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NEW APPROACHES IN THE DIAGNOSIS OF *TAENIA SOLIUM* CYSTICERCOSIS AND TAENIASIS

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SUMMARY

Taenia solium cysticercosis is now recognized as a priority in Mexico and a number of other developing countries, both in public health and in economic terms. Recognition of the problem has been greatly aided in recent years by new developments in molecular diagnostics. In this paper data are presented on ELISA for the detection of anti-cysticercus antibodies and of parasite antigens in patients with neurocysticercosis and in cysticercotic pigs. Also, several biological fluids were evaluated: cerebrospinal fluid (CSF), serum and saliva, all of which have proved useful. CSF is, however, the most appropriate for detection of antibodies and antigens in patients and serum in pigs. In addition, saliva may be especially used in epidemiological surveys. The electroimmunotransfer blot technique (EITB) for antibody detection in patients and pigs has also proved highly sensitive and due to the use of an enriched fraction of glucotproteins, EITB is also highly specific. Cloned cDNA sequences from *T. solium* are now being assessed as an alternative source of antigens for immunodiagnosis.

Two methods for the diagnosis of the adult stage of *T. solium* are also undergoing standardization. These are an ELISA for the detection of parasite antigens in fecal samples and DNA hybridization techniques for the detection of eggs in stools. Both assays have promising results and should now be assessed in larger numbers of clinical and epidemiological samples.

RÉSUMÉ : **Nouvelles approches pour le diagnostic de la cysticercose et du teniasis à *Taenia solium*.**

La cysticercose à *Taenia solium,* est aujourd'hui reconnue comme une priorité au Mexique et dans quelques autres pays en voie de développement, au point de vue sanitaire aussi bien qu'économique. L'identification de ce problème a été grandement facilitée pendant les dernières années par le récent développement du diagnostic moléculaire. Dans ce travail, sont présentés les résultats basés sur la technique d'ELISA pour la détection d'anticorps anticysticerque et d'antigènes parasitaires chez des malades et des porcs atteints de cysticercose. On a aussi étudié plusieurs liquides biologiques : le liquide cérébrospinal (LCS), le sérum et la salive; ils se sont tous révélés positifs. Chez l'homme c'est le LCS qui est cependant le plus approprié pour la détection d'anticorps et d'antigènes et chez le porc c'est le sérum; de plus, la salive peut être spécialement utilisée pour les études épidémiologiques. La technique de blot électroimmunotransfert (EITB) pour la détection d'anticorps chez l'homme et le porc s'est montrée également très sensible, et par l'utilisation d'une fraction enrichie en glycoprotéines, l'EITB est même spécifique. Des séquences de cDNA clônées de *T. solium* sont actuellement à l'essai comme une possible source d'antigènes pour l'immunodiagnostic.

Deux méthodes pour le diagnostic du stade adulte de *T. solium* sont en voie de réalisation. Ce sont un ELISA pour la détection d'antigènes parasitaires dans les selles et une technique d'hybridation de DNA pour la détection d'œufs dans les matières fécales. Les deux techniques ont donné des résultats prometteurs et devraient maintenant être essayées à grande échelle pour des travaux cliniques et épidémiologiques.

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Neurocysticercosis

Neurocysticercosis is a parasitic disease caused by the larval stage of the cestode *Taenia solium*. It is common in many underdeveloped countries of Latin America, Asia and Africa (Mahajan, 1982). *Taenia* eggs are probably ingested in environments where an individual carrying the intestinal tapeworn lives, as well as with contaminated food. Eggs hatch in the digestive tract, embryos penetrate the mucosa, circulate and develop into cysticerci in several locations, mainly subcutaneous tissue, muscle, eye and the central nervous system (CNS) (Schmidt and Roberts, 1977). Neurocysticercosis may be a serious long lasting disease or an acute and sometimes fatal problem. Also, up to 80 % of asymptomatic cases have been reported in autopsy findings (Rabiela *et al.*, 1979). Symptomatology is non-specific in the CNS where it is associated mainly with endocraneous hypertension and convulsive crisis (Sotelo *et al.*, 1985). Depending upon the number, location and state of

the parasites in the brain, treatment is based on cestocidal drugs (praziquantel or albendazole), symptomatic drugs (steroids or anticonvulsants), or surgery in order to excise the cysts or to drain the cerebrospinal fluid to the peritoneum through a Hakim or Pudenz Shunt (Flisser, 1989; Madrazo *et al.,* 1983; Sotelo *et al.,* 1984).

