Summary & Conclusions
In the present study, human hydatid cyst is used as a source of antigen for identification of diagnostically relevant protein and for their use in serodiagnosis. The SDS-PAGE characterization of human hydatid cyst fluid, protoscolex and cyst wall revealed 43kDa, 37kDa and 24kDa as the most dominant and consistently demonstrable proteins. A 92kDa band was present in the cyst wall and protoscolex antigens but not in the cyst fluid antigen.

The EITB using crude extract of cyst fluid, protoscolex and cyst wall antigens showed immunodominant bands of Mr 43kDa, 37kDa, 24kDa and 9kDa. In addition to all these bands, a marked 92kDa component is found to be present in both protoscolex and cyst wall antigens but absent in the cyst fluid antigen.

Urinary parasitic antigen as a potential source of antigen in serodiagnosis is a recent approach. The isolation of parasite antigen excreted in the urine and their use as antigen following purification has opened a new approach in the serodiagnosis of CE. To our knowledge, the present study constitutes the first attempt for the purification of diagnostically relevant hydatid protein from the urine specimen for use in serodiagnosis of CE.

EITB analysis of the urine of confirmed CE cases showed 24kDa protein to be of diagnostic relevance. For the first time, the hydatid antigen excreted in the urine is eluted and further characterized based on immunochemical reactivity. Immunochemical study of 24kDa urinary hydatid protein along with 24kDa hydatid cyst wall protein showed both to be glycoprotein, containing con A specific sugar and galactose residues. Both hydatid cyst wall proteins and urinary hydatid proteins are periodate-sensitive, heat stable and sensitive to proteolytic treatment.
2. In the present study, ELISA and Dot-ELISA using cyst fluid, protoscolex and cyst wall antigens, Dot-ELISA using 24kDa urinary and cyst wall protein; and EITB using cyst wall antigen were used for the detection of hydatid antibodies in serum.

Dot-ELISA using protoscolex and cyst fluid antigens are found to be useful for the serodiagnosis of CE whereas Dot-ELISA or ELISA using cyst wall antigen is found to be equally sensitive for the serodiagnosis of CE. The result of present study suggest that protoscolex and cyst wall apart from the cyst fluid can be used as alternate source of antigen in the serodiagnosis of CE.

In the present study, 24kDa urinary hydatid proteins and 24kDa hydatid cyst wall, eluted from the concentrated urine of confirmed CE patients and from the hydatid cyst wall respectively, employed in the Dot-ELISA is observed to be equally sensitive for detection of IgG antibodies in the serum. The results showed that 24kDa hydatid proteins of urine or the cyst wall could be used as antigen in the serodiagnosis of CE.

The EITB carried out using hydatid cyst wall antigen detected three specific proteins: 92kDa, 24kDa and 9kDa. These proteins are found to be specific and of diagnostic value in the CE.

Further to clarify whether or not 24kDa consists of an 8kDa component of antigen B, the detailed characterization of various antigens of the hydatid cyst wall by DNA cloning approaches is in progress. Preliminary results of the study has shown that the polyclonal rEAgB8/2 antibodies were reactive to the 24 kDa molecule by EITB.

In the present study, all the serological tests except ELISA using cyst wall antigen and Dot-ELISA using protoscolex antigen showed higher sensitivity.
with sera from patients with multiple hydatid cysts than with sera from patients with single hydatid cyst.

3. The production of monoclonal antibodies is important both in improving the sensitivity and specificity of the serological tests for diagnosis of CE.

In the present study, cyst wall antigen is used for purifying hydatid specific protein by affinity column containing rabbit anti-hydatid IgG coupled with CNBr activated sepharose. The result showed the fraction F4 to contain hydatid specific protein Mr24kDa along with 37kDa, a non-specific protein.

Monoclonal antibodies raised against fraction F4 showed 12 reactive clones (117av, 107bv, v11, v1b, 8av, 118av, 109bv, 107av, 104cv, 5cv, 201bv and 123v). The specificity of clones checked by the EITB showed four clones (117av, 107bv, v11 and v1b), against 24kDa, hydatid specific protein. These four clones were checked for cross reactions with other parasite antigen by Dot-ELISA. The result showed clone v11 is found to be specific with higher affinity.

The monoclonal antibodies (MAb.v11) obtained from fraction 4 of the hydatid cyst wall are selected for subsequent use in the sandwich ELISA and Dot-ELISA for detection of hydatid antigens in the serum and in the urine for the diagnosis of the CE.

4. Detection of hydatid antigen is more useful as it helps in differentiating current infection from the past and also in post-surgical follow up of CE cases. The present study demonstrated the presence of circulating hydatid antigens in the sera of patients with CE by the sandwich ELISA. Dot-ELISA using both polyclonal and monoclonal antibodies and the EITB using polyclonal antibodies.
Sandwich ELISA using either polyclonal or monoclonal antibodies is found to be highly sensitive than the Dot-ELISA using either polyclonal or monoclonal antibodies for detection of hydatid antigen in the serum.

Results of the study also show that both the sandwich ELISA and Dot-ELISA employing polyclonal antibodies is more sensitive than the tests using the monoclonal antibodies for detection of hydatid antigen in the serum.

To best of our knowledge, this is the first attempt where EITB is used to detect circulating hydatid antigen in the serum for diagnosis of CE. The EITB detected proteins of Mr 92kDa, 30kDa and 24kDa of diagnostic interest in the serum from patients with CE.

The present study is the first study of the kind where in purified polyclonal antibodies and monoclonal antibodies are used to develop a sandwich ELISA and Dot-ELISA for detection of hydatid antigen in the urine for diagnosis of CE.

In this study, Dot-ELISA showed the best diagnostic efficacy (75%) than the sandwich ELISA (diagnostic efficacy-73.33%). Dot-ELISA gave the least cross-reaction with other parasites and thus it has a good potential as a screening test which can be used in endemic area with poorly equipped laboratories.

EITB is evaluated for the first time to analyze the specific and cross reactive antigens in the urine for diagnosis of CE. The EITB analysis using polyclonal hydatid antibodies revealed three specific proteins of 24kDa, 14kDa and 10kDa, of diagnostic importance.

The sensitivity of sandwich ELISA, Dot-ELISA and EITB for detection of antigen in the serum was observed to be higher than the sensitivity of sandwich ELISA. Dot-ELISA and EITB for detection of antigen in the urine for diagnosis of CE.
Overall Conclusion

Cystic echinococcosis in human is a re-emerging disease of public health importance. The rapid novel method such as Dot-ELISA developed in this laboratory may be useful for routine laboratory diagnosis as well as large scale seroprevalence studies of CE in endemic countries. The use of urine as a specimen may be very helpful for field set up. The use of serological tests such as ELISA or Dot-ELISA for detection of hydatid antibodies or antigen may be useful as screening tests for monitoring post-surgical or chemotherapeutic CE cases. The serological tests are of preferred choice in situation wherein imaging techniques are either not available or the results drawn out of it is inconclusive.