The Primer 3 amplified two fragments of 0.8 and 0.5 kb were \textit{S. bovicanis} (Plate-32). In the case of \textit{S. bovifelis} two fragments of size 1.0 and 1.1 kb were obtained. There were no DNA fragments present in the host DNA.

In the present study, \textit{S. bovicanis} DNA produced only single fragment of size 0.5 kb whereas \textit{S. bovifelis} DNA amplified 0.7, 0.9 and 1.0 kb (Plate-33) for primer 4. In host DNA none of the fragments were visible. The similarity co-efficient between the two species by RAPD-PCR was found to 0.375(Table-16).

5.0 DISCUSSION

Sarcocystosis an occult protozoan disease is now recognized as an emerging coccidian disease in livestock particulary in cattle (Shah, 1983). The \textit{Sarcocystis} species in cattle are known to produce morbidity and mortality in naturally occurring and experimentally induced infections.
The clinical signs in acute bovine Sarcocystosis include anorexia, poor growth, decreased milk production, abortion, cachexia and sometimes death (Dubey et al., 1989). The chronic infection is characterized by downgrading or condemnation of infected carcasses. The *Sarcocystis* species transmitted through canids produces sporocysts that are disproportionately much larger and hence increases the infection potential of the definitive host to the intermediate host to a great extent. The sporocysts can remain viable in the environment up to one year under varying conditions of temperature and moisture (Shah, 1983). Sarcocystosis is prevalent throughout the year and prevalence rate may reach up to 100 per cent. Many workers in India have reported Sarcocystosis in cattle (Srivastava et al., 1977; Juyal et al., 1981; Jain and Shah, 1988; Pandit et al., 1994; Mohanty et al., 1995; Arun, 2005) and have recorded its prevalence throughout the year.

Diagnosis of Sarcocystosis in live animals is difficult as clinical signs are non-specific and characteristic lesions are absent in infected animals (Dubey et al., 1989). However, the diagnosis of infection and specific identification of species is essential to reduce the dissemination and transmission, zoonotic impact and economic losses in terms of decreased growth rate, abortion, condemnation of infected carcasses etc.

Comprehensive surveys on infections by *Sarcocystis* species are limited to a large extent by the time and effort required to diagnose infections. Conventional methods viz., squash preparations, histological
techniques etc., are time consuming, labour intensive and fails to detect light infections (Lunde and Fayer, 1977; Pandit et al., 1993). The tissue digestion method was found to be more sensitive and rapid, it was found to result in considerable cellular debris that hindered detection of *Sarcocystis* bradyzoites (Collins et al., 1980). Therefore such techniques are not suitable for use in large-scale programmes nor for use in detecting infection in live animals.

Alternative methods of diagnosis of infections therefore been explored and several immunological tests have been tried for the detection of specific antibodies against *Sarcocystis* species in experimental and naturally infected animals. (Weiland et al., 1982). Various immunological tests have been attempted such as Indirect fluorescent antibody technique and double immunodiffusion (Tadros et al., 1974), indirect haemagglutination and gel diffusion test (Lunde and Fayer, 1977), single radial immunodiffusion (Juyal et al., 1990), Immunoelectrophoresis, counter current immunoelectrophoresis, double immunodiffusion, radial immunoelectrophoresis (Pandit et al., 1993), Dot- ELISA (Tenter, 1988; Singh et al., 2004) and enzyme linked immunosorbent assay (Gasbarre et al., 1984; O’Donghue and Weyerter, 1983; Shi and Zhao, 1987; Savini et al., 1997; Arun, 2005). Though these tests are found to be simple and sensitive, the specificity and sensitivity of the tests varies significantly depending on the type of antigen used, type of test employed and cross
reactivity within the *Sarcocystis* species (Gasbarre *et al.*, 1984; Pandit *et al.*, 1993).

Based on the previous observations and limitations the present study was undertaken to critically evaluate the accuracy, sensitivity and specificity of ELISA with partially purified soluble antigen in diagnosing infections in naturally infected animals.

