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Multi-immunodot for rapid differential diagnosis of eosinophilic meningitis due to parasitic infections

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Abstract

A multi-dot enzyme-linked immunosorbent assay (ELISA) was developed for the rapid and simple differential diagnosis of eosinophilic meningitis due to helminth infections. Ultrafiltered, purified antigens of *Parastrongylus* (=*Angiostrongylus*) *cantonensis, Gnathostoma spinigerum* and *Taenia solium* metacestodes, the most common parasites that invade the central nervous system and cause eosinophilic pleocytosis, were dotted onto a single nitrocellulose membrane strip. Antigen-coated strips, when blocked with 5% skimmed milk and dried, were stable for at least 6 months at 4°C. With peroxidase conjugated anti-human immunoglobulins and 4-chloro-1-naphthol as a substrate, antibodies in the corresponding patients' sera were clearly detected on the membrane strip as well-defined blue dots. Although cross-reactions between *P. cantonensis* and *G. spinigerum* antigens were observed with the use of partially purified antigens, the darkest dot correlated well with the infecting parasites in all cases. This fast, easy and economical multiple dot-blot ELISA method is useful for the differential diagnosis of eosinophilic meningitis caused by parasitic helminths, as semi-purified antigens can be easily obtained by ultrafiltration and used. Further improvements using highly specific parasite antigens may make this multi-immunodot test more suitable for wide-scale use in field studies and diagnostic laboratories.

Introduction

Eosinophilic meningitis is typically associated with certain helminth infections in which the nervous system is involved. The most common helminth parasites that invade the central nervous system (CNS) are *Parastrongylus* (=*Angiostrongylus*) *cantonensis*, *Gnathostoma spinigerum* and *Taenia solium* metacestodes. These parasites produce similar signs and symptoms in the CNS. Typically, the infections are associated with peripheral eosinophilia, and a high eosinophil count in the cerebrospinal fluid (CSF).

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Parastrongylus cantonensis, G. spinigerum and *T. solium* metacestodes are common causes of parasitic eosinophilic meningitis in Thailand and in parts of Southeast Asia (Daengsvang, 1980; Shmutzhard *et al.*, 1988; Nawa, 1991; Ito, 1992; Kliks & Palumbo, 1992; White, 2000; Thongchareon, 2001; Gorgolas *et al.*, 2003). As clinical symptoms are not solely diagnostic and patients with these infections require different drugs for appropriate and effective treatment, definitive diagnoses of individual infections are required for prompt and adequate treatment.

Although many signs and symptoms of eosinophilic meningitis caused by these helminth parasites are similar, specific immunological tests can help in differential diagnosis. An immunoblot for specific antibodies has been reported to distinguish infections with *P. cantonensis,*

G. spinigerum, and cysticerci of *T. solium*. A 31-kDa glycoprotein of *P. cantonensis* was identified as a specific band for human parastrongyliasis (angiostrongyliasis) in an immunoblot test (Eamsobhana *et al.*, 1997, 1998). Likewise, a 24-kDa glycoprotein of *G. spinigerum* was found to be a diagnostic band for human gnathostomiasis (Tapchaisri *et al.*, 1991). Similarly, the more specific banding patterns of cysticercosis corresponded to low molecular weight antigens of 10–26 kDa of *T. solium* cysticerci (Ito *et al.*, 1998). However, a rapid and easy single test, which can be applied concurrently for the diagnosis of different parasites through the detection of specific antibodies, is not practical.

A novel, fast and easy diagnostic method, the dot-blot ELISA, which is much simpler to perform than the immunoblot, has been successfully used with purified, specific parasite antigens for the detection of *P. cantonensis*-associated eosinophilic meningitis or eosinophilic meningoencephalitis in humans (Eamsobhana *et al.*, 2003). With this technique, a positive result is indicated by a coloured spot on a nitrocellulose sheet and is easily distinguished with the naked eye. The test has also been shown to be comparable to immunoblot for specific diagnosis of *P. cantonensis* infections (Eamsobhana *et al.*, 2004).

