REVIEW OF LITERATURE
Antigenic Analysis of C. cellulosae

In spite of the reports on immunologic approaches to the diagnosis of neurocysticercosis in man, there is remarkably little known about the physicochemical characteristics of antigens of C. cellulosae and other parasites in the family Taenidae. Serological activities have been associated with a variety of crude fractions prepared from extracts of both adults and metacestodes, but in only a few instances have further steps towards purification been taken.

Comparative analysis of diagnostic antigens of members of the Taenidae was first studied by Biguet et al., (1965) and Capron (1968). They showed about sixteen to twenty three antigens in T. saginata, which were present in six other parasites (cestodes). However, an antigen 5, a species specific to Echinococcus granulosus was identified by immunoelectrophoresis. This was known to occur in T. solium, T. hydatidigena, T. ovis and E. multilocularis and is the most highly characterized antigenic component of members of the family Taenidae.

Of 20 to 22 antigens in T. solium and T. saginata (Capron et al., 1968; Geerts et al., 1979), a few showed some promise of specificity for immunodiagnosis, but there has been little success in exploiting these findings so far.

Certain host components that enter the cystic stages of most taenid larvae (Hustead and Williams, 1977) or bind to their surface membranes (Williams and Arcos, 1977; Kwa and Liew, 1978), have to be identified and eliminated from consideration in the purification of metacestode antigens. Antigenic components that are shared between host and parasite, such as blood group antigens (Russe et al., 1974), can also be a complicating factor in serodiagnosis (Ben-Ismail et al., 1980).
Immediate type of hypersensitivity reactions to tapeworm metacestode antigens has been reported (Williams and Sandeman, 1982). This is true for antigen H of T. solium (Flisser et al., 1980), thermostable, lipo-protein antigen B of E. granulosus (Dessaint et al., 1975) and 50 Kd of T. taeniaeformis. However, despite the high sensitivity of diagnostic allergic reactions, specificity in intradermal tests is commonly problematical, suggesting that allergenic determinants are shared by related helminth parasites.

Host protective antigens of T. taeniaeformis have been characterized to homogeneity by Kwa and Liew (1978). This kind of study has not been established with other Taenia spp. Earlier, attempts were made to analyse antigens of T. solium metacestodes of its anatomical parts using simple techniques like immunodiffusion and immunoelectrophoresis (Flisser et al., 1982). With the increasing awareness of the problem of diagnosis of this disease (NCC), few studies reported on characterization of antigens of diagnostic importance using sensitive methods like sodium dodecyl sulfate (SDS), polyacrylamide gel electrophoresis (PAGE), enzyme linked immunoelectrotransfer blots (EITB) (Larralde et al., 1986; Gottstein et al., 1986; Tsang et al., 1989).

Immunodiffusion

The precipitin reaction of antigen and antibody complexes in agar gel described by Oudin in 1946 was initially applied for the analysis of antigenic relationship amongst scolex, wall and cyst fluids antigenic preparations (Flisser et al., 1979). Using a rabbit hyperimmune sera, in a double immunodiffusion (DID) techniques, immunochemical identity was observed among fluid, scolex and wall protein antigens, while all the
antigens from the wall were shared with the scolex. Further some antigens which were specific for the scolex or fluid were detected. The cyst vesicular fluid seemed to have given false positive results 50% of the time when tested against human sera (Flisser et al., 1982).

The differences in the antigens of cysts were prepared from various batches of infected pork and stability of those antigenic extracts were determined by DID and single radial immunodiffusion (SRID) respectively using rabbit hyperimmune serum (Flisser et al., 1982). The degree of similarity among antigens of different batches of cyst was ranged from 30-100% and potency of antigenic preparation appeared to have been retained over a period of one year, if it was stored at -20°C.

