6.0 SUMMARY

Bovine Sarcocystosis an emerging protozoan disease has been found to be associated with pathogenic conditions in both animals and man. The disease is characterized by poor feed efficiency, reduced growth, low milk yield, abortion, condemnation or downgrading of meat containing grossly visible sarcocysts and sometimes death. Sarcocystosis being an occult infection cannot be diagnosed by using conventional parasitological techniques and morphological identification is said to be unreliable.

Therefore, the present study was conducted wherein Enzyme linked immunosorbent assay (ELISA) and Enzyme immuno transfer blot (EITB) were used for detection of anti-sarcocystic antibodies. The immunoreactive peptides were identified by EITB in *S.bovicanis* and *S.bovifelis* soluble antigen extract. The protein profile of soluble extract was studied by SDS-PAGE. To determine genotypic variation between *S.bovicanis* and *S.bovifelis*, RAPD-PCR was conducted.

Initially the two *Sarcocystis* species were identified based on morphological characters. Then the soluble antigen extracts of *S.bovicanis*
and *S. bovifelis* were prepared and they had protein concentration of 550µg and 920µg per ml of antigen, respectively. The host tissue soluble antigen had protein concentration of 2mg/ml of antigen.

The working dilutions of conjugate, antigen and serum of both *S. bovicanis* and *S. bovifelis* were determined by checkerboard titration method. The cut off values for *S. bovicanis* and *S. bovifelis* were 0.210 and 0.221 respectively in ELISA.

The specific antibodies of *S. bovicanis* and *S. bovifelis* were detected in 146 (48.66%) and 46 (15.33%) cattle serum samples by ELISA. The sensitivity and specificity was found to be 76.4 and 66.6 per cent for *S. bovicanis*, respectively. The sensitivity of 81.8 and specificity of 71.4 per cent was recorded for *S. bovifelis*. The cross reaction was observed between the *Sarcocystis* spp. of cattle and with *S. capracaani* of goat.

In *S. bovicanis* soluble antigen, a total of 10 polypeptides were detected by SDS-PAGE ranging between 170 kDa to 12 kDa. Eleven polypeptides were identified in *S. bovifelis* ranging from 100 kDa to 15 kDa. Nine polypeptide bands were stained in the host tissue ranging from 80 kDa to 11 kDa.

In *S. bovicanis*, *S. bovifelis* and host tissue antigen 10, 11 and 9 polypeptides were identified, respectively. Out of these, three peptides of molecular weight 100, 82 and 15 kDa were found common between
S. bovicanis and S. bovifelis. The host antigen showed only one band to be common with S. bovifelis and no band was shared with S. bovicanis.

The polypeptides detected on western blots using HIS of S. bovicanis with anti bovine IgG conjugate at 1:1000 dilution ranged between 100 to 12 kDa. A total of six polypeptides of size 100, 82, 52, 36, 15 and 12 kDa were identified on blots. When S. bovicanis soluble extract was probed against S. bovifelis HIS only two polypeptides of size 48 and 15 kDa were recognized. It did not react with any of the peptides from host tissue extracts.

S. bovifelis blots when treated with homologous hyperimmune serum the polypeptides of size 100, 82, 49, 45, 43, 31, 22 and 14 kDa reacted with the serum. In S. bovifelis, 82, 49 and 14 kDa polypeptides were immunoreactive when probed by HIS raised against S. bovicanis. None of the polypeptides were recognized in host tissue antigen when probed against HIS of S. bovifelis. S. bovicanis and S. bovifelis blots did not react with normal sera from non-infected cattle.

In known positive and negative homologous serum samples of S. bovicanis the polypeptides in the range of 170, 83, 82, 48, 36 and 15 kDa were identified. The 170 kDa and 36 kDa peptides were present in all the serum samples with 100 percent sensitivity. However, other immunodominant proteins viz., 83, 48, 36, 29 and 15 kDa could not be recognized in all the positive sera. In S. bovifelis immunoreactive peptides of
size 76, 68, 67, 45, 38, 35, 32 and 31 kDa were identified. The polypeptides of molecular weight 76, 68 and 38 were identified in all the positive samples with 100 per cent sensitivity. None of the polypeptides were identified in negative sera.

EITB revealed specific antibodies against *S. bovicanis* and *S. bovifelis* in 175 (58.3%) and 89 (29.6%) cattle, respectively. The polypeptides 170 kDa and 36 kDa were identified in all the *S. bovicanis* positive serum samples. The sensitivity and specificity was found to be 87.5 and 81.8 per cent respectively for *S. bovicanis*. The polypeptides of size 76 kDa and 38 kDa were commonly identified in all the serum samples were positive for *S. bovifelis*. The sensitivity of 93.5 per cent and specificity of 87.5 per cent was recorded for *S. bovifelis*.

In RAPD-PCR assay all the four random primers directed the amplification of atleast a single DNA fragment with genomic DNA of *S. bovicanis* and *S. bovifelis*. Primer 1 amplified two fragments of size 1.3 and 1.2 kb for *S. bovicanis* and for *S. bovifelis* four fragments 1.1, 1.0, 0.85 and 0.75 kb were amplified. A second primer produced DNA fragments of 0.4, 0.6 and 0.7 kb for *S. bovicanis* and fragments of 1.2, 1.3 and 1.5 kb were amplified for *S. bovifelis*.

Primer 3 in *S. bovicanis* amplified two fragments of 0.8 and 0.5 kb. In the case of *S. bovifelis* two fragments of size 1.0 and 1.1 kb were obtained. *S. bovicanis* DNA produced only single fragment of size 0.5 kb whereas
S. bovifelis DNA amplified 0.7, 0.9 and 1.0 kb for primer 4. In host DNA none of the fragments were amplified with all the four primers.

Hence, ELISA and EITB can be effectively used for detection of anti-Sarcocystis antibodies in live animals. The SDS-PAGE analysis of soluble extract of bovine Sarcocystis species revealed that both the species share some common antigenic determinants including host antigen extract. RAPD-PCR assay results suggested that the primers used in the present study can be used as diagnostic probes for differentiation of S. bovicantis and S. bovifelis. Further, it may be particularly useful to study the taxonomy and epidemiology of members of the family Sarcocystidae.