In the present study, the diagnostic potential of a simple dot-immunobinding method was evaluated. A single membrane strip dotted with *P. cantonensis, G. spinigerum* and cysticerci of *T. solium* antigens was used for detecting corresponding specific antibodies in sera of patients with parastrongyliasis, gnathostomiasis and cysticercosis. Parasite antigens partially purified by ultrafiltration, a membrane separation technique, were used in the assay to determine their merit as specific antigens.

Materials and methods

Parasites and parasite antigens

Adult worms of *P. cantonensis* (Thailand strain) were obtained from the pulmonary arteries of rats previously infected for at least 6 weeks with *P. cantonensis* infective larvae collected from experimentally infected snails (*Biomphalaria glabrata*). *Gnathostoma spinigerum* third-stage larvae were collected from the liver of naturally infected eels purchased from local markets in Bangkok. *Taenia solium* cysticerci were obtained from a naturally infected pig. Adult worms or larvae of each parasite were cleared of debris, washed and homogenized separately in a small volume of sterile normal saline with a glass tissue grinder. The suspension was sonicated and left overnight at 4°C. Soluble antigen was obtained as the supernatant after centrifugation at 5000 rpm for 15 min. The protein content of the extract was determined using a protein assay kit II (Bio-Rad Laboratories, USA).

Purification of parasite antigens

Isolation of specific antigenic components from crude somatic worm extracts of *P. cantonensis*, *G. spinigerum* and *T. solium* cysticerci was accomplished by ultrafiltration using low protein-binding ultrafiltration membranes, i.e. 100K and 3K Nanosep (Pall Gelman Laboratory, USA). In brief, 500 μl of a crude parasite extract were centrifuged at 5000 rpm for 15 min through a 100K Nanosep (molecular weight cut-off of 100,000 Daltons) and the resulting filtrate was collected. Fifty μl of phosphate-buffered saline (PBS) pH 7.4 were then added to the non-filtrate and the mixture was centrifuged as above. This step was repeated twice. All filtrates were pooled and re-concentrated as described above using a 3K Nanosep (molecular weight cut-off of 3000 Daltons). The final filtrate was discarded and the protein of interest was retained on the membrane surface. A protein assay kit II (Bio-Rad Laboratories, USA) was used to determine the protein content. All *P. cantonensis*, *G. spinigerum*, and *T. solium* cysticerci soluble proteins were purified using procedures described above and were used as parasite antigens in a multi-dot immunoassay for detecting specific antibodies to the corresponding parasites.

Serum samples

Serum samples were obtained from ten patients each with parastrongyliasis, gnathostomiasis and cysticercosis. For parastrongyliasis patients, five were parasitologically proven cases (three with cerebral parastrongyliasis from whom *P. cantonensis* larvae were recovered from the CSF and two with ocular parastrongyliasis from whom immature *P. cantonensis* worms were recovered from the eye chambers). The other five patients were presumptive cases, diagnosed as parastrongyliasis based on clinical symptoms and history of exposure to infection, as well as having high antibody titres detected by ELISA. The confirmed gnathostomiasis serum was that of a patient from whom a *G. spinigerum* larva was recovered from the skin on the abdomen, while the nine presumptive gnathostomiasis sera were from patients with intermittent cutaneous migratory swelling as well as having high antibody titres by ELISA. Of the ten patients with cysticercosis, nine were diagnosed pathologically as subcutaneous cysticerci and one with neurocysticercosis. The diagnosis was based on computerized axial tomography (CAT) brain scan and confirmed by histopathology.

Antigen cross-reactivity studies were also carried out on sera from ten patients each with toxocariasis, filariasis, paragonimiasis and malaria. All patients with filariasis, paragonimiasis and malaria were diagnosed parasitologically. Ten cases of toxocariasis were serologically positive on ELISA using excretory–secretory antigens of second-stage larvae of *Toxocara canis*. The normal control group of sera was obtained from ten healthy adults who were negative for any parasitic infection at the time of blood collection. All serum samples were kept at −20°C until use.

