Cystic echinococcosis (CE), caused by *Echinococcus granulosus* is recognized as one of the world’s major zoonotic disease, causing considerable human morbidity and mortality. The diagnosis, treatment as well as control of this cosmopolitan disease are difficult. The farming dogs have been established as a risk factor for *E. granulosus* infection since they usually have close contact with livestock and farmers. The disease is endemic in many parts of India, particularly in sheep rearing states of North India like Himachal Pradesh and Jammu & Kashmir. Keeping these points in view, the present study was aimed to investigate the epidemiology of CE in Himachal Pradesh followed by identification of immunodominant peptides for vaccine and diagnostic use and screening of amide based compounds for anti-echinococcal activity.

A total of 1000 serum samples were collected during 2012 -2014 from Regional Hospital of Solan and other clinics of Solan District. Anti hydatid IgG and IgG subclass antibodies were determined in the serum samples by indirect ELISA. Further, the immunoblot and DD5 assay of ELISA IgG positive sample was performed. For genotyping, 14 human Hydatid cysts were collected from Surgery Department of Regional Hospital, Solan. The genomic DNA was extracted by using Qiagen DNA extraction kit. Further, the PCR amplification and sequencing analysis for *Nicotinamide adenine dinucleotide dehydrogenase-1 (ND-1)* and *Cytochrome Oxidase-1 (Cox-1)* gene was carried out for molecular characterization of strains. Further, the promiscuous T cell and B cell epitopes were also identified using *in-silico* approach targeting vaccine candidate antigens: Eg95, Reticulon-4, LDL-receptor, Tetraspanin and Glutathione-S-transferase proteins of *E. granulosus* and docked with HLA Class I and Class II alleles. Molecular docking of the predicted T cell epitopes was carried out with the HLA alleles in order to ascertain the binding pattern of the peptides with HLA alleles. Further, we also identified some diagnostic peptides specific for *E. granulosus* targeting Tetraspanin-1, Heat Shock proteins- Hsp-90, Hsp-8, Phosphoenol Pyruvate Carboxykinase, Antigen 5 and Antigen B. A library of 30 amide based compounds was prepared and docked with the potent drug targets of *E. granulosus* viz. Fatty acid binding protein, kinase A, Glucose 6 phosphate dehydrogenase and Aquaporin. The best five compounds predicted after docking analysis were synthesized in the laboratory and screened for anti-echinococcal activity.
IgG antibody was observed to be most prevalent and highly produced antibody in response to CE with a prevalence rate of 5.6%. Among the subclasses of IgG, the prevalence of anti-hydatid IgG2 antibody was highest - 42.80%, followed by IgG3 - 21.4%, IgG1 - 19.64% and IgG4 - 16.07%. The immunoblot results obtained for IgG subclasses suggested that the 37kDa subunit of Arc 5 was more reactive against IgG subclasses as compared to 20kDa subunit. Also the 16kDa antigen was highly reactive against IgG antibody and its subclasses, showing 58.9% reactivity towards IgG, 50% for IgG1, 42.8% for IgG2, 27.7% for IgG3 and 100% for IgG4. Hence, 16 kDa and 37 kDa antigens may be used as diagnostic markers for the detection of CE. IgM antibody showed 100% reactivity with 58 kDa antigen and 75% reactivity with 66kDa antigen and didn’t show any reactivity with arc 5 antigen subunits. The reactivity of IgM with 58 kDa and 66kDa antigens suggested that these antigens could be used as diagnostic markers for the early diagnosis of CE. In addition, the prevalence of IgE antibody was also found high accounting for 64.28% (36/56 samples). The results observed for IgE suggests that CE is also associated with allergic reactions thereby eliciting the IgE antibody in patients. Sero-epidemiological study revealed that the Solan district of Himachal Pradesh is a mesoendemic region for CE.

The PCR-sequencing analysis of hydatid cysts revealed that the majority of strains prevalent in this region are belonged to G1 genotypes (84.61%, sheep strain) followed by G3 genotype (15.38%, buffalo strain). Thus the study revealed that the G1 genotype of E. granulosus is predominantly prevalent in Solan district of H.P.

The overall in-silico analysis performed for identification of vaccination peptides showed that Tsp-1 and LDL-receptor could be the most important targets for vaccine development against CE. Such predicted peptides could be used for subunit vaccine design against CE. Further, 8 peptides (7 peptides from Hsp-90 and 1 from Tsp-1) specific for E. granulosus were also identified which could be used for diagnostic purposes and may prevent the cross reaction with other helminthic parasites like Schistosoma and Taenia, the major problem encountered so far in diagnosis.

Among the amide based compounds tested for antiechinococcal activity, N-p-tolyl-1-naphthamide (compound 10) showed the most potent protoscolicidal activity with IC$_{50}$ value of around 29.51µg/ml, followed by 2,3,4,5,6-pentafluoro-N-p-tolylbenzamide (IC$_{50}$ = 39.55µg/ml),
4-pentyl-N-p-tolylbenzamide ($IC_{50} = 44.98 \mu g/ml$), N-p-tolyl-4-(trifluoromethoxy) benzamide ($IC_{50} = 66.51 \mu g/ml$) and 3,5-dinitro-N-p-tolylbenzamide ($IC_{50} = 77.02 \mu g/ml$) respectively. The compounds should further be tested in animal models for complete validation of our findings which we will be carrying out in our future prospective. The overall results obtained in the present study will further help the researchers in better understanding the nature of the disease and will help in developing effective strategies to control the disease in this area.