Towards Precise and Rapid Diagnosis of Eosinophilic Meningitis due to the Rat Lungworm *Angiostrongylus cantonensis*

Praphathip Eamsobhana, Ph.D, D.A.P.&E.

Department of Parasitology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand.

Siriraj Med J 2010;62:84-89

E-journal: http://www.sirirajmedj.com

Angiostrongylus cantonensis, the rat lungworm, now referred to as *Parastrongylus cantonensis*[1] (Fig 1) is the most common cause of human eosinophilic meningitis or eosinophilic meningoencephalitis worldwide. In Thailand and many countries in Southeast Asia and Pacific Islands, this neurotrophic nematode is endemic and the disease is well recognized.[2] Rat is the definitive host of *A. cantonensis*. The first-stage larvae hatch in the lungs of rats and migrate via the trachea and gut into its faeces. Mollusks are the intermediate hosts. When rat excrement is ingested by mollusks the first-stage larvae molt twice in the intermediate host to become the infective third-stage larvae. Humans, an accidental host, get infected by ingestion of infective larvae in raw or improperly cooked snail dishes. Other paratenic hosts such as freshwater shrimps, toads, frogs, crabs and monitor lizards can also be a source of the parasite infection, as can vegetables contaminated with mollusk slime or excretions.[2,3]

Fig 1. *Angiostrongylus cantonensis* adult worms - top: female; bottom: male.

In humans, infective larvae migrate via the bloodstream to the central nervous system where they cause severe inflammatory responses and symptoms usually occur 4-23 days after exposure. The illness is characterized by severe headache and meningism, and painful paraesthesias are well described. The clinical spectrum usually ranges from mild disease to meningitis, and less frequently with encephalitis.[3-5] Treatment is primarily supportive, with repeated spinal taps to relieve symptoms of persistent headache due to increased intracranial pressure. Anthelmintics can worsen symptoms due to exacerbation of the inflammatory reaction to dying worms and increase the severity of the disease. The value of therapy with corticosteroids alone or in conjunction with albendazole or mebendazole is not yet fully defined.[3]

The definitive diagnosis of angiostrongylid eosinophilic meningitis is made by direct demonstration of *A. cantonensis* larva(e) or young adult(s) in the cerebrospinal fluid of patients. Although a large number of patients have been reported with the infection, only few cases were confirmed by the finding of worms in the cerebrospinal fluid.[2,6] The diagnosis is therefore mainly presumptive based on clinical settings and a history of specific eating, as well as laboratory findings. Characteristically there is pleocytosis in the spinal fluid with the eosinophil count ranging from 26% to 75%, and the peripheral eosinophilia typically ranging from 5% to 63%.[4,5] Computed tomography (CT) scans and magnetic resonance imaging (MRI) techniques may reveal the presence of lesions in the brain and are useful for following up the disease or monitoring the complications, but they cannot serve as the basis for differential diagnosis.[7-10]

The difficulty in obtaining a definitive diagnosis is one reason why immunological means have played an

important role for the confirmation of human infection. Methodologies exist today to detect both antibodies, which indicate present or past infection, and circulating antigens, which indicate current infection, providing reliable and useful adjuncts to the clinical diagnosis of cerebral angistrogyliasis cantonensis. These will therefore result in appropriate treatment and effective management of the disease. Today, the enzyme-linked immunosorbent assay (ELISA), dot-blot ELISA, and immunoblot are test formats that have received the most attention. Many more immunological detection formats which are rapid, precise, cheap and easy to be used, e.g. a simple eye-read rapid test, are awaiting comprehensive assessment and application. Modern molecular biology, such as PCR-based diagnostic techniques, is being assessed towards the development of alternative assays and it appears likely that these new diagnostic tests will find broad application for routine use as well as for field studies in the near future.

Antibody detection methods

An increase in antibody titer can be used as evidence of recent infection and also a likelihood of existing infection. Serological test using antibody-detection assays has now become widely accepted as the most appropriate diagnostic approach. Over the years, a number of traditional immunological tests, e.g. intradermal test, complement fixation test, indirect haemagglutination test, indirect immunofluorescence antibody test, immunoelectrophoresis, and gelatin latex agglutination test, have been developed to support the clinical diagnosis, in which crude somatic antigens or partially purified antigens of *A. cantonensis* adult worms, brain-stage larvae or excretory-secretory products were used with satisfactory sensitivity and specificity.[2,11,12] To date, almost all traditional tests have now been replaced by the ELISA and immunoblotting which are commonly performed in routine laboratory diagnosis of patients suspected to be infected with *A. cantonensis*.