Multi-dot ELISA procedure

The optimal antigen concentration per dot and the appropriate serum dilution were pre-determined by a chequerboard titration; 0.5 μg of each parasite protein per

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dot was chosen when incubated with their corresponding positive sera.

Nitrocellulose membrane (Bio-Rad Laboratories, Richmond, California), 0.45 μm pore size, was cut into strips measuring 1.5 × 5.0 cm. Three circular windows were made on the membrane strip using a plastic mask with a hole in the centre. The strips were soaked for 5 min in phosphate-buffered saline (PBS), pH 7.4 and then air-dried on filter paper. On to the centre of each circular window on the strip, 2 μl of ultrafiltered antigen of *P. cantonensis*, *G. spinigerum* and *T. solium* metacestodes diluted in PBS, were deposited separately as a discrete dot (0.5 μg protein per dot) and allowed to dry for 1 h at ambient temperature. The membrane strips were then immersed in blocking solution (5% skimmed milk in PBS) with gentle shaking for 1 h. After washing three times, by shaking for 5 min during each wash in PBS-Tween 20, the strips were kept at 4°C until use.

During use, the strip was incubated for 1 h in the test serum (diluted 1 in 200 with 1% bovine serum albumin (BSA) in PBS) and washed as described above. The washed membrane was immersed in the enzyme conjugate, peroxidase rabbit anti-human immunoglobulins (Dakopatt, Denmark) diluted to 1:1000 with 1% BSA in PBS, for 1 h. It was then washed as above and transferred to a substrate solution containing 30 mg of 4-chloro-1-naphthol (Pierce Chemical Company, USA) in 10 ml of absolute methanol mixed with 100 μl of 30% H_2O_2 in 100 ml of PBS, pH 7.4. After the appearance of the colour, the strip was washed several times with distilled water. The development of a well-defined blue dot in the window on the test strip was considered as positive. The sample was considered to be negative when no colour dot was evident. Each serum sample was tested in triplicate to confirm the reproducibility of the results.

Results

The results of the multi-immunodot test using various parasite antigens dotted onto a membrane strip against a given patient's serum are shown in fig. 1. All patients' sera with parastrongyliasis, gnathostomiasis and cysticercosis produced positive bluish purple dots against their corresponding parasite antigens. Sera of cysticercosis, however, reacted less strongly. None of the other sera with toxocariasis, paragonimiasis, filariasis and malaria, and the ten control sera from normal parasite-free individuals produced a positive blue dot.

In most of the patients' sera with parastrongyliasis and gnathostomiasis, there were cross-reactions between the antigens of *P. cantonensis* and *G. spinigerum*. Nevertheless, each serum reacted most strongly with the corresponding parasite antigen to produce a positive result, but produced only pale blue dots with the other parasite antigen, hence all serum samples tested could be diagnosed precisely. No cross-reactions were observed in sera from patients with cysticercosis against *P. cantonensis* and *G. spinigerum* antigens.

Discussion

Parastrongylus cantonensis-associated eosinophilic meningitis in humans has been commonly reported worldwide (Cross, 1987; Alicata, 1991; Prociv *et al.*, 2000; Tsai *et al.*, 2001; Slom *et al.*, 2002). Humans become accidental hosts when they ingest the third larval stage in raw or undercooked molluscs or in fresh vegetables contaminated with infected molluscs (Lim & Ramachandran, 1979; Bhaibulaya, 1991). Larvae penetrate the wall of the gut and migrate to the brain, spinal cord and sometimes the eye. The most common symptom is headache, and paraesthesiae is frequently reported.

