

Eosinophilic meningitis caused by *Angiostrongylus cantonensis*: an emergent disease in Brazil

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Eosinophilic meningitis (EoM) is an acute disease that affects the central nervous system. It is primarily caused by infection with the nematode Angiostrongylus cantonensis. This infection was previously restricted to certain Asian countries and the Pacific Islands, but it was first reported in Brazil in 2007. Since then, intermediate and definitive hosts infected with A. cantonensis have been identified within the urban areas of many states in Brazil, including those in the northern, northeastern, southeastern and southern regions. The goals of this review are to draw the attention of the medical community and health centres to the emergence of EoM in Brazil, to compile information about several aspects of the human infection and mode of transmission and to provide a short protocol of procedures for the diagnosis of this disease.

Key words: eosinophilic meningitis - *Angiostrongylus cantonensis* - emergent in Brazil - algorithm

Nomura and Lin first described eosinophilic meningitis (EoM) caused by *Angiostrongylus cantonensis* in 1944, based on the observation of a nematode in the cerebrospinal fluid (CSF) of a patient with meningitis (Prociv et al. 2000). EoM caused by *A. cantonensis* may also be referred to as cerebral angiostrongyliasis (CA) or neuroangiostrongyliasis.

A. cantonensis typically occurs in southeast Asian countries and the Pacific Islands. However, this scenario began to change with the first reported occurrence in the Americas in 1981 when infected rats and snails were found in Cuba (Aguiar et al. 1981). Since that time, the occurrence of *A. cantonensis* has been reported in the United States of America, Jamaica and Ecuador (Kim et al. 2002, Slom et al. 2002, Wang et al. 2008, Pincay et al. 2009); it has recently been found in Brazil (Caldeira et al. 2007, Lima et al. 2009, Espírito-Santo et al. 2013).

Although intermediate and definitive hosts infected with *A. cantonensis* have been identified in Brazil, few cases of human infection have been reported to date. This situation could be interpreted simply as due to a lack of information about the parasite and its occurrence in Brazil. When CSF eosinophilia is detected, angiostrongyliasis should be considered in the differential diagnosis.

This review focuses on the distribution of *A. cantonensis* in Brazil and provides basic information about the biology of this parasite, as well as the clinical aspects, diagnosis and recommended treatment of infection.

Biological aspects of A. cantonensis infection - Adult *A. cantonensis* worms are not found in infected humans. *Rattus norvegicus* is the main definitive host in which adult worms live inside the pulmonary arteries and produce the infective stage larvae (L1) for molluscs. In addition to *R. norvegicus*, other rodents may have a role as definitive hosts (Alicata 1965). These larvae are released inside the bronchial tree, swallowed and eliminated with the rats' faeces. Molluscs become infected both by ingestion of L1 or penetration of that larval form through the mollusc tegument. The larvae undergo two moults and produce the third stage (L3) that is eventually released along with the mollusc mucous secretions. Humans become infected after ingestion of L3 larvae; the parasites migrate to the central nervous system (CNS) where they moult twice (L4 and L5) and eventually die in the meninges.

Planarians, frogs, fishes, crabs, shrimps and lizards may serve as paratenic hosts of *A. cantonensis*. The parasite does not develop within these hosts, but they may be sources of infection (Ash 1968, Radomyos et al. 1994, Tsai et al. 2011).

Occurrence of A. cantonensis in Brazil - Naturally infected definitive and intermediate hosts have been found in several municipalities from northern to southern Brazil. *A. cantonensis* larvae from molluscs or adult worms in rats have been detected in the states of Paraná, São Paulo (SP), Rio de Janeiro (RJ), Pará, Bahia, Santa Catarina and Rio Grande do Sul (Figure, Table I) (Caldeira et al. 2007, Maldonado Júnior et al. 2010, Simões et al. 2011, Carvalho et al. 2012, Cognato et al. 2013).

