RECENT ADVANCES IN SERODIAGNOSIS FOR CYSTICERCOSIS

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Abstract. Neurocysticercosis (NCC) caused by infection with the larval stage of *Taenia solium* is an important cause of neurological disease worldwide. Up to the present, many studies on characterizing species-specific antigens of *T. solium* have been done and several high quality antigens for serodiagnosis are available. Hence the research on serodiagnosis has been shifted to the next phase, stable production of diagnostic antigens using molecular techniques. In order to establish an enzyme-linked immunosorbent assay (ELISA) using recombinant proteins, we carried out molecular cloning and identified four diagnostic antigen candidates (Agl, AglV1, Ag2, and Ag2Vl). Recombinant proteins, except Ag2Vl, were successfully expressed using an *Escherichia coli* expression system. Immunoblot analysis using NCC patient sera detected recombinant proteins. But as reactivity to rAgl was too weak, Ag1 was not suitable for the immunodiagnosis antigen. Therefore Ag1V1 and Ag2 were chosen for ELISA antigens and AglVl/Ag2 chimeric protein was expressed. Of 49 serum samples from NCC patients confirmed to be seropositive by immunoblot analysis, 44 (89.7%) were positive by ELISA. Serum samples from patients with other parasitic infections did not recognized Ag1V1/ Ag2 chimeric protein. Ag1V1 / Ag2 chimeric protein obtained in this study is of value for differential immunodiagnosis.

INTRODUCTION

The larval stage of the pork tapeworm *Taenia solium* is responsible for cysticercosis. Humans are accidentally infected with *T. solium* by ingestion of eggs excreted with the feces of individuals harboring the adult tapeworm in the intestinal tract. The larvae migrate throughout the body, invade skeletal muscle, subcutaneous tissue, or the central nervous system causing neurocystlcercosis (NCC). This disease is crucial as an emerging disease in developing countries (Schantz *et al,* 1992, 1998; Craig *et al,*1996; Simanjuntak *et al,* 1997; White, 1997; Wandra *et al,* 2000).

Diagnosis of NCC has been based on clinical criteria, computed tomography (CT), and nuclear magnetic resonance imaging (MRI). The imaging techniques are useful and accurate for diagnosis but there is a possibility to overlook the infection when the number of parasites is few and/or the figures are not clear or not typical. Moreover these techniques are too expensive and inaccessible in most areas where NCC is endemic. Therefore, the development of immunodiagnosis test that detects species-specific antibodies (Gottstein *et al,* 1986; Larralde *et al,* 1986; Baily *et al,* 1988; Tsang *el at,* 1989; Sloan *el at,* 1995; Ito *et al,* 1998) or antigens (Brandt *et al,* 1992) either in sera or in cerebrospinal fluid (CSF) is urgently required. For these reasons, efforts have been directed toward characterizing species-specific antigens of *T. solium* metacestodes. The issue to be overcome is that antibody detection largely depends upon the quality of available antigens. Table 1 shows a brief history of research works for characterizing species-specific antigen. Gottstein *et al* (1986) reported the species-specific-antigens (8 and 26 kDa proteins) in crude extract of *T. solium* metacestodes. Parkhouse and Harrison (1987) described the glycopioteins in cyst fluids of *T. solium* and *Taenia saginata* using lentil-lectin affinity chromatography. Tsang *et al* (1989) (Centers for Disease Control and Prevention, Atlanta) characterized the glycoproteins in crude extract of metacestode using lentil-lectin affinity chromatography and described the usefulness of glycoproteins (seven glycoproteins ranged from 13 to 50 kDa) for differential serodiagnosis based on immunoblot analysis but not ELISA (Table 2). These immunodiagnostic antigens have been widely accepted for serodiagnostic purpose. Nevertheless due to the existence of cross-reactive components, these antigens were not applicable to ELISA system suitable for sero-epidemiological studies. Ito *et al* (1998) developed a simple method to purify diagnostic antigens (10-26 kDa antigens under reducing condition) by preparative isoelectric-focusing electrophoresis (IEFE) from cyst fluid available for both EL1SA and immunoblot analysis and demonstrated the sensitivity and specificity for differential serodiagnosis of NCC. These antigens were utilized for diagnosis of pigs (Ito *et al,* 1999c). Chung *et al* (1999) carried out the molecular

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Table 1
A brief summary of research works for characterizing species-specific antigens.

