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Review article

Diagnosis of human angiostrongyliasis

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Background: Angiostrongyliasis is caused by *Angiostrongylus cantonensis* or rat lungworm. Definitive diagnosis via recovery of the worm is uncommon. The diagnosis is done by a combination of methods.
Objective: Review of available methods used in the diagnosis of angiostrongyliasis.
Methods: We carried out a systematic review to document the available methods used in the diagnosis of angiostrongyliasis using MEDLINE as the main database supplemented by other resources.
Results: At present, only a few cases of this disease are diagnosed by parasitological methods. Thus, more commonly, a diagnosis of *A. cantonensis* infection is made by the combination of clinical manifestations and a history of ingestion of intermediate or paratenic hosts. In addition, serodiagnosis, including an enzyme linked immunosorbent assay (ELISA), and immunoblot test are useful in assisting diagnosis of this disease.
Conclusion: Further development of diagnostics for human angiostrongyliasis is needed for definite diagnosis of angiostrongyliasis

Keywords: Angiostrongyliasis, *Angiostrongylus cantonensis*, diagnosis, eosinophilic meningitis

Human angiostrongyliasis is a food-borne parasitic zoonosis caused by the larval stage of nematode *Angiostrongylus cantonensis* or rat lungworm. It has been reported in several parts of the world, particularly from Southeast Asia to the Pacific Islands [1-3]. To date, at least 2,872 cases of the disease have been documented worldwide and over half of these (1,337) were in Thailand [4]. Although four species of Metastrongyloidea worms are found in Thailand, namely, *A. cantonensis* [5], *A. siamensis* [6], *A. malaysiensis* [7] and *Thaistrongylus harinasuti* [6], only *A. cantonensis* is known to cause the disease. It is considered the primary causative agent of eosinophilic meningitis or meningoencephalitis in humans infected with helminth parasites. Moreover, this parasite can be the cause of ocular angiostrongyliasis [8-11].

Humans are an accidental host who become infected by ingesting third-stage larvae (L3) from infected snails or slugs or contaminated uncooked vegetables. Then, the L3 larvae migrate to the brain, spinal cord, and nerve roots, causing eosinophilia in both the spinal fluid and peripheral blood. Because humans are not the normal definitive host, larvae are unable to complete their development into the adult stage. Thus, no progeny larval worms are seen microscopically during examination of human fecal samples. However, adult worms are occasionally reported in young children [12, 13]. The most common clinical manifestations of angiostrongyliasis are severe headache, vomiting, paresthesia, weakness, and visual disturbances. Most patients recover fully, although severe infections can lead to coma and even death [14, 15].

A definitive diagnosis of human angiostrongyliasis is made by finding the worms in cerebrospinal fluid (CSF) or eyeball of patients, but such direct identification is rare. A diagnosis based on clinical signs or symptoms can be difficult because the symptoms can mirror those produced by other parasitic diseases, such as cysticercosis and gnathostomiasis [16]. A presumptive clinical diagnosis of angiostrongyliasis can usually be made where infections are endemic such as northeastern Thailand. Computerized tomography (CT) [17] and magnetic resonance imaging (MRI)

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[18-20] have been reported to help in the diagnosis of human angiostrongyliasis. However, no specific imaging features can differentiate angiostrongyliasis from other helminthic infections producing similar clinical symptoms on CT or MRI. Thus, a final diagnosis of this disease in humans requires a combination of findings including a history of eating raw or undercooked snails or yellow tree monitors, clinical symptoms, and/or the presence of eosinophils in CSF or peripheral blood. Pleocytosis in CSF with the eosinophil counts ranging from 26 to 75% and the peripheral eosinophilia typically ranging from five to 65% may aid diagnosis of this disease [21, 22]. Supporting diagnostic evidence can be provided by evaluation of antibodies/antigens in blood and/or CSF samples.

Diagnosis of human angiostrongyliasis

A summary of methods in diagnosing human angiostrongyliasis is shown in **Table 1**.