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Several species of land snail can be served undercooked as a dish that is known in French cuisine as escargot. This term is also applied to commonly consumed molluscs, such as *Helix pomatia* (L.), *Helix aspersa* (L.) and *Helix lucorum*. *Achatina fulica* (Bowdich, 1822), the “giant African snail”, is considered to have a high CA transmission potential because of its extensive presence in many parts of Brazil and its susceptibility to *A. cantonensis* infection (Thiengo et al. 2007, Vitta et al. 2011, Carvalho et al. 2012). Infected *A. fulica* and other land and freshwater molluscs have been found in many parts of Brazil (Table I).



Distribution map of *Angiostrongylus cantonensis* and angiostrongyliasis cases in Brazil. AL: Alagoas; AM: Amazonas; BA: Bahia; CE: Ceará; ES: Espírito Santo; MA: Maranhão; MG: Minas Gerais; PA: Pará; PB: Paraíba; PE: Pernambuco; PI: Piauí; PR: Paraná; RJ: Rio de Janeiro; RN: Rio Grande do Norte; RS: Rio Grande do Sul; SC: Santa Catarina; SE: Sergipe; SP: São Paulo.

Human infection - Humans acquire *A. cantonensis* infection by eating raw or undercooked foods, such as snails, slugs, crustaceans (shrimp and crabs), frogs and bush meat (lizards). Condiments, salads, herbs and fruit juices (Tsai et al. 2004) may also contain L3 larvae from crushed molluscs or from mollusc slime trails (Bonetti & Graeff-Teixeira 1998). Infection may also occur by accidental ingestion during hand manipulation of molluscs in fisheries and/or during garden upkeep. Food from domestic vegetable gardens and ecological fairs may pose a higher risk of contamination, as the lack of industrial processing and pesticides may favour mollusc infection.

After ingestion, L3 larvae penetrate the intestinal walls, gain access to the bloodstream and migrate to the CNS. The presence of L3-L5 larvae in the meninges induces an eosinophilic inflammatory response.

The pathophysiology of CA is attributed primarily to larval movement and also involves proteolytic enzymes and pro-inflammatory and cytotoxic agents that

are released by eosinophil granules. These enzymes and agents exacerbate cerebral tissue lesions together with vascular insufficiency due to inflammation, which may elicit permanent neurotrophic damage. This inflammatory process leads to larval death in the meninges, which in turn exacerbates inflammation.

Clinical manifestations - CA is an acute disease that may be asymptomatic and it is characterised by the spontaneous disappearance of symptoms within a few weeks; though very rarely, it can lead to severe sequelae (Graeff-Teixeira et al. 2009). This disease is fatal in at least 3% of all cases (Eamsobhana & Yong 2009).

The prepatent period may range from one day to three months post-infection, with symptoms occurring within two weeks in most cases (Sawanyawisuth et al. 2009). The clinical manifestations of CA depend on larval localisation; heavy infestations can produce encephalitis with severe neurological symptoms, coma and even death. Involvement of the spinal cord can produce radiculitis (Cooke-Yarborough et al. 1999).

The classical triad pattern of meningitis (headache, neck stiffness and fever) is frequently observed in patients with EoM, but all three symptoms are not always present. An extensive analysis of symptoms in 484 patients revealed that a headache was present in 99% of the patients, neck stiffness in 64% and fever in only 37% of the patients (Punyagupta et al. 1975). Other symptoms, such as diplopia or blurred vision (38%), nausea (38%) and vomiting (49%) may be present. Less commonly reported symptoms include muscle weakness, orbital/retro-orbital pain, ataxia, abdominal pain, body and extremity aches, convulsions, facial paralysis, somnolence and urinary retention/incontinence (Wang et al. 2008, Graeff-Teixeira et al. 2009, Sawanyawisuth et al. 2009). Ocular infection is a rare clinical manifestation (~1%) that may lead to permanent lesions and vision loss (Sawanyawisuth et al. 2007). Eosinophilic pneumonitis occurs in approximately 6% of the cases (Jin et al. 2008).