### 5.1 Immunodiagnosis of Sarcocystosis in cattle

#### 5.1.1 Antigens


Among these antigens, previous workers have reported that partially purified cystozoite soluble extract is more commonly used because of ease in its preparation and abundance in its availability (Pandit *et al.*, 1993). In addition, with purified soluble antigen the cross-reactivity and false positive cases may be reduced between the species. However, merozoite antigens have not been utilized for serodiagnostic purposes due to difficulty in
obtaining merozoites in sufficient quantity from early developmental stages (Pandit et al., 1993). The acute stage will last for few days, hence merozoite antigen fails to detect the antibodies against Sarcocystis in chronic infections (Smith and Herbet, 1986; Granstrom et al., 1990). Mertens et al. (1996) employed recombinant antigens which overcome the high levels of cross reactivity among different Sarcocystis species and found to be costly, time consuming and appeared undesirable as single peptide is included for the diagnosis. The cystozoite soluble extract from both macrosarcocyst and microsarcocyst was employed in serodiagnosis of Sarcocystosis in animals in the present study.

The protein concentration of the S.bovicantis and S.bovifelis was found to be 550 and 920 μg per ml, respectively during this study. However, Lunde and Fayer (1977) obtained the protein concentration of 8mg/ml who had employed pepsin digestion followed by purification with Hank’s balanced salt solution. Shi and Zhao (1987) had obtained protein concentration of 897 μg per ml in soluble antigen of macrosarcocyst of Sarcocystis which is in agreement with the present findings. However, Pandit et al. (1993) had obtained a low protein concentration of 260 and 232 μg per ml in one batch and slightly higher concentration of 920 μg and 660 μg per ml in second batch for S.cruzi and S.hirsuta soluble antigen respectively. In the above study pepsin digestion followed by Percoll purification was used. In this method in the digestion process with pepsin resulted in considerable cellular debris (Collins et al., 1980). A large volume
of Percoll was required for separation and it proved to be uneconomical. The total brazdyzoites may not be procured from the above method.

In the present study, discontinuous Percoll gradient method was employed for purification of bradyzoites which clearly separated the host tissue. During the extraction method, the stirring of infected finely diced meat along with PBS and glass beads released the microcysts easily from the muscle fibres. Hence, by this method it was possible to obtain more than 95 per cent of the bradyzoites and it contained very less amount of host tissue which was subsequently eliminated by Percoll gradient method (Tenter, 1988). However, the wide range in the protein concentration could have been due to differences in the method of extraction of cyst, antigen preparation and methodology used for the estimation of protein concentration. Further, the use of preservatives and protease inhibitors added viz., PMSF are known to greatly influence the protein concentration of the antigens.

5.1.2 Detection of anti-Sarcocystis antibodies by ELISA

Munday (1975) and Shukla and Victor (1976) showed that complement fixation test was more confirmatory for the serodiagnosis of Sarcocystosis in bovines than other tests like staining, post mortem examination and histopathological studies. Indirect haemagglutination test was found to more sensitive than agar gel diffusion in detection of
antibodies using zoite soluble antigen (Lunde and Fayer, 1977). In contrast, O’Donoghue and Ford (1986) reported that Complement fixation test and Indirect fluorescent test cannot be used for detection of serum antibodies against *Sarcocystis* species in sheep because most of the species cross-react as these are genus specific but not species specific. However, Pandit *et al.* (1993) found Counter immunoelectrophoresis (CIEP) to be more sensitive than SRID, DID, IEP, precipitation tests for diagnosis of field cases in bovine Sarcocystosis.

Gasbarre *et al.* (1984) indicated that detection of *Sarcocystis* specific IgG antibodies in cattle are more specific that IgM antibodies as it last for few days where as IgG response persists for longer periods. In addition, ELISA is sensitive to competitive binding by more abundant IgG classes. Similarly, Smith and Herbert (1986) concluded that IgG ELISA is more sensitive and specific for the detection of specific anti-*Sarcocystis* antibodies. It was observed that use of density gradient purified *Sarcocystis* antigen is more sensitive and specific in antibody detection with less cross reaction between the *Sarcocystis* species.