The ELISA has been used as the standard against which new tests are compared. It is less subjective in reading and more sensitive than other tests. A variety of ELISA has been in routine diagnostic and seroepidemiological use. An ELISA for IgG1 antibodies is able to distinguish eosinophilic meningitis individuals with *A. cantonensis* infection.[13] Similarly, the intrathecal synthesis pattern of IgG1+IgG2 and IgE can also contribute to the diagnosis of eosinophilic meningitis and meningoencephalitis due to *A. cantonensis*.[14,15]

Immunodiagnosis utilizing crude extracted worm antigens or partially purified antigens of *A. cantonensis*, although a convenient way to diagnose this infection, has a considerable degree of false positive reaction with other parasitic infections. The introduction of antigen preparations which contain a more restricted range of specific epitopes has overcome the problem of cross-reactivity that occurs in immunodiagnostic methods. For the past several years, progress has been made in the identification of the antigens that are specifically diagnostic for *A. cantonensis* infection in humans. These include a 31-kDa glycoprotein antigen from the adult worm,[16-18] a 29-kDa antigen from the young adult worm,[19] and a 32-kDa protein obtained by elution from SDS-PAGE gels.[20] The differences in the estimated molecular weight of the specific antigens may be perhaps due in part to the parasite strain variability or technical differences in SDS-PAGE procedures and molecular weight calculations and there is no good reason at present to believe that those differences are real.

A 204 kDa young adult worm antigen purified by immuno-affinity chromatography has been reported to be a specific antigen.[21] More recently, a 104-kDa antigen has been demonstrated to be larva-specific and a 33-kDa antigen to be specific for the female adult worm.[22] In addition, the differences among the subclasses of IgG in angiostrongyliasis cantonensis patients have been noted with IgG4 directed primarily against a 29-kDa antigen.[23,24]

Most of the work to date on the various specific antigens recognized by immunoblotting has been on the antigens of 29, 31 or 32 kDa. Partial purification of the specific 31-kDa antigen with gel filtration through Sephacryl S-200 has resulted in improved specificity.[25] When used in the ELISA to detect antibodies in the sera of patients with angiostrongyliasis cantonensis, the sensitivity reaches 100% and the specificity 98%. A more extensive purification procedure using electroelution from SDS-polyacrylamide gel, results in 100% diagnostic sensitivity and specificity on testing in ELISA.[26] To date, immunoblotting has definitely improved antibody detection for routine diagnosis of human angiostrongyliasis. At the Parasitology Laboratory, Department of Parasitology, Faculty of Medicine Siriraj Hospital, Mahidol University (Bangkok, Thailand), a standard ELISA using crude somatic antigens is used for routine screening and all ELISA-positive specimens are tested by im-munoblot for confirmation. A serum reacting with a specific 31-kDa band is indicative of angiostrongyliasis cantonensis (Fig 2).

Although ELISA and immunoblot are useful laboratory tests for human angiostrongyliasis, the instability of reagents and the need for sophisticated equipment are among the factors limiting their use in the field. A simple version of the dot-blot ELISA has shown promise in fulfilling the requirements of a simple field test.[27-29] This test, which uses purified 31-kDa antigen (Fig 3) has been developed for detecting *A. cantonensis* specific antibody, attaining 100% sensitivity and 100% specificity.[28-29] The immunodot assay using

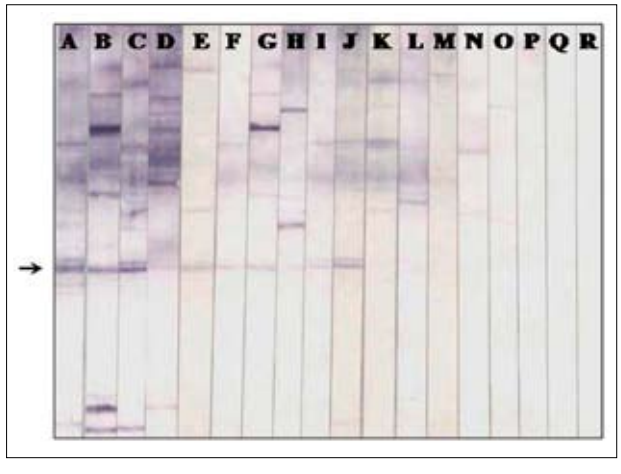

Fig 2. Immunoblots of serum samples of patients with angiostrongyliasis (A-J), gnathostomiasis (K), toxocariasis (L), filariasis (M), paragonimiasis ((N), cysticercosis (O) and malaria (P) against crude extract of *Angiostrongylus cantonensis*. Q, R: normal control sera. Arrow indicates 31-kDa band.

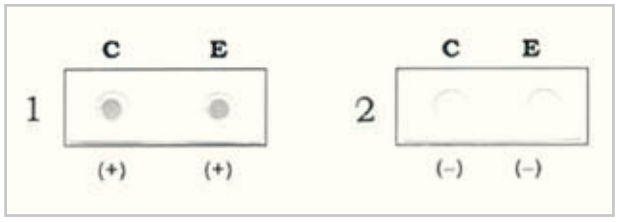

Fig 3. Dot-blot ELISA on nitrocellulose membrane for detection of specific antibodies in sera of patients using crude somatic extract (C) and electroeluted 31-kDa antigen (E) of *Angiostrongylus cantonensis*. 1: deeply coloured dots show positive reaction; 2: lightly coloured or uncoloured dots show negative reaction.

