1.0 INTRODUCTION

*Sarcocystis* an apicomplexan protozoan once regarded as a non-pathogenic parasite has been found associated with disease conditions in both animals and man. The economic loss has been estimated to be in terms of millions of dollars to the cattle industry due to the condemnation or downgrading of meat containing grossly visible sarcocysts (Fayer and Dubey, 1986). However, economic losses from clinical and subclinical infections are difficult to calculate because it is difficult to differentiate affected and non-affected animals. The losses due to poor feed efficiency, reduced growth, low milk yield, reproductive problems and clinical disease could be enormous. Diagnosis of Sarcocystosis in live animals is usually not attempted. Sarcocystosis is now recognized as an emerging protozoan disease in animals (Srivastava et al., 1977) and man (Juyal and Bhatia, 1989; Shah, 1995) and it has generated interest among veterinary protozoologists all over the world. *Sarcocystis hominis* although non-pathogenic to cattle as the intermediate host, it causes intestinal and muscular Sarcocystosis in man as the definitive host and is zoonotic in nature.

Sarcocystosis in cattle is a coccidian infection caused by three species viz., *Sarcocystis bovicanis* (*Sarcocystis cruzi*), *Sarcocystis bovifelis* (*Sarcocystis hirsuta*) and *Sarcocystis bovihominis* (*Sarcocystis hominis*) (Tenter, 1995). The developmental cycles of *Sarcocystis bovicanis*, *S.bovifelis* and *S.bovihominis* have canids, felids and man, respectively as their definitive hosts in which sexual reproduction and sporocyst formation occur in the small intestine. Cattle act as a intermediate host for all the three species. Generally, species transmissible through canids (*S.cruzi*) are
more pathogenic to cattle than those transmissible through other definitive hosts such as felids, man etc., (\textit{S.bovifelis} and \textit{S.hominis}).

The severity of clinical Sarcocystosis is dependent on dose of the sporocysts ingested by the cattle. The size or weight of the host appear to be not relevant to resistance or susceptibility of clinical disease. However, stress may play an important role in the severity of illness and the susceptibility to infection. Pregnancy, lactation, poor nutrition, weather or stress may influence the severity of clinical Sarcocystosis.

Cattle develop acute Sarcocystosis when 200,000 or more sporocysts are ingested at a given time. Four weeks after infection, cattle develop high temperature, anorexia, diarrhoea, weight loss, weakness, muscle twitching, infertility, abortion in pregnant animals, prostration and sometimes death.

Acute Sarcocystosis in calves affect growth rate as the disease has a negative impact on feed intake during the acute phase by decreasing somatotropin and increasing the somatomedin levels. Increase in cytokine (Cachectin) level causes cachexia and suppresses lipoproteinase activity resulting in elevation of triglycerides in plasma (Fayer and Elsasser, 1991). The infected animals show severe decrease in milk production and abortion in pregnant animals. Body weight was reduced in Sarcocystis infected cattle by 1.4 times and 37.0 per cent less milk production was observed when compared to uninfected cows (Fayer \textit{et al.}, 1983). In the acute phase, infected animals show an oligocythemic anemia, leukocytic shift to the left,
increased serum levels of creatinine, phosphokinase, lactic dehydrogenase and glutamic oxaloacetic transaminase.

Pathologic changes are most severe when schizonts are found throughout the body including vascular endothelium. The haemorrhages on the serous surface of viscera in cardiac and skeletal muscles are observed (Frontis piece). Sarcocystosis leads to infiltration with mononuclear cells, neutrophils, eosinophils, and giant cells (Dubey et al., 1989).

The infection is prevalent throughout the year and has high prevalence worldwide with almost 100 percent infection rates in adult cattle in North America (Fayer and Dubey, 1986; Fayer and Elsasser, 1991) and 62.0 per cent in slaughtered cattle in Bangalore, Karnataka state (Arun, 2005).