1986 Gottstein *et al*	Demonstration of species-specific and cross-reactive components of *Taenia solium* metacestode antigens.
1987 Parkhouse and Harrison	Cyst fluid and surface associated glycoprotein antigens of *Taenia* sp metacestodes.
1989 Tsang *et al*	An enzyme-linked immunoelectrotransfer blot assay and glycoprotein antigens for diagnosing human cysticercosis *(Taenia solium).*
1998 Ito *et al*	Novel antigens for neurocysticercosis: simple method for preparation and evaluation for serodiagnosis.
1999 Chung *et al*	A recombinant 10-kDa protein of *Taenia solium* metacestodes specific to active neurocysticercosis.
2000 Sako *et al*	Molecular characterization and diagnostic value of *Taenia solium* low-molecular-weight antigen genes.

Table 2
The comparison of immunodiagnostic antigens prepared at CDC[a] and Asahikawa Medical College.

Antigens	Method	Source	Immunoblot	ELISA
CDC[b]	Lentil-lectin affinity chromatography	cyst fluid + cyst tissue	yes	no[d]
Asahikawa[c] Medical College	Isoelectric focusing electrophoresis	cyst fluid	yes	yes

[a]: Centers for Disease Control and Prevention, Atlanta; [b]: Tsang *et al* (1989);
[c]: lto *et al* (1998); [d]: Contamination of cross-reactive components.

cloning of 10-kDa protein, homologue to immunodiagnostic antigen of *Taenia crassiceps* (Zarlenga *et al,* 1994), specific to active NCC.

At present, we can obtain the species-specific antigens from *T. solium* materials available for antibody detection methods. However preparation of serodiagnostic antigens is under many restrictions. We need to find naturally infected pigs or to maintain infected pigs, which is not practicable. So the next stage is the establishment of stable production system of stable production system of immunodiagnostic antigens using molecular techniques.

In this paper, we described our current work on molecular cloning of immunodiagnostic antigen genes and expression of recombinant proteins (Sako *et al,* 2000).

Cloning and characterization of diagnostic antigen candidate genes

To identify immunodiagnostic antigen genes, *T. solium* metacestode expression cDNA library was immunoscreened with sera from rabbits immunized with low-molecular-weight antigens as described by Ito *et al* (1998). Immunoscreening allowed the selection of two clones expressing protein epitopes recognized by immunized-rabbit sera and NCC patients sera. After DNA hybridization screening using a cDNA clone as a probe, four clones named Ag1, Ag1V1, Ag2, and Ag2Vl respectively, having full-length cDNA were isolated (Fig 1). These clones ranged from 325 to 415 bp in length and encoded polypeptides with 85 to 112 amino acids and with predicted molecular masses of 9.6 to 13 kDa. These clones showed 53-94% similarity at the amino acid level (Table 3). Putative *N*-linked glycosylation sites were found at positions 22,59 and 82 in Agl, and at positions 29 and 83.in Ag1Vl, but not in clones Ag2 and Ag2Vl. All clones had N-terminal hydrophobic regions, which were thought to be a signal sequence, and each signal sequence cleavage site was predicted by the method described by Nielsen *et al* (1997). After