Recovery of the worm

The fourth-stage larvae or young adults of *A. cantonensis* can be found in the eye or cerebrospinal fluid (CSF). This was first reported in Thailand by Bunnag and colleagues [23] who found young adult worms in the CSF of a patient with eosinophilic meningitis. Ten years later, Punyagupta [21] reported the recovery of larval worms in 19 of 484 Thai suspected patients. This was also reported by Chen [24] who found larval worms in 27 of 259 patients. Of these 27 patients, 25 had larval worms recovered from their CSF, while in the other patients, larvae were found in the anterior chamber of the eye. However, in other study, no larval worms could be recovered from 116 suspected patients [25]. Recently, a methodology using intraocular infiltration of preservative-free lidocaine to paralyze the worms has greatly increased the ease with which the otherwise highly motile worm could be identified [26].

Table 1. Summary of methods used in diagnosis of human angiostrongyliasis

Diagnostic method	Outcome	References
1. Recovery of the worm (Definitive diagnosis)	1. 19 of 484 patients were positive by detection of *A. cantonensis* larvae.	[21]
	2. 27 out of 259 patients were positive by larval worm detection.	[24]
2. Laboratory findings	1. CSF glucose is usually normal.	[12]
	2. Eosinophil count in CSF ranges 26-75% and peripheral blood eosinophils range 5-63%.	[21, 22]
3. Computerized tomography (CT) scans and magnetic resonance imaging (MRI) scans	Could not find characters that differentiated angiostrongyliasis from other neurological parasitic diseases.	[14, 17]
4. ELISA test		
4.1 Using crude somatic extract of female *A. cantonensis* for IgG detection in serum	Sensitivity = 100%, Specificity = 66.8%	[40]
4.2 Using somatic antigen of *A. cantonensis* for detection of IgG subclass in serum	Sensitivity = 100%, Specificity = 100% in IgG1 Sensitivity = 85.7%, Specificity = 88.9 in total IgG	[42]
5. Immunoblot test		[40]
5.1 31 kDa band from crude extract female worms	Sensitivity = 69.2%, Specificity = 82.4%	[51]
5.2 29 kDa band from young adult female worm somatic extract	Sensitivity = 55.6%, Specificity = 99.6%	[52]
5.3 29 kDa band from young adult somatic extract	Sensitivity = 75%, Specificity = 95% in IgG4	[53]
5.4 31 kDa crude soluble extract from adult worm	Strongly recognized with sera from angiostrongyliasis patients	
6. Supportive diagnosis	1. Medical history combination with the clinical manifestations related with severe headache, eosinophilic meningitis or meningoencephalitis	[4, 14, 57]
	2. Detection of antibody or antigen from blood or CSF should be aid in diagnosing human angiostrongyliasis	

Laboratory findings

When eosinophilia is detected in peripheral blood or CSF, the possibility of this disease is high. With differential counts, the white blood counts range from normal to slight increases in eosinophils (from 5 to 63% in blood). In cases with headache, cerebrospinal pressure is moderately increased and is usually proportional to the intensity of symptom. The pressure ranges from normal to 280 mm of water. The most common appearance of the CSF in patients with eosinophilic meningitis is a slight cloudiness "coconut milk". Xanthochromia is observed in some cases. The protein concentrations ranges from normal to moderately elevated while glucose is usually normal [12]. Pleocytosis with eosinophilia is seen in all cases of angiostrongyliasis. The average cell count in CSF ranges from 200 to 5,000 cells/ml and the eosinophil count ranges from 26 to 75%, while counts of peripheral blood eosinophils constitute 5 to 63% [21, 22]. Eosinophilia has been shown to be related to interleukin-5 stimulation and Th2-cytokine production [27].

Computerized tomography (CT) scans and magnetic resonance imaging (MRI)

Studies of diagnosis in eosinophilic meningitis have been conducted using CT, but were inconclusive because the specific characters differentiating this disease from other helminthic infections that produce similar signs and symptoms could not be identified by CT [17].

MRI scans have been used to aid in a presumptive diagnosis. Absences of focal lesions are distinguished *A. cantonensis* meningitis from other helminthic infections of the central nervous system. Tsai et al. [18] reported that meningeal and basal ganglion enhancement was noted on MRI scans in several patients. Later, Podwall et al. [19] showed that brain MRI in a patient with angiostrongyliasis revealed two areas of gadolinium enhancement in the subcortical white matter and increased signals on the fluid attenuated inversion recovery pulse sequence (FLAIR) corresponding to those areas. Abnormalities were shown on MRI in three cases of meningoencephalitis, one case of encephalitis and one of myelomenigitis due to *A. cantonensis*. Lesions of the brain and spinal cord were diffuse or scattered and appeared as similar or slightly reduced signal intensities on T1-weighted images (T1WI), high signal intensities on T2-weighted images (T2WI), and FLAIR images [20]. In summary, CT scans and MRI techniques are useful for following up the disease and excluding other causes, but they cannot serve as the basis for a definitive diagnosis.