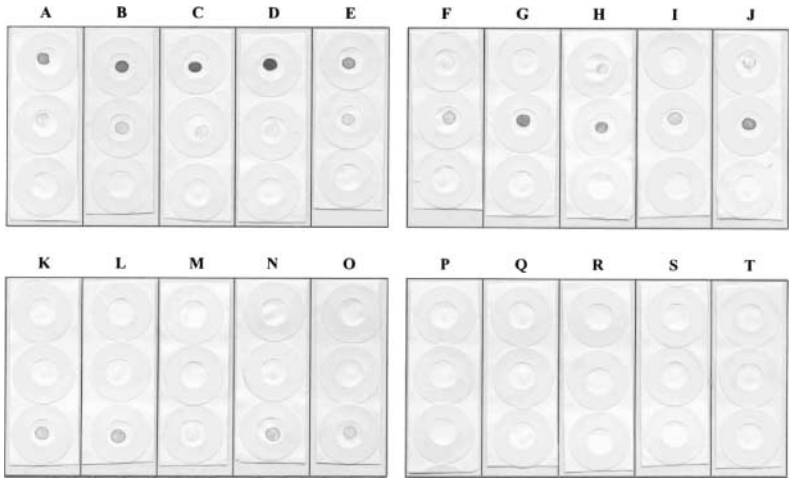

Fig. 1. Reactions of multi-dot ELISA using ultrafiltered, purified antigens of *Parastrongylus cantonensis*, *Gnathostoma spinigerum* and *Taenia solium* metacestodes, on nitrocellulose membrane strips for the detection of specific antibodies from sera of patients with parastrongyliasis (A–E), gnathostomiasis (F–J) and cysticercosis (K–O), and sera from patients with toxocariasis (P), filariasis (Q), paragonimiasis (R), and malaria (S), and normal control serum (T). Deep coloured dots show a positive reaction; no colour is considered as negative. Antigen dots from top to bottom are those of *P. cantonensis*, *G. spinigerum*, and *T. solium* metacestodes, respectively.

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Parasitologically confirmed cases are rare as the parasite can be recovered only infrequently from the CSF of patients. The diagnosis of parastrongyliasis in a patient with acute eosinophilic meningitis is generally supported by a history of mollusc ingestion.

Gnathostomiasis is most often the result of the ingestion of the third-stage larvae of the nematode *G. spinigerum*, although several other species also cause human disease (Lo Re & Gluckman, 2002; Nawa, 2002; Moore *et al.*, 2003). Larvae may be found in raw or undercooked meat of freshwater fish, chicken, frogs, or in contaminated water. Any organ system can be involved; the most common manifestation of infection is localized, intermittent, migratory swelling in the skin and subcutaneous tissues. Both *P. cantonensis* and *G. spinigerum* are common causes of parasitic eosinophilic meningitis, resulting from their random migration into the CNS. Low-grade fevers, headache, and non-focal neurological symptoms and signs are typical. Computerized tomography in CNS disease may reveal evidence of meningeal inflammation in half of the cases (Jaroonvesama, 1988).

Tissue-invading larval stages (cysticerci) of the pork tapeworm, *T. solium*, in the CNS cause neurocysticercosis. Cysticercosis is acquired by ingesting tapeworm eggs shed in human faeces. Although cysticerci may be localized throughout the body, many clinical manifestations are related to their presence in the CNS, where they can invade the parenchyma, the subarachnoid spaces, and the ventricular system, causing seizures, headache, hydrocephalus, and other neurological dysfunctions (White, 1997). The most sensitive diagnostic tool is brain imaging by computerized axial tomography (CAT) and magnetic resonance imaging (MRI) (Jaroonvesama, 1988; White, 1997, 2000).

The potential value of immunodiagnosis becomes greatest in these parasitic infections since the parasites do not fully mature in humans and only the larval stages are found. They cannot be diagnosed easily by parasitological means. Speiser (1982) developed a multi-antigen ELISA as a screening test for tissue-dwelling parasites. With the use of a single serum dilution against several antigens in the same plate, the reaction can be compared and cross-reactions become obvious. However, compared with ELISA, dot-blot ELISA does not require specific equipment, and positive reactions can be observed by the naked eye with similar reliability (Tellez-Giron *et al.*, 1987; Eamsobhana *et al.*, 2003, 2004). This may make the test more appropriate and cost-effective in developing countries.

