellular assay for the diagnosis of bovine tuberculosis. The development of a sandwich enzyme immunoassay (EIA) for bovine IFN-γ (Rothel et al., 1990) has now made it possible to obtain result from the IFN-γ assay in 24 hrs.

In the present study, values of IFN-γ assay on day 120 and 150 was compared between control and recombinant DNA vaccinated group and found was highly significantly different (P < 0.001), whereas, difference in values of IFN-γ assay of control and rDNA vaccinated calves on 90 days was moderately significant (P < 0.01), and it was non-significant on 30 and 60 days (P > 0.05) of post vaccination.

Similarly, the difference between values of the IFN-γ assay from conventional BCG and recombinant-DNA vaccinated calves after day 30, 60 and 90 post vaccination was non-significant (P>0.05). But difference was highly significant when values of control and BCG vaccinated calves compared after 60th day (P<0.001) and it was moderately significant after day 30 post vaccination (P < 0.01), whereas, difference between Control
and rBCG vaccinated calves IFN-γ was highly significant (P<0.001) after day 30, but between conventional BCG and rBCG vaccinated calves, the difference was highly significant after day 60 and 90th day (P<0.001) and was significant after day 0 and 30.

Data from a number of studies suggest that IFN-γ is the major effector cytokine against mycobacterial infections: however, mechanisms of protective immunity are more complex, as IFNγ is also associated with disease progression (Vordermeier et al., 2002. Wedlock et al., 2003). In cattle, previous work has shown that the best association between IFN-γ response and protection occurs when vaccination induces a strong IFN-γ response to bovine PPD at 2 to 4 weeks (30 days) after vaccination, which is followed by a sharp peak of IFN-γ production for few week after vaccination and then declines as the antigen is eliminated or controlled (Wedlock. et al., 2000). This type of response pattern was detected in this work especially in animals vaccinated with the rBCG and conventional BCG vaccine where the IFN-γ response started from 30 days post vaccination, which become stronger after 60 days post vaccination and reached peak on 90 days post vaccination and to some extent reflected protection against bovine tuberculosis. Whereas, it was noticed on 120 and 150 days post vaccination in DNA vaccination study.

The rDNA vaccinated group and rBCG vaccinated group exhibited a strong IFN-γ response only after 120 days after booster and 60days
after primary vaccination respectively, but this response continued to increase and became the highest response seen in any of the groups. Therefore, a rapid increase in IFN-γ response after vaccination, followed by a subsequent decrease, may be crucial indicator of protection rather than the absolute amount of IFN-γ produced. In addition, IFN-γ producing effector cells may be short lived and in the absence of continued strong antigenic stimuli may not develop into long-term effector memory cells (Corner et al., 1990).

IFN-γ, a key cytokine in control of *Mycobacterial* infection is produced by both CD4+ and CD8+ T cells. IFN-γ might augment antigen presentation, leading to recruitment of CD4+ T-lymphocytes and/or cytotoxic T-lymphocytes, which might participate in mycobacterial killing. Although IFN-γ production alone is insufficient to control *Mycobacterial* infection, it is required for the protective response to this pathogen. IFN-γ is the major activator of macrophages and it causes macrophages to inhibit the growth of *Mycobacteria in vitro*. IL-4 could bring about *in vitro* killing of mycobacteria by macrophages either alone or in synergy with IFN-γ in the murine system. IFN-γ GKO mice are most susceptible to virulent *M. tuberculosis* (Cooper et al., 1993).