In the present study out of 300 serum samples examined for anti-*Sarcocystis* antibodies, 146(48.66%) and 46(15.33%) were found to be positive for *S.bovicans* and *S.bovifelis*, respectively by ELISA. In the study of Klima (1979) antibody titres >1/32 in 86.0 per cent of 100 cattle were found and 96 per cent were found to be infected by a trypsin digestion technique. Shi and Zhao (1987) reported the seroprevalence of *Sarcocystis*
species in cattle to be 79.25 per cent by ELISA, 64.78 and 77.36 per cent by macro and microscopic examination of infected organs and microscopic examination of digest material, respectively.

The cross reactions between the *Sarcocystis* species has been reported by several authors (O'Donoghue and Weyreter, 1983; Shi and Yuan, 1987; Tenter, 1988; Savini *et al*., 1994; Arun, 2005). However, no cross reactivity between *S. tenella* and *S. arieticanis* was observed by Mertens *et al.* (1996) who employed recombinant STC29/GST fusion protein in detection of *S. tenella*.

In the present study crossreaction was observed between cattle and goat species as well as within the *Sarcocystis* species of cattle. The crossreaction was found between *S. bovicanis* and *S. bovifelis*. Further, *S. bovifelis* showed crossreaction with *S. capracanis* of goat where as *S. bovicanis* did not cross react. This could be possibly due sharing of common epitopes/antigenic determinants between the closely related *Sarcocystis* species. In addition, the cystozoite antigen used was only partially purified.

In the present study, the sensitivity and specificity of ELISA was found to be 76.4 and 66.6 per cent for *S. bovicanis* and 81.8 and 71.4 per cent for *S. bovifelis* respectively. The false positive reaction was observed in eleven cattle in which the micro and macro sarcocyst of *S. bovicanis* and *S. bovifelis* were not found, respectively. On the other hand false negative
results were found against *S.bovicanis* and *S.bovifelis* in four and seven infected cattle. The sensitivity and specificity of the ELISA in the present study was found to be lower which might have been due to more number of false positive and false negative cases. There was a difference in the cut off values of 0.213 and 0.221 for *S.bovicanis* and *S.bovifelis* contrary to 0.24 reported by Shi and Zhao (1987). However, Tenter (1988) had found a higher specificity and sensitivity. Similarly, the higher specificity and positive predictive values of 100 per cent were reported by Tenter *et al.* (1992) for detection of *T.gondii* antibodies using recombinant antigens compared to present study where soluble extract was used. This may be attributed to the differences in antigen preparation and type of antigen used. Arun (2005) recorded slightly higher sensitivity in detection of *S.capracanis* compared to the present findings.

This is the first report where the comparison was made in detection of *S.bovicanis* and *S.bovifelis* antibodies by ELISA. The present findings showed that the seroprevalence of *S.bovicanis* is high compared to *S.bovifelis* infection in cattle.

### 5.2 Protein profile of *S.bovicanis* and *S.bovifelis*

Bovine Sarcocystosis is considered to be an important protozoan disease but the antigenic profile of the various *Sarcocystis* species is not properly understood and there is scanty information on the antigenic nature of *Sarcocystis* species in cattle (Dubey *et al.*, 1989). However,
attempts were made to know the antigenic composition of different stages of *S.bovicanis* (Burgess *et al*., 1988; Dubey *et al*., 1989). There is no report on the antigenic nature of *S.bovifelis*. Therefore, Sodium do-decyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was employed to study the protein profile.

During this study, the SDS-PAGE analysis of *S.bovicanis* soluble antigen revealed a total of 10 polypeptides ranging from 170 kDa to 12 kDa. Whereas in *S.bovifelis* a molecular weight range from 100 kDa to 15 kDa was detected. In host tissue antigen a total of nine polypeptides were noticeable ranging from 80 kDa to 11kDa. Three peptides were found common between *S.bovicanis* and *S.bovifelis*, only one peptide between *S.bovifelis* and host antigen. None of the peptides were found common between *S.bovicanis* and host antigen.

