SUMMARY
6.0 SUMMARY

*Echinococcus granulosus*, the dwarf tapeworm of dog, is the causative agent of cystic echinococcosis in domestic animals and man, an important zoonotic disease widely distributed throughout the world. Echinococcosis in dogs cannot be differentiated from other taeniosis by using conventional parasitological techniques and morphological identification is not reliable and confirmatory based on coproscopic examination.

Two major diagnostic methods have been extensively used in dogs, purgation with arecoline compounds and necropsy of the small intestine. Although the specificity of purgation can be 100 per cent, it is time-consuming, biohazardous, has variable sensitivity and requires trained personnel.

The eggs of taeniid cestodes are morphologically indistinguishable by light microscopy due to extreme morphologic similarity and identification by microscopic examination of the faeces is risky and non-specific.

Therefore, the present study was undertaken for detection of *Echinococcus granulosus* infection in dogs by using immunological and molecular techniques. Enzyme linked immunosorbent assay (ELISA) and Enzyme immuno transfer blot (EITB) were used for detection of anti-echinococcosis antibodies. The protein profile of somatic, excretory secretory and fecal supernatant antigen of *E.granulosus* was studied by SDS-PAGE and the immunoreactive polypeptides were identified by EITB. The copro-PCR was
used for accurate identification of *E.granulosus* and for differentiating it from other *Taenia* species of dogs.

Two different antigens viz., somatic and excretory secretory antigens were prepared from *E.granulosus* adult worm. The faecal supernatant antigen was obtained from the positive faecal sample. The protein concentration of E/S and somatic antigen was found to be 540μg and 940μg per ml of antigen, respectively. The faecal supernatant antigen had protein concentration of 1150μg/ml of antigen. The somatic antigen of *T.hydatigena* and *D.caninum* had the protein concentration of 1250 and 1450μg per ml of antigen respectively.

The counter immunoelectrophoresis (CIEP) was evaluated for the diagnosis of *Echinococcus granulosus* in dogs by using somatic and E/S antigen. The sensitivity was found to be 90 and 80 percent and the specificity was 100 per cent with both E/S and somatic antigen of *Echinococcus granulosus*, respectively.

The ELISA was standardized and working dilutions of conjugate, antigen and sera for both somatic and excretory secretory antigens of *E.granulosus* were determined by checkerboard titration method. The cut off values for somatic and E/S antigen were 0.486 and 0.427 respectively in ELISA.

The specific antibodies of *E.granulosus* to somatic and E/S antigen were detected in 62 (24.8 per cent) and 81 (32.4 per cent) dog serum
samples by ELISA. The sensitivity and specificity of ELISA with somatic antigen was found to be 100 and 71.60 per cent respectively. However the sensitivity and specificity of ELISA with E/S antigen was found to be 100 and 79.20 per cent.

The study of protein profile by SDS-PAGE with somatic antigen resulted in a total of 11 polypeptides ranging between 114 kDa to 16 kDa. The major bands were in the range of 94, 66, 45, 34, 24 and 16 kDa. A total of twelve polypeptides were identified in excretory/secretory Ag ranging from 110 kDa to 14 kDa. The major bands included 98, 66, 45, 38 and 34 kDa. Twenty-one polypeptide bands were found in the somatic Ag of *T. hydatigena* ranging from 104 kDa to 14 kDa. Twenty-two polypeptide bands were found in the somatic Ag of *D. caninum* ranging from 101 kDa to 12 kDa.

Of the polypeptides identified in somatic and excretory/secretory antigen only four peptides of molecular weight 66, 45, 34 and 24 kDa were found to be common between somatic and excretory/secretory antigen of *E.granulosus*. *T. hydatigena* showed only two bands of 66 and 34 kDa common with somatic antigen of *E.granulosus* where as three bands of 66, 34 and 14 were common with excretory/secretory antigen of *E.granulosus*. However somatic antigen of *D.caninum* shared only two bands of 84 and 68 kDa with somatic antigen of *E.granulosus* and three
bands of 78, 30 and 14 kDa with excretory/secretory antigen of *E.granulosus*.

