CYSTICERCOSIS : IgG-ELISA EVALUATIONS OF PEAK1 ANTIGEN AND <30 kDa ANTIGEN OF DELIPIDIZED EXTRACT OF *TAENIA SOLIUM* METACESTODES

Paron Dekumyoy[1], Jitra Waikagul[1], Sirivan Vanijanonta[2], Malinee Thairungroj[1], Minoru Nakao[3], Yasuhito Sako[3], Sonoyo Watanabe[3] and Akira Ito[3]

[1]Department of Helminthology, [2]Hospital for Tropical Diseases, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand; [3]Department of Parasitology, Asahikawa Medical College, Asahikawa, Japan

Abstract. The antigenicity of ether-delipidized *Taenia solium* metacestode extract (DLPAg) was investigated by IgG-ELISA. The antigen showed higher antigenicity than that of non-delipidized antigen (NDLPAg). Then the DLPAg was subjected to Sephacryl S-200 gel chromatography and a partially purified antigen (DLPP1Ag) was identified as the promised antigen by IgG-ELISA using 25 sera from cysticercosis cases, 177 cases of 24 heterologous infections, and healthy controls. Sensitivity was 52% and specificity was 91.8% at the cut-off value ($\overline{X}$+ 7SD), 0.399. Cross-reactivity occurred with 17 cases of eight diseases: cystic echinococcosis (7/11), taeniasis (1/16), gnathostomiasis (2/8), strongyloidiasis (1/12), angiostrongyliasis (1/12), paragonimiasis heterotremus (2/15), opisthorchiasis (1/9) and fascioliasis (2/7). When DLPP1Ag was fractionated through Ultra free centrifugal tube (retained 30 kDa) and Amicon (PM10), MWCOP1Ag (<30-10> kDa) was obtained; the antigen showed better results than DLPP1Ag with 88% sensitivity and 95.6% specificity at the cut-off value ($\overline{X}$ + 4SD), 0.264. Nine cases of six diseases cross-reacted with this antigen: cystic echinococcosis (2/11), gnathostomiasis (2/8), trichinellosis (2/12), toxocariasis (1/5), schistosomiasis (1/6), and fascioliasis (1/7). MWCOP1Ag gave higher sensitivity than that of DLPP1Ag but some cross-reactivity occurred.

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 reports.

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

Correspondence: Paron Dekumyoy, Department of Helminthology, Faculty of Tropical Medicine, Mahidol University, 420/6 Rajvithi Road, Bangkok 10400, Thailand.
Tel: +66 (0) 2354-9100 ext 1820; Fax : +66 (0) 2643-5600
E-mail: tmpdk@mahidol.ac.th

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 and healthy controls. The delipidized antigen gave better ODs-ELISA than those of the non-delipidized antigen (Dekumyoy *et al*, 1998).

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 Sephacryl 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, capillariasis, hookworm infections, strongyloidiasis, trichinellosis, toxocariasis, bancrofti filariasis, angiostrongyliasis, 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: cystic echinococcosis, onchocercosis, toxocariasis, fascioliasis, schistosomiasis, paragonimiasis westermani, and HIV. Thirty healthy controls, who were free of infection by examination using parasitological techniques. One extra sample was from Asahikawa Medical College, Japan.

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-phenylanin 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 NDLPAg 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 45,000*g* 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% NaN_3, 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, MWCOP1Ag (<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-

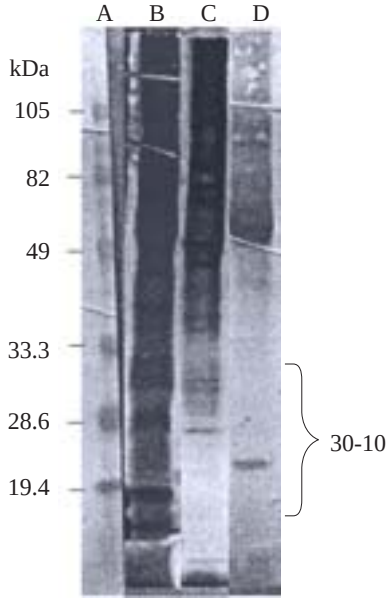

Fig 1–Reactions of the DLPP1Ag with the serum antibody of neurocysticercosis (B), cystic echinococcosis (C), and healthy controls (D). LMW standards are in lane A.