Wide spread use of tuberculosis vaccines in domestic animals depends on the development of diagnostic tests which can readily differentiate between vaccinated and non-vaccinated individuals. The
present work used a whole-blood IFN-γ assay to detect immune response to different mycobacterial vaccines such as recombinant DNA and r-BCG vaccines in comparison with conventional BCG vaccine. Since vaccination of cattle with recombinant DNA induced protection in this study only after 120 days post vaccination, a high immune response was recorded to selected mycobacterial antigens expressing rBCG vaccinated animals which subsequently maintained. The majority of the vaccinated calves produced moderate responses to recombinant protein (Rv3881c protein) in the IFN-γ assay but very weak response in the unstimulated blood samples and in unvaccinated animals. 150 days after vaccination with recombinant DNA vaccine, there were marked difference in the IFN-γ response to recombinant (Rv3881c protein) antigen in non vaccinated and vaccinated group. Similarly results were also found within 30 to 60 days in animals vaccinated with r-BCG and conventional BCG vaccine. This is in correlation with the findings of Bryce et al., 1999.

Bryce et al., (1999) reported that antigen-specific reactions can be viewed in experimental animals known to be vaccinated, all positive and suspect results for the IFN-γ assay were regarded as positive. All other reactions were regarded as negative results. The sensitivity and specificity of the IFN-γ assay used in the experimental model compared well to the recorded results of the IFN-γ when used in domestic cattle.
5.3 CD4 and CD8 T-cells response (Flowcytometry)

In the present study, the ratio of CD4 and CD8 cells in the 60 days post vaccination was same as earlier in the DNA group. In the subsequent 90 days post vaccination, the CD4 and CD8 cells were found ranging from 6 to 21% and 4.2 to 8.9%, 6 to 15.3% and 3.7 to 9.7%, and 11 to 19% and 2.5 to 9.8% in control group, DNA vector group and rDNA vaccine group respectively. On 120 and 150 days post vaccination, there was marginal increase in the CD8 cells in the rDNA vaccine group compared to the other two groups. Similarly, in BCG group, the cells were increased on the 30 days of post vaccination. This clearly indicates that the CMI response was started in the host. The immune response to tuberculosis is described as a ‘double-edged sword’, as it protects the host against disease, but also assists the pathogen by causing the tissue damage required for effective transmission. There is emerging evidence that different sub-classes of T helper cells may be involved in the protective and destructive responses. The decision as to which sub-class of T helper cells are called in to act in a particular individual, and to what extent, is perhaps dependent on the genetic profile of the individual rather than the nature of tubercle bacilli. Excessive elaboration and/or activity of IFN-γ may be responsible for necrosis and fibrosis.

Investigations into the sources of IFN-γ in bovine tuberculosis have shown that both CD4+ and CD8+ T cells are significant producers of this
cytokine (Liébana et al., 1999). The cell mediated immune response plays predominant roles in containing mycobacterial pathogens in human and bovine tuberculosis. Type-1 T-cell responses are thought to correlate with protection in human and bovine tuberculosis, and the balance of Type 1/Type 2 cytokines (IFN-γ, IL-4) in cattle is more similar to human profile (Welsh et al., 2005). Activated bovine CD4+ and CD8+ T cell subset also express homologues of perforin and granulysin, granule protein with important role in immunity to tuberculosis (Endsley et al., 2004). Immunology of tuberculosis is a complex subject. Depending upon the genetic make-up of animal and environmental factors, the entry of tubercle bacilli into body may lead to no response at all, protective immunity, excessive tissue damage and disease or different spectra of tuberculosis in between these. There is evidence that T cells are the main cells involved in CMI and macrophages are the executors of the bacilli.

Effector activities of CMI response include the production of cytokines and the cytolysis of infected cells. Cytotoxicity, as a general mechanism for pathogen control, can involve apoptosis of infected cells through Fas/Fasl interaction or lysis/apoptosis of infected cell resulting from release of cytotoxic granule protein (Flyn, 2006). In TB, a disassociation between lysis of infected cells and reduction of Mtb CFU by CD8 T cells indicates functional separation of receptor and granule lytic mechanisms (Canady et al., 2001). In the present study, the results of flowcytometry reveals that the CD4:CD8 ratio was maintained at 2:1
level throughout the study but the percentage of cells increased up to 29 and 25 respectively at 120 to 150 days after recombinant DNA vaccine administration and increased up to 32 and 25 respectively at 60 to 90 days after inoculation of recombinant BCG vaccine compared to other groups.