Ag1

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          10         20         30         40         50         60
GAACAACCTGTAGAATGCGTGCCTACATTGTGCTTCTCGCACTCACTGTTTTCGCAGTGG
                   M  R  A  Y  I  V  L  L  A  L  T  V  F  A  V  A
          70         80         90         100        110        120
CGGTTTCGGCCGAGAAAAATAAAACGGATGGCGTTGGAAAGAGTATTAAGAATTGGATAG
 V  S  A  E  K  N  K  T  D  G  V  G  K  S  I  K  N  W  I  E
          130        140        150        160        170        180
AATTTGTCCACAGATTCTTCTACGAAGACCCAATTGGAAAACAAATTGCTCAACTCGCAA
 F  V  H  R  F  F  Y  E  D  P  I  G  K  Q  I  A  Q  L  A  K
          190        200        210        220        230        240
AGGACTGGAATGAAACAGCGCCGGAGGCTAGATGCAAAGTGCGGACGTTACTGGCTGAGA
 D  W  N  E  T  A  P  E  A  R  C  K  V  R  T  L  L  A  E  N
          250        260        270        280        290        300
ACCGCAGAGGTCTCAAGAACAAAACCGCTTAACTTGCCAACTTTTATGCGCTCTTCTCTT
 R  R  G  L  K  N  K  T  A
          310        320
CACGAATAAATGCTAATTAATGCTT
      ######
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Ag1V1

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          10         20         30         40         50         60
AGAATGCGTGTCTACATTGTGCTTCTCGCTCTCACTGTATTCGTAGTGGCGGTTTCGGCC
    M  R  V  Y  I  V  L  L  A  L  T  V  F  V  V  A  V  S  A
          70         80         90         100        110        120
GAGAAAAACAAACCGAAGTGTGATGGAAATAGTACTAAGAAAGAGATAGAATTTATCCAC
E  K  N  K  P  K  C  D  G  N  S  T  K  K  E  I  E  F  I  H
          130        140        150        160        170        180
AATTGGTTCTTCCACGATGACCCGATTGGAAATCAAATTGCTCAACTCGCAAAGGATTGG
N  W  F  F  H  D  D  P  I  G  N  Q  I  A  Q  L  A  K  D  W
          190        200        210        220        230        240
AAGGTAGCAATGCTGAAAGCCAAAGGCGAAATTCGGGCGTCACTGGCTGAGTACTGCAGA
K  V  A  M  L  K  A  K  G  E  I  R  A  S  L  A  E  Y  C  R
          250        260        270        280        290        300
GGTCTGAAGAACAAAACTGCTTAACTTGTCAACTTTCATGCGTTCTTCTCTTCACCAATA
G  L  K  N  K  T  A                                        ####
          310        320        330
AATGCTGATTAACAAAAAAAAAAAAAAA
##
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Ag2

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          10         20         30         40         50         60
AGAATGCGTGCCCACATTTTGCTTCTCGCTCTCAGTGTTTTCGTAGTGGCGGTGTCGGCC
    M  R  A  H  I  L  L  L  A  L  S  V  F  V  V  A  V  S  A
          70         80         90         100        110        120
AAGGAAACTAAACCAGAGGACGTGGTAAAGAATATTAAGAAAGGGATGGAAGTTGTCTAC
K  E  T  K  P  E  D  V  V  K  N  I  K  K  G  M  E  V  V  Y
          130        140        150        160        170        180
AAATTTTTCTACGAAGACCCGTTGGGAAAGAAAATAGCTCAACTCGCAAAGGACTGGAAG
K  F  F  Y  E  D  P  L  G  K  K  I  A  Q  L  A  K  D  W  K
          190        200        210        220        230        240
GAAGCAATGTTGGAAGCCAGAAGCAAAGTGCGGGCGTCACTGGCTGAGTACATCAGAGGT
E  A  M  L  E  A  R  S  K  V  R  A  S  L  A  E  Y  I  R  G
          250        260        270        280        290        300
CTCAAGAACGAAGCTGCTTAACTTGTCAACTTTCATGCGTTCTTCTCTTCACGAATAAAT
L  K  N  E  A  A                                        ######
          310        320
GCTCATTAATAAGAAAAAAAAAA
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Ag2V1