However, the smaller molecular weight polypeptides (10,000-18,000 dalton) were detected by Abbas and Powell (1983) with surface antigens of *S.muris*. The samples containing some weakly stained high molecular weight polypeptides were also reported. Whereas Speer *et al*. (1986) reported similar profile of polypeptides ranging from 15 to 215 kDa from merozoite antigen of *S.cruzi*. Similarly, Speer and Burgess (1988) reported a number of proteins ranging from 15-215 kDa from *invitro* cultured merozoite antigen. Fractionation of proteins in *S.fusiformis* of buffalo by Khulbe *et al*. (1989) also revealed various fractions.
A large number of peptides ranging from 18.5 to 200 kDa was reported by Granstrom et al. (1990) who used *S.cruzi* bradyzoite extracts. Twelve bands were detected in the newborn calf extract in comparison with *S.cruzi* extracts. These findings were in agreement with the present study. However, a total of 61 distinct bands identified in the above study could be possibly due to differences in the antigen preparation, the type or stage of antigen used and the staining method employed. More number of polypeptides were found in silver nitrate staining which is said to be more sensitive as per Laemmlli (1970) when compared with Coomassie blue staining.

In the present study, the presence of three common bands between *S.bovicanis* and *S.bovifelis* indicated that both the *Sarcocystis* species are closely related. The differences in the banding patterns compared to previous reports could be due to type of antigen used. The percentage of resolving gel may also influence the separation of polypeptides based on molecular size.

This is the first report on protein profile of soluble extracts of *S.bovifelis* in comparison with *S.bovicanis* and host tissue antigen.

### 5.3 Enzyme Immuno Transfer Blot (EITB)

The usefulness of serological tests in the diagnosis of *Sarcocystis* infections has been limited by the high antigenic cross reactivity among different *Sarcocystis* species. A strategy to overcome the limitations of
immunological tests is based on the identification of species specific immunoreactive peptides (Tenter, 1995). Some studies on the electrophoretic and chromatographic analyses of protein extracts derived from cystozoites of *S. tenella*, *S. arieticanis* or *S. gigantea* and from merozoites of *S. neurona* indicated that species-specific proteins and antigens exist in these species (Tenter et al., 1989; Tenter et al., 1991, Granstrom et al., 1993). Hence EITB was carried out to identify the species specific immunoreactive polypeptides in *S. bovicanis* and *S. bovifelis*.

The immunoreactive polypeptides detected on western blots in the present study in *S. bovicanis* when probed with homologous hyperimmune serum raised in calves included those of 100, 82, 52, 36, 15 and 12 kDa. Only two polypeptides were identified when probed with heterologous hyperimmune serum raised against *S. bovifelis*.

However when *S. bovifelis* blots were treated with homologous hyperimmune serum, a total of eight immunoreactive peptides were identified. Three polypeptides were detected when probed with heterologous hyperimmune serum of *S. bovicanis*. Similarly, polyclonal antisera derived from animals that have been infected experimentally with the species of *Sarcocystis* had revealed a high cross reaction with antigens of *Toxoplasma gondii* (Uggla and Buxton, 1990) which is concurrent with the present study. Host tissue blots showed no immunoreactive peptides against HIS of *S. bovicanis* and *S. bovifelis*. 
Similar to the above findings, Speer and Burgess (1988) reported polypeptides ranging from 20-74 kDa with *S. cruzi* blots exposed to surface reactive monoclonal antibodies. In contrast, Burgess *et al.* (1988) detected only single 45 and 500 molecular weight bands in bradyzoites probed against anti-merozoite monoclonal antibodies (mAbs) and non-surface reactive anti-merozoite mAb’s. However, six bands reacted in sporozoites with the anti-merozoite mAbs. This study revealed that the different stages of *Sarcocystis* such as bradyzoites, sporozoites and merozoites bear similar epitopes.

Similar to the above findings, in the present study a total of five peptides were found to be reactive when *S. bovicanis* and *S. bovifelis* blots probed with heterologous HIS. This cross-reactivity could be possibly due to sharing of antigenic determinants and also the use of polyclonal antisera during this study.

More number of proteins were detected on *S. cruzi* western blots ranging from 10.5 to 240 kDa by Granstrom *et al.* (1990) who used cattle sera obtained from natural infection. Out of 39 distinct bands a total of twenty protein bands were detected consistently with antibovine IgG conjugate and cattle sera. Whereas 6 of 16 bands were recognized on blots using cattle serum, monoclonal anti-bovine IgE and anti-mouse conjugate. The significant variation in the above study could be attributed to use of specific monoclonal antibodies. Therefore, the species specific diagnosis of *Sarcocystis* stages can only be made when specific monoclonal antibodies
are used. However, the repertoire of monoclonal antibodies that are directed against *Sarcocystis* species of livestock is limited (Tenter, 1995). Species specific monoclonal antibodies have so far been developed against *S. tenella* and *S. gigantea* (O’Donoghue and Rommel, 1992).