Diagnosis - CA is clinically diagnosed by a history of headaches, larval exposure within three months and evidence of eosinophilia in > 10% of the white blood cells in CSF (Ramirez-Avila et al. 2009, Sawanyawisuth et al. 2009). The laboratory examinations are discussed below. An initial complete blood count assessment is recommended in patients suspected of having EoM. Peripheral eosinophilia is common; an eosinophil count > 798 in an individual at risk (e.g., due to a history of raw mollusc consumption) has shown 76.6% sensitivity, 80.2% specificity, a 58.1% positive predictive value and an 90.5% negative predictive value, which support the diagnosis of CA (Sawanyawisuth et al. 2010).

Imaging examination - Magnetic resonance imaging (MRI) has been recommended for the diagnosis of CA because it has identified abnormalities in many patients. Analyses of 74 patients who were infected with *A. cantonensis* using intravenous administration of gadolinium chelate revealed that the neuropathology of angiostrongyliasis includes cerebral congestion and thickened leptomeninges; multiple 150 µm microcavities in the brain and spinal cord correlated with the migration

TABLE I

Naturally infected hosts of *Angiostrongylus cantonensis*, municipalities and states where infected species were found in Brazil

Species	States	Municipalities
Molluscs		
<i>Achatina fulica</i>	AM	Barcelos
	BA	Alcobaça, Ilhéus
	CE	Baturité
	ES	Cariacica, Serra
	PA	Belém
	PR	Paranaguá
	PE	Cabo de Santo Agostinho, Olinda, Recife
	RJ	Angra dos Reis, Barra do Piraí, Niterói, Nova Iguaçu, Rio de Janeiro, Queimados, São Gonçalo
	SC	Florianópolis, Joinville, Navegantes
	SP	Jundiaí, Rio Claro, São Paulo
<i>Bradybaena similis</i>	BA	Ilhéus
	ES	Cariacica, Serra
	SC	Navegantes
	SP	Santos
<i>Pomacea lineata</i>	PE	Escada
<i>Sarasinula linguaeformis</i>	SP	São Paulo
<i>Sarasinula marginata</i>	BA	Salvador
	ES	Cariacica, Vila Velha
	PA	Belém
	RJ	Niterói
	SP	Santos, São Paulo
<i>Subulina octona</i>	BA	Ilhéus
	ES	Cariacica
	PA	Belém
	PE	Recife
	SP	Santos, São Sebastião
Rats		
<i>Rattus norvegicus</i>	PA	Belém
	RJ	São Gonçalo
	RS	Porto Alegre
<i>Rattus rattus</i>	PA	Belém

AM: Amazonas; BA: Bahia; CE: Ceará; ES: Espírito Santo; PA: Pará; PR: Paraná; PE: Pernambuco; RJ: Rio de Janeiro; RS: Rio Grande do Sul; SC: Santa Catarina; SP: São Paulo (Caldeira et al. 2007, Maldonado Júnior et al. 2010, Thiengo et al. 2010, Simões et al. 2011, Carvalho et al. 2012, Cognato et al. 2013, Espírito-Santo et al. 2013, Moreira et al. 2013).

and traffic patterns of larvae (Jin et al. 2005, 2008). Microhemorrhages, dilated perivascular spaces and Wallerian degeneration were also observed (Jin et al. 2005, 2008). However, in 55% of the patients with clinical symptoms of CA, no abnormalities were observed with MRI. Another MRI study involving 13 patients revealed varying degrees of meningeal enhancement and abnormal globus pallidus enhancement, but localised lesions were not observed (Tsai et al. 2003).

MRI is a valuable tool for the evaluation of CNS lesions. Abnormalities such as single or multiple enhancing lesions of the leptomeninges and brain parenchyma

have been observed using MRI, but this modality cannot provide a definitive diagnosis without the concomitant use of other laboratory methods.