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% NaN_3-0.008% 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 MWCOP1Ag 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 $\bar{X}$ (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 MWCOP1Ag 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 $\bar{X}$ (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

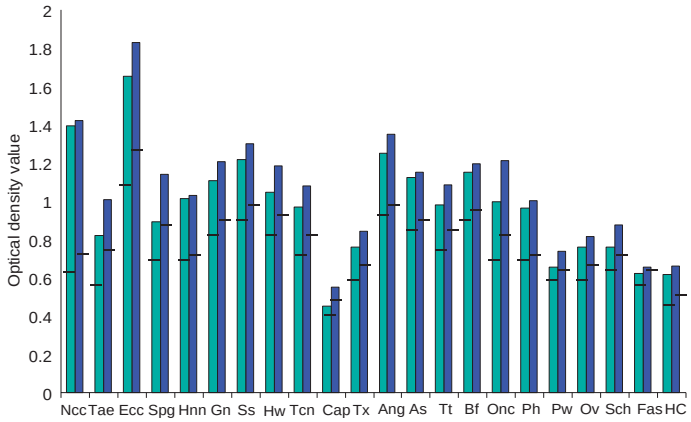

Fig 2–Histogram of antigenicity of *Taenia solium* metacestodes extract, NDLP and DLP, with antibodies of NCC, other helminthic infections and healthy controls by indirect ELISA. Serum samples were neurocysticercosis (Ncc), taeniasis (Tae), echinococcosis (Ecc), sparganosis (Spg), hymenolepiasis nana (Hnn), gnathostomiasis (Gn), strongyloidiasis (Ss), hookworm infection (Hw), trichinellosis (Tcn), capillariasis (Cap), toxocariasis (Tx), angiostrongyliasis (Ang), ascariasis (As), trichuriasis (Tt), *bancrofti* filariasis (Bf), onchocercosis (Onc), paragonimiasis heterotremus (Ph), paragonimiasis westermani (Pw), opisthorchiasis (Ov), schistosomiasis (Sch), fascioliasis (Fas) and healthy control (HC). An open block was NDLPAg and closed block was DLPAg. The mean OD presented as bar (▬).

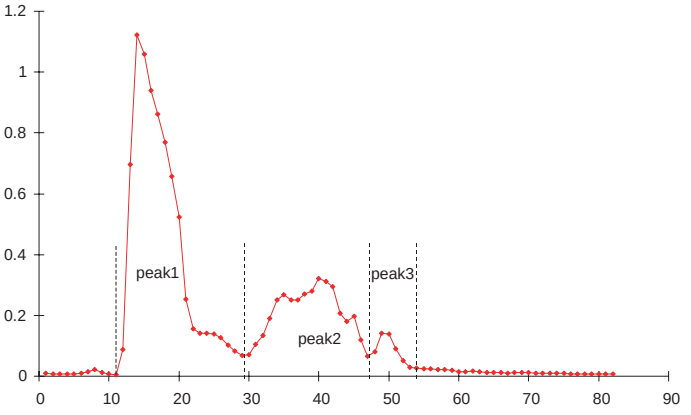

Fig 3–Profile of partial purification of delipidized antigen by Sephacryl S-200 gel chromatography, obtained three fractionated peaks, P1, P2 and P3.

neurocysticercosis cases and 95.6% specificity. Nine cases of six heterologous diseases showed false positive results (Table 1). The optical density values of neurocysticercosis samples were 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 ($p < 0.001$).