This is the first study wherein the immunoreactive peptides were detected in *S. bovicanis* and *S. bovifelis* using partially purified cystozoite soluble antigen extract and polyclonal antisera.

**5.4 Serodiagnosis of Sarcocystosis in cattle by EITB**

The serodiagnosis of Sarcocystosis by ELISA by Tenter (1988), Savini et al. (1994) and Shi and Yaun (1987) had indicated cross reactivity with the *Sarcocystis* species of different host species and with in the *Sarcocystis* species of cattle. Western blotting enabled the identification of all the positive and negative samples (Cook et al., 2001). Therefore immunotransfer blot was employed to detect *Sarcocystis* species specific antibodies.

Out of 300 blood samples examined for *S. bovicanis* and *S. bovifelis* by EITB, specific antibodies were detected in 175 (58.3%) and 89 (29.6%) serum samples in the present study. In the field samples, 170 and 36 kDa peptides were detected in *S. bovicanis* positive samples whereas 76 and 38
kDa was detected in *S. bovifelis*. During this study the sensitivity and specificity of 87.5 and 81.8 per cent was observed for *S. bovicanis*. However, higher sensitivity (93.5%) and specificity (87.5%) was recorded for *S. bovifelis*.

The differences in this study could be possibly due to the type of cyst material used. In the case of *S. bovicanis*, microcysts were used for antigen preparation where as macrocysts were used for *S. bovifelis*. However, Saville *et al.* (1997) recorded an overall seroprevalence of 53.6 per cent for *S. neurona* in horses by identifying the 10.5, 13 and 22.4 kDa on western blots. Dubey *et al.* (1999) determined antibodies against *S. neurona* and *T. gondii* in 36 and 16 of 101 horses, respectively by immunoblots. Antibodies against *Neospora caninum* were not found in serum of any of the 101 horses analysed by immunoblots. Western blot was employed by Cook *et al.* (2001) for the detection of *S. neurona* antibodies in the serum of young horses.

There are no previous reports on serodiagnosis of *S. bovicanis* and *S. bovifelis* by EITB in cattle. This appeared to be the first attempt on serodiagnosis of bovine Sarcocystosis by EITB. The results indicated that EITB can be effectively used for detection of anti-*Sarcocystis* species antibodies in field serum samples. EITB can be employed for species specific antibodies detection in live animals. This in turn could curtail negative effects on growth rate, abortion, downgrading of infected carcasses etc., thereby increasing the health status and productivity of cattle.
5.5 Comparison of ELISA and EITB

The sensitivity and specificity for serodiagnosis of *S. bovicanis* by ELISA was found to be 76.4 and 66.6 per cent, respectively. A higher sensitivity and specificity was recorded for *S. bovifelis*. However for *S. bovicanis* and *S. bovifelis* the sensitivity and specificity by EITB was found to be 87.5 and 93.5 per cent and 81.8 and 87.5 per cent, respectively.

In the present study, the anti-*Sarcocystis bovicanis* and *S. bovifelis* antibodies were detected in 146 and 46 serum samples by ELISA respectively. In comparison to ELISA, 175 and 89 serum samples showed positive reaction for *S. bovicanis* and *S. bovifelis* specific antibodies by EITB. This shows that EITB is more sensitive and specific test for serodiagnosis of bovine Sarcocystosis.

However, Tenter (1988) had showed specificity of 94 per cent and a very high sensitivity by ELISA. Similarly, the higher specificity and positive predictive values of 100 per cent were reported by Tenter *et al.* (1992) for detection of *T. gondii* antibodies using recombinant antigens compared to present study where soluble extract was used. Arun (2005) recorded slightly higher sensitivity in detection of *S. capracanis* compared to the present findings in goats.