Microscopy - A definitive diagnosis of CA is very rarely based on the finding of parasitic larvae in a wet-mount preparation of a fresh CSF sample. Biopsy can also rarely provide a diagnosis; one such case has been reported (Petjom et al. 2002). The patient was first diagnosed with an intramedullary spinal cord tumour, but histopathological analysis of a surgically excised tissue sample revealed eosinophilic infiltration and *A. cantonensis* larvae (Petjom et al. 2002).

Laboratory investigation - The examination of CSF from patients with EoM reveals large numbers of eosinophils. The diagnosis of EoM is usually based on a CSF eosinophil count $\geq 10\%$ of the cells or 10 eosinophils/mL. Importantly, at least two very atypical and fatal cases of encephalitis caused by *A. cantonensis* infection have occurred without CSF eosinophilia, but high levels of eosinophils were detected in the blood from these patients. Autopsies showed meningeal infiltration of eosinophils and the very rare development of adult worms in the lung arteries in both cases (Cooke-Yarborough et al. 1999, Lindo et al. 2004).

The infected CSF is clear, with no colour or turbidity, which allows the clinician to readily distinguish CA from other infections that may cause yellow coloration and viscosity, such as bacterial infections and some fungal infections. This characteristic also enables distinction from an infection by *Gnathostoma* spp nematodes that are common in Asian countries and that produce bloody CSF due to haemorrhagic parenchymal lesions. The CSF from patients with CA may also appear slightly cloudy, resembling coconut juice, which is pathognomonic of angiostrongyliasis (Sawanyawisuth & Sawanyawisuth 2010). Biochemical analyses frequently show normal levels of glucose and protein; an elevated protein level is more common than a low glucose level (Punyagupta et al. 1975), most likely due to larval and tissue degradation that is mediated by eosinophil degranulation.

Immunology - Given the infrequency of a parasitological diagnosis of CA, e.g., the detection of larvae in CSF sediment, the use of indirect tests was studied. The majority of proposed assays use purified antigens, rather than crude extracts. In 1986, Chen (1986) observed that different fractions of purified antigens from juvenile or adult worms increased the sensitivity of ELISAs, though these antigens cross-reacted with *Toxocara canis* infection.

Subsequently, Akao et al. (1992) described two bands with molecular weights of 29-31 kDa from female adult worms that were potentially useful for an immunodiagnosis. Nuamtanong (1996) tested a whole crude antigen from female worms with ELISA and achieved 100% sensitivity and 66.8% specificity regarding a diagnosis of CA. The author also tested the 31-kDa band using western blotting (WB) methods and observed cross-reactivity with trichinellosis, trichuriasis and opisthorchiasis in sera from infected patients.

Eamsobhana et al. (2001) purified the 31-kDa component and reported that it demonstrated 100% sensitivity and specificity in a dot blot assay. The same group (Eamsobhana et al. 2013) recently proposed the use of the 31-kDa antigen in a dot immunogold filtration assay (DIGFA), which is more rapid while also demonstrating 100% sensitivity and specificity.

Another indirect approach to EoM diagnosis is the detection of *A. cantonensis* circulating antigen. Shih and Chen (1991) obtained a monoclonal antibody that recognises an 91-kDa component from the excretion and secretion products of cultivated L3 larvae. In enzyme-linked fluorescent assays, the antibody detected the 91-kDa component in serum and CSF samples from infected

patients with 88% sensitivity (Shih & Chen 1991). In the same way, Chye et al. (1997) developed a monoclonal antibody that detects an antigen with a molecular weight of 204-kDa from L5 juveniles. This antibody showed greater sensitivity in CSF samples rather than serum samples.

These antigens are not widely available. Eamsobhana and Yong (2009) proposed the use of a purified 31-kDa antigen for an in-house kit preparation, but its use is restricted to Thailand. Our laboratory has sought to develop recombinant 31-kDa antigens and make them widely available for diagnostic purposes. Some progress has been made; the composition, coding sequences and dependence on glycidic moieties for immunogenicity of the 31-kDa component have been identified (Morassutti et al. 2012). However, its expression in eukaryotic and prokaryotic systems has not yet produced satisfactory results (Morassutti et al. 2013). Although recombinant proteins are not available, the crude antigen has been employed for ELISAs and WB assays.