Patients with neurocysticercosis do not participate in the maintenance of the life cycle and thus are accidental intermediate hosts. Nevertheless, neurocysticercosis is a public health problem in many countries. The natural intermediate host is the pig which acquires cysticercosis because it ingests human feces contaminated with *T. solium* eggs.

The life cycle is perpetuated when human beings ingest raw or undercooked pork meat harboring a living cysticercus; the parasite evaginates in the digestive tract and develops into an adult tapeworn. Around 12 weeks later, the carrier starts to release gravid proglottids, each one containing approximately 60,000 eggs. Individuals may harbour a *Taenia* for over 20 years and contaminate continuously the environment.

In contrast to neurocysticercosis, swine cysticercosis and human taeniasis are usually asymptomatic diseases. While several methods have been standardized and applied in diagnosis of neurocysticercosis, detection of swine cysticercosis is currently by sanitary inspection. Diagnosis of human taeniasis is by coproparasitoscopic techniques. Both methods have low sensitivity and the latter also lacks specificity (Gemmel *et al.,* 1983).

The purpose of this presentation is to describe the immunodiagnostic methods we use for human cysticercosis as well as some alternatives for diagnosis of swine cysticercosis and human taeniasis.

Immunodiagnosis of human cysticercosis

We standarized an enzyme linked immunosorbent assay (ELISA) for antibody detection. As antigen source a crude extract of swine cysticerci or purified antigen B (Guerra *et al.,* 1982) are used; the latter is the most frequently recognized antigen by the sera of patients with neurocysticercosis (Flisser *et al.,* 1980). As second antibody, a conjugate of anti-human IgG coupled to alkaline phosphatase is used (Espinoza *et al.,* 1986). Antibodies are detected in serum and cerebrospinal fluid (CSF) and the sensitivity of the assay is 80 % in serum samples and 90 % in CSF. This assay has now been used in routine work as a support to clinical diagnosis for several years. With the crude extract there is cross reactivity with serum samples from patients with other parasitic diseases but such cross-reactions are decreased when antigen B is used (Espinoza *et al.,* 1986). In countries where hydatid disease is also found, a positive serum result in ELISA will only indicate a non-specific cestode infection and final diagnosis has to be confirmed by other methodologies and clinical symptomatology. A positive CSF result is considered confirmatory in patients with neurological symptomatology (Alarcon de Noya *et al.,* 1989). As an alternative source of highly specific antigen, several putatively specific cDNA sequences from *T. solium* have been cloned in phage lambda gt11 and expressed in *E. coli.* These were selected by differential screening with antibodies from human cestodiases cases (McManus *et al.,* 1990). The clones are currently under study to assess their diagnostic value. One of the six lysogenic clones assayed to date, reacts with pools of sera and CSF from patients with neurocysticercosis. This and the other expressed peptides will be tested shortly using individual sera and CSF for sensitivity.

Recently we standarized the detection of anti-cysticercus IgG in human saliva, since IgG is present in saliva because it transudates from serum by the gingival spaces (Challacombe, 1980). We searched for antibodies in saliva and in serum by the electroimmunotransfer blot (EITB) developed at the CDC with *T. solium*-specific glycoprotein antigens (Tsang *et al.,* 1989) and we compared this assay with ELISA. EITB gave 100 % sensitivity in serum and 70 % in saliva while ELISA gave 82 % sensitivity in saliva and only 74 % in serum (Feldman *et al.,* in press). These differences are probably due to the serum and saliva dilution used and the source of antigen. Saliva* is always used undiluted, while serum is diluted 1: 100 for EITB and 1: 2,000 for ELISA. A crude parasite extract is used in ELISA while in EITB a purified fraction of glycoproteins is employed.