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          10         20         30         40         50         60
GTAGAATGCGTGCCCACATTTTGCTTCTCGCTCTCAGTGTTTTCGTAGTGGCGGTGTCGG
      M  R  A  H  I  L  L  L  A  L  S  V  F  V  V  A  V  S  A
          70         80         90         100        110        120
CCGAGTGGGTGCCCATTTCGAGGGTCTACATAGCCTCATGCAAGACCTACTACATGCTCC
  E  W  V  P  I  S  R  V  Y  I  A  S  C  K  T  Y  Y  M  L  Q
          130        140        150        160        170        180
AATTAAAACGCTTTTTTGCCTTTAGGGAAACTAAACCAGAGGACGTGGTAAAGAATATTA
 L  K  R  F  F  A  F  R  E  T  K  P  E  D  V  V  K  N  I  K
          190        200        210        220        230        240
AGAAAGGGATGGAAGTTGTCTACAAATTTTTCTACGAAGACCCGTTGGGAAAGAAAATAG
  K  G  M  E  V  V  Y  K  F  F  Y  E  D  P  L  G  K  K  I  A
          250        260        270        280        290        300
CTCAACTCGCAAAGGACTGGAAGGAAGCAATGTTGGAAGCCAGAAGCAAAGTGCGGGCGT
  Q  L  A  K  D  W  K  E  A  M  L  E  A  R  S  K  V  R  A  S
          310        320        330        340        350        360
CACTGGCTGAGTACATCAGAGGTCTCAAGAACGAAGCTGCTTAACTTGTCAACTTTCATG
 L  A  E  Y  I  R  G  L  K  N  E  A  A
          370        380        390        400        410
CGTTCTTCTCTTCACGAATAAATGCTCATTAATAAGAAAAAAAAAAAAAAAAAAA
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Fig 1- Nucleotide and amino acid sequences of four-antigen candidate clones. Features within the sequences are denoted by the following: the underlines at the N-terminal mark the putative signal sequence; N-glycosylation sites are boxed; cysteine residues are circled; amino acid sequence conserved among four clones is italicized; sharp marks indicated the putative polyadenylation signals.

Table 3
Amino acid sequence homologies (%) among four-antigen candidate clones.

	Ag1	Ag1V1	Ag2	Ag2V1
Ag1	-	61.6	62.8	60.5
Ag1V1		-	55.8	52.9
Ag2			-	94.2
Ag2V1				-

cleavage of the signal sequence, the predicted molecular mass of mature polypeptide ranged from 7.0 to 10.0 kDa. A sequence homology research revealed that all clones were related to the cysticercosis-specific antigen of *T solium* (Chung *et al,* 1999), the immunodiagnostic antigen of *T. crassiceps* (Zarlenga *et al,* 1994) and antigen B of *Echinococcus* sp (Shepherd *et al,* 1991; Fernandez *et al,* 1996).

To estimate the copy number of isolated genes per genome, Southern blot analysis was performed (data not shown). Genomic DNA derived from *T. solium* metacestodes was digested with restriction enzyme (*Eco*RI, *Hind*III, and *Bam*HI*)* and electrophoresed, transferred onto a nylon membrane and probed with the Agl cDNA insert. Several bands (at least four bands) in each digest were detected under mildly stringent washing condition (55°C for 30 minutes) and this hybridization pattern did not change under highly stringent washing condition (65°C for l hour). None of the cDNA clones possessed a recognition sites for restriction enzymes used in Southern blot analysis within the transcribing regions. In order to confirm whether recognition sites for restriction enzymes used in Southern blot analysis existed within each gene in genomic DNA, PCR was performed on *T. solium* genomic DNA using specific primer sets that amplified from the initiation codon to the termination codon of each gene, and restriction enzyme digestion analysis was performed (data not shown). Each primer set specific to Ag1, Ag1V1, Ag2, and Ag2Vl gene generated DNA fragments 390 bp in length, approximately 130 bp larger than predicted from the cDNA sequences while Ag2V I had approximately 50 bp larger than predicted from the cDNA sequence. Those PCR products were not digested by restriction enzymes used in Southern blot analysis which indicated that no restriction enzyme recognition sites presented within genes in genomic DNA. Complex banding patterns generated in Southern blots of *T. solium* genomic DNA digested with restriction enzymes which could not cut within each gene were consistent with either the existence of multiple copies of these clones throughout the genome of parasite or of a gene family. The latter might be supported by the fact that these clones showed similarity to each other.