Diagnosis of acute Sarcocystosis is not possible since the clinical signs are non-specific and parasitemia during the acute phase of the disease may be brief or too low for easy detection (Fayer and Dubey, 1986). Sarcocystosis is an occult infection and cannot be diagnosed by using conventional parasitological techniques. Serodiagnostic techniques are used to detect specific antibodies or circulating antigens. However, concentration of native diagnostic antigens vary between the soluble antigen of different species employed in the serodiagnostic tests.
Different serological tests have been attempted for the diagnosis of Sarcocystosis such as Single Radial Immunodiffusion (SRID), Counter current Immunoelectrophoresis (CIEP), Enzyme Linked Immunosorbent Assay (ELISA) (Singh et al., 2004), Sodium do-decyl sulphate polyacrylamide Gel Electrophoresis (SDS-PAGE) and Western Blotting (Granstrom et al., 1990). Gel electrophoresis has proved to be of considerable value in differentiating parasitic strains and elucidating the nature of the soluble proteins present in parasites (Atkinson and Collins, 1981; Bullini, 1984).

Morphology remains practical, inexpensive and the mainstay for accurate identification of many parasites but in the case of Sarcocystis species, the morphological identification is said to be unreliable (Obendorf and Munday, 1987). Further the morphological features of the species may vary during cyst development with the type, location and degenerative state of the host cell parasitized as well as with the methods used for examination or fixation (Dubey et al., 1989).

Similarly, high cross-reactivity among different Sarcocystis species infecting the same intermediate host has hampered the development of species specific immunological methods for detection of Sarcocystis species. Currently it is not possible to differentiate between pathogenic and non-pathogenic Sarcocystis species in intermediate hosts. Further, the usefulness of serological tests for the diagnosis of Sarcocystis infections has
been limited by the high antigenic cross-reactivity among different Sarcocystis species.

Antigens have been derived from cystozoites / merozoites of Sarcocysts and these antigenic preparations are frequently contaminated with debris of host tissue. The Polymerase chain reaction (PCR) based assays using ssrRNA/DNA have been standardized for specific identification and rapid diagnosis of infections with Sarcocystis thereby overcoming the limitations of morphological studies and serodiagnostic tests (Tenter, 1995).

The comparison of ssrRNA gene sequences and the advent of DNA based diagnostic assay have facilitated rapid, accurate identification of micro and macro sarcocyst using species-specific nucleotide sequences (Tenter, 1995). The use of RAPD technique to amplify short regions of genome of an organisms without prior sequence information has great potential in identifying genetic markers, tagging genes and performing population studies and as a valuable source of species-specific DNA fragments which may be used as diagnostic probes (Welsh and McClelland, 1990).

DNA probes have been developed from unique gene or intergenic sequences for differentiation of pathogenic species from non-pathogenic Sarcocystis species in live animals. The RAPD-PCR is becoming a valuable
tool for rapidly differentiating closely related species and strains, for genetic mapping and for generating PCR fingerprints.

Although research work had been focussed on S. bovicanis the overall study of the immunology and diagnosis of S. bovifelis has not been attempted. Therefore, a comparative study on Sarcocystis species in cattle was undertaken with the following objectives:

* To study the protein profile of soluble extract of macro and micro sarcocysts of cattle by Sodium do-decyl sulphate Polyacrylamide gel electrophoresis (SDS-PAGE).

* To identify the immunodominant peptides in soluble extracts by Enzyme Immuno Transfer Blot (EITB).

* To standardize Enzyme Linked Immunosorbent Assay (ELISA) and Enzyme Immuno Transfer Blot (EITB) using soluble extracts of sarcocysts for serodiagnosis of Sarcocystosis in cattle.

* To determine genotypic variation of Sarcocystis species by Random Amplified Polymorphic DNA- Polymerase Chain Reaction (RAPD-PCR).

2.0 REVIEW OF LITERATURE

The literature pertaining to the immunodiagnosis and molecular diagnosis of Sarcocystosis in cattle with special reference to Enzyme Linked