PREDICTIVE FACTORS FOR *GNATHOSTOMA* SEROPOSITIVITY IN PATIENTS VISITING THE GNATHOSTOMIASIS CLINIC AT THE HOSPITAL FOR TROPICAL DISEASES, THAILAND DURING 2000-2005

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Abstract. This was a retrospective study of patients having *Gnathostoma* antibody testing at the Hospital for Tropical Diseases, Bangkok during 2000-2005 to investigate predictive factors for *Gnathostoma* seropositivity in patients attending the Gnathostomiasis Clinic. Out of 849 patients tested, 531 (62.5%) were *Gnathostoma* seropositive. The median absolute eosinophil counts were 464 (0-16,796) and 326.5 (0-10,971) cells/mm^3 in seropositive and seronegative patients, respectively (p<0.001). Differences in a history of cutaneous swelling, the habit of eating raw meat, eosinophilia (>500 cells/mm^3), and the frequency of cutaneous swellings between seropositive and seronegative patients were all statistically significant. Patients with a history of eating raw meat and a history of cutaneous swelling were at 2.1 and 1.8 times more likely to be *Gnathostoma* seropositive, respectively. Logistic regression analysis showed eosinophilia was not a predictive factor for *Gnathostoma* seropositivity.

Key words: *Gnathostoma*, predictive factor, seropositivity, Thailand

INTRODUCTION

Human gnathostomiasis, a tissue parasitic disease caused by advanced third stage larvae of *Gnathostoma spinigerum*, is prevalent in Thailand. Gnathostomiasis is caused by eating raw fresh water fish, frogs, snakes, chickens, ducks or birds containing *Gnathostoma* larvae. Clinical presentations include cutaneous migratory swelling, cutaneous creeping eruption, acute radicular pain with migration signs, and meningoencephalitis (Suntharasamai, 2003). The most common clinical presentation in patients attending the Gnathostomiasis Clinic at the Hospital for Tropical Diseases, Bangkok, Thailand, is cutaneous migratory swelling. However, the presentation of cutaneous migratory swelling is not specific for gnathostomiasis. The differential diagnoses for cutaneous migratory swelling includes loiasis, which is prevalent in West and Central Africa (Grove, 2000), paragonimiasis, sparganosis, other tissue parasitic infestations, and localized angioedema due to allergic

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reactions. Several serological tests for the detection of antibodies against *Gnathostoma* antigens have been developed for the confirmation of clinical gnathostomiasis. (Suntharasamai *et al*, 1985; Dharmkrong-At *et al*, 1986; Tada *et al*, 1987; Maleewong *et al*, 1988; Tapchaisri *et al*, 1991).

Unfortunately, all currently available serodiagnostic tests, including the immunoblot technique, are not helpful for differentiating recent and past *Gnathostoma* infection. This study aimed to investigate predictive factors for *Gnathostoma* seropositivity in patients attending the Gnathostomiasis Clinic.

MATERIALS AND METHODS

This was a retrospective case-control study carried out at the Gnathostomiasis Clinic, Hospital for Tropical Diseases in Bangkok and the Immunodiagnostic Unit for Helminthic Infections, Department of Helminthology, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand.

The study population consisted of 849 patients whose medical records were reviewed for clinical baseline and laboratory data, including presenting symptoms, diagnosis, eating habits, immunoblot analysis results, CBC results and stool examinations (both simple smear and formalin ether concentration techniques) done within 4 weeks prior to or after the performance of immunoblot analysis. Eosinophilia was defined as an absolute eosinophil counts ≥500 cells/mm.

Antigen preparation

Third-stage larvae of *G. spinigerum* were collected from the livers of freshwater eels purchased from local vendors in Bangkok. The eel livers were separated from the intestines and washed several times with tap water until no appearance of blood. The livers were chopped into small pieces and digested with artificial gastric juice (1% pepsin-HCl solution) at 37ºC. After two hours of incubation, the suspension was passed through a screen and collected. The suspension collected was left to sediment, then washed several times with distilled water and examined for the presence of larvae under a stereomicroscope. All larvae were washed several times with distilled water and kept at -75ºC, or used directly in a further step. Parasites were ground with alumina powder in distilled water and the suspension was sonicated using probe No. 418, at magnification No. 4 (Sonicator Heat Systems, Lakewood, NJ), for 10 minutes at intervals of 1 minute. The suspension was then centrifuged at 10,000 rpm for 60 minutes, and the supernatant was collected and determined for protein content determined by Coomassie Blue Plus Protein Assay Reagent Kit (Pierce, Rockford, IL).