Immunoelectrophoresis (IEP)

When antigens of scolex, wall and vesicular fluid were analysed by immunoelectrophoresis (IEP) against their respective hyperimmune rabbit antisera, the results supported the findings of DID (Flisser et al., 1982). Eleven antigens were characterized in a crude porcine cyst homogenate employing simple immunoelectrophoresis (IEP) against rabbit hyperimmune serum (Flisser et al., 1980). Up to eight different precipitation bands were found with different frequencies when a crude procine cyst extract antigen was reacted with patient's sera (Flisser et al., 1980). Antigens A, E and C were more frequently precipitated by cysticercotic patients sera, as compared to antigens D, G, H and F. However, the most consistently precipitable antigen recognized by human sera was a component designated 'antigen B'. This led to purification of antigen B (Guerra et al., 1982) and its evaluation in the immunodiagnosis of neurocysticercosis (Espinoza et al., 1982) wherein, they could reduce the background noise of non-specificity.
Very few studies have made an attempt on characterization of antigenic components of *C. cellulosae* by SDS-PAGE. Although Espinoza, *et al.*, (1982) reported that 20 protein bands were seen in crude cyst homogenate on SDS-PAGE, the characterization of the antigenic components appears to be incomplete as immunological and biochemical identity of these antigens was not elucidated. However, partially purified antigen B seemed to have two components having molecular weights 105 and 95 Kd, which were identified as glycoproteins with a pI between 5.0-5.3 (Guerra *et al.*, 1982). The immunological reactivity of fully characterized antigen B was evaluated in ELISA studies which resulted in 85% sensitivity in CSF and 76% in serum (Espinoza *et al.*, 1982). But there are no experimental evidences to show that this sensitivity (or reactivity) was due to the activity of antigen B alone or other minor contaminants present in the preparation.

For the demonstration of most specifically active and non cross-reactive components of *Cysticercus cellulosae* a systematic study was conducted by Gottstein, *et al.*, (1986), in which 5-20% gradient SDS-PAGE revealed a complex protein mixture ranging from 250 Kd to 8 Kd (by extrapolation, relative molecular mass). Further two polypeptides namely 26 Kd and 8 Kd did not have any cross-reactivity to even the closely related germs *echinococcus* sps. and other nematodes and trematodes. These two components appear to have superiority over antigen B as the antigen B had the cross-reactivity with *echinococcus* sps. in ELISA (Espinoza *et al.*, 1982).
After the evaluation of all morphological compartments of parasite as of antigen, Larralde, et al., (1986) found that the vesicular fluid was a stable and reliable source of antigen. On SDS-PAGE analysis, fluid revealed about 15 protein bands ranging from 246 Kd to 10 Kd antibody response was most frequently found with 103 Kd protein on immunoblots and this protein did not share any antigenic determinants of antigen B. Apparently, antigen B appeared to be absent in vesicular fluid.

Recently, Tsang et al. (1989) have purified seven glycoprotein antigenic components from crude homogenate of procine cysts using a lentin-lectin affinity chromatographic technique. Evaluating these antigens namely GP50, GP42-39, GP24, GP21, GP18, GP14 and GP13 in an enzyme linked immunoelectro transfer blot (EITB) technique, they observed that these antigens were non-cross-reactive and highly specific for diagnosis of neurocysticercosis. GP42 and GP24 proteins were most constantly recognized. There is apparently little difference between the recognition patterns of CSF compared to serum.

Immunodiagnostic Studies in Neurocysticercosis

In the recent years, immunodiagnosis has improved in the detection of anticysticercal antibodies in serum and CSF. A number of techniques have been described in the detection of antibodies, they are - complement fixation test (CFT), indirect or passive haemagglutination test (IHA or PHA), enzyme linked immunosorbent assay (ELISA), immunofluorescence antibody assay (IFA) and radioimmunoassay (RIA). Although formerly tests were unreliable (Espinoza et al., 1982; McCormick et al., 1982), it is becoming increasingly sensitive and specific. Attempts also have been made in the detection of cysticercal antigens by enzyme linked immunosorbent assay and dot immunobinding assays.
Fixation Test (CFT)