purified antigen is as sensitive and nearly as specific as the immunoblots with a 31-kDa specific band.[27] Also, the test is much easier to perform than an immunoblot analysis. An in-house dot-ELISA kit with purified 31-kDa antigen has been evaluated to have an overall diagnostic sensitivity of 100% and specificity of 100% for human angiostrongyliasis cantonensis.[28] This dot-blot ELISA kit has performed well in a blinded multi-laboratory evaluation, without cross-reactions with sera of patients infected with other commonly occurring human parasites.[29] Moreover, this diagnostic test kit has been used effectively for field studies in endemic areas, under the parasite control project conducted by the Department of Disease Control, Ministry of Public Health, Thailand, for community screening of clinically suspected eosinophilic meningitis due to *A. cantonensis*.[30,31] In addition, a multi-dot ELISA on a single nitrocellulose membrane strip (Fig 4) has been developed and evaluated for the rapid and simple differential diagnosis of eosinophilic meningitis due to helminth infections using ultrafiltered, purified antigens of *A. cantonensis*, *Gnathostoma spinigerum* and *Taenia solium* metacestodes, the most common parasites that invade the central nervous system and cause eosinophilic pleocytosis.[32] Although there are weak cross-reactions among the parasite antigens of *A. cantonensis* and *G. spinigerum*, these do not interfere with judgment as the darkest dot which indicates the infecting parasite is apparent in all cases. The advantage of this method is that semi-purified specific parasite antigens can be used with reliability.

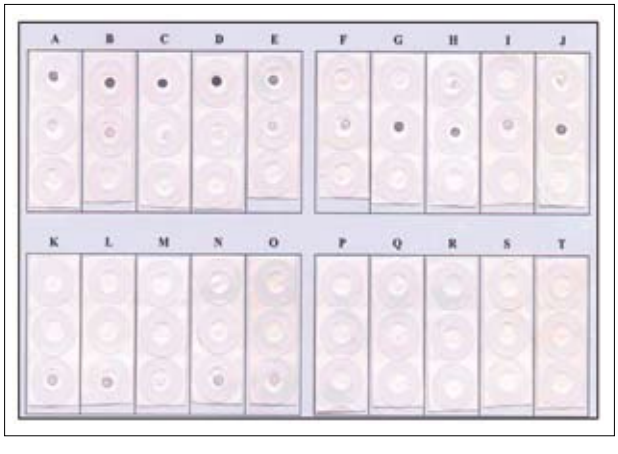

Fig 4. Multi-dot ELISA using ultra-filtered purified antigens of *A cantonensis* (top dot), *G. spinigerum* (middle dot) and *T. solium* metacestodes (bottom dot), on nitrocellulose membrane strip for detection of specific antibodies in sera of patients with angiostrongyliasis (A-E), gnathostomiasis (F-J), cysticercosis (K-O), toxocariasis (P), filariasis (Q), paragonimiasis (R) and malaria (S), and normal control serum (T). Deep coloured dot shows positive reaction; no colour indicates negative reaction.

Although ELISA, dot-blot ELISA, and the immunoblot test are well accepted as the best diagnostic assays available today for angiostrongyliasis, their technical difficulties associated with the procedures and time constraint, limit their use in epidemiological surveys. Other novel tests utilizing a more rapid and simpler assay format that would retain the same sensitivity and specificity are being developed and evaluated. A new 3-min rapid dot immunogold filtration assay (DIGFA) has been developed to detect the specific IgG antibody of *A. cantonensis* in infected humans.[33] This rapid immunodiagnostic test uses protein A conjugated with colloidal gold as the detection marker instead of enzyme conjugates. In the vertical flow test format, *A. cantonensis* antigens are attached on a special pad for DIGFA, and serum applied, followed by colloidal gold conjugated protein A to give a desired color change for indication of a positive or negative reaction. The diagnostic utility of this rapid dot assay vis-à-vis ELISA was evaluated in a cohort of 166 individuals with *A. cantonensis* infection and trichinellosis, schistosomiasis, cysticercosis, clonorchiasis, fasciolopsiasis and tuberculosis. Preliminary results showed promise with 90.5% sensitivity and 98% specificity.[33] Consequently, the developed test is being adapted and assessed for routine diagnostic use at the Parasitology Laboratory, Siriraj Hospital, Mahidol University. The DIGFA format can be performed with minimal facilities, making it an extremely attractive option for epidemiological studies. Validation studies under field conditions deserve further investigation. Moreover, further improvements to this assay, especially the capability to also differentiate other clinically related parasites in a multidot test format, and development into an end-user format where only a drop of sample and, if necessary, buffer needs to be added to the test pad would broaden its applicability. It is likely that this 3-min DIGFA test will finally replace the 2-hr dot-ELISA for both routine use and community screening of eosinophilic meningitis patients in areas where *A. cantonensis* is endemic. This approach is also promising in terms of future diagnostic test kits.