An alternative for immunodiagnosis of human cysticercosis is the detection of secretory/excretory parasite molecules which, when detected, confirm the presence of the parasite. We standarized a double antibody capture ELISA with monoclonal or polyclonal antibodies against *Taenia* antigens for detection of parasite products in human CSF. These assays gave up to 72 % sensitivity (Correa *et al.,* 1989). When antigen B was searched in CSF only 12 % of positive cases were found, probably because antigen B, in the CSF, forms immune complexes.

Immunodiagnosis of swine cysticercosis

No ante-mortem immunological techniques are used for the diagnosis of swine cysticercosis. In our experience, ELISA with a crude extract was less sensitive with pig serum than with human serum, whereas EITB with *T. solium*-specific glycoprotein antigens was 100 % sensitive and 100 % specific in pig sera (Torres, 1990).

Since the presence of parasite antigens unambiguously demonstrates a current infection, an ELISA for antigen detection was standardized with the use of monoclonal antibodies directed against a surface/secretion glycoprotein of *T. saginata* (Harrison *et al.,* 1989); 79 % positivity prior to cestocidal treatment and 91 % positive pig serum samples after praziquantel treatment were obtained (Rodriguez-del-Rosal *et al.,* 1989).

The next step is to evaluate the feasibility of performing

* Saliva is an accessible body fluid, safer to handle and offers a non-invasive, painless sampling procedure, which might be specially useful for epidemiological surveys.

immunodiagnosis in the serum of pigs sent to the abattoir in order to demonstrate the usefulness of either assay in routine ante-mortem pig inspection. Pigs positive for cysticercosis can then be treated with a cestocidal drug and cured (Torres, 1990) in order to avoid condemnation of infected pork meat.

Diagnosis of human taeniasis

Two approaches have been followed for the diagnosis of human taeniasis. Firstly detection of parasite specific antigen in host faeces. Initially *Hymenolepis diminuta* in the rat was used as a model (Allan and Craig, 1989). Antigen levels started to rise before patency and cleared rapidly post treatment with praziquantel.

When a similar test was developed for human taeniasis *T. solium* and *T. saginata* samples were cross reactive using polyclonal rabbit antisera but no cross reaction was shown with any other gut helminths including *Hymenolepis nana* (Allan *et al.,* 1990). Antigen levels have been shown to be present in the absence of eggs and to reach a plateau several weeks before the onset of patency in *Taenia pisiformis* infections of dogs (Kinder *et al.,* unpublished observations).

A second approach is the detection of eggs in feces using DNA hybridization techniques. Total genomic *T. solium*, *T. saginata* and *T. pisiformis* DNA were used as probes and hybridized with eggs or DNA of the three species dotted onto nitrocellulose membranes in separate wells. Encouraging results were obtained because one egg could be detected in the *T. saginata* system and no cross-reaction between *T. saginata* and the *T. pisiformis* eggs or DNA occurred (Flisser *et al.,* 1988). Specificity was also found in the reaction of th *T. saginata* probe with *T. solium* eggs or DNA but no specificity could be obtained when *T. solium* DNA was used as a probe. Specificity may be related to the number of repetitive DNA sequences in both species. The presence of repetitive sequences was tested by restriction enzyme analysis of DNA samples from both *Taenia saginata* and *Taenia solium*. One to three repetitive fragments ranging in size from about 2-20 Kb were detected in *T. saginata* DNA digested with 5 of the 7 enzymes tested. In contrast, in order to observe repetitive fragments in *T. solium* DNA it was necessary to digest a larger quantity of DNA. These results are consistent with a higher number of repetitive DNA sequences in *T. saginata* which may account for the differential specificity in the egg assay (Vallejo, 1990).

In conclusion, antibody detection by ELISA for clinical cases of neurocysticercosis is a useful diagnostic aid mainly to corroborate computed tomography or magnetic resonance findings or to support diagnosis when this equipment is not available. Serum, CSF or even saliva are adecuate samples for the detection of anti-cysticercus IgG. EITB performed with *T. solium* specific glycoprotein antigens is very useful for this purpose due to its high specificity and sensitivity, although it is a more sophisticated technique. Recombinant proteins are under study as an alternative source of antigens. Antigen detection in human and swine cysticercosis is a novel method for diagnosis but the approach has still to be assessed using large numbers of samples. Techniques for the diagnosis of human taeniasis are being developed and should be further assessed in feces obtained from clinical cases and epidemiological surveys.

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