The complement fixation test was one of the earliest successful tests used in parasitology. Since the description of test in 1909 by Weinberg using an alcoholic extract of cysticerci as antigens, a number of workers have adopted the test with different cysticercal antigens in immunodiagnosis of neurocysticercosis. In 1911, Moses used, for the first time, the aqueous extract of cysticerci in CFT to diagnose in man. Pessoa e Silveira (1930) applied CFT in CSF with good results. In 1942, Neito developed CFT using alcoholic extract of procine cyst as antigen. He used pooled normal human serum as a source of both complement and as well as a source of antibody in the indicator system. False positive results were seen in 3 to 9% of patients with neurosyphilis (Neito, 1956). Mahajan and his colleagues in 1975, developed a CFT using alcohol extract of cyst of human origin as source of antigen. They further evaluated CFT with IHA, the sensitivity of CFT was found to be less (78.1%) compared to IHA (87.5%). According to Sotello (1985) CFT still appears to be highly sensitive and specific test for the diagnosis of NCC. The diagnostic accuracy of NCC could be increased to 95% by performing CFT along with IgM-ELISA (Sotelo, 1987).

Indirect or Passive Haemagglutination Assays (IHA or PHA)

The haemagglutination of cysticercal antigens sensitized using tanned sheep cells was first introduced for the detection of anticysticercal antibodies by Proctor, et al (1966). Subsequently, various workers have used IHA with different antigenic preparations such as crude saline extract of cyst (Proctor et al., 1966; Mahajan, 1974), adult worm antigens (Mahajan et al., 1974) and cyst vesicular fluid (Larralde et al., 1986), for detecting anticysticercal antibodies in serum. Mahajan and colleagues
(1974) evaluated antigens prepared from Cysticercus cellulosae and Taenia solium adult worm by IHA, and observed that cysticercus antigen was superior to adult worm antigens in terms of sensitivity and specificity in the serodiagnosis of cysticercosis. On comparison of IHA with CFT, the former could detect 87.5% of cysticercosis cases, whereas CFT could detect only 78.1% of cases, thereby showing superiority of PHA over CFT (Mahajan et al., 1975). Larralde et al. (1986) coupled vesicular fluid antigens to sheep blood cells through glutaraldehyde bridges. Further using cyst vesicular fluid antigen, the differences between PHA and enzyme linked immunosorbent assay (ELISA) was found to be minimal in detecting anti-cysticercal antibodies (Larralde et al., 1986). McCormick et al. (1982) reported that IHA could diagnose only 60% of cerebral cysticerci cases in their series of study. However, the details of the methodology were not discussed. Even though, PHA test suffers from the problems of variation in the erythrocytes used, cross-reactivity with other tapeworms, Kagan (1980) feels that IHA is the test of choice for cysticercosis.

Enzyme Linked Immunosorbent Assays (ELISA)

To improve the serological diagnosis of neurocysticercosis by reducing the influence of non-specific reactivity, many workers have described most sensitive immunological assays like ELISA, using different cysticercal antigens (Arambulo et al., 1978; Diwan et al., 1982; Coker-Vann et al., 1984; Espinosa et al., 1982; Estarda-Kuhn, 1985; Rosas, et al., 1986; Mohammad et al., 1984; Larralde et al., 1986; Espinosa et al., 1986 Nascimento, et al., 1987 and Bailey et al., 1988).

Arambulo et al., (1978) described ELISA for human cysticercosis, and reported positivity rate of 78% in Mexican patients suspected of having cysticercosis. They did find cross-reactivity in patients with schisto-
romal or echinococcal infections. Diwan et al (1982) described a different ELISA system wherein, normal pork muscle antigen was used as control antigen in parallel with cysticercus antigen. This enabled them to distinguish clearly between the sera of cysticerci patients from that of normal sera. With this system they reported that 79% of the sera and 80% of cerebrospinal fluids were positive for antibody to cysticercus antigen, but did find cross-reactivity in sera of patients with echinococcosis, schistosomiasis and angiostrongyliasis. In addition an attempt was made to reduce the influence of non-specific reactivity by employing a partially purified chromatofocussed antigens (Coker-Vann et al., 1984), but the cross-reactivity with echinococceus continued to persist.