Molecular tests - Immunodiagnosis is not possible in the early stages of infection when anti-*Angiostrongylus* immunoglobulins have not been produced. In contrast, nucleic acids are present in CSF samples once nematode-specific cellular debris has been released into the subarachnoid space. Researchers initially sought to develop molecular tests for the identification of infected intermediate hosts for epidemiological studies and to differentiate *Angiostrongylus* larvae from those of many other nematodes that may infect molluscs. Caldeira et al. (2003) developed a polymerase chain reaction (PCR)-restriction fragment length polymorphism test based on the amplification of ribosomal DNA internal transcribed spacer (ITS)2, followed by restriction enzyme cleavage. The authors suggested that this test could be used to diagnose CA (Caldeira et al. 2003). However, the US Centers for Disease Control and Prevention (CDC), in collaboration with a parasitology group from the Pontifical Catholic University of Rio Grande do Sul (PUCRS), Brazil, developed a sensitive quantitative real-time PCR method based on the amplification of ribosomal ITS1 that can detect DNA from the equivalent of less than one larva (Qvarnstrom et al. 2010). The CDC and PUCRS applied this real-time PCR test to human CSF samples and found that it was sufficiently sensitive to detect an amount of nucleic acid that was equivalent to less than one larva; these preliminary results show promise for the identification of infection in humans (Qvarnstrom et al. 2010).

CA in Brazil - In 2006, a human immunodeficiency virus-positive patient from RJ with a history of raw mollusc consumption presented with EoM. An ELISA test showed positivity for the *A. cantonensis* crude antigen. An extensive laboratory investigation led to the confirmation of no other aetiological diagnoses, suggesting that this case was the first observation of *A. cantonensis* infection in Brazil.

In 2007, two intoxicated men aged 22 and 39 years from Cariacica, in the southeastern state of Espírito Santo (ES), ate a raw snail (*Sarasinula marginata* was identified as the predominant species in that area) that was cut into two pieces, on a bet. After four days, the men developed

TABLE II

Diagnosis of *Angiostrongylus cantonensis* infection in Brazilian individuals - Laboratory of Molecular Parasitology of Pontifical Catholic University of Rio Grande do Sul

Case origin city (state)	Positive diagnosis/ suspected cases
Curitiba (PR)	0/1
Distrito Federal (ES)	0/1 3/5
Porto Alegre (RS)	1/1
Porto Velho (RO)	0/11
Recife (PE)	9/27
(RJ)	2/4
São José dos Pinhais (PR)	1/1
Sao Paulo (SP)	18/33
Total	34/84

ES: Espírito Santo; PE: Pernambuco; PR: Paraná; RJ: Rio de Janeiro; RO: Rondônia; RS: Rio Grande do Sul; SP: São Paulo.

abdominal and cervical pain, severe headache, myalgia, arthralgia, neck rigidity, disorientation, dysarthria and limb paralysis and presented at two different hospitals. The CSF eosinophil counts were 20-45%. CA was diagnosed via reverse-transcription (RT)-PCR analysis of CSF samples (Garcia et al. 2008). Another case in ES involved a 20-month-old child from the city of Vila Velha who presented with the same symptoms (Caldeira et al. 2007).

A fatal case of EoM involving a 26-year-old woman was reported in 2009 in the city of Olinda, in the state of Pernambuco. The patient presented with 87% CSF eosinophilia, joint pain and somnolence. The diagnosis of CA was confirmed by RT-PCR analysis of a CSF sample (Lima et al. 2009).

In 2010, an 11-year-old boy in the city of São Paulo (SP) presented with a history of a headache for seven days, no fever, mild neck stiffness and 36% CSF eosinophilia. RT-PCR analysis was negative for *A. cantonensis* infection, but infection was confirmed after 135 days of hospitalisation by positive serum conversion using an ELISA approach (Espírito-Santo et al. 2013). Many other cases of EoM have been diagnosed subsequently (Table II).