Expression of recombinant antigens and evaluation for diagnostic value using ELISA

In order to obtain recombinant proteins, an *E. coli*-based expression system was established. Recombinant proteins without N-terminal hydrophobic region were expressed as thioredoxin (TRX)/His tag fusion proteins. In a preliminary observation, the expression of recombinant Ag2VI (rAg2VI) was successful, but its yield was too low due to its cytotoxicity against *E. coli*. The other three recombinant proteins (rAg1, rAg1V1 and rAg2) were, therefore, selected for further experiment. In immunoblot analyses, recombinant proteins were recognized by NCC patient sera but not by AE patient sera (Table 4). Notably, NCC patient sera recognized rAg1 but its reaction was too weak. This indicated that antigenicity of Ag1 polypeptide was low in natural infection and it was not suitable for diagnostic applications. For this reason, rAg1 V1 and rAg2 were chosen as diagnostic polypeptides. In a preliminary observation, when recombinant rAg1 V1 fusion protein or rAg2 fusion protein was used as an ELISA antigen, little high background was observed owing to reactivity of sera to TRX protein. So we needed to express recombinant proteins without TRX protein for ELISA antigens. But in such a case the size of recombinant protein inevitably became small (29 kDa to 7 kDa). We speculated the possibilities that small molecules were not immobilized on the surface of ELISA plate efficiently and sometimes the B cell epitopes were concealed lead to decrease sensitivity. In addition, if rAg1V1 and rAg2 are expressed and purified separately, we must consider the possibility of quality and quantity differences between rAg1Vl and rAg2. So the expression ofAglVl/Ag2 chimeric protein (Fig 2) was carried out to overcome speculated problems above-mentioned. This Ag1V1/ Ag2 chimeric protein contained His tag for purification but not TRX. In immunoblot analysis, Ag1 V 1/ Ag2 chimeric protein was strongly recognized by NCC patient sera (Table 4).

To assess the diagnostic value of Ag1V1/Ag2 chimeric protein, we further tested its immuno-reactivity by ELISA using individual sera from patients with various parasitic infections (Table 5). A positive reaction to AgIV1/Ag2 chimeric protein was observed in 89.7% (44/49 cases) of sera from NCC patients confirmed to be seropositive by immunoblot analysis (Ito *et al,* 1998; 1999a) based on a cutoff value of 0.17 (the mean OD_{405} plus four standard deviations for normal human controls). Table 6 shows the correlation

EKNKPKCDGNSTKKEIEFIHNWFFHDDPIGNQIAQLAKDWKVAMLKAKGE 50
IRASLAEYCKETKPEDVVKNIKKGMEVVYKFFYEDPLGKKIAQLAKDWKE 100
AMLEARSKVRASLAEYIRGLKNEAA 125

Fig 2- Amino acid sequence of Agl Vl/Ag2 chimeric protein. Amino acids underlined were derived from Agl V1 (^{20}E to ^{78}C) clone and the remains were derived from Ag2 (^{20}K to ^{85}A) clone.

Table 4
Antigenicities of recombinant proteins by immunoblot analyses.

Serum	TRX[a]	Ag1	Ag1V1	Ag2	Ag1V1/Ag2
Normal	-	-	-	-	-
NCC	-	+/-	++	++	++
AE	-	-	-	-	-

[a] : Negative control protein; - : No reaction
+/- : Very weak reaction; ++ : Strong reaction

between immunoblot/ELISA using purified native glycoproteins (Ito *et al*, 1999b; Ohsaki *et al*, 1999) and ELISA using Ag1V1/Ag2 chimeric protein (Sako *et al*, 2000) (eight imported NCC patients in Japan). Out of five patients with a single cyst, two were negative by ELISA using Ag1V1/Ag2 chimeric protein. By immunoblot analysis using native diagnostic antigens, one of two negative cases by ELISA was also negative and another showed a very weak reaction. All patients with multiple cysts were positive by both native and recombinant antigens. This result indicates the good correlation between serodiagnosis using native antigens and Ag1V1/Ag2 chimeric protein. However, the sensitivity of Ag1V1/Ag2 chimeric protein-ELISA was lower that that of native antigens-based immunodiagnosis. Immunoblot analysis detected 98% of parasitologically proven cases with multiple cysts, whereas it was less sensitive (between 60 and 80%) in cases with a single cyst

Table 5
Results of ELISA using Ag1V1/Ag2 chimeric protein.