During the past decade, specific antigens of many helminths, including *P. cantonensis*, *G. spinigerum* and *T. solium* metacestodes have been identified. Chaicumpa *et al.* (1991) reported a 24-kDa antigen to be specific antigen of *G. spinigerum*. Eamsobhana *et al.* (1997, 1998) demonstrated that a glycoprotein component of 31 kDa was the specific antigen of *P. cantonensis*. More recently, the protein components with molecular weight of 10–30 kDa of *T. solium* metacestodes were reported by Dekumyoy *et al.* (2004) to be potent immunogens and had potential in the immunodiagnosis of human neurocysticercosis. As the sensitivity and specificity of a test are dependent on the antigens used, a purified antigen in an antibody detection assay for the diagnosis should be explored. However, several steps required in purification methods are complicated and will cause protein loss. Therefore a simple and easy purification method for obtaining a substantial supply of specific antigens is required.

Diafiltration is a technique using ultrafiltration membranes to fractionate different size biomolecules in macromolecular solutions. This purification method is easy to perform and relatively inexpensive. There have been previous reports using diafiltration to obtain more specific parasite antigens of *G. spinigerum* and *T. solium* for immunodiagnosis. Sugaroon *et al.* (2002) demonstrated in sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) that the 24-kDa band of *G. spinigerum* can be identified in the filtrate after ultrafiltration through a 100K Nanosep. Likewise, Dekumyoy *et al.* (2004) used Ultrafree centrifugal tube (retained 30 kDa) and Amicon (PM10) on partially purified protein of *T. solium* metacestodes to obtain the more specific antigens of 10–30 kDa for use in ELISA.

In the present study, helminth specific proteins were separated by diafiltration. A low protein-binding ultrafiltration membrane with a molecular cut-off of 100 kDa (100K Nanosep) was used to retain macromolecules which are non-specific proteins for *P. cantonensis*, *G. spinigerum* and *T. solium* metacestodes. The resulting filtrate, which contained low molecular weight proteins including specific proteins of each parasite, was re-concentrated using a second ultrafiltration membrane of molecular cut-off of 3 kDa (3K Nanosep). Specific proteins of *P. cantonensis* (31 kDa), *G. spinigerum* (24 kDa) and *T. solium* metacestodes (10–30 kDa) were retained on the membrane surface. Purified proteins were then used as parasite antigens for dotting on to a single membrane strip, and used for identification of specific helminth antibodies in the sera of infected patients.

The present attempt to detect specific antibodies to various antigens concurrently on a multi-dot strip was successful. The advantage of this method is that semi-purified antigens can be used with reliability. Although cross-reactions among the parasite antigens (*P. cantonensis* and *G. spinigerum*) were still observed, these did not interfere with judgement, as the darkest dot which indicated the infecting parasite was apparent in all cases. Furthermore, antigen-dotted nitrocellulose membranes are stable for at least six months at 4°C (data not shown), so the membrane strips can be kept in a refrigerator until use. Using the stored membrane, results can be obtained in less than 3 h. Further improvements using highly specific parasite antigens may make this multi-immunodot test more suitable for diagnostic laboratories and large-scale use in field studies. The method could also perhaps be automated for use in epidemics.

The present method, based on the presence of specific antibody, however, will not detect recent infections. As only ten sera from infected subjects of each disease group were used, the findings need to be verified with a large-scale study. Such a study should also address the possible occurrence of false negatives due to genetic factors.

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References

Alicata, J.E. (1991) The discovery of *Angiostrongylus cantonensis* as a cause of human eosinophilic meningitis. *Parasitology Today* **7**, 151–153.

Bhaibulaya, M. (1991) Snail borne parasitic zoonoses: angiostrongyliasis. *Southeast Asian Journal of Tropical Medicine and Public Health* **22** (suppl.), 189–193.

