Cystic echinococcosis (CE), caused by tapeworm *Echinococcus granulosus* is recognized as one of the world’s major zoonotic disease, causing considerable human morbidity and mortality. Domestic dogs have long been identified as the main infection source for human beings. 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. The present study was aimed to investigate the epidemiology of CE in Solan district of Himachal Pradesh along with the identification of the immunodominant peptides using reverse vaccinology approach and as well as the analysis of antiechinococcal activity of synthetic amide based compounds.

Sero-prevalence of CE was investigated by using ELISA, Western Blotting and DD5 assay. A total of 1000 serum samples were analysed of which 406 samples were of males and 594 samples were of females. Sero-epidemiological study revealed that the Solan district of Himachal Pradesh is a meso-endemic region for CE. In the present study, 5.6% (56/100) samples were found positive for anti-hydatid IgG antibody. Out of 406 male samples collected, 4.1% (17/406) were found positive for IgG while it was detected 6.5% (39/594) in females. Hence the females were observed on risk as the frequency of prevalence of IgG antibodies was found high among them in comparison to males. The highest prevalence was observed in age group 41-60 (6.8%) in males while in females, the highest prevalence was observed in age group 61-80 (25.3%). Thus the overall results indicates that females are more frequently exposed to the infection in comparison to males as the females are closely associated with house hold pet animals. The reason of higher prevalence of IgG antibody in females may be due to the close contact with livestock along with pet dog, which increase the exposure and risk of acquiring the infection by females. The frequency of infection was high among the people residing on highlands of Solan District, in comparison to the foothills and plane geographical areas.

All the 56 IgG positive samples were further subjected to immunoblot analysis by using IgG and its subclasses (IgG1, G2, G3 and G4), IgM and IgE antibodies. The immunoblot analysis suggests that IgG is highly reactive antibody and produced on large
scale in response to the hydatid fluid antigen. The overall results obtained for IgG subclasses suggested that the 37kDa subunit of Arc 5 is 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 with 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. In addition, the prevalence of IgE antibody was also found high accounting for 64.28% (36/56 samples). It showed reactivity with 37kDa (52.7%) and 66kDa (69.4%) antigens. The results observed for IgE suggests that CE is also associated with allergic reactions thereby eliciting the IgE antibody in patients.

Further the samples found positive by ELISA were also subjected for DD-5 assay. Of the 56 positive samples analysed, only 14 samples showed the bands of precipitation by DD5 assay. The study suggested that the DD-5 assay was least sensitive among all the diagnostic techniques applied for the detection of CE with a very low sensitivity of around 25%. However, the specificity of DD5 assay was observed 100%, due to lack of precipitation bands with all the non-hydatid sera tested.

A total of 14 human hydatid cyst samples were collected and subjected to PCR. The cox-1 and nd-1 genes were used for the genotyping of CE by using PCR sequencing analysis. The PCR products were obtained only in 13 cyst samples while the PCR product was not obtained in one sample even after repeated attempts. The sequences obtained were further subjected to various bioinformatics tools to know the genotypes and evolutionary pattern. The PCR-sequencing analysis of 13 hydatid cysts of cox-1 and nd-1 gene revealed that the majority of strains prevalent in this region belonged to G1 genotypes (84.61%), however two cysts belonged to G3 (15.38%) genotype (buffalo strain). The study revealed that G1 genotype is predominantly prevalent in Solan district of H.P. The prevalence of G1 genotype among the residents of Solan confirm the transmission cycle of CE between dog and sheep as it is sheep rearing state.

Considering the need for efficient immunodiagnosis of CE, six antigens, Antigen 5, Antigen B, Heat shock proteins- Hsp-8, Hsp-90, Phosphoenolpyruvate carboxykinase, and Tetraspanin-1 of E. granulosus were selected for prediction of B and
T cell epitopes using \textit{in-silico} tools. In the present study, eight T cell peptides specific for \textit{E. granulosus} (7 peptides from Hsp-90 and 1 from Tsp-1) were identified which may prove as an important marker for the diagnosis of CE and might be prevent the misdiagnosis and mismanagement encountered so far by decreasing the chances of cross reactivity with antigens of other helminthic parasites.

