INTRODUCTION

The consumption of infective eggs of Taenia solium causes an initiation of an oncosphere and further develops into cysticerci in the human body. When the oncosphere invades the brain, it causes neurocysticercosis (NCC). Neurocysticercosis is a major public health problem and occurs in most Latin American, and African countries (White, 2000). Migration of people and their pigs is a possible cause of the spread of neurocysticercosis into new areas. An increased number of epileptic seizures, possibly caused by neurocysticercosis, was observed in Irian Jaya (Subianto et al., 1978; Wandra et al., 2000). Neurocysticercosis had been reported in industrialized countries such as Australia, the United States, France, etc, because of people returning from endemic countries and immigrants (Walber et al., 1991; Schantz et al., 1992; Rousseau et al., 1999; Roman et al., 2000). The increase in detected cases results in part from good technology, such as high quality instruments and immunological methods.

In retrospective studies of neurocysticercosis in Thailand from 1979-1988, ninety-eight cases were found at Prasat Neurological Hospital. There was a trend of increasing numbers of cases detected due to the use of CT scan (Jitsukon and Towanabut, 1989). However, CT scan is not available in most hospitals. Up until now, only a few cases of neurocysticercosis have been reported.

Immunodiagnosis of diseases is designed to prove infection. The process is dependent on the antigens used, availability of homologous samples of both serum and cerebrospinal fluid for the test, and the serological techniques performed. Different degrees of cross-reaction are observed with...
a number of diverse heterologous serum samples. The varying antibody levels of infected patients are factors in neurocysticercosis serodiagnosis. This may be due to the anatomical position of cysts in the brain, number and forms of cysts, such as, the vesicular form corresponding to live parenchymal larvae, cystic form corresponding to dying parasitic larvae, and parenchymal calcified cysts, etc (Zini et al 1990; Wilson et al 1991; Simac et al 1995).

A critical point, in immunodiagnostic techniques, is to have a good antigen. Several kinds of antigens are derived from adult worms, whole metacestodes or cystic fluid of *T. solium* (Coker-Vann et al, 1984; Baily et al, 1988; Tsang et al, 1989; Ito et al, 1998). Antigens derived from other species of *Taenia* have been used to detect the antibody of neurocysticercosis (Morakote et al, 1992; Vaz et al, 1997; Bueno et al, 2000).

An unwanted part of a crude antigen can be eliminated by deglycosylation and delipidization, which may increase the sensitivity and specificity of a test. Delipidization of the extract of a crude antigen for *T. solium* metacestodes was found by using 3M KCl solution. This chemical solution caused a reduction in antigenicity, decreasing the sensitivity of the test to an unacceptable level (Flisser et al, 1980; Plancarte et al, 1994). In contrast, a preliminary comparison of antigenicity was done between ether-delipidized and non-delipidized *T. solium* metacestode antigens and serum samples of neurocysticercosis (Morakote et al, 1992; Vaz et al, 1997; Bueno et al, 2000).

Due to non-specific symptoms and the variation of antibody responses to active and inactive forms of cysts, specific tests should be developed, by using different preparations of antigens, which react with a large number of cysticercosis and neurocysticercosis cases, as well as with heterologous samples. The infection should be distinguished from other neurological diseases by using both serological and non-serological techniques. This study aimed at providing ether-delipidized antigens of *T. solium* metacestodes for the serodiagnosis of neurocysticercosis. The antigens were fractionated by Sephadryl S-200 gel chromatography and by further molecular weight cut-off techniques. First, the effects of ether on the antigen was observed by comparing the OD-ELISA results of the ether-delipidized and non-delipidized extracts. Second, an evaluation of the fractionated antigens of both techniques was performed by indirect ELISA.

**MATERIALS AND METHODS**

**Serum samples**

Two hundred and thirty-two human sera of neurocysticercosis and other infections were categorized in this study. Neurocysticercosis cases were diagnosed by a combination of computerized axial tomography (CAT) brain scans, soft tissue surveys, clinical syndromes, and biopsies. The serum samples of neurocysticercosis were from seven Thai patients from the Hospital for Tropical Diseases, Bangkok, one Thai case from Prasat Neurological Hospital, one case of a Myanmar patient from a refugee camp in Thailand, and 16 cases from the Service de Parasitologie et Mycologie, Groupe Hospitalier Pitié-Salpêtrière, France. An extra case was kindly provided by Professor Akira Ito, Asahikawa Medical College, Japan.