Immunodiagnosis

Somatic and metabolic antigens of *A. cantonensis* were used to test against serum and CSF collected from nine patients with eosinophilic meningitis. Positive reactions were obtained in sera and CSF in all cases using the somatic antigens. With metabolic antigens, eight out of nine gave a positive reaction and one case gave a weak reaction [28].

Tungkanak et al. [29] reported that four serum and five CSF samples out of 17 from patients with eosinophilic meningitis were positive using somatic antigens from adult worms. With the same type of antigen, Kamiya et al. [30] found that 14 out of 15 (93.3%) serum samples from patients with eosinophilic meningitis were positive and seven of eight CSF samples were positive. In 1983, Sato and Otsuru [31] showed that nine serum samples from suspected cases of angiostrongyliasis had IHA titers ranging from 1:64 to 1:8,192 except for one case who had a titer of less than 1:16. With CSF samples from three patients with angiostrongyliasis, the IHA titers ranged from 1:32 to 1:1,024. Chen [32] used an antigen purified by DEAE-cellulose chromatography and reported that the serum IHA titers of four patients with the history of eating raw *Achatina fulica* were 1:128 in two patients and 1:256 in the other two cases.

Bouthemy et al. [33] demonstrated that 13 of 16 serum samples collected from eight patients with eosinophilic meningitis were positive in the IEP test against the crude extract of adult worms of *A. cantonensis* with the number of bands ranging from one to four, while 10 of 16 serum samples were positive against the crude extract of larvae of *A. cantonensis* with the number of bands ranging from one to three. However, cross-reactivity with other helminthic infections such as *Dipetalomema viteae*, *Toxocara canis* and *Ascaris suum* also was observed in the IEP test. Using an extract of adult females of *A. cantonensis*, the serum samples from 12 of 15 (80%) patients with eosinophilic meningitis were positive in the IEP test, while sera from 100 blood donors and 6 opisthorchiasis cases gave a negative result [34]. In addition, 13 of 20 (65%) patients with eosinophilic meningitis were positive in the IEP test using an extract of adult female worms with the number of bands

ranging from one to four. Cross-reactivity with sera from patients with gnathostomiasis also was observed in the IEP test, although only one band was displayed [35]. Subsequently, Sato and Otsuru [31] showed that sera from six cases, which gave positive gel diffusion test results against an adult worm *A. cantonensis* extract, were also positive in the IEP test using the same antigen with the number of bands ranging from one to six.

Shih and Chen [36] reported that TD2 and 3A5 monoclonal antibodies, which recognized the circulating 91 kDa antigen were used in a sensitive enzyme-linked fluorescent assay (ELFA) for the immunodiagnosis of human angiostrongyliasis. By this technique, TD2 and 3A5 monoclonal antibodies were coated on the assay plates to detect the circulating antigen from sera of experimentally infected rats; two fluorescence unit (F.U.) peaks appeared in sera from infected rats collected 18 and 44 days after infections. Using the same method, specimens from 35 patients (showing clinical symptoms and eosinophilic meningoencephalitis) were tested; all CSF samples and most sera (88%) were positive.