Immunoblot technique

A 13% separating gel of SDS-polyacrylamide gel carried the SDS-treated proteins in a single well. After electrophoresis transfer, a nitrocellulose sheet was treated with blocking buffer containing 2% skim milk, 0.02% NaN_3-PBS, pH 7.4. The blot was cut into small strips, which contained 15 μg of antigen per strip. The strips were then reacted with serum samples diluted to 1:50 in 0.05% Tween20, 0.02% NaN_3-PBS, pH 7.4, on a rocking platform at room temperature for 14-16 hours. The strips were incubated with diluted peroxidase conjugate anti-human IgG (SouthernBiotech, Birmingham, AL) (1:1,000) in 0.05% Tween20-PBS, pH 7.4, for 2 hours, washed, and color developed with reagent containing 2, 6-dichlorophenol indophenol. The excess reaction was removed by washing with distilled water (Dekumyoy *et al*,

Table 1
Factors that may predict the results of *Gnathostoma* antibody detection by immunoblot.

Characters	*Gnathostoma*		*p*-value
	Seropositive No. (%)	Seronegative No. (%)	
Sex			
Male	190 (61.1)	121 (38.9)	0.568
Female	340 (63.3)	197 (36.7)	
History of cutaneous swelling[a]			
Present	311 (69.0)	140 (31.0)	<0.001
Absent	219 (55.2)	178 (44.8)	
History of eating raw meat[b]			
Yes	468 (66.0)	241 (34.0)	0.001
No	25 (43.9)	32 (56.1)	
Eosinophilia[c]			
Yes	203 (66.8)	101 (33.2)	0.044
No	232 (59.0)	161 (41.0)	
Frequency of cutaneous swelling[d]			
≤1/month	140 (77.3)	41 (22.7)	0.049
>1/month	56 (65.1)	30 (34.9)	
Location of cutaneous swelling[e]			
Head and neck	48 (60.0)	32 (40.0)	0.053
Extremity	139 (74.7)	47 (25.3)	
Trunk or mixed location	30 (66.7)	15 (33.3)	
Result of stool examination for intestinal parasite[f]			
Positive	23 (69.7)	10 (30.3)	0.462
Negative	164 (61.4)	103 (38.6)	

[a]There was a patient whose clinical manifestation on cutaneous swelling was not recorded.
[b] There were 766 patients with known eating habits; 493 and 273 patients in *Gnathostoma* seropositive and seronegative, respectively.
[c] There were 697 patients who had CBC results; 435 and 262 patients were *Gnathostoma* seropositive and seronegative, respectively.
[d]There were 267 patients, who had more than 1 episodes of cutaneous swelling, whose frequencies of cutaneous swelling were recorded.
[e]There were 311 patients whose locations of cutaneous swelling were recorded.
[f]There were 187 and 113 *Gnathostoma* seropositive and seronegative patients, respectively.

2002). For standard control, a strip of each blotted nitrocellulose sheet was treated with monoclonal antibody (kindly provided by Prof Wanpen Chaicumpa, Department of Parasitology, Faculty of Medicine, Siriraj Hospital, Mahidol University, Thailand) against 24 kDa antigen detected with peroxidase conjugated anti-mouse IgG (KPL, Galthersbury, MD) at 1:1,000.

Statistical analysis

Clinical and laboratory data were analyzed by SPSS for windows. Data were summarized as mean (SD) for normally distributed quantitative data, median (range) for non-normally distributed quantitative data and number (percentage) for categorical data. Group comparison was done with the *t*-test for quantitative

Table 2
Predictive factors for *Gnathostoma* seropositivity.

Predictive factors	Odds ratio	95% CI	*p*-value
History of eating raw meat[a]	2.106	1.14-3.88	0.017
History of cutaneous swelling[a]	1.840	1.32-2.56	<0.001
Eosinophilia[a]	1.176	0.83-1.65	0.354

[a]Not present was used as a reference.

data with normal distribution, the Mann-Whitney *U*-test for non-normal distribution, and the chi-square test or Fisher's exact test where appropriate, for categorical data. Logistic regression was performed to confirm an association with various predicting factors with *G. spinigerum* seropositivity.

This study was approved by the Ethics Committee of Faculty of Tropical Medicine, Mahidol University.

RESULTS

Eight hundred forty-nine patients were enrolled in this study; the median age was 37 (5-79) years. Three hundred eleven (36.6%) patients were male. Of those 849 patients, 451(53.3%) patients had cutaneous swellings. The clinical diagnoses of patients with no cutaneous swelling included urticaria (23.8%), tactile hallucinations/tingling sensation (9.2%), delusions of parasitosis (1.3%), localized pain in an extremity (0.7%), anxiety (0.1%), pruritus without skin lesions (2.4%), dermatitis (2.4%), skin nodules (0.7%), headaches (0.9%), arthralgia/arthritis (0.8%), asymptomatic eosinophilia (0.6%), allergies (0.6%), myalgia (0.5%), healthy (1.9%), creeping eruption (0.2%), cellulitis (0.1%), hordeolum (0.1%), parotitis (0.1%), cancer (0.1%), and feeling numbness (0.1%). In one patient with possible connective tissue disease, it was not known whether cutaneous swelling was present or absent. Of 766 patients whose eating habits were known, 709 (92.6%) ate raw meat and only 57 (7.4%) did not eat raw meat.