Espinoza et al (1982) described the method of ELISA using partially purified antigen, namely, antigen B, wherein, the sensitivity was found to be 85% in CSF samples and 73% of sera of neurocysticercosis patients. In another study, antigen B did show cross-reactivity with sera of patients with hydatidosis (Plancarte et al., 1987). However, using antigen B, Espinoza et al (1982) could find false negative results to the tune of 27% in sera and 15% in CSF samples.

Using delipidized antigens of C. cellulosae, Mohammad et al (1984) increased the diagnostic accuracy of ELISA to 94.7% in CSF and 100% in serum. On comparison with RIA, ELISA appeared too simple and inexpensive to perform with equal sensitivity and specificity (Mohammad et al., 1984). The performance of ELISA was improved by treating polyvinyl chloride microtitre plates with Poly-D-lysine hydrobromide and glutaraldehyde, which increased the binding of relevant antigens in crude homogenate extract of C. cellulosae (Estrada and Kuhn, 1985).
Espinoza et al. (1986), characterized the humoral immune response by ELISA, using a crude extract and two partially purified antigens (viz., antigen B and cysticercus surface glycoprotein) of *Taenia solium* cysticerci. It was observed that IgG was the predominant class of antibody, which could be detected in CSF and serum to all cysticercal antigens. This finding supported the earlier reports (Flisser et al., 1980; Diwan et al., 1982; Estrada and Kuhn, 1985).

In sharp contrast, Rosas et al. (1986) compared the sensitivity and specificity of IgG-ELISA and IgM-ELISA and found that IgM type of immunoglobulins in CSFs of NCC patients. The specificity and sensitivity of IgM-ELISA was found to be 95% and 87% respectively and for IgG-ELISA 92% and 76% respectively in CSF using a cyst antigen derived from the bladder wall.

Higher levels of sensitivity and reproducibility was achieved using cystic vesicular fluid as source of stable and reliable antigen in the detection of anticysticercal antibodies in human sera of cysticercosis patients in contrast to partially purified or the whole cyst sonified antigens by Larralde et al. (1986).

Recently, a significant amount of specificity (100%) was obtained with ELISA using antigens purified on a immunoaffinity column tagged with monoclonal antibodies produced to scolex protein antigens (Nascimento et al., 1987). A 100% sensitivity and specificity was achieved by ELISA using antigens purified with monoclonal antibodies SP 80, SP 10a and SP 10b when compared to 91% sensitivity with crude scolex protein antigens.
For the first time, an ELISA was evaluated using antigens prepared from morphological parts of the C. cellulosae (viz., fluid, scolex and wall). The results of this study indicated variability in serological responses to different antigens of cysticerci and fluid antigen had better discriminating power between reactive and non-reactive sera (Baily et al., 1988).

Immunofluorescence (IF)

Reports on application of indirect immunofluorescence antibody assay has not been successful and appeared to be subjective and variable in immunodiagnosis of NCC (Rydeniski et al., 1975).

Radioimmunoassay

Miller and his colleagues (1984), for the first time performed RIA using a cysticercus antigen obtained from CDC, Atlanta, Georgia. The nature of antigen used was not mentioned. The diagnostic application of RIA was found to be limited and RIA has its own peculiar combination of sensitivity and specificity.

On comparison of RIA with ELISA, both tests seems to have good correlation \( r = 0.96; \ P < 0.001 \). ELISA found to have similar sensitivity and specificity in the immunodiagnosis of NCC (Mohammad, et al., 1984).