In 2013, a mentally deficient young male from the city of Viamão, adjacent to Porto Alegre (RS), presented with EoM. ELISA and WB analyses revealed a positive CA infection (unpublished observations). This case extended the area of EoM occurrence to the southernmost state in Brazil.

Although only a few cases of EoM due to *A. cantonensis* infection have been reported since the emergence of angiostrongyliasis in Brazil, our laboratory has supported many Brazilian health centres in the diagnosis of this disease. More than 80 examinations have been performed to date and at least 34 cases (including unpublished cases) have yielded positive ELISA results that

were confirmed by RT-PCR or WB analyses (Table II). These data draw attention to the potential for identifying additional cases in the near future.

Differential diagnosis of EoM - Although *A. cantonensis* is the main causative agent of EoM, the disease may have other aetiological agents, including parasitic infection by *Gnathostoma* spp, cysticercus, *Schistosoma* spp, *Toxocara* spp and *Trichinella* spp, among others, as well as fungal, bacterial and viral infections [for a review, see Graeff-Teixeira et al. (2009)]. EoM also occurs in 30% of patients with intraventricular shunt malfunctions (Bezerra et al. 2011).

Treatment of CA - The treatment of CA consists primarily of reducing the inflammatory response and relieving pain. The majority of hospitals in areas where the disease is most prevalent have adopted the administration of oral corticosteroids (prednisolone, 60 mg/kg/day) for 14 consecutive days (Sawanyawisuth 2008, Tseng et al. 2011). Intravenous administration may be necessary when the patient is unconscious.

The effectiveness of anthelmintic drugs for the treatment of CA remains unclear. A single placebo-controlled clinical trial has been conducted; it demonstrated the efficacy of treatment with albendazole (15 mg/kg twice a day for 14 days) in 57 patients (Jitpimolmard et al. 2007). However, Prociv et al. (2000) postulated that anthelmintic administration causes greater damage due to the increased inflammatory reaction that is triggered by massive parasitic death. Thus, the concomitant use of anthelmintic and anti-inflammatory drugs (prednisolone, 60 mg/day, and albendazole, 15 mg/kg/day, or mebendazole, 10 mg/kg/day, for two weeks) has been investigated (Chotmongkol et al. 2004).

Table III summarises the treatment regimens for EoM that is caused by *A. cantonensis* infection. A severe headache may persist for months in patients who receive only analgesic treatment. The administration of corticosteroids alone or in combination with anthelmintic therapy (e.g., albendazole or mebendazole) can significantly shorten the duration of a headache. However, a recent trial found no significant difference in the outcomes of corticosteroid and corticosteroid plus albendazole regimens (Chotmongkol et al. 2009). As mentioned above, anthelmintic therapy may not be favourable due to prolonged inflammatory processes. In clinical practice, a two-week course of corticosteroids is recommended in the absence of an obvious contraindication, such as diabetes or immunodeficiency. Minor side effects, such as transient facial oedema, may occur during the course of treatment. An open-label study using a one-week corticosteroid course showed a similar mean duration of headache (4.8 days) compared with other regimens (Sawanyawisuth et al. 2004), but the headache relapsed within two weeks in approximately 15% of the patients.

Supportive treatment should be provided to maintain patients' hydration and analgesic drugs should be administered for pain relief. Repeated lumbar punctures as needed to decrease intracranial pressure, thereby reducing pain symptoms, have also been recommended (Graeff-Teixeira et al. 2009). The severity of a headache declines rapidly following a lumbar puncture (Sawanyawisuth et al. 2004).