Of the 849 patients tested for *Gnathostoma* antibodies by immunoblot technique, 531 (62.5%) and 318 (37.5%) patients were *Gnathostoma* seropositive and seronegative, respectively. The median ages of patients in the former and latter groups were 37 (8-79) and 37 (5-79) years, respectively (p = 0.722). The median absolute eosinophil count was significantly higher in the seropositive group [464 (0-16,796) *vs* 326.5 (0-10,971) cells/mm^3 p<0.001].

There were significant differences between the *Gnathostoma* seropositive and seronegative groups in the presence of cutaneous swelling, raw meat eating habits, eosinophilia (absolute eosinophil count >500 cells/mm^3), and frequency of cutaneous swellings. There was no significant difference in location of cutaneous swelling between *Gnathostoma* seropositive and seronegative patients (Table 1).

Logistic regression analysis revealed patients with a history of eating raw meat and a history of cutaneous swellings were 2.1 and 1.8 times more likely to be *Gnathostoma* seropositive, respectively. However presence of eosinophilia was not associated with *Gnathostoma* seropositivity (Table 2).

DISCUSSION

Patients came to the Gnathostomiasis Clinic for various reasons. Cutaneous swelling, pruritus and skin rashes are common clinical presentations. We used serological testing to help confirm the diagnosis but it may not be able to distinguish past from present infection. In this study we looked at risk behaviors, clinical manifestations and laboratory findings to find associations with *Gnathostoma* seropositivity.

We found around 90% of patients attending the Gnathostomiasis Clinic had a history of raw meat consumption, hence they were at risk for many parasitic infections including gnathostomiasis. The prevalence of gnathostomiasis in Thailand has been estimated to be 0.4 % (Suntharasamai, 1987).

In Thailand, the differential diagnosis for cutaneous swelling includes allergic related localized angioedema, gnathostomiasis, insect bite reactions, and trauma related cutaneous swelling. With gnathostomiasis, a cutaneous swelling usually lasts longer than 24 hours and is often migratory. To diagnose gnathostomiasis, a history of eating raw meat, especially raw fresh water fish, snake, frog, or poultry, which are intermediate hosts or paratenic hosts for *Gnathostoma*, and *Gnathostoma* seropositivity by immunoblot are required, in addition to the clinical symptom of cutaneous migratory swelling.

Unfortunately, cross-reactivity with other parasites in some patients with a diagnosis of paragonimiasis, trichinosis, opisthorchiasis, cysticercosis, strongyloidiasis, or fascioliasis, has been reported with the immunoblot test (Tapchaisri *et al* 1991; Laummuanwai *et al*, 2007). Therefore, we aimed to investigate predictive factors for *Gnathostoma* seropositivity in patients attending the Gnathostomiasis Clinic. Hopefully, these predictive factors will help clinicians confidently diagnose gnathostomiasis.

The study found that 219 (55.2%) of patients with no cutaneous swelling were *Gnathostoma* seropositive. These included 2 patients with creeping eruption, 3 patients with asymptomatic eosinophilia, 115 patients with urticaria, and 99 patients with other clinical diagnoses. The rationale for *Gnathostoma* seropositive may be subclinical infection, unrecognized cutaneous swelling or false positivity.

This study showed a history of eating raw meat and a history of cutaneous swelling were predictive factors for *Gnathostoma* seropositivity. The findings are consistent with current clinical practice.

Eosinophilia, or absolute eosinophil count ≥ 500 cells/mm^3, are not necessary for diagnosing gnathostomiasis because it is not always present (Sirikulchayanonta and Viriyavejkul, 2001; Dekumyoy *et al*, 2002; Magana *et al*, 2004). Eosinophilia is not specific for gnathostomiasis, since it may be associated with various diseases or conditions (Tefferi, 2005). However, this study revealed significant differences in absolute eosinophil counts and eosinophilia between *Gnathostoma* seropositive and seronegative patients. Nevertheless, eosinophilia was not a predictive factor for *Gnathostoma* seropositivity.

A disadvantage of this study was the data were incomplete. First, cutaneous swelling was not usually evident on physical examination but relied on the patients' verbal report. Second, there was no information regarding duration of each swelling episode. In case of rapidly resolved cutaneous swelling, allergy associated localized angioedema should be considered. Third, some patients were seen after a

single episode of non-migratory cutaneous swelling. These factors caused problems when an investigator looked for a classic clinical presentation of cutaneous gnathostomiasis. Fourth, not all patients were interviewed in detail for habits of eating raw intermediate or paratenic hosts for gnathostomiasis. In theory, eating raw meat, such as pork or beef, does not put one at risk for gnathostomiasis. Therefore the data collection may not reflect true risk exposure to *Gnathostoma* parasite.

Selection bias should be considered as well, because the majority of the patients seen at the Gnathostomiasis clinic were referral cases and were very much concerned about gnathostomiasis.

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