Antigen detection methods

One major limitation of antibody-based assays is that antibodies may not be detected in a certain proportion of individuals who have active disease. In order to overcome the limitation, several attempts have been made to develop antigen-based tests in the belief that the detection of antigens would correlate with the presence of live and active *A. cantonensis* within the human host. The detection of specific *A. cantonensis* antigens in the serum or cerebrospinal fluid provides a more rapid confirmation of current or active infection. To detect defined *A. cantonensis* antigens in patient's blood during acute infection, alternative antigen capture assays have employed monoclonal antibodies directed against parasite specific antigens with relatively high specificity and reasonably good sensitivity.[2,16,34]

Panels of monoclonal antibodies that are reactive toward the somatic extract antigens of *A. cantonensis* have been generated - e.g. several monoclonal antibodies

against the adult worm antigens;[35] and four specific IgG monoclonals against the young adult worm antigens.[36] However, they show low specificity and sensitivity.[37] In more recent years, specific monoclonal antibodies to *A. cantonensis* have been produced for clinical diagnosis of active angiostrongyliasis cantonensis.[38] The AW-3C2 MAb has been used as a capture reagent in sandwich ELISA to detect specific circulating antigen in the sera of angiostrongyliasis cantonensis patients with 100% specificity and 50% sensitivity.[38] A more simple test, immunodot, has also been developed using specific *A. cantonensis* monoclonal antibody (AW-3C2).[39] Although the diagnostic specificity approaches 100%, the sensitivity is around 60%.[39] Furthermore, this simple immunodot test can be used in field surveys. Blood collected on filter papers may also be eluted and used to detect antigens of the parasite.[2]

Although standardization of reagents appears to be less of a problem, the sensitivity of the antigen detection assay needs to be enhanced. As low sensitivity of the tests may arise with the binding of a single antibody to a single antigenic epitope, using a panel of monoclonals which belong to different subclasses of IgG to react with different epitopes on the same circulating antigen may overcome the problem of sensitivity.[40] Very promising results have been achieved using double monoclonal antibodies (AcJ1 and AcJ20) with a specificity of 100% in serum and cerebrospinal fluid from patients with eosinophilic meningitis or meningoencephalitis with worms recovered from them.[40] Recently, three specific monoclonals (2A2, 3F1, 4H2) against the adult worm show a positive detection rate of 86.4%.[41,42]

A more sensitive method for antigen detection by means of a specific antibody-DNA conjugate, the immuno-polymerase chain reaction (immuno-PCR), is one such technique incorporating a molecular method (this will be discussed in detail in the next section), for the identification of the circulating antigens of *A. cantonensis* fifth-stage worm. Immuno-PCR technology uses a reporter DNA target that is amplified to diagnostic concentrations by PCR. Using the hybridomas AcJ1 and AcJ20, the immuno-PCR detects a circulating 204-kDa AcL5 antigen in human patients with eosinophilic meningitis or meningoencephalitis with 100% specificity and 98% sensitivity.[43]

It is likely that many more monoclonal antibodies of correct binding specificity, affinity, avidity and stability are still required. On the other hand, the failure of generated monoclonal antibodies to detect parasite antigens in infected patients may be related to the immune complex formation. Antigen epitopes may be blocked by native antibodies leading to this immune complex formation. Accordingly, when monoclonal antibodies are used, small quantities of circulating antigen may escape detection. Furthermore, a balanced dilution between antigen and antibody concentrations needs to be properly determined in order not to compromise the sensitivity of the detecting assay.

Since the development of ELISAs for the detection of *A. cantonensis* antigens in clinical samples, the potential of a rapid and simple field-applicable test has been recognized. However, initiatives up to now have been hampered by the lack of commercial interest, funds, and availability of the right technology. Although an immunodot assay for the detection of *A. cantonensis* antigens in serum or CSF of infected patients has been developed, this assay involves a number of steps and thus cannot be regarded as truly user-friendly and field applicable.

In the last few years, various rapid lateral-flow assays have been developed for many different applications. The lateral-flow formats use the principle of lateral flow through a nitrocellulose strip of the sample mixed with a colloidal carbon conjugate of a panel of specific monoclonal antibodies. This test is rapid as well as easy to perform and requires no technical equipment or special personnel training. This assay can easily be developed to an end-user format. By using available technology and know-how, an assay that is simple, user-friendly, and field applicable can be adapted for diagnosis of human angiostrongyliasis.

Currently, at the Department of Parasitology, Siriraj Hospital, a monoclonal-based rapid chromatographic immunoassay based on antibodies tagged with colloidal gold which bind to *A. cantonensis* specific antigens in the sera of infected individuals, is under development. Moreover, since infections with multiple parasite infections are the norm in the developing countries, including Thailand, there is a need for a diagnostic parasitology laboratory to ascertain accurate diagnosis. The development of a rapid reagent strip/card approach to distinguish eosinophilic meninigitis due to other clinically related parasites, i.e. *G. spinigerum* and *T. solium* metacestodes is also awaited, as the chemotherapy and pathogenesis of these parasites differ.

Molecular methods

Techniques in molecular diagnostics for parasitic infections usually incorporate nucleic-acid-based assays to detect parasites or products of parasites. In general, a reporter DNA or RNA molecule, probe or primer, is used to either amplify the nucleic acid in a polymerase chain reaction (PCR) or to detect DNA or RNA sequences of parasites by hybridization.

Nucleic acid probe-based diagnosis can be an alternative to immunodiagnosis. This method has the advantage of a direct assay of current infection. The characterization of several genes of *A. cantonensis* recently has paved the way for the use of molecular probes for the diagnosis of angiostrongyliasis cantonensis in humans.[44] With the rising challenge for diagnostics, a molecular approach is now seen as a potential new tool for angiostrongyliasis diagnosis.