Twenty-four diseases from 177 cases were diagnosed either by parasitological examinations (Kato’s thick smear, fecal culture or direct technique), clinical manifestations, imaging techniques and/or serodiagnosis. The majority of the serum samples were deposited at the Department of Helminthology, Faculty of Tropical Medicine, *ie*, cystic echinococcosis, taeniasis, sparganosis, hymenolepiasis nana, gnathostomiasis, capillarisis, hookworm infections, strongyloidiasis, trichinellosis, toxocariasis, bancrofti filariasis, angiostrongylia, ascariasis, trichuriasis, paragonimiasis heterotremus, opisthorchiasis, schistosomiasis, creeping eruption (negative for hookworm infection and strongyloidiasis), blastocystosis, HIV, and lung infections (negative for eggs and adult worms of *Paragonimus*) in sputum and feces). Other serum samples were provided by colleagues from many countries: *T. solium* metacestodes

**Taenia solium metacestodes**

Eight hundred cysts of *T. solium* were ob-
tained from a naturally infected pig. Cysts were separated from debris under a stereomicroscope and washed with PBS, pH 7.4 containing 0.02% sodium azide solution. Four kinds of antigens were prepared using the procedures described below.

**Non-delipidized antigen (NDLPAg)**

Cysts were ground with PBS, pH 7.4 containing enzyme inhibitors [0.1 mM phenylmethylsulphonylfluoride (PMSF) and 0.1 mM N-tosyl-L-phenylalanin chlomethylketone (TPCK)] and alumina paste. The process was done in an iced box. The homogenate was further sonicated by probe No. 419B, magnification No. 4 (Sonicator Ultrasonic Processor XL), at 1-minute intervals for 30 minutes. The supernatant was collected after centrifuging at 45,000 g for 2 hours at 4ºC (High speed centrifuge, HITACHI) and dialysed against distilled water containing 0.02% sodium azide. The Coomassie Plus Protein Assay Reagent Kit (Pierce, USA) determined total protein content.

**Delipidized antigen (DLPAg)**

An equal volume of NDLP Ag and cold ether were vigorously mixed and then swirled on a rotating shaker for a 15-minutes interval. The suspension was subjected to ether evaporation by simple airing. The supernatant was then obtained by centrifugation at 45,000g for 2 hours, at 4ºC. The protein content was determined as above.

**Gel chromatographic antigen**

A partially purified antigen was prepared from the separation of DLPAg through a Sephacryl S-200 column. The gel bed was packed to 80 cm in a 100-cm column. The packed gel was presaturated with the liquid phase (PBS, pH 7.4) at a flow rate of 1x and speed number 9 for 120 drops of PBS per fraction. This step was carried out for 1 day before loading the antigen sample. Twenty-five milligrams of antigen were passed into Sephacryl S-200 in the same manner as above. The contents of each fraction were assayed against PBS by a spectrophotometer (UV-160A, Shimadzu) at 280 nm. The optical density of a fraction was plotted to obtain a peak of fractions by a Power Point program. All fractionated antigens of one peak were pooled, lyophilized and then reconstituted with a small volume of distilled water and dialyzed against distilled water containing 0.02% NaN₃, at 4ºC for 24 hours with 2 changes of distilled water. Each peak was assayed for its protein content. All peaks were tested by the indirect ELISA system. The DLPP1Ag was decided as a promising peak after indirect ELISA.