Three presumptive cases angiostrongyliasis in U.S. Marines, who had a history of eating *A. fulica* intermediate hosts in Okinawa, Cross [37], were tested with ELISA coated with antigens from the fourth-stage larvae recovered from the brains of experimentally infected rats. The test was positive and yielded titres of 6.6, 13.5, and 33.2 for these patients compared to a control value of 2.5. The author also reported ELISA values ranging from 13.1 to 71.7 in confirmed angiostrongyliasis cases compared to controls and values with other helminthic infections of 0-6.3. Subsequently, using the ELISA with the fourth-stage larva antigen preparation, Cross and Chi [38] reported that the serum titers range of patients were 4.5 to 23.1 for patients with suspected angiostrongyliasis, 12.7 to 34.4 for parasitologically-confirmed cases and 1.3 to 2.2 for negative controls. Jaroonvesama et al. [25] reported that 96% of the sera from 116 patients with eosinophilic meningitis from the northeastern part of Thailand yielded positive-ELISAs. Chen [39] also evaluated metabolic and purified antigens of adult or juvenile *A. cantonensis* by ELISA. Serum and CSF samples from both rats and patients gave higher ELISA values against these antigens than those obtained from negative control samples. However, cross-reactions were seen in serum samples from *Toxocara canis* infected individuals. Nuamtanong [40] used a crude somatic extract of female *A. cantonensis* to compare the sera from human angiostrongyliasis patients by IgG-ELISA with a large number of patients with other diseases. Sensitivity was 100% while specificity was 66.8%, including positive and negative predictive values were 100% and 27.1%, respectively. An alternative antigen from *A. costaricensis* adult worms was tested with 27 sera of *A. cantonensis* infections using the IgG-ELISA, and specificity and sensitivity at 0.261 cut-off values were 73% and 95.6%, respectively [41]. To study reactivity of immunoglobulins and immunoglobulin sub-classes, Intapan et al. [42] investigated the total IgG, IgG1, IgG2, IgG3, IgG4, IgA, and IgM specific antibodies against *A. cantonensis* somatic antigen in angiostrongyliasis sera from proven and from clinically-suspected cases by indirect ELISA. It was found that IgG1 antibody had the highest sensitivity and specificity of the IgG group, while the IgM and IgA responses were generally poor predictors of infection in both groups of cases. Yen and Chen [43] purified adult- and young adult-*A. cantonensis* crude antigens by immuno-affinity chromatography to detect antibodies in serum and CSF samples of human eosinophilic meningitis or meningoencephalitis by ELISA. The levels of IgG, IgA, IgM, and IgE antibodies to *A. cantonensis* in these patients were higher than in uninfected patients. Chye et al. [44] repeated the method of Yen and Chen [43] but also used SDS-PAGE, which showed only a single band with a molecular weight of 204 kDa. Likewise, this antigen reacted with antibodies in serum and CSF samples from patients with meningoencephalitis or eosinophilic meningitis by ELISA, and demonstrated that ELISA values in the patients group were significantly higher than those of control subjects.

Eamsobhana [45, 46] produced the Aw-3C2 monoclonal antibody (IgM type), which recognized a circulating antigen of angiostrongyliasis in sera using a sandwich ELISA and dot-blot ELISA. The specificity of this monoclonal antibody was 100% in both tests while sensitivity in sandwich ELISA was 50% (5/10) and 60% (6/10) in dot-blot ELISA. Chye et al. [47] used two monoclonal antibodies (AcJ1 and AcJ20) in a sandwich ELISA to detect a circulating antigen from immature adults of *A. cantonensis* with a molecular mass of ~204 kDa. When comparing serum and CSF samples from the patients with parasite-confirmed infections and clinical histories of eosinophilic meningitis or meningoencephalitis, the

specificity and sensitivity in CSF samples were higher than those of the sera from the same patients.

The multi-dot enzyme-linked immunosorbent assay (ELISA) has been used for rapid and simple differential diagnosis of eosinophilic meningitis due to helminth infections. Ultrafiltered, partially purified antigens of *Parastrongylus cantonensis*, *Gnathostoma spinigerum*, and *Taenia solium* metacestodes were dotted onto a single nitrocellulose membrane strip. With peroxidase conjugated anti-human IgG and 4-chloro-1-naphthol as a substrate, the antibodies in the patients' sera were clearly seen as well-defined blue dots. Although weak cross-reactions between *P. cantonensis* and *G. spinigerum* antigens were observed with the use of partially purified antigens, this did not interfere with determination of specific parasite infections in all cases [48]. Immuno-PCR was developed as a sensitive method for detection of antigen by means of two specific antibodies and amplification of DNA linked with a specific antibody by PCR. A specific antibody is biotinylated and another is strepavidinated, which is subsequently conjugated with a biotinylated-plasmid DNA. A monoclonal antibody was used to capture a circulating *A. cantonensis* antigen in the serum sample. A DNA label generated by PCR amplification with biotinylated primer was bound by use of streptavidin to a biotinylated third antibody. The circulating antigens sandwiched by monoclonal antibody were detected by PCR amplification of the DNA label. Like ELISA, immuno-PCR was used to detect the circulating 204-kDa AcL5 antigen in the sera of patients with eosinophilic meningitis or meningoencephalitis with 98% sensitivity, and 100% specificity [49].

