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Effect of Helminthic Infections Together with Mite Allergen Exposure on the Risk of Wheeze in Preschool Children

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SUMMARY Although some studies have indicated that helminthic infections and house dust mite exposure may have an alleviating effect on wheeze, an interaction effect of both risk factors has not been examined in Thailand. The objective of this study was to investigate whether exposure to helminthic infections together with house dust mite allergen was associated with wheeze in children aged 18-36 months, living in an area of southern Thailand where helminthic infections are endemic. This study was undertaken as a part of The Prospective Cohort Study of Thai Children which recruited 1,076 children born between December 2000 and November 2001. A home dust sample was collected once when the infants were 12-18 months old to measure house dust mite allergen (*Der p1*). Questionnaire data on wheeze and a stool specimen for soil-transmitted helminth analysis were collected at age 18-36 months. Prevalence of exposure to house dust mite allergen (*Der p1*) > 10 μg/g dust was 31.8%. Soil-transmitted helminths were present in 25.0%, predominately *Ascaris lumbricoides*. Hookworm infection was associated with a physician's diagnosis of wheeze (OR 4.20, 95% CI 1.45-12.10) and hospitalized wheeze (OR 5.40, 95% CI 1.26-23.01). Hospitalized wheeze was significantly higher in subjects exposed to *Der p1* 2-10 μg/g dust. Helminthic infections were not associated with a significant interaction effect with mite allergen against a risk of wheeze. Our survey confirms that hookworm infection and mite allergen exposure are independent risk factors for childhood wheeze and there is no evidence of important interaction between the two.

Helminthic infection is a major problem in the developing world where approximately 25% of the populations are infected with at least one helminth.[1] Ascariasis is the most common helminthic infection and the estimated global burden is one billion people.[2] In southern Thailand, a recent study reported the prevalence of soil-transmitted helminthic infection was 24.1%.[3]

To date, any association between parasitic infections, allergen sensitizations and the symptom of wheeze remains unclear. In developed countries, the level of specific IgE and positive skin test to house dust mite (*Dermatophagoides pteronyssinus*) are strong risk factors for the development of recurrent wheeze and asthma,[4-6] but this relationship has not been confirmed for developing countries. For example, in Ethiopia, positive skin tests for house dust mite and cockroach (*Blattella germanica*) were found to be unrelated to wheeze, whereas the ascaris infection was associated with a reduced risk of wheeze in the previous year (adjusted OR 0.5, 95% CI 0.3-0.9).[7] This dissociation between allergic skin

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test results and wheeze may be modified by the presence of parasites. In an experimental mice model, chronic exposure to soil-transmitted helminths, particularly *Ascaris lumbricoides* and hookworms, reduced experimental-induced allergic inflammation of airways.[8] However, there was a contrasting result from 2,164 children aged 8-18 years in rural China, where infection with *A. lumbricoides* increased both the risk of asthma (OR 1.85, 95% CI 1.37-2.49) and the number of skin tests positive for aeroallergens (OR 1.25, 95% CI 1.13-1.37).[9]

Adding to the uncertainty of these inconsistent findings, there is a scarcity of information about younger children who, if infected, may have different outcomes from older children or adults. The purpose of the current study was to investigate exposure to helminthic infections together with house dust mites as a risk factor for wheeze in children aged 18 to 36 months, living in an area of southern Thailand where helminthic infections are endemic. The results of the current study may contribute to better care of children with wheeze in these areas.

MATERIAL AND METHODS

Study area and population

This study was undertaken as part of The Prospective Cohort Study of Thai Children (PCTC). The PCTC is being done as a population-based birth cohort study which follows all births of the year 2001 from four rural districts in different regions of Thailand. This report is confined to Thepa District of Songkla Province in the southern part of Thailand, a rural area with available data on soil-transmitted helminthic infections. Agriculture and fishing are the main occupations here, and there is no serious air pollution. The temperature is 25-35^{o}C year round, with precipitation around 1,500 mm/year.

The National Ethics Committee of the Thailand Ministry of Public Health approved the study. The study was explained to the registered families who were invited to participate. Verbal permission was obtained followed by written informed consent.