**Molecular weight cut-off antigen (MWCOAg)**

The cross-reactivity was observed between the antigen of the DLPP1Ag with cystic echinococcosis and that of those sera which frequently react with many kinds of antigens derived from both whole cysts and the cystic fluid of *T. solium* metacestodes. The DLPP1Ag was then separated by multi-well 4-20% gradient gel (Tefco, Tokyo, Japan), and transferred onto a PVDF membrane. The individual blot was reacted with 1:50 diluted sera of neurocysticercosis, cystic echinococcosis, and a healthy control, which were provided by Professor Akira Ito. The reaction was again performed with casein buffer, and with a secondary antibody (anti-human IgG, 1:1,000). The immuno-reaction was treated with a substrate solution, 4-chloro-1-naphthol (Ito et al., 1993). From Fig 1, it was found that the DLPP1Ag lower than 33.3 kDa (compared with prestained LMW protein standards, Bio-Rad) gave a stronger reactivity to the neurocysticercosis serum than those of cystic echinococcosis and healthy controls. Some reactions of cystic echinococcosis and normal controls were seen at lower MW than 19.4 kDa (standard). Serum antibodies of neurocysticercosis and cystic echinococcosis strongly cross-reacted with the antigen > 33.3-105 kDa of the LMW standards. The DLPP1Ag (650 mg) was filtered through an Amicon (PM10, cut-off 10 kDa) to separate out those with lower MWs than 10 kDa. The filtrate was filtered by Ultrafree-4 Centrifugal Filter Units (containing a cut-off of 30 kDa, Millipore) at 2,000 g for 20 minutes. The non-filtrate was added up to 300 µl with distilled water, mixed by micropipette and recentrifuged as above. This step was repeated two times. All filtrates were pooled together, and then concentrated by the miniplus (cut-off 10 kDa membrane, Amicon). The antigen, MWCOPIAg (<30-10> kDa) was assayed for protein content and its antigenicity by indirect ELISA.

**Antibody detection by indirect ELISA**

Microelisa-wells (Nunc) were sensitized with the diluted antigen in carbonate-bicarbonate buffer, pH 9.6 at 37ºC overnight. The unbound antigens were eliminated with a washing solu-
tion (0.05% Tween20-PBS). Coating the unbinding sites of wells was done by 1% bovine serum albumin. Test serum samples were diluted with a washing solution containing 0.02% NaN₃-0.008% bromphenol blue and put in triplicate wells. The immune complexes were then combined with a diluted rabbit anti-human immunoglobulin G peroxidase, Dakopatt) in a washing solution. The reactions were visualized with substrate (ABTS, Sigma) after a 30-minute incubation and added to 1%SDS to stop the reaction. Optical density values were measured at 405 nm.

In the study of the effect of ether, comparing the antigenicity between NDLPAg and DLPAg, the same conditions of the test were performed: 2.5 µg/ml of antigen concentration, 1:400 serum dilution and 1:2,000 secondary antibody dilution, which is reduced to half the strength of previous research (Dekumyoy et al, 1998). The optimal antigen (DLPP1Ag) concentration, conjugate and serum dilutions were found to be 5 µg/ml, 1:2,000 and 1:400, respectively. The MWCO1Ag used 2 µg/ml, a serum dilution of 1:200 and secondary antibody of 1:2,000.

**RESULTS**

**Comparison of the NDLPAg and DLPAg by IgG-ELISA**

Both the NDLPAg and DLPAg reacted with all sera of neurocysticercosis and other helminthic serum samples in comparing of their antigenicity. Based on the ELISA-histogram, the DLPAg showed superior antigenicity to the NDLPAg as observed by the higher OD values and mean OD values of the DLPAg (Fig 2).

**Gel chromatographic antigens**

The separation of DLPAg through the Sephacryl S-200 column obtained 3 main peaks (Fig 3). The first peak (DLPP1Ag) showed a good discrimination between ODs of diluted pooled serum controls of positive and negative sera after checkerboard titration. The P2 and P3 antigens could not discriminate ODs from the controls.

**Evaluation of the DLPP1Ag and MWCO P1Ag for the detection of neurocysticercosis**

The DLPP1Ag determined the negative-positive discriminating threshold by ELISA and the threshold value was \( X(0.140) + 7SD(0.259) = 0.399 \). Thirteen of the 25 neurocysticercosis cases gave higher absorbance values than the cut-off value. Evaluation of the diagnostic sensitivity yielded 52%, while the specificity was at 91.8%. Cross-reactivity among heterologous serum samples (n = 177) was calculated to be 9.6% (17/177) at the threshold value (Fig 4a). The optical density values of the neurocysticercosis samples were compared with those of healthy controls and of heterologous serum samples by the \( t \)-test. The absorbance data of neurocysticercosis were significantly higher than those of both kinds of serum samples (p < 0.001). False positives occurred in 17 cases among eight kinds of helminthic infections (Table 1).