Akao et al. [50] reported that four sera of angiostrongyliasis patients strongly recognized the antigens prepared from adult female worm of *A. cantonensis* at molecular weights of 31 and 29 kDa using an enzyme-linked immunoelectrotransfer blot technique (EITB). Nuamtanong [40] showed that the 31 kDa component was better than the 29 kDa antigen in recognizing sera from angiostrongyliasis patients, although cross-reaction occurred in trichinellosis, trichuriasis, and opisthorchiasis sera. Sensitivity, specificity, and positive and negative predictive values were 69.2%, 82.4%, 46.2%, and 92.5%, respectively. In 2001, Maleewong et al. [51] used the antigenic components of an *A. cantonensis* young adult female somatic worm extract (FSE) in total IgG tests with sera and CSF from patients with clinical angiostrongyliasis. The prominent antigenic band had a molecular mass of ~29 kDa, which reacted with five out of nine and 28 out of 85 sera from patients with parasitologically confirmed and clinical angiostrongyliasis. The sensitivity, specificity, and positive and negative predictive values of the immunoblot analysis of this antigenic band were 55.6%, 99.4%, 83.3%, and 97.4%, respectively. Later, Intapan et al. [52] demonstrated that detection of IgG4 was high in angiostrongyliasis sera of parasite-proven patients and clinically suspected cases with eosinophilic meningitis. Again, an antigenic band of 29 kDa reacted with antibodies of those patients by immunoblotting at an accuracy, sensitivity, specificity and positive and negative predictive values of the test gave 89.2%, 75%, 95%, 89.7%, and 90.4%, respectively. This test was able to discriminate between human angiostrongyliasis, gnathostomiasis, and cysticercosis, three diseases that produced similar symptoms.

Using immunoblot analysis of the crude soluble extract from adult worms of *Parastrongylus cantonensis* against sera from patients with parastrongyliasis strongly reacted with isolated antigens, especially a prominent band of 31 kDa. There was no cross reactivity with sera from patients with gnathostomiasis, toxocariasis, filariasis, paragonimiasis, cysticercosis or malaria, nor were crossreactions detected with sera from normal healthy individuals [53]. Recently, Vitta et al. [54] used an 81-kDa recombinant protein from *A. cantonensis* in detecting antibody of serum patients. Its sensitivity, specificity, positive and negative predictive values in immunoblot test was 93.5, 91.5, 79.0, and 97.5%, respectively. Although some cross-reactivity of the antigen was observed among gnathostomiasis, bancroftian filariasis, ascariasis, echinococcosis, paragonimiasis and opisthorchiasis, sera of 14 other infections were all negative.

In summary, the immunodiagnosis of human angiostrongyliasis in earlier studies aimed to verify the clinical manifestations and parasitological methods. Included among these are tests involving complement fixation [28], indirect hemagglutination [29-31], immunoelectrophoresis [31, 33], enzyme-linked fluorescence [36], enzyme-linked immunosorbent assays [37-40, 43, 46, 47, 55] and immunoblot assays [40, 50], all of which focused on the detection of specific *A. cantonensis* antibodies or antigens in blood and/or CSF of animals and/or humans. Unfortunately, the antigens or antigen preparations used in these

immunodiagnostic methods also cross-reacted with a variety of other helminth infections including gnathostomiasis, toxocariasis, ascariasis, trichinellosis, trichuriasis, opisthorchiasis, and paragonimiasis [39, 40]. The cross-reaction may be a result of helminth-proteins sharing epitopes of two or more parasites. Another possibility is that patients with these cross-reactive tests might previously have been infected with *A. cantonensis*.

In contrast to earlier studies, Chye et al. [44] reported that a 204-kDa antigen from the L5 larval stage of *A. cantonensis* was purified by immuno-affinity chromatography incorporating a specific monoclonal antibody, and this antigen was then used in an ELISA for detection of specific antibody levels in serum and CSF of patients with eosinophilic meningitis due to *A. cantonensis* infection. The antigen showed no cross-reaction with patient sera in four cases of *Paragonimus westermani* infection, two cases of *Fasciolopsis buski* infection, 6 cases of *Clonorchis sinensis* infection, five cases of *Dipylidium caninum* infection and two cases of *Wuchereria bancrofti* infection. In a later study, Eamsobhana et al. [56] using a 31-kDa antigen from *A. cantonensis* adult worms purified by electroelution from SDS-PAGE gels, demonstrated a 100% sensitivity and specificity in three confirmed angiostrongyliasis patients and serum samples from tissue parasitic infections, respectively, by dot-blot ELISA. In addition, a 29-kDa protein band isolated from young adult somatic extracts of *A. cantonensis* has also been shown to have immunodiagnostic potential based on its reactivity with IgG4 isotype antibodies in sera from patients [52]. The accuracy, sensitivity, specificity, and positive and negative predictive values were 89.2%, 75%, 95%, 85.7%, and 90.4%, respectively. This antigen showed cross-reacted with one of five serum samples from cysticercosis patients. Although these antigens showed great promise for the immunodiagnosis of angiostrongyliasis, the tests still gave variable results in the diagnosis of individual clinical cases and clearly indicated a need for identification of more specific antigens to better improve to immunodiagnostic testing for this parasite.