So far there is no human vaccine reported for the prevention of CE. Only one vaccine based on antigen Eg95 and that too for other intermediate host (animal host-sheep, dog) has been reported. For regions which are highly endemic for the disease, a vaccine for humans is urgently needed. The vaccine candidates included in the present study were Low Density Lipoprotein (LDL) receptor, Tetraspanin-1, Glutathione-S-transferase (GST), Reticulon-4 and Eg95. The published gene sequences retrieved from data base were screened to predict promiscuous peptides by using \textit{In-silico} tools. The promiscuous peptides (T cell epitopes) for HLA Class I and Class II were predicted by using sequence based prediction servers like NetMHCpan 2.4, NetMHC3.4, IEDB-ANN and IEDB-SMM servers, NetMHCIIpan 3.0, ProPred and MultiPred. Only the high-affinity binding T cell epitopes (IC$_{50}$ value $<50$ nM) were selected for further investigations. The criteria for selecting the promising T cell epitopes, was that, the peptide should be promiscuous, have least IC$_{50}$ value, present on the surface of the protein, should be charged and predicted by at least three out of five servers used. The highest number of high affinity HLA class I T cell epitopes were identified for Eg95 (19), followed by LDL (18), Tsp-1 (15), Reticulon-4 (9) and GST (8), whereas for HLA class II, the highest number of high affinity epitopes were identified for GST (45), followed by Eg95 (21), LDL (14), Tsp-1 (14) and Reticulon-4 (11). However, any promiscuous peptide of HLA Class I was not observed, therefore, we analyzed only HLA-A* 02:01 restricted epitopes, the most predominant allele worldwide.

The most promising T cell epitopes predicted were docked with the worldwide predominant HLA class I allele, HLA-A* 02:01 and HLA Class II allele, HLA-DRB1* 01:01 respectively by using AutoDock Program. The overall \textit{in-silico} analysis performed revealed that Tsp-1 and LDL-receptor could be the most important targets for vaccine development against CE. The reason is that they are highly expressed proteins during the
infection stage in intermediate host and also essential for carrying out multiple functions essential for the parasite survival and growth.

Furthermore, the protoscolicidal activity of amide based compounds was investigated. A total of 30 amide based compounds (drug molecules) were prepared using chemdraw software and various physico-chemical and electronic parameters which influence the physico-chemical properties of drug molecules were analyzed. Then all 30 compounds were docked with the selected drug targets of *E. granulosus*. These were Kinase A, Glucose-6-phosphate-1-dehydrogenase, Aquaporin-4 (Na+ channel) and Fatty acid Binding Protein. The compounds 3,5-dinitro-N-p-tolylbenzamide (compound 6), N-p-tolyl-1-naphthamide (compound 10), N-p-tolyl-4-(trifluoromethoxy) benzamide (compound 16), 4-pentyl-N-p-tolylbenzamide (compound 18) and 2,3,4,5,6-pentafluoro-N-p-tolylbenzamide (compound 20) were predicted as best compound which possesses protoscolidal activity after docking analysis and were selected for synthesis for *in-vitro* protoscolidal activity against CE.

The cytotoxicity of the synthesized compounds was analyzed by using human hepatic cancer (HepG2) cell lines. The 4-pentyl-N-p-tolylbenzamide (compound 18) exhibited the lowest cytotoxicity on HepG2 cell lines since the survival rate of cells was approximately 54% on the third day with the concentration of 125µg/ml, followed by N-p-tolyl-1-naphthamide (compound 10) - 40%, N-p-tolyl-4-(trifluoromethoxy) benzamide (compound 16) - 38%, 3,5-dinitro-N-p-tolylbenzamide (compound 6) - 36% and 2,3,4,5,6-pentafluoro-N-p-tolylbenzamide (Compound 20) - 32% respectively.

All the five compounds synthesized exhibited significant antiechinooccocal activity. The most potent protoscolidal activity was observed for N-p-tolyl-1-naphthamide (IC$_{50}$ = 29.51µg/ml), followed by 2,3,4,5,6-pentafluoro-N-p-tolylbenzamide (IC$_{50}$ = 39.55µg/ml), N-p-tolyl-4-(trifluoromethoxy) benzamide (IC$_{50}$ = 66.51µg/ml), 4-pentyl-N-p-tolylbenzamide (IC$_{50}$ = 44.98µg/ml) and 3,5-dinitro-N-p-tolylbenzamide (IC$_{50}$ = 77.02µg/ml) respectively.

The observations obtained in the present study indicates that Solan district of Himachal Pradesh is a meso-endemic area for CE. The seroprevalence of CE was observed to be 5.6%. Among the diagnostic techniques applied, ELISA was observed highly sensitive for the diagnosis of CE followed by Western Blotting and DD5 assay.
Further the genotyping of *E. granulosus* was carried out to know the genetic type of strains prevalent in this region as the strain variation is the major limitation factor for effective diagnosis and chemotherapy of CE. The G1 genotype is frequently prevalent strain of *E. granulosus* in Solan district. The frequency of G1 genotype was observed high followed by G3 genotype. The effective diagnosis and treatment of CE for prevention of disease is required. *In silico analysis of vaccine candidates antigens provide clue that an effective vaccine may be developed based on promiscuous peptides predicted from* immunodominant epitopes as well as these peptides may be used to developed diagnostic tool for the rapid diagnosis of CE. Furthermore the *in vitro* screening of amide based compounds have been determined that these compounds may be used for the effective treatment of CE. The present study had limitation that we could not validate these promiscuous peptides for their immunogenic nature which provide the important clue for vaccine and diagnostic importance, further study is required.