Chaicumpa, W., Ruangkunaporn, Y., Nopparatana, C., Chongsa-Nguan, M., Tapchaisri, P. & Setasuban, P. (1991) Monoclonal antibody to a diagnostic Mr 24,000 antigen of *Gnathostoma spinigerum*. *International Journal for Parasitology* **21**, 735–738.

Cross, J.H. (1987) Public health importance of *Angiostrongylus cantonensis* and its relative. *Parasitology Today* **3**, 367–369.

Daengsvang, S. (1980) *A monograph on the genus Gnathostoma and gnathostomiasis in Thailand*. Southeast Asian Medical Information Foundation Center, Tokyo.

Dekumyoy, P., Waikagul, J., Vanijanonta, S., Thairungroj, M., Nakao, M., Sako, Y., Watanabe, S. & Ito, A. (2004) Cysticercosis: IgG-ELISA evaluations of peak 1 antigen and <30 kDa antigen of delipidized extract of *Taenia solium* metacestodes. *Southeast Asian Journal of Tropical Medicine and Public Health* **35**, 1–9.

Eamsobhana, P., Mak, J.W. & Yong, H.S. (1997) Identification of *Parastrongylus cantonensis* specific antigens for use in immunodiagnosis. *International Medical Research Journal* **1**, 1–5.

Eamsobhana, P., Tungtrongchitr, A., Wanachiwanawin, D. & Yoolek, A. (1998) Characterization of a 31-kDa specific antigen from *Parastrongylus cantonensis* (Nematoda: Metastrongylidae). *International Medical Research Journal* **2**, 9–12.

Eamsobhana, P., Yoolek, A. & Punthuprapasa, P. (2003) Dot-blot ELISA for the immunological detection of specific antibody to *Parastrongylus cantonensis*. *Tropical Biomedicine* **20**, 1–6.

Eamsobhana, P., Yoolek, A., Punthuprapasa, P. & Suvouttho, S. (2004) A dot-blot ELISA comparable to immunoblot for the specific diagnosis of human parastrongyliasis. *Journal of Helminthology* **78**, 287–291.

Gorgolas, M.D., Santos, O., Connor, F., Garete, T., Troyas, R.M.G., Lopez-Unzu, A., Grobusch, M.P. & Fernandez-Guerrero, M.L. (2003) Cutaneous and medullar gnathostomiasis in travellers to Mexico and Thailand. *Journal of Travel Medicine* **10**, 358–361.

Ito, A. (1992) Cysticercosis in Asia-Pacific regions. *Parasitology Today* **8**, 182–183.

Ito, A., Plancarte, A., Ma, L., Kong, Y., Flisser, A., Cho, S.Y., Liu, Y.H., Kamhawi, S., Lightowlers, M.W. & Schantz, P.M. (1998) Novel antigens for neurocysticercosis: simple method for preparation and evaluation for serodiagnosis. *American Journal of Tropical Medicine and Hygiene* **59**, 291–294.

Jaroonvesama, N. (1988) Differential diagnosis of eosinophilic meninigitis. *Parasitology Today* **4**, 262–266.

Kliks, M.M. & Palumbo, N.E. (1992) Eosinophilic meningitis beyond the Pacific Basin: the global dispersal of a peridomestic zoonosis caused by *Angiostrongylus cantonensis*, the nematode lungworm of rats. *Social Science Medicine* **34**, 199–212.

Lim, B.L. & Ramachandran, C.P. (1979) Ecological studies of *Angiostrongylus malaysiensis* (Nematoda: Metastrongylidae) in Malaysia. pp. 27–48 *in* Cross, J.H. (*Ed.*) *Studies on angiostrongyliasis in Eastern Asia and Australia*. NAMRU-2. Special Publication No. 44, Taipei, Taiwan.

Lo Re, V. & Gluckman, S.J. (2002) Eosinophilic meningitis due to *Gnathostoma spinigerum*. *Journal of Infection* **45**, 117–120.

Moore, D.A.J., McCroddan, J. & Dekumyoy, P. (2003) Gnathostomiasis: an emerging imported disease. *Emerging Infectious Diseases* **9**, 647–650.

Nawa, Y. (1991) Historical review and current status of gnathostomiasis in Asia. *Southeast Asian Journal of Tropical Medicine and Public Health* **22** (suppl.), 217–219.

Nawa, Y. (2002) *Gnathostoma* and gnathostomiasis: then and now. pp. 8–11 in *Proceedings of the First International Meeting on Gnathostomosis*, 14–16 March 2002 Sinaloa, Mexico, Culiacan.

Prociv, P., Spratt, D.M. & Carlisle, M.S. (2000) Neuroangiostrongyliasis: unresolved issues. *International Journal for Parasitology* **30**, 1295–1303.

Shmutzhard, E., Boongird, P. & Vejjajiva, A. (1988) Eosinophilic meningitis and radiculomyelitis in Thailand, caused by CNS invasion of *Gnathostoma spinigerum* and *Angiostrongylus cantonensis*. *Journal of Neurology, Neurosurgery and Psychiatry* **51**, 80–87.

Slom, T.J., Cartese, M.M., Gerber, S.I., Jones, R.C., Haltz, T.H., Lopez, A.S., Zambrano, C.H., Sufit, R.L., Sakolvaree, Y., Chaicumpa, W., Herwaldt, B.L. & Johnson, S. (2002) An outbreak of eosinophilic meningitis caused by *Angiostrongylus cantonensis* in travelers returning from the Caribbean. *New England Journal of Medicine* **346**, 668–675.

Speiser, F. (1982) Serodiagnosis of tissue dwelling parasites: application of a multi-antigen enzyme-linked immunosorbent assay (ELISA) for screening. *Annales de la Société Belge de Médecine Tropicale* **62**, 103.

Sugaroon, S., Saksirisampant, W., Kraivichian, K., Suwansaksri, J. & Wiwanitkit, V. (2002) Preparation of *Gnathostoma* protein by ultrafiltration method using Nanosep membrane. *Southeast Asian Journal of Tropical Medicine and Public Health* **34** (suppl. 2), 121–122.

Tapchaisri, P., Nopparatana, C., Chaicumpa, W. & Setasuban, P. (1991) Specific antigen of *Gnathostoma spinigerum* for immunodiagnosis of

human gnathostomiasis. *International Journal for Parasitology* **21**, 315–319.

Tellez-Giron, E., Ramos, M.C., Dufour, L., Alvarez, P. & Montante, M. (1987) Detection of *Cysticercus cellulosae* antigens in cerebrospinal fluid by dot enzyme-linked immunosorbent assay (Dot-ELISA) and standard ELISA. *American Journal of Tropical Medicine and Hygiene* **37**, 169–173.

Thongcharoen, P. (2001) Emerging and re-emerging infectious disease. Situation in Thailand. pp. 241–275 in *Proceeding of the National Seminar on Emerging Infectious Diseases*, Bangkok, Thailand, Department of Communicable Diseases Control, Ministry of Health.

Tsai, H.C., Liu, Y.C., Kunin, C.M., Lae, S.S., Chen, Y.S., Lin, H.H., Tsai, T.H., Lin, W.R., Huang, C.K., Yen, M.Y. & Yen, C.M. (2001) Eosinophilic meningitis caused by *Angiostrongylus cantonensis*: report of 17 cases. *American Journal of Medicine* **111**, 109–114.

White, A.C. Jr (1997) Neurocysticercosis: a major cause of neurological disease worldwide. *Clinical Infectious Diseases* **24**, 101–113.

White, A.C. Jr (2000) Neurocysticercosis: updates on epidemiology, pathogenesis, diagnosis, and management. *Annual Review of Medicine* **51**, 187–206.

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