Vaccination strategy based on conventional BCG and recombinant BCG injection shown result in significant enhancement in all parameters. In comparison, vaccination with recombinant-DNA also enhanced all the parameters but level of response in all study parameter was lower than the earlier two vaccines. However, there were negligible difference between rDNA and conventional BCG groups.

Recent studies have shown that high level of cell-mediated immunity can be stimulated by consecutive use of DNA vaccines (Ramshaw and Ramsay, 2000). Sensitization of the herd to environmental mycobacteria may have contributed to the low level of protection observed with rDNA in this trial. It is possible that differences in the DNA vaccine, the type of BCG strain may explain the differences in the efficacy. The vaccine efficacy determined in mouse models of tuberculosis does not necessarily equate with that found in cattle (Skinner et al., 2003).
5.4 Cytokine Interleukin-4 capture ELISA

Th1 and Th2 cells operate in a reciprocal fashion, whereby cellular and humoral immune responses are mutually antagonistic. Th2 cells are thus associated with exacerbation and rapid lesion formation in several models of chronic infectious disease (Thoen and Bloom 1995).

In the present study, the results of the cytokine capture IL-4 ELISA test of BCG vaccine group revealed that initially OD reading was ranging between 0.019 to 0.056 and mean of all three sub group was around 0.040, but after injecting the vaccine, at 30 days, the mean OD values was increased to 0.064. Similarly at 60 and 90 days after vaccination, the mean reading was further increased to 0.074 and 0.085. On comparison with DNA group, in unvaccinated animal, the OD values were ranging between 0.024 to 0.031 with mean of 0.029 in rDNA calves, 0.018 to 0.041 in vector and control calves. But it was increased to 0.035 at 30 days, 0.047 at 60 days 0.074 at 90 days and 0.119 at 150 days post vaccination. This indicated that vaccine might have stimulated Th1 CMI response.

At 60 and 90 days post vaccination, the OD values were increased in the rBCG vaccinated animal group than other group. In DNA group, till the end of the 30 days after injecting rDNA vaccine, the OD value was below the 0.05, but only after 90 days post vaccination, it was increased to 0.074, which indicates that the vaccine might have stimulated
humoral response at later stage. But notably, the OD values of all the animals group were almost at the same level and the difference in the sample on 30, 60, 90 120 and 150th day from control, vector, BCG, rBCG and rDNA vaccinated calves was non-significant (P>0.05). In cattle, previous work has shown that a strong IFN-γ response to bovine PPD occurs after vaccination, which followed by a sharp peak of IFN-γ and then declines in few weeks after vaccination which is associated with increase in interleukin-4 cytokine as the antigen is controlled and processed (Wedlock et al., 2000). This indicates that, in the present study CMI response was stimulated and it was attained peak level at 90 days in BCG group and at 150 days in DNA group.

Although humoral antibodies are not considered of importance in protecting the host from virulent tubercle bacilli, certain monoclonal antibodies prolonged survival in M. bovis infected mice and were also associated with reduction in granuloma size (Glatman-Freedman, 2003). Th2 responses and IL-4 in tuberculosis are subjects of some controversy. In human studies, a depressed Th1 response, but not an enhanced Th2 response was observed in PBMC from TB patients (Lin et al., 1996; Robinson et al., 1994). Elevated IFN-γ expression was detected in granuloma within lymph nodes of patients with tuberculous lymphadenitis, but little IL-4 mRNA was detected (Lin et al., 1996). These results indicated that in humans a strong Th2 response is not associated with tuberculosis.
Data from mice studies (Cooper et al., 1993) suggest that the absence of a Th1 response to *M. tuberculosis* does not necessarily promote a Th2 response and an IFN-γ deficiency, rather than the presence of IL-4 or other Th2 cytokines, prevents control of infection. In a study of cytokine gene expression in the granuloma of patients with advanced tuberculosis by in situ hybridization, IL-4 was detected in 3 of 5 patients, but never in the absence of IFN-γ expression. The presence or absence of IL-4 did not correlate with improved clinical outcome or differences in granuloma stages or pathology. (Alamelu Raja, 2004).