	+ve/test	%
NCC	44/49	89.7
AE	0/35	0
CE	0/10	0
OP	0/70	0

NCC : neurocysticercosis
AE : alveolar echinococcosis
CE : cystic echinococcosis
OP : other parasitic diseases: clonochiasis (10), sparganosis (10), fascioliasis (8), paragonimiasis (32), schistosomiasis (10)

Table 6
Correlation[a] between immunoblot /ELISA using native purified antigen[b] and ELISA using recombinant protein[c].

NCC cases		IB / ELISA (native antigen)	ELISA (Ag1V1/Ag2 chimeric protein)
5 with a single cyst	Positive	4[b]	3
	Negative	1[d]	2
3 with multiple cysts	Positive	3[b]	3
	Negative	0	0

a : Sera were from eight imported NCC patients in Japan; b : Ito *et al*, (1998; 1999b)
c : Sako *et al* (2000); d : Ohsaki *et al* (1999)

(Wilson *et al*, 1991). This suggested that a single cyst was not always efficient to stimulate host responses that produced measurable antibody to diagnostic antigens. In addition, since Ag1V1/Ag2 chimeric protein used in this study was expressed using an *E. coli* system, this protein was not glycosylated. Native antigens might be highly glycosylated, and the carbohydrates were thought to be key antigenic parts for immunodiagnostic sensitivity. We, however, speculated that the carbohydrate components showed similarity among various parasites and were not suitable targets for differential immunodiagnosis. Indeed, when native antigens purified by affinity chromatography using monoclonal antibodies to *T. solium* low-molecular-weight antigen were used as ELISA antigens, some serum samples from alveolar echinococcosis (AE) patients showed a strong reaction (our unpublished observation). In our experiments (Table 5), serum samples from other parasitic infection patients (AE, cystic echinococcosis, clonochiasis, sparganosis, fascioliasis, paragonimiases, schistosomiasis) did not recognize recombinant protein (100% specificity). Therefore, it is expected that Ag1V1/Ag2 chimeric protein is a valuable target antigen for differential diagnosis.

Hereafter, we will determine the B cell epitopes on these molecules for the development of synthetic peptide-based ELISA system that may be suitable for supplying stable and high quality diagnosis.

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REFERENCES

Baily GG, Mason PR, Trijssenar FE, Lyons NF. Serological diagnosis of neurocysticercosis: evaluation of ELISA tests using cyst fluid and other components of *Taenia salium* cysticerci as antigens. *Trans R Soc Trop Med Hyg* 1988;82:295-9.

Brandt JR, Geerts S, De Deken R, *et al*. A monoclonal antibody-based ELISA for the detection of circulating excretory-secretory antigens in *Taenia saginafa* cysticercosis. *Int J Parasitol* 1992;22: 471- 7.

Chung JY, Bahk YY, Huk S, Kang SY, Kong Y, Cho SY. A recombinant 10-kDa protein of *Taenia solium* metacestodes specific to active neurocysticercosis. *J Infect Dis* 1999;180:1307-15.

Craig PS, Rogan MT, Allan JC. Detection, screening and community epidemiology of taeniid cestode zoonoses: cystic echinococcosis, alveolar echinococcosis and neurocysticercosis. *Adv Parasitol* 1996;38:169-250.

Fernandez V, Ferreira HE, Femandez C, Zaha A, Nieto A. Molecular characterisation of a novel 8-kDa subunit of *Echinococcus granulosus* antigen B. *Mol Biochem Parasitol* 1996;77:247-50.

Gottstein B, Tsang VC, Schantz PM. Demonstration of species-specific and cross-reactive components of *Taenia solium* metacestode antigens. *Am J Trop Med Hyg* 1986;35:308-13.

Ito A, Ma L, Schantz PM, *et al*. Differential serodiagnosis for cystic and alveolar echinococcosis using fractions of *Echinococcus granulosus* cyst fluid (antigen B) and *E. multilocularis* protoscolex (EM18). *Am J Trop Med Hyg* 1999a;60:188-92.