A PCR-technique has been described for the DNA detection of abdominal angiostrongyliasis caused by *A. costaricensis* in clinical samples from patients in Brazil.[45] The primers were constructed based on a mRNA sequence that encodes a 66-kDa native protein of the *A. cantonensis* adult worm, and amplified a 232-bp fragment from serum samples of three patients with histopathologically confirmed angiostrongyliasis costaricensis.

Likewise, a similar application of the primers has been studied in the case of *A. cantonensis* infection. Initial trials using cerebrospinal fluid from Thai patients with serologically suspected angiostrongyliasis cantonensis conducted at the Department of Parasitology, Faculty of Medicine Siriraj Hospital, revealed an amplified fragment of approximately 300 bp in four out of ten patients (Fig 5). Preliminary results are encouraging. Nonetheless, more cerebrospinal fluid samples from parasitologically confirmed *A. cantonensis* infected patients as well as clinical samples from other clinically related

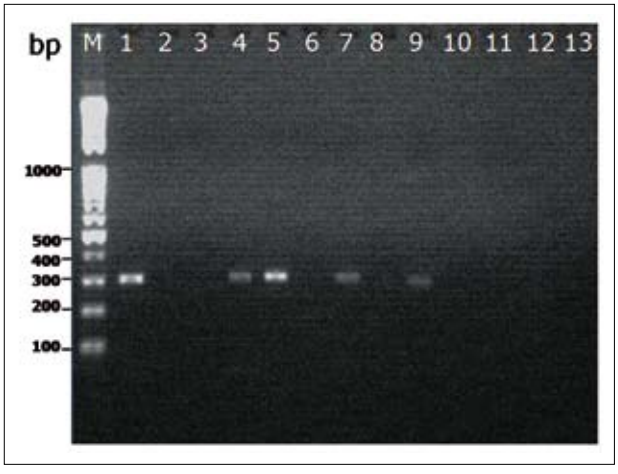

Fig 5. Gel electrophoresis showing positive PCR products from CSF samples of four infected patients with serological positive angiostrongyliasis cantonensis (Lanes 4, 5, 7, 9). Lane 1, positive control; Lane 2, negative control (master mix); Lane M, molecular weight markers (100-bp ladder).

parasitic infections are needed for confirmation of the specificity and sensitivity of PCR in diagnostic use. It may be possible in future studies to develop more specific primers/probes and to increase the sensitivity of this assay by signal amplification using polymerase chain reaction technologies.

The importance of a molecular diagnostic tool for detecting and identifying *Angiostrongylus* species from natural samples is well recognized. Methods for distinguishing field and laboratory strains/isolates of the *Angiostrongylus* nematode are needed in diagnostic laboratories in endemic areas. The ability of PCR to amplify the hybridization target sequence will aid in studying the snail intermediate hosts and animal hosts of the parasite by detecting the parasites that have been previously overlooked. A molecular method using PCR-direct sequencing has recently been developed using the *Angiostrongylus* 18S rRNA gene as a genetic marker to detect and confirm the presence of the third-stage larvae of *A. cantonensis* in infected mollusks collected in the environment.[46] Simultaneously, a similar PCR-DNA sequencing study using the 5' end of the small subunit (SSU) ribosomal (r) RNA gene as a marker has been reported to identify the third juvenile stage of *A. cantonensis* in infected black slug *Laevicaulis altae* from The Philippines.[47] The phylogenetic position of other non-angiostrongylid nematodes isolated was also determined.[47] Likewise, a study in Brazil has employed a polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) directed to the internal transcribed spacer 2 (ITS2) region and using the restriction enzyme *Cla* I to confirm the identity of the third-stage larvae recovered from naturally infected snails as *A. cantonensis*.[48] These observations can also lead to a useful application of molecular assays to differentiate *Angiostrongylus* species and subspecies and subsequently to the direct diagnosis of angiostrongyliasis in patients. Furthermore, efforts to control angiostrongyliasis in endemic areas also require a thorough knowledge of the ecology and epidemiology of the parasite. Large-scale epidemiologic studies should also be enhanced by this technique, given the potential to screen many samples at one time.

Until now, DNA probe-based diagnosis has been limited to highly repeated DNA or RNA sequences. However, the PCR has eliminated the need for such repeated sequences and should allow in future for the development of DNA probes specific for certain characteristics of the parasite, such as virulence. Further work is still needed concerning the development of more specific probes for all *Angiostrongylus* species. The ability of the PCR to increase the sensitivity of these specific probes will make its application a necessity in the future. Further refinements of this methodology will certainly be of immense diagnostic and epidemiologic value.