A total of eight polypeptides were identified in faecal supernatant antigen ranging from 76 kDa to 17 kDa. The major bands included 54, 45, 37 and 34 kDa.

The immunoreactive polypeptides detected on western blots with somatic antigen of *E.granulosus* using homologous known positive serum with anti dog IgG conjugate at 1:1000 dilution ranged between 84 to 16 kDa. A total of four polypeptides of size 89, 66, 45 and 16 kDa were identified on blots. The E/S antigen of *E.granulosus* showed six polypeptides of size 98, 82, 66, 45, 34 and 24 kDa on blots. A total of four polypeptides of size 66, 45, 34 and 17 kDa were identified on blots with faecal supernatant antigen. The immunoreactive polypeptides of 66 and 45 kDa was common among the three antigens of *E.granulosus* used in the present study.

The polypeptides in the excretory secretory antigen of 66, 45 and 34 kDa were detected in all 52 (20.80 per cent) positive serum samples whereas in somatic antigen three polypeptides of 84, 66 kDa and 45 kDa were detected in all 41 (16.40 per cent) positive serum samples. The sensitivity and specificity of E/S and somatic antigen was found to be 100 and 88.40 per cent and 100 and 83.60 per cent, respectively. The polypeptides 45 kDa and 34 kDa were detected in all the positive serum
samples by EITB with faecal supernatant antigen, specific antibodies were detected in 47 (18.80 per cent). The sensitivity and specificity was found to be 100 and 86.80 per cent, respectively.

Cross reaction was observed between \textit{E.granulosus}, \textit{T.hydatigena} and \textit{D.caninum}. Two polypeptides of size 69 and 42 kDa were detected with \textit{T.hydatigena} and only one polypeptide of 35 kDa was detected in \textit{D.caninum} somatic Ag, when reacted with the serum of \textit{E.granulosus}. These polypeptides were found specific to \textit{T.hydatigena} and \textit{D.caninum} but not observed with \textit{E.granulosus} antigens.

The latex agglutination test was conducted with a total of 250 serum samples using \textit{E.granulosus} F/S antigen. In these serum samples when subjected to Latex agglutination test 53 (21.2 per cent) samples gave positive agglutination reaction. The sensitivity and specificity of latex agglutination was found to be 100 and 78.80 per cent, respectively.

The specific antibodies of \textit{E.granulosus} were detected by Dot-ELISA with fecal supernatant antigen. Out of 250 serum samples examined, the specific antibodies were detected in 45 (18.0 per cent). The sensitivity and specificity was found to be 100 and 82.0 per cent, respectively.

Copro-PCR was conducted with 19 samples which were positive for Taenia eggs by copromicroscopy using two sets of \textit{E.granulosus} specific primers including Eg 1f, Eg 1r and JB 3f, JB 4.5r. The Eg 1f, Eg 1r primer amplified single band of 255 bp which was specific to \textit{E.granulosus} in ten
samples which were positive for *E.granulosus* at necropsy and no bands were observed in nine faecal samples which were positive for *Taenia* eggs by copromicroscopy. The other primer JB 3f, JB 4.5r which amplified a single band of 440 bp was also specific to *E.granulosus* in ten samples which were positive for *E.granulosus* at necropsy and no bands were observed in nine faecal samples which were positive for *Taenia* eggs by copromicroscopy. The Copro-PCR was found to be 100 percent sensitive in the detection of *E.granulosus* eggs in the faecal samples.

It was concluded that ELISA and EITB could be effectively used for specific detection of *Echinococcus* infection in dogs. The SDS-PAGE analysis of different antigens used in the present study revealed that both *E.granulosus* and *T.hydatigena* shared some common antigenic determinants including *D.caninum*. Copro-PCR assay indicated that both the primers used in the present study could be used for differentiation of *E.granulosus* from *T.hydatigena*. 