Ito A, Nakao M, Ito Y, *et al.* Neurocysticercosis case with a single cyst in the brain showing dramatic drop in specific antibody titers within 1 year after curative surgical resection. *Parasitol Int* 1999b; 48:95-99.

Ito A, Plancarte A, Ma L, *et al.* Novel antigens for neurocysticercosis: simple method for preparation and evaluation for serodiagnosis. *Am J Trop Med Hyg* 1998;59:291-4.

Ito A, Plancarte A, Nakao M, *et al*. ELISA and immunoblot using purified glycoproteins for serodiagnosis of cysticercosis in pigs naturally infected with *Taenia solium. J Helminthol* 1999c; 73:363-5.

Larralde C, Laclette JP, Owen CS, *et al.* Reliable serology of *Taenia solium* cysticercosis with antigens from cyst vesicular fluid: ELISA and hemagglutination tests. *Am J Trop Med Hyg* 1986; 35:965-73.

Nielsen H, Engelbrecht J, Brunak S, von Heijne G. Identification of prokaryotic and eukaryotic signal peptides and prediction of their cleavage sites. *Protein Eng* 1997;10:1-6.

Ohsaki Y, Matsumoto A, Miyamoto K, Neurocysticercosis without detectable specific antibody. *Intern Med* 1999;38:67-70.

Parkhouse RM, Harrison LJ. Cyst fluid and surface associated glycoprotein antigens of *Taenia* sp

metacestodes. *Parasite Immunol* 1987;9:263-8.

Sako Y, Nakao M, Ikejima T, Piao XZ, Nakaya K, Ito A. Molecular characterization and diagnostic value of *Taenia solium* low-molecular-weight antigen genes. *J Clin Microbiol* 2000;38:4439-44.

Schantz PM, Moore AC, Munoz JL, *et al*. A Neurocysticercosis in an Orthodox Jewish community in New York City. *N Engl J Med* 1992; 327:692-5.

Schantz PM, Wilkins PP, Tsang VCW. Immigrants, imaging, and immunoblots: the emergence of neurocysticercosis as a significant public health problem. In: Shelds WM, Craig WA, Hughes JM, eds. Emerging InIections 2 Washington DC: ASM Press, 1998:213-42.

Shepherd JC, Aitken A, McManus DP. A protein secreted *in vivo* by *Echinococcus granulosus* inhibits elastase activity and neutrophil chemotaxis. *Mol Biochem Parasitol* 1991;44:81-90.

Simanjuntak GM, Margono SS, Okamoto M, Ito A. Taeniasis/Cysticercosis in Indonesia as an emerging disease. *Parasitol Today* 1997;13:321-3.

Sloan L, Schneider S, Rosenblatt J. Evaluation of enzyme-linked immunoassay for serological diagnosis of cysticercosis. *J Clin Microbiol* 1995; 33:3124-8.

Tsang VCW, Brand JA, Boyer AE. An enzyme-Iinked immunoelectrotransfer blot assay and glycoprotein antigens for diagnosing human cysticercosis *(Taenia solium). J Infect Dis* 1989;159:50-9.

Wandra T, Subahar R, Simanjuntak GM, *et al*. Resurgence of cases of epileptic seizures and burns associated with cysticercosis in Assologaima, Jayawijaya, Irian Jaya, Indonesia, 1991-95. *Trans R Soc Trop Med Hyg* 2000;94:46-50.

White AC Jr. Neurocysticercosis: a major cause of neurological disease worldwide. *Clin Infect Dis* 1997;24:101-13.

Wilson M, Bryan RT, Fried JA, *et al*. Clinical evaluation of the cysticercosis enzyme-linked immunoelectrotransfer blot in patients with neurocysticercosis. *J Infect Dis* 1991;164:1007-9.

Zarlenga OS, Rhoads ML, al Yaman FM. A *Taenia crassiceps* cDNA sequence encoding a putative immunodiagnostic antigen for bovine cysticercosis. *Mol Biochem Parasitol* 1994;67: 215-23.