CONCLUSION

With continued interest in the rapid diagnostics for parasite identification, clinical laboratories are now reviewing their test ordering options with regard to commercial kits that can be incorporated into their routine laboratory testing protocols. Currently, although the conventional ELISA and immunoblot for routine diagnosis and a dot-blot ELISA adapted for field use, as well as a multi-immunodot for differential diagnosis of clinical suspected angiostrongyliasis have revolutionized diagnosis of eosinophilic meningitis due to *A. cantonensis* infection worldwide, the use of an enzyme immunoassay format, which requires multiple reagent additions, washing steps and incubations, is time-consuming, labor-intensive and requires laboratory infrastructure. The challenge is to develop more rapid and simpler tests using a non-enzymatic immunoassay that retain the exquisite sensitivity and specificity of enzyme immunoassay for diagnosis of angiostrongyliasis. A 3-min dot immuno-colloidal gold technique which is rapid and easy to perform has been developed and is now being evaluated for routine and field use. Preliminary results with such a format are promising, indicating that the final goal of a field-applicable rapid test based on parasite antibody detection for the diagnosis of angiostrongyliasis cantonensis is within sight.

The ability to concurrently detect and distinguish among clinically related parasites that cause eosinophilic meningitis in clinical specimens with a multi-immunodot provides another very useful diagnostic tool, and this may be accomplished with the more rapid non-enzymatic format, dot-immunogold test. The procedure is rapid, simple to perform, requires minimal training, and can be used for a single specimen or batch-testing approaches. The multiple parasite panels will provide diagnostic laboratories with a simple, convenient, alternative method for performing simultaneous, discrete detection of various related parasites in patient specimens

PCR-based assays have been described in research settings for the diagnosis of abdominal angiostrongyliasis caused by *A. costaricensis* and cerebral angiostrongyliasis due to *A. cantonensis*. The method still needs to be evaluated in comparison with other conventional parasitological or immunological methods in clinical and field setting. Additionally, it is also important to note that laboratory results should be used in conjunction with clinical manifestations and exposure history for accurate diagnosis of eosinophilic meningitis due to *A. cantonensis*.

ACKNOWLEDGEMENTS

The SEAMEO-TROPMED Network, the Siriraj China Medical Board Grant, the Princess Mother's Medical Volunteer Foundation of Thailand, and the Department of Disease Control, Ministry of Public Health, Thailand, are acknowledged for financial support. The comments and suggestions by Emeritus Professor Dr. HS Yong, University of Malaya have greatly improved the manuscript and are gratefully acknowledged. I thank the publisher and Chief Editor Professor Bill Cameron of IJID for permission to reproduce figures 2, 3 and 4 that appeared in Eamsobhana P, Yong HS. Immunological diagnosis of human angiostrongyliasis cantonensis. International Journal of Infectious Diseases 2009;13:425-431.

REFERENCES

1. Ubelaker JE. Systematics of species referred to the genus *Angiostrongylus*. J Parasitol. 1986 Apr;72(2):237-44.
2. Eamsobhana P. The rat lungworm *Parastrongylus* (= *Angiostrongylus*) *cantonensis*: parasitology, immunology, eosinophilic meningitis, epidemiology and laboratory diagnosis. Bangkok: Wankaew (IQ) Book Center Co Ltd, 2006.
3. Cross JH, Chen ER. Angiostrongyliasis. In: Black SJ, Seed JR, eds. Foodborne parasitic zoonoses. USA: Springer Science, 2007:263-92.
4. Bhaibulaya M. Snail borne parasitic zoonoses: angiostrongyliasis. Southeast Asian J Trop Med Public Health. 1991 Dec;22 Suppl:189-93.
5. Punyagupta S, Juttiyudata P, Bunnag T. Eosinophilic meningitis in Thailand. Clinical studies of 484 typical cases probably caused by *Angiostrongylus cantonensis*. Am J Trop Med Hyg. 1975 Nov;24(6 Pt 1):921-31.
6. Punyagupta S. Angiostrongyliasis: clinical features and human pathology. In: Cross JH, ed. Studies on angiostrongyliasis in Eastern Asia and Australia. NAMRU-2-SP-44. Taipei, Taiwan: US Army Medical Research Unit No. 2, 1979:138-50.
7. Jin E, Ma D, Liang Y, Ji A, Gan S. MRI findings of eosinophilic myelomeningoencephalitis due to *Angiostrongylus cantonensis*. Clin Radiol. 2005 Feb;60(2):242-50.
8. Kanpittaya J, Jitpimolmard S, Tiamkao S, Mairiang E. MR findings of eosionphilic meningoencephalitis attributed to *Angiostrongylus cantonensis*. Am J Neuroradiol. 2000 Jun-Jul;21(6):1090-4.
9. Tiewcharoen S, Keerasuntonpong A, Eamsobhana P, Danpukdee K, Benjarassamerote S. Eosinophilic meningitis due to *Angiostrongylus cantonensis*. Siriraj Hosp Gaz 2002;54:797-802.
10. Tsai HC, Liu YC, Kunin CM, Lai PH, Lee SSJ, Chen YS, et al. Eosinophilic meningitis caused by *Angiostrongylus cantonensis* associated with eating raw snails: correlation of brain magnetic resonance imaging scans with clinical findings. Am J Trop Med Hyg. 2003 Mar;68(3):281-5.
11. Ko RC. Application of serological techniques for the diagnosis of angiostrongyliasis: In: Ko RC, ed. Current Concepts in Parasitology. Hong Kong: The University of Hong Kong Press; 1989:101-10.
12. Tharavanij S. Immunology of angiostrongyliasis. In: Cross JH, ed. Studies on angiostrongyliasis in Eastern Asia and Australia. NAMRU-2-SP-44. Taipei, Taiwan: US. Naval Medical Research Unit No. 2, 1979:151-64.
13. Intapan PM, Maleewong W, Polsan Y, Sawanyawisuth K, Chotmongkol V. Specific IgG antibody subclasses to *Angiostrongylus cantonensis* in patients with angiostrongyliasis. Asian Pac J Allergy Immunol. 2002 Dec; 20(4):235-40.
14. Dorta-Contreras AJ, Noris-Garcia E, Escobar-Perez X, Duenas-Flores A, Mena-Lopez R. IgG subclasses intrathecal synthesis patterns in eosinophilic meningoencephalitis due to *Angiostrongylus cantonensis*. Rev Neurol. 2003 Mar 16-31;36(6):506-9.
15. Dorta-Contreras AJ, Noris-Garcia E, Escobar-Perez X, Padilla-Docal B. IgG1, IgG2 and IgE intrathecal synthesis in *Angiostrongylus cantonensis* meningencephalitis. J Neurol Sci. 2005 Nov 15;238(1-2):65-70.
16. Eamsobhana P. Immunological studies on the rat lung-worm *Angiostrongylus cantonensis* (Nematoda: Metastrongylidae). PhD Thesis. Kuala Lumpur: University of Malaya; 1994.
17. Eamsobhana P, Tungtrongchitr A, Wanachiwanawin D, Yong HS, Mak JW. Characterization of a 31-kDa specific antigen from *Parastrongylus cantonensis* (Nematoda: Metastrongylidae). Int Med Res J 1998;2:9-12.
18. Nuamtanong S. The evaluation of the 29 and 31 kDa antigens in female *A. cantonensis* for serodiagnosis of human angiostrongyliasis. Southeast Asian J Trop Med Public Health. 1996 Jun;27(2):291-6.
19. Maleewong W, Sombatsawat P, Intapan PM, Wongkham C, Chotmongkol V. Immunoblot evaluation of the specificity of the 29-kDa antigen from young adult female worms *Angiostrongylus cantonensis* for immunodiagnosis of human angiostrongyliasis. Asian Pac J Allergy Immunol. 2001 Dec;19(4):267-73.
20. Li H, Chen XG, Shen HX, Chen DX, Qiu YR, Hu XJ. Value of antigen with molecular mass of 32 000 in immunodiagnosis of *Angiostrongylus cantonensis*. Di Yi Jun Yi Da Xue Xue Bao. 2005 Apr;25(4):380-3.
21. Chye SM, Chang JH, Yen CM. Immunodiagnosis of human eosinophilic meningitis using an antigen of *Angiostrongylus cantonensis* L5 with molecular weight 204 kD. Acta Trop. 2000 Feb 25;75(1):9-17.
22. Li H, Chen XG, Shen HX, Peng HJ, Zhao XC. Antigen analysis of *Angiostrongylus cantonensis* in different developmental stages. Zhongguo Ji Sheng Chong Xue Yu Ji Sheng Chong Bing Za Zhi. 2005 Feb 28;23(1): 36-9.
23. Intapan PM, Maleewong W, Polsan Y, Sawanyawisuth K, Chotmongkol V. Evaluation of human IgG subclasses antibodies in the serodiagnosis of angiostrongyliasis. Parasitol Res. 2003 Apr;89(6):425-9.
24. Kittimongkolma S, Intapan PM, Laemviteevanich K, Kanpittaya J, Sawanyawisuth K, Maleewong W. Eosinophilic meningitis associated with angiostrongyliasis: clinical features, laboratory investigations and specific diagnostic IgG and IgG subclass antibodies in cerebrospinal fluid. Southeast Asian J Trop Med Public Health. 2007 Jan;38(1):24-31.