Due to the high number and high ODs of cystic echinococcosis cases which cross-reacted with the DLPP1Ag, the MWCO1Ag was provided to eliminate the cross-reactive molecules with heterologous sera. The cut-off value was 0.264, which could discriminate the negative-positive threshold at \( X(0.144) + 4SD(0.120) \). Twenty-two of 25 neurocysticercosis cases gave higher optical density values than the cut-off value (Fig 4b). This value gave 88% sensitivity for
Cysticercosis: Evaluations of DLPP1Ag by IgG-ELISA

Comparison of cross-reactivity of the DLPP1Ag and MWCO P1Ag

The DLPP1Ag and MWCO P1Ag gave false positives with 17 and 9 sera of eight and of six heterologous diseases, respectively. Five sera of four diseases, which previously cross-reacted with the DLPP1Ag became true negatives with the MWCO P1Ag: angiostrongyliasis, strongyloidiasis, taeniasis, opisthorchiasis, and paragonimiasis heterotremus. It was found that three diseases: schistosomiasis, toxocariasis and trichinellosis, became false positives with the antigen. Only two cases of cystic echinococcosis cross-reacted with this antigen, however, the ODs were near the cut-off value of 0.264 except fascioliasis (0.429) with MWCO P1Ag (Table 1).

DISCUSSION

In the previous studies on delipidization, ether-extracted Schistosoma japonicum antigens were studied by a group of Japanese researchers and all delipidized antigens showed high sensitivities for patients by serodiagnosis (Sato et al., 1970; Sawada et al., 1970). NaCl-delipidized extracts of T. solium and its cysticerci gave 77.6% sensitivity in 49 suspected cases of cysticercosis, but some cases of echinococcosis and schistosomiasis showed cross-reactivity (Arambulo et al., 1978). Using freon-delipidization, T. solium cysticerci extract was prepared and concentrated, compared with those of healthy controls and of heterologous serum samples by t-test. The optical density data of neurocysticercosis were significantly higher than ODs of other of serum samples (p < 0.001).
but only glycoprotein antigens were tested by immunoblot (Tsang et al, 1989). The present study showed superior results for DLPPAg compared to those of NDLPPAg by ELISA-histogram. Though healthy controls also increased their ODs-ELISA, compared with the NDLPPAg, a sephacryl-purified DLPP1Ag showed low sensitivity (12/25; 52%) of ELISA at holding high specificity (91.8%). Thirteen cases of cysticercosis gave false negative reactions. Seventeen cases of eight diseases cross-reacted with DLPP1Ag. Seven cases of cystic echinococcosis gave high ODs. These results indicated that many cross-reactive molecules of DLPP1Ag reacted with antibodies of these echinococcosis cases. By immunoblot, this antigen performed strong cross-reaction of serum antibody of cystic echinococcosis at high reactive molecules of over 33.3 kDa. This evidence encouraged ELISA-false positive of echinococcosis.

The DLPP1Ag was therefore eliminated this cross-reactive part, and MWCOP1Ag (<30-10> kDa) obtained. Nine cases of six heterologous diseases gave false positive results with the antigen by 0.264 of cut-off value. The MWCOP1Ag reduced numbers of ECC cases and ODs. Two cases (one case of Thai patient infected while working in the Middle East and another obtained from France) cross-reacted with the antigen. False positives found at ODs of 0.269 and 0.281, but the ODs were close to the cut-off value, 0.264. It is difficult to eliminate cross-reactions with both crude and some purified cysticercus antigens, frequently occur with anti-sera from echinococcosis cases as proven in many other studies (Coker-Vann et al, 1984; Moro et al, 1992; McManus and Leggatt, 1993; Retamal et al, 1995). In contrast to isoelectric focusing technique, the fractionated antigens from T. solium cysticerci were highly specific and sensitive for the serodiagnosis of neurocysticercosis, especially in differentiating from alveolar or cystic echinococcosis by ELISA. This antigen contained three main antigenic bands (10-26 kDa) with some minor antigens (Ito et al, 1998). Our
mass antigen was lower than 30 kDa due to the cut-off filter membrane. The antigen reduced the numbers of false positive but it still contained antigenic impurity. When using the DLPP1Ag, one each of angiostrongyliasis, strongyloidiasis, taeniasis, and two of paragonimiasis heterotremus were false positives. Those cases became true negatives when reacted with the MWCOP1Ag. The MWCOP1Ag initially induced ODs of one each of schistosomiasis (0.300) and toxocariasis (0.286), and two of trichinellosis (0.295, 0.306) becomes false positives. Fascioliasis was reduced from two to one false positives case, which showed its OD was lower than those with the DLPP1Ag.