TABLE III
Outcomes of various treatment regimens in eosinophilic meningitis caused by *Angiostrongylus cantonensis*

Outcomes	Patients (n)	Headache at 14 days n (%)	Median duration of headache (days)	Reference
Placebo	55	25 (45.5)	13 (1-56)	Chotmongkol et al. (2000)
One week steroid	52	8 (15)	4.8 ^a	Sawanyawisuth et al. (2004)
Two weeks steroid	55	5 (9.1)	5 (1-60)	Chotmongkol et al. (2000)
Two weeks albendazole	34	7 (20.6)	8.9 ^a	Jitpimolmard et al. (2007)
Two weeks steroid with albendazole	26	3 (11.5)	4	Chotmongkol et al. (2004)
Two weeks steroid with albendazole	53	0 (0)	3 (1-14)	Chotmongkol et al. (2009)
Two weeks steroid with mebendazole	41	4 (9.8)	3	Chotmongkol et al. (2006)

a: indicated mean value.

Ocular angiostrongyliasis - Ocular angiostrongyliasis manifests in approximately 1% of patients with *A. cantonensis* infection and may not be associated with EoM (Sawanyawisuth et al. 2007, Sinawat et al. 2008). This disease is not fatal, but it can permanently damage the affected eye. Prommindaroj et al. (1962) first observed the presence of parasites in the eyes of patients in Thailand in 1962. The main symptom of ocular angiostrongyliasis is blurred vision, which may persist for four days to eight weeks. A diagnosis is made by the indirect ophthalmoscopic observation of worms or by morphological identification of the parasite following surgical removal.

The surgical removal of parasites is recommended in the cases involving eye infections, but lesions may damage vision irreversibly (Sawanyawisuth et al. 2007). Oral and topical corticosteroid treatments can prevent the deterioration of vision that is due to the inflammation caused by laser or surgical procedures, but intravenous methylprednisolone, topical prednisolone, laser treatment and surgery did not result in improved vision in most cases (Sawanyawisuth et al. 2007, Sinawat et al. 2008).

Development of coma - Diffuse encephalitis occurs in a small proportion of patients, causing a permanent comatose condition with a high mortality rate (Chotmongkol & Sawanyawisuth 2002). The risk factors for this encephalitic condition are age, fever and duration of headache, with adjusted odds ratios (95% confidence intervals) of 1.22 (1.05-1.42), 37.05 (1.59-862.35) and 1.26 (1.03-1.55), respectively (Sawanyawisuth et al. 2009).

These data suggest that a missed diagnosis of EoM increases the risk of coma by 26% per day with prolonged headache. Pathological analysis has identified several larvae in the brain tissue of patients who develop a coma. According to the *A. cantonensis* life cycle, larvae should live in the subarachnoid spaces. A longer disease duration may increase the chance of larval migration to brain tissue and the consequent development of coma.

Prophylaxis - Personal hygiene and proper food preparation are essential to avoid *A. cantonensis* infection. The L3 larvae of *Angiostrongylus* spp remain infective even after 17 days of refrigeration at 5°C and mathematical models have estimated that approximately 80 days are

required to eliminate the risk of infection by chilling as the sole larvicidal method (Richinitti et al. 1999). The use of bleach at a concentration of 1.5% at room temperature seems to be more promising, as it was shown to eliminate 97% of infective larvae (Zanini & Graeff-Teixeira 2001).

Snail or slug inclusions can be very small and go unnoticed during food preparation, especially when food is crushed or chopped. A classic study conducted in Guatemala during an abdominal angiostrongyliasis outbreak that was caused by a congeneric species, *Angiostrongylus costaricensis* (Morera & Céspedes 1971), determined that the risk of infection was primarily associated with the consumption of fresh mint and shrimp (Kramer et al. 1998). Mint is used in the preparation of ceviche, a typical dish from the Pacific coastal areas of South America where seafood is prepared with herbs, including mint. In an outbreak of CA in Jamaica, Caesar salad was identified as the source of infection (Slom et al. 2002).

Brazilian food habits do not typically include exotic or raw meat, which may prevent the occurrence of a large number of cases of human *A. cantonensis* infection. In this setting, small children, people with mental dysfunctions, food handlers and gardeners may represent important risk groups. The control of mollusc populations is of health and environmental importance, especially with respect to the huge problem that is caused by the widespread dissemination of *A. fulica*.