It has been reported that PBMC from TB patients, when stimulated in vitro with PPD, release lower levels of IFN-γ and IL-2, as compared to tuberculin positive healthy subjects (Huygen et al., 1996). Other studies have also reported reduced IFN-γ increased IL-4 secretion (Sanchez et al., 1994) or increased number of IL-4 secreting cells (Suther et al., 1974). These studies concluded that patients with tuberculosis had a Th2-type response in their peripheral blood, whereas tuberculin positive patients had a Th1-type response (Alamelu, 2004).

Cell mediated immune response play predominant roles in containing mycobacterial pathogens in human and bovine tuberculosis. Type-1 T cell responses are thought to correlate with protection in human and bovine tuberculosis and the balance of Type-1/Type-2
cytokines (IFNγ/IL-4) in cattle is more similar to human profile (Welsh et al., 2005). Effector activities of CMI response include the production of cytokines and the cytolysis of infected cells. Cytotoxicity, as a general mechanism for pathogen control, can involve apoptosis of infected cells through Fas/Fasl interaction or lysis/apoptosis of infected cell resulting from release of cytotoxic granule protein (Flyn, 2006). Ameni et al., 2006 reports that, the level of IFN-g and intradermal tuberculin test to the mycobacterial antigens (PPD-B) was significantly lower in Aris cattle, a Zebu B. tarus indicus breed, than in Holstein cattle (B. tarus tarus) kept under the same husbandry conditions.

All the animals from the present vaccine study tested negative for FMD on the ELISA, indicating that serum antibody against the disease was not present in all the animals. As previously discussed, antibody production is regulated by IL-4 which is produced by the TH2 response. In the light of the fact that very low levels of circulating antibody against the bovine tuberculosis antigens and also against vaccines was present and that TH1 and TH2 responses operate in reciprocal fashion, similarly, in this study also, serum IL-4 against the mycobacterial recombinant antigen (Rv3881c protein) was not present in all the animals and their OD value was below the 0.1 in specific cytokine capture ELISA test, further. There was no difference of IL-4 levels in the all the animals groups was found. Similar finding was also observed by Lawler, 1996, that for successful vaccination of animals against bovine tuberculosis,
both cellular and humoral immune response is necessary rather than cellular alone.

5.5 CONCLUSION

The present study was undertaken to compare two types of Tuberculosis vaccines, namely recombinant-DNA with conventional-BCG, and recombinant-BCG vaccines in five groups of 8 calves each, aged approximately 3-6 months. They were injected intramuscularly with 1000ug of recombinant-DNA expressing Rv3881c protein or subcutaneously with 1x10^6 CFU of recombinant-BCG or conventional BCG vaccine. A control group received PBS via the same route and vector control group received plasmid PJW4303 used as vector for rDNA vaccine. The efficacy of a live r-BCG, BCG vaccine and recombinant DNA was tested in a group of calves from herds with known negative tuberculosis status.

In the present experiment, the cell mediated (Th-1) immune status was monitored using the IFN-γ assay, enumeration of CD4 and CD8 T-cells by Flowcytometry study and humoral (Th-2) immune response by Cytokine IL-4 capture ELISA. Laboratory IFN-γ assay tests were able to distinguish between infected and non-infected animals from an early stage. The immune responses of individual animals were found to be much higher in the recombinant-BCG than BCG vaccinated calves. Whereas, the immune responses in calves for recombinant DNA vaccine
was lower than BCG vaccine. The differences between immune responses to recombinant-BCG to other groups were significant and it can be concluded that under the prevailing conditions the recombinant-BCG vaccine was able to show higher immune status in calves against the *Bovine tuberculosis*. However, to assess the protective response of the vaccine, challenge studies has to be taken up in controlled conditions in homologous experimental animals.