The interpretation of ELISA results by sensitivities was as follows: 79% and 61% sensitivities from reactions between PBS-extracted metaccestodes and NCC sera from Mexico and from Irian Jaya, respectively (Diwan et al, 1982), 70% sensitivity (Coker-Vann et al, 1984) and 65% for Diaz et al (1992). The purified antigens from the metaccestodes also gave vary sensitivities, such as 73% by using purified antigen B (Espinoza et al, 1982) and 80% from the partially purified chromatography antigen (Coker-Vann et al, 1984). With recent antigen preparation techniques, recombinant antigens can be produced in unlimited materials eg, from cDNA of T. solium called Ag1V1/Ag2 chimeric protein, which was 89.7% positive to NCC and was not positive to other parasitic infections (cystic and alveolar echinococcosis, clonorchiasis, sparganosis, fascioliasis, paragonimiasis and schistosomiasis) (Sako et al, 2000). A recognized recombinant antigen, called stS15, produced from the cDNA of T. solium. It reacted with a majority of sera from patients with cysticercosis (53%) but did not react with other helminth infections (cystic and alveolar echinococcosis, schistosomiasis, filariasis, trichinellosis, ascariasis, dracunculiasis) or normal controls (Green et al, 2000). By literatures, antigens produced from T. solium, other Taenia spp and recombinant antigens can induce high sensitivity and specificity but not 100% of both evaluations. The false negatives of neurocysticercosis may be an affect of the inactive form [low (1-2) calcified cysts] that have low levels of antibody (Zini et al, 1990; Wilson et al, 1991). Neither ELISA nor immunoblot could detect a case of NCC where a histopathological examination revealed a cyst of T. solium (Ito et al, 1999; Ohhsaki et al, 1999). The presentation of neurocysticercosis on computed tomography showed a single, small enhancing lesion (SSEL’s) in 37 patients, but EITB showed positive results in only 18 patients.

<table>
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<tr>
<th>Diseases (No.)</th>
<th>Cystic echinococcosis (11)</th>
<th>Taeniasis (16)</th>
<th>Gnathostomiasis (8)</th>
<th>Strongyloidiasis (12)</th>
<th>Trichinellosis (12)</th>
<th>Toxocariasis (5)</th>
<th>Angiostrongyliasis (12)</th>
<th>Opisthorchiasis (9)</th>
<th>Paragonimiasis heterotremus (15)</th>
<th>Schistosomiasis (6)</th>
<th>Fascioliasis (7)</th>
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<td>2</td>
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<td>1</td>
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<td>25</td>
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<td>0.295, 0.306</td>
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Table 1
Comparison of false positives (FP) between heterologous sera and antigens, the DLPP1Ag and MWCOP1Ag at their cut-off values, 0.399 and 0.264, respectively.
(48.64%) and ELISA showed positive in 21 cases (56.76%). This differed from multilesion neurocysticercosis, where EITB and ELISA showed 100% and 80% sensitivities, respectively. It was suggested that the low sensitivities in SSEL’s were probably due to an insufficient immune stimulation (Singh et al, 1999). The production of an effective antigen is needed for serodiagnosis of all types of neurocysticercosis. Cross-reactivity is an important factor in the establishment of a specific diagnosis. Many kinds of parasitic infections need to be included to determine the accuracy of a test.

The present study shows the MWCOP1Ag of T. solium metacestodes has a high sensitivity (22/25; 88%) and specificity (95.6%) by ELISA when compared with the DLPP1Ag. This antigen almost eliminates the cross-reactivity of cystic echinococcosis.

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