The sensitivity and specificity in the present study was found to be higher with macrocyst soluble antigen compared to microcyst soluble extract. These differences could be attributed to the different methods
employed in the preparation of antigen. Tissue homogenization of macrocyst was carried out in contrast to freezing and thawing for microcyst as the microcysts were small in size and difficult to homogenize. This may result in exposure of very small number of antigenic determinants thereby reducing the immune response in raising the HIS.

5.6 Differentiation of *Sarcocystis* species by Random amplified polymorphic DNA (RAPD-PCR)

The accurate identification and diagnosis of *Sarcocystis* species by conventional techniques is time consuming, labour intensive and is difficult to diagnose in live animals. Although *Sarcocystis* species are known to be very specific to definitive host, there are reports to indicate that different *Sarcocystis* species may infect the same host in experimentally induced infections (Long and Joyner, 1984). Hence, the host transmission assays fail to establish host specificity and cannot be relied completely (MacPherson and Gajadhar, 1993). The currently employed *Sarcocystis* specific serological tests could not differentiate between infections with pathogenic species and those with mild or non-pathogenic species because of cross reactivity (Tenter, 1995). These tests failed to overcome the cross-reactivity between the species or closely related genus. Since microscopic or macroscopic examination of *Sarcocystis* is possible only at post-mortem
and serological tests are not species-specific, a diagnostic probe for the detection and differentiation of pathogenic *Sarcocystis* species was considered essential.

The phylogenetic analysis of *Sarcocystis* species of sheep, goats, cattle and mice has been performed using rRNA sequences (Tenter et al., 1992). Similarly, Fenger et al. (1994) differentiated *S.neurona* from other members of family Sarcocystidae using small sub unit (SSU) rRNA. The synthetic oligonucleotides derived from species-specific SSU rRNA sequences of *S.muris*, *S.cruzi* and *S.tenella* were used for detection of these parasites in RNA hybridization assays (Gajadhar et al., 1992; Holmdahl et al., 1993). Though ribosomal RNA molecules are the most abundant macromolecules in cellular organisms. Some authors believed that diagnostic assays targeting naturally abundant SSU rRNA would be much more sensitive and accurate than assays targeting the SSU rRNA gene (Waters and McCutchan, 1990; Gajadhar et al., 1992).

However, the differentiation of *Sarcocystis* species under natural conditions requires a test system that is applicable to the examination of a broad range of different species. Consequently SSU rRNA is not a stable target and is degraded by enzymatic digestion. In addition, standardization of RNA hybridization assays is difficult and not suitable for epidemiological studies (Gajadhar et al., 1992; MacPherson and Gajadhar, 1993). These studies involved the use of radioactively labelled oligonucleotide probes to obtain high sensitivities (Gajadhar et al., 1992; Holmdahl et al., 1993).
which restricted the use of these tests to a limited number of specialized laboratories. Therefore, it was found to be impractical for many diagnostic applications and prolonged epidemiological studies.

Consequently, a different rationale was used to develop a PCR based diagnostic test for species-specific detection and differentiation of the *Sarcocystis* species (Frederick *et al.*, 1991; Joachim *et al.*, 1996; Ndiritu *et al.*, 1996). A series of species specific oligonucleotide was generated that can be used as primers for specific amplification of SSU rRNA gene fragments from genomic DNA templates of the homologous parasite species by PCR.

Later, the identification of hypervariable regions in the 18s rDNA of *Sarcocystis* species made possible the development of species–specific and genus specific DNA probes (Holmdahl *et al.*, 1993; Ellis *et al.*, 1995; Jeffries *et al.*, 1997). However the PCR methods required short DNA sequences that could be identified quickly and should be either unique for a single organism or polymorphic allowing species identification based upon a DNA electrophoretic mobility pattern (MacPherson and Gajadhar, 1994). The major disadvantage with the PCR is the requirement of nucleotide sequence information from which primers are designed. Hence Welsh and McClelland (1990) and Williams *et al.* (1990) independently introduced a novel PCR assay which did not require nucleotide sequence information from which to design primers. Therefore, to overcome the cross reaction and to clearly
differentiate the pathogenic and non-pathogenic *Sarcocystis* species of cattle RAPD-PCR assay was performed during this study.