Symptoms and therapy of human angiostrongyliasis

The severity of the disease depends on the number of worms, their location in the body and the immune system of the patient. The most common symptoms of the infection are severe headache, nausea, vomiting, neck stiffness, seizures, and other neurological abnormalities. **Table 2** shows the clinical manifestations of eosinophilic meningitis or meningoencephalitis, which have been reported by several authors. The symptoms of several patients may persist from two weeks to two months. Paresthesis rarely persisted for year. Permanent sequelae such as facial palsy, spasticity, epilepsy, and permanent blindness were occasionally reported. The mortality rate is very low. Most patients usually recover within a month.

To date, there are no specific treatments for angiostrongyliasis and its accompanying eosinophilic meningitis or meningoencephalitis, although there are several reports of trials [58]. Corticosteroid can be used to treat patient with eosinophilic meningitis. Treatment of ocular angiostrongyliasis is surgical.

Conclusion

A definitive diagnosis can be made by finding the worm at the site of infection, but this only occurs in some patients. A history of eating raw or improperly cooked snails, slugs, prawns, crabs, frogs and yellow tree monitors or eating raw vegetables contaminated with infective third-stage larvae may aid in diagnosing. Clinical findings, dietary history of eating improperly cooked intermediate or paratenic hosts and serological tests are useful in assisting diagnosis of human angiostrongyliasis. The ELISA method is widely used for detecting antibodies in serum of patients. The 29 and 31 kDa antigens from *A. cantonensis* have been shown to be good indicators of infection by immunoblotting. However, cross-reaction with other parasitic infections still occurs in both ELISA and immunoblotting. Thus, further development of diagnostics for human angiostrongyliasis is still needed to overcome these problems.

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Table 2. Symptoms of patients with eosinophilic meningitis or meningoencephalitis

Symptoms	Percent of patients				
				Chen et al. [60]	
	Yii [12] (125 cases)	Tsai et al. [18] (17 cases)	Slom et al. [59] (12 cases)	Outbreak 1 (47 cases)	Outbreak 2 (8 cases)
Headache	86	100	100	93.6	100
Nausea or Vomiting	83	24 or 24	or 67	or 19.1	or 100
Somnolence or Lethargy	82	-	-	8.5	87.5
Blurred vision or Diplopia	10	12	92	-	-
Fever	80	65	42	-	-
Constipation	76	-	-	-	-
Malaise	71	-	-	-	-
Anorexia	64	-	-	-	-
Cough	54	-	-	-	-
Subjective neck stiffness	40	47	83	-	75
Abdominal pain	34	18	-	-	-
Paresthesia	28	12	50	63.8	37.5
Weakness of extremity	17	-	-	-	-
Muscle twitching	13	-	-	-	-
Strabismus	10	-	-	-	-
Sneezing	10	-	-	-	-
Coma	10	-	-	-	-
Irritation	8	-	-	-	-
Paralysis of extremity	6	-	-	-	-
Urinary incontinence or intention	6	-	-	-	-
Rhinorrhea	5	-	-	-	-
Diarrhea	-	-	17	-	-
Profuse salivation	4	-	-	-	-
Convulsion	3	-	-	-	-
Muscle weakness		47	37	-	-
Orbital/retro-orbital pain	-	41	-	-	-
Ataxia	-	6	-	-	-
Skin rash	-	24	-	-	-
Hyperesthesia	-	-	75	-	-
Fatigue	-	-	83	14.9	87.5
Muscle pain	-	-	50	91.5	100
Skin eruption	-	-	-	21.3	-
Skin itch	-	-	-	27.7	-

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