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 MWCOP1Ag: 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,

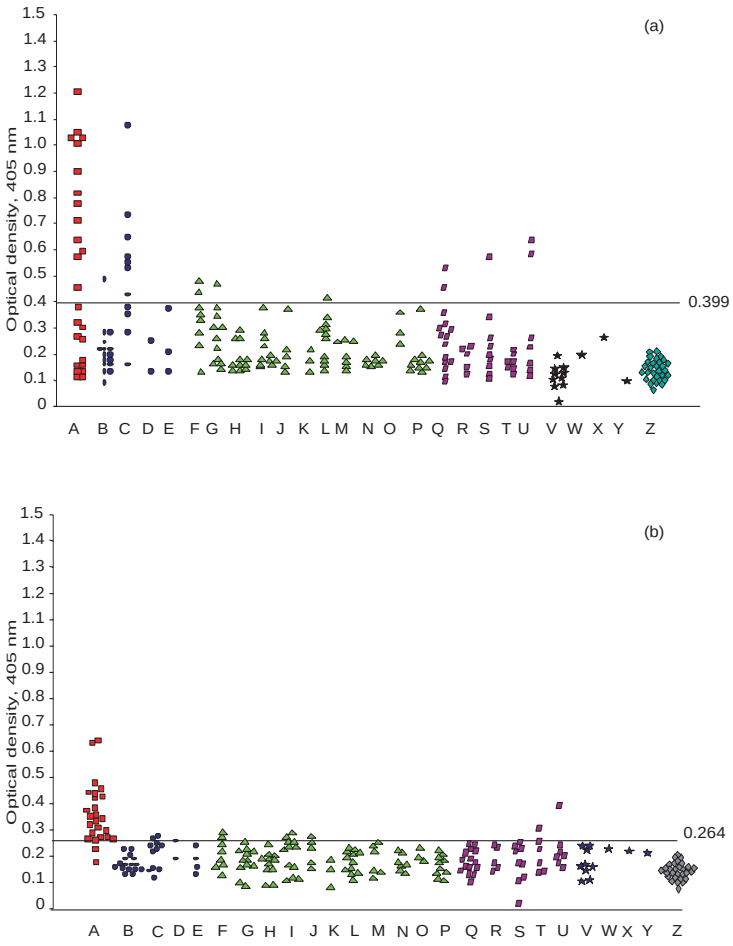

Fig 4–Scatter patterns of ELISA optical density using the DLPP1Ag (a) and MWCOP1Ag (b) against serum samples; A = neurocysticercosis, B = taeniasis, C = cystic echinococcosis, D = sparganosis, E = hymenolepiasis nana, F = gnathostomiasis, G = strongyloidiasis, H = hookworm infection, I = trichinellosis, J = toxocariasis, K = capillariasis, L = angiostrongyliasis, M = ascariasis, N = trichuriasis, O = onchocercosis, P = bancrofti filariasis, Q = paragonimiasis heterotremus, R = paragonimiasis westermani, S = opisthorchiasis, T = schistosomiasis, U = fascioliasis, V = HIV, W = creeping eruption, X = *Blastocytis hominis* infection, Y = other lung infection, Z = healthy serum controls.

but only glycoprotein antigens were tested by immunoblot (Tsang *et al*, 1989). The present study showed superior results for DLPAg compared to those of NDLPAg by ELISA-histogram. Though healthy controls also increased their ODs-ELISA, compared with the NDLPAg. 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 echniococcosis at high reactive molecules of over 33.3 kDa. This evidence encouraged ELISA-falsed positive of echinococosis.

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 serdiagnosis 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

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.

Diseases (No.)	IgG-ELISA					
	DLPP1Ag			MWCOP1Ag		
	FP	%FP	ODs	FP	%FP	ODs
Cystic echinococcosis (11)	7	63.64	0.417-1.097	2	18.18	0.269, 0.281
Taeniasis (16)	1	6.25	0.472	-	-	-
Gnathostomiasis (8)	2	25	0.431, 0.484	2	25	0.270, 0.299
Strongyloidiasis (12)	1	8.33	0.456	-	-	-
Trichinellosis (12)	-	-	-	2	16.67	0.295, 0.306
Toxocariasis (5)	-	-	-	1	20	0.286
Angiostrongyliasis (12)	1	8.33	0.420	-	-	-
Opisthorchiasis (9)	1	11.11	0.549	-	-	-
Paragonimiasis heterotremus (15)	2	13.33	0.424, 0.508	-	-	-
Schistosomiasis (6)	-	-	-	1	16.67	0.300
Fascioliasis (7)	2	28.57	0.478, 0.606	1	14.28	0.429
Total number	17			9		

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 metacestodes 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 metacestodes 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 (Greene *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; Ohsaki *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

(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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