In the present study, all the four primers directed the synthesis of more than one fragment and each primer revealed unique DNA banding patterns for both *S. bovicanis* and *S. bovifelis*. RAPD-PCR in this study revealed a clear cut difference in the fragment pattern between *S. bovicanis* and *S. bovifelis*. In the case of host DNA none of the bands/fragments could be detected. This indicated that the all the four primers used did not directed the synthesis of host DNA fragments.

Similar findings were reported by MacPherson and Gajadhar (1994) who differentiated *S. cruzi* DNA combined with bovine DNA using RAPD-PCR. Five primers of various length and sequences were used and multiple DNA fragments ranging from 2.2 to 0.5 kb were found. An intense band with an electrophoretic mobility corresponding to approximately 0.8 kb was shown to be specific for *S. cruzi* and was absent in *T. gondii* or *S. campestris*. Guclu *et al.* (2004) used RAPD technique to amplify short regions of cattle Sarcocystis species and observed separate clusters for *S. bovicanis*, *S. bovihominis* and *S. bovifelis*. They observed only one fragment of 0.3 kb for OSA-04 primer in *S. bovihominis*, where as OSA-06 showed amplification of 0.3 and 0.25kb fragment for *S. bovihominis* and *S. bovifelis*, respectively. In primers OSA-06, OSA-07 and OSA-08 more than two DNA fragments were shown be amplified for *S. bovicanis*, *S. bovifelis* and *S. bovihominis*. 
However, analysis of seven *Eimeria* species by RAPD showed that DNA fragments ranging from 200 to 2200 bp were synthesized in the different reactions (MacPherson and Gajadhar, 1993) and the percent GC content of primers and the number of fragments amplified were correlated. Granstrom *et al.* (1994) identified a 550 bp DNA fragment unique to *S.neurona* that was not found in *S.cruzi, T.gondii*, three *Eimeria* species and bovine DNA. In the study no cross reactivity between the isolate and other coccidian species by hybridization could be seen. A RAPD-PCR derived marker was shown to be useful in differentiation of pathogenic and non-pathogenic *Sarcocystis* species of sheep where a fragment of about 1280 bp from genomic DNA of *S.tenella* was synthesized but not from genomic DNA of *S.gigantea* or *T.gondii* (Joachim *et al.*, 1996). However, Marsh *et al.* (1996) distinguished *S.neurona* from other closely related protozoal parasites using *S.neurona* specific DNA sequences in the nuclear small subunit ribosomal RNA (nss-rRNA).

In RAPD assays unique DNA banding patterns were observed that could be exploited to identify different species (MacPerson and Gajadhar, 1993). In general, those primers that had the highest GC content were responsible for multiple DNA fragments after PCR. During this study, the primer B with 61 per cent GC and 18 nucleotides in length produced six DNA fragments. In contrast, the primer A with 20 nucleotides (45% GC) also amplified six fragments. However primers that were 16 and 22 nucleotides in length (C and D respectively) with more than 50 per cent GC
produced fewest DNA fragments. The above finding was in concurrence with MacPherson and Gajadhar (1993) and Guclu et al. (2004) who recorded similar results with seven *Eimeria* species and *Sarcocystis* of cattle, respectively.

The fingerprints generated in this study showed variation in the band intensity, with each primer atleast one DNA fragment was amplified more efficiently than others in the same reaction. Similarly, MacPherson and Gajadhar (1993) observed variation in the intensity of DNA fragments produced. The more intense bands could be probably due to priming within repeated regions of the *Sarcocystis* genome which would result in more copies being produced during PCR. The shorter primers consistenly produced DNA fragment patterns containing at least one DNA fragment. This suggested that shorter oligonucleotides might generate more characteristic DNA fingerprints.

In addition the two primers primer C and primer D which were newly designed resulted in amplification of *S.bovicanis* and *S.bovifelis* genomic DNA. Hence, these results suggest that the primers used in the present study can be used as diagnostic probes for *S.bovicanis* and *S.bovifelis*. The RAPD-PCR assay of this study may be particularly useful to study the taxonomy and epidemiology of members of the family Sarcocystidae. Instead of relying on ultrastructural or transmission studies for the identification of tissue coccidia in cattle, investigators may use DNA assays to accurately determine the presence of *Sarcocystis* species in cattle.