Data collection

Networks of well-established village health volunteers were employed to follow new births in the study area between December 2000 and November 2001. The mother's education and family history of allergic diseases were recorded at birth. Data on tobacco smoke exposure were collected through a home visit and face to face interviews when the infant who was the subject of the study was aged approximately 12 months. A home dust sample was collected once when the infant was 12-18 months old, by vacuuming the infant's sleeping area for 2 minutes with a vacuum cleaner (1.3 kW, Samsung). The samples were stored at –20^{o}C until they were transported to the laboratory of the Department of Parasitology, Mahidol University, in Bangkok. A weight of 0.1 gm of fine dust of each sample was extracted overnight in 2 ml of PBS-Tween 20. Extracts were stored at -40^{o}C before analysis. Concentrations of the mite allergen (*Der p 1*) were determined using ELISA (Indoor Biotechnologies, Cardiff, UK) according to the manufacturer's instructions. Fresh stool specimens were collected from each infant at 18-36 months of age, and a helminth egg examination was performed using the Kato-Katz smear technique.[10]

Outcome variables

At the time of stool collection, information on the number of wheeze episodes during the previous six months was recorded. Each "episode" was distinct, in that it had to have been separated from any other episode by the infant being free of symptoms for at least 2 weeks before and after, with additional confirmation by a physician's examination or hospitalization. Both a physician's diagnosis and hospitalization for wheeze were noted as the main outcomes.

Statistical analysis

Median and range were computed for continuous variables and percentage for categorical variables. For risk factor analysis, the outcome was modeled using logistic regression with helminthic infections and mite allergen as the main explanatory variables. Significant effects of the explanatory variables and the interaction terms were subjected to the likelihood ratio test. Statistical analyses were carried out using Intercooled Stata software (Stata Corporation). The statistical significance level was set at 0.05.

RESULTS

Subject characteristics

The study originally recruited 1,076 children born during the study period, which covered approximately 95% of the total births in the area during that time. Most mothers had only primary school education. Family histories of allergy or exposure to tobacco smoke were 5.7% and 75.8%, respectively (Table 1).

Mite allergen exposure

Dust samples to test for mite allergens were obtained from 781 houses (72.6%). The main reasons for failing to obtain a sample were no electricity and no pillow or mattress for dust collection (usually the infant slept only on a thin cloth). A comparison between groups with missing and available dust samples showed no significant difference in demographic characteristics. The median mite allergen level (*Der p1*) was 2.92 μg/g dust (range 0-145 μg/g dust). Approximately a third (31.8%) of the samples had *Der p1* higher than 10 μg/g dust.

Table 1 Characteristics of the study population

	N/total*	%
Male	557/1,076	51.8
Mother's education		
Primary school	710/1,076	66.0
Secondary school	279/1,076	25.9
College/bachelor	87/1,076	8.1
Family history of allergy	49/858	5.7
Exposure to tobacco smoke at home	800/1,054	75.8
Der p1 (μg/g dust)		
< 2	360/781	46.1
2-10	173/781	22.1
➢ 10	248/781	31.8
Helminthic infection		
Ascaris lumbricoides	148/815	18.2
Trichuris trichiura	75/815	9.2
Hookworm	43/815	5.3
Wheeze in past six months		
Reported by parent	116/739	15.7
Confirmed by physician	81/739	11.0
Hospitalization	33/739	4.5

*Variation among variables according to availability of the collected data (see text).

Helminthic infections

With a minimum of three attempts we were able to collect stool samples from 815 infants (75.7%). The maternal education level of those whose stool samples could be obtained was significantly lower than the group from which we were unable to obtain a sample. In those who we did manage to obtain a sample, the prevalence of helminthic infection was 25.0%, with *Ascaris lumbricoides*, *Trichuris trichiura* and hookworm being found in 18.2%, 9.2% and 5.3%, respectively. Mixed infections were found in 6.8%. For positive specimens, the median egg counts were 13,984 eggs/g feces (range 115-291,318) for *A. lumbricoides*, 483 eggs/g feces (range 23-176,226) for *T. trichiura,* and 230 eggs/g feces (range 46-14,214) for hookworm.

Association between helminthic infections and mite allergen on risk of wheeze

Six-month prevalences based on parent's report, physician's diagnosis and hospitalized wheeze were 15.7%, 11.0% and 4.5%, respectively. From univariate analysis, hookworm infection was significantly associated with parent's report wheeze (OR 2.52, 95% CI 1.16-5.46) and physician's diagnosis wheeze (OR 2.89, 95% CI 1.25-6.67). The presence of mite allergen was a significant predictor for hospitalized wheeze. There was no linear relationship noted between the risk of wheeze and the dose of exposure to mite allergens (data not shown). For this reason, allergen levels were classified into three categories: < 2, 2-10 and >10 μg/g dust. The cutoff-points have been noted to be associated with increased levels of risk for asthma.[11] Hospitalized wheeze was most common in subjects exposed to *Der p1* 2-10 μg/g dust (Table 2). In cross-tabulation between helminthic infection and the physician's diagnosis wheeze after stratification by *Der p1* levels (Table 3), we found a significant association between hookworm infection and physician diagnosis of