Proposed diagnostic algorithm for CA - Based on our knowledge of angiostrongyliasis studies, we developed an algorithm to help diagnose potential new cases of CA: step 1 - The syndromic diagnosis of meningitis. Fever, headache and neck stiffness are the classical main elements for the diagnosis of meningitis. Meningitis that is caused by *Angiostrongylus* infection may manifest as a headache alone; step 2 - The syndromic diagnosis of EoM. A CSF examination can reveal increased cellularity and eosinophil numbers in infected patients. Some authors have considered the presence of a single eosinophil leukocyte to be abnormal, whereas others have defined CSF eosinophilia as affecting > 10% of the CSF leukocytes (Kuberski 1979, Weller 1993). For epidemiological surveillance in a new area of occurrence, such as Brazil, the presence of a single eosinophil leukocyte is the best

criterion for the investigation of CA; step 3 - The consideration of an aetiological diagnosis of CA. If possible, the sediment from a CSF sample following step 2 should be reexamined to search for moving *Angiostrongylus* larvae. This finding is extremely rare, but it is the basis for a confirmed aetiological diagnosis of angiostrongyliasis; step 4 - The collection of epidemiological data that strongly support *A. cantonensis* as the cause of EoM. Such data include reported ingestion of raw molluscs or manipulation of snails and slugs during gardening. The patient's recent travel history, particularly to endemic areas such as Southeast Asia or the Pacific Islands, and consumption of raw exotic food should also be explored; step 5 - Immunodiagnosis and DNA detection. The following tests, which are available in the Brazilian reference diagnosis laboratory, should be performed: immunoglobulin G ELISA using the crude antigen, followed by WB analysis for the detection of the 31-kDa antigen and real-time PCR for the detection of DNA in CSF samples.

Collection of CSF and serum samples - The following instructions are useful to collect and send serum and CSF samples for laboratory tests: at least 250 µL of CSF should be frozen immediately at -20°C for serology and PCR analysis. Venous blood (5 mL) should be collected without an anticoagulant. After spontaneous or accelerated blood clotting by incubation at 37°C in a water bath for 20 min, the blood samples should be centrifuged and the serum (supernatant) removed. Store the serum in aliquots of approximately 100 µL with proper identification of the patient's name and date.

Analysis of infected molluscs - The following instructions are useful to collect and send land snails for parasitological examination: it is much easier to collect terrestrial molluscs early in the morning or at night because they are active. It is important to use gloves to avoid direct contact with the snails while collecting them. As they must be sent alive for the detection of nematode larvae, confirm that they are all alive before wrapping each snail using a paper towel or another similar material. Then, put them all into a plastic bag or a jar and place the package into an appropriate box that is used to transport biological samples. It is not necessary to include ice or anything else that keeps them refrigerated. Include a label with the local collection site and date of collection, in addition to the name of the collector and the epidemiological data, if possible.

CA is an emerging disease in Brazil and the medical community needs to be aware of its presence in order to diagnose novel cases. Cell differentiation analysis should be performed on patients with meningeal syndrome and increased CSF cell counts. The presence of eosinophils in CSF samples should prompt serological testing and PCR analysis of angiostrongyliasis and other less common aetiologies. A protocol that can be applied at sentinel hospitals and laboratories that perform CSF examinations is being established across the country as a measure to monitor the occurrence of CA. A second important set of criteria comes from the assessment of CA risk, including a history of raw mollusc or fish consumption, recent garden manipulation or other types of contact with molluscs or paratenic hosts. Clinicians must also be aware

that people who have travelled to endemic areas may return to Brazil with *A. cantonensis* infection. CA should be suspected in a patient with a severe headache and a positive risk history, even when an eosinophil is not detected in a CSF sample. Increased awareness of the presence of *A. cantonensis* and thus potential infection, in a new geographic area may result in a much larger number of diagnoses and improved knowledge of the distribution and public health importance of this disease.

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