25. Eamsobhana P, Tungtrongchitr A, Wanachiwanawin D. A partially purified specific antigen of Parastrongylus cantonensis for immunodiagnosis of human parastrongyliasis. Int Med Res J 2000;4:23-9.
26. Eamsobhana P, Yoolek A, Suvouttho S, Suvuttho S. Purification of a specific immunodiagnostic *Parastrongylus cantonensis* antigen by electroelution from SDS-polyacrylamide gels. Southeast Asian J Trop Med Public Health. 2001 Jun;32(2):308-13.
27. Eamsobhana P, Yoolek A, Punthuprapasa P, Suvouttho S. A dot-blot ELISA comparable to immunoblot for the specific diagnosis of human parastrongyliasis. J Helminthol. 2004 Dec;78(4):287-91.
28. Eamsobhana P, Yoolek A, Kreethapon N. Blinded multi-laboratory evaluation of an in-house dot-blot ELISA kit for diagnosing human parastrongyliasis. Southeast Asian J Trop Med Public Health. 2003 Mar;34(1):1-6.
29. Eamsobhana P, Yoolek A, Punthuprapasa P. Dot-blot ELISA for the immunological detection of specific antibody to *Parastrongylus cantonensis*. Trop Biomed 2003;20:1-6.
30. Chaiyaserth P, Poosikeaw P, Pooribuncha L, Phapirom S. Prevalent study of eosinophilic meningitis in Khon Kaen Province. Khon Kaen: Klungnana-vitaya Press, 2002:27-31.
31. Chaiyaserth P, Poosikeaw P, Pooribuncha L, Phapirom S. Retrospective studies on eosinophilic meningitis in Wang-yai district, Khon Kaen Province. Khon Kaen: Klungnana-vitaya Press, 2002:21-28.
32. Eamsobhana P, Ongrotchanakun J, Yoolek A, Monkong N, Punthuprapasa P, Dekumyoy P. Multi-immunodot for rapid differential diagnosis of eosinophilic meningitis due to parasitic infections. J Helminthol. 2006 Sep;80(3):249-54.
33. Gan XX, Wang Y, Guo JX, Yang FZ, Zhang LL, Eamsobhana P. Rapid detection of specific IgG antibody for *Angiostrongylus cantonensis* by dot immunogold filtration assay. Chin J Zoonoses. 2007;23:345-7.
34. Eamsobhana P, Yong HS. Immunological diagnosis of human *angiostrongyliasis* due to Angiostrongylus cantonensis (Nematoda: Angiostrongylidae). Int J Infec Dis. 2009 Jul;13(4):425-31.
35. Yen CM, Chen ER, Kojima S, Kobayashi M. Preparation of monoclonal antibody against *Angiostrongylus cantonensis* antigen. Southeast Asian J Trop Med Public Health. 1989 Mar;20(1):119-24.
36. Chang JH, Yen CM, Chen ER. Characterization of monoclonal antibody to young-adult worms of *Angiostrongylus cantonensis*. Hybridoma. 1990 Oct;9(5):465-71.
37. Shih HH, Chen SN. Immunodiagnosis of angiostrongyliasis with monoclonal antibodies recognizing a circulating antigen of mol. wt. 91,000 from *Angiostrongylus cantonensis*. Int J Parsitol. 1991 Apr;21(2):171-7.
38. Eamsobhana P, Mak JW, Yong HS. Detection of circulating antigens of *Parastrongylus cantonensis* in human sera by sandwich ELISA with specific monoclonal antibody. Southeast Asian J Trop Med Public Health. 1995 Dec;26(4):712-5.
39. Eamsobhana P, Yong HS, Mak JW, Wattanakulpanich D. Detection of circulating antigens of *Parastrongylus cantonensis* in human sera by dot-blot ELISA and sandwich ELISA using monoclonal antibody. Southeast Asian J Trop Med Public Health. 1997 Sep;28(3):624-8.
40. Chye SM, Yen CM, Chen ER. Detection of circulating antigen by monoclonal antibodies for immunodiagnosis of angiostrongyliasis. Am J Trop Med Hyg. 1997 Apr;56(4):408-12.
41. Liang SH, Huang HC, Pan CW, Tan F. Detection of *Angiostrongylus cantonensis* circulating antigen by monoclonal antibodies. Zhonghua Yi Xue Za Zhi. 2005 Nov 16;85(43):3057-61.
42. Tan F, Pan CW, Liang SH, Huang HC. Preparation and preliminary application of monoclonal antibodies against adult worm of *Angiostrongylus cantonensis*. Zhongguo Ji Sheng Chong Xue Yu Ji Sheng Chong Bing Za Zhi. 2005 Aug 30;23(4):209-12.
43. Chye SM, Lin SR, Chen LY, Chung LY, Yen CM. Immuno-PCR for detection of antigen to *Angiostrongylus cantonensis* circulating antigen fifth-stage worms. Clin Chem. 2004 Jan;50(1):51-7.
44. Bessarab IN, Joshua GWP. Stage-specific gene expression in *Angiostrongylus cantonesis*: characterization and expression of an adult-specific gene. Mol Biochem Parasitol. 1997 Sep;88(1-2):73-84.
45. da Silva AC, Graeff-Teixeira C, Zaha A. Diagnosis of abdominal angiostrongyliasis by PCR from sera of patients. Rev Inst Med Trop S Paulo. 2003 Sep-Oct;45(5):295-7.
46. Qvarnstrom Y, Sullivan JJ, Bishop HS, Hollingsworth R, da Silva AJ. PCR-based detection of *Angiostrongylus cantonensis* in tissue and mucus secretions from molluscan hosts. Appl Env Microbiol. 2007 Mar;73(5): 1415-9.
47. Fontanilla IKC, Wade CM. The small subunit (SSU) ribosomal (r) RNA as a genetic marker for identifying infective 3rd juvenile stage *Angiostrongylus cantonensis*. Acta Tropica. 2008 Feb;105(2):181-6.
48. Caldeira RL, Carvalho OS, Mendonca CLFG, Graeff-Teixeira C, Silva MCF, Ben R, et al. Molecular differentiation of *Angiostrongylus costaricensis*, A. cantonensis and A. vasorum by polymerase chain reaction-restriction fragment length polymorphism. Mem Inst Oswaldo Cruz. 2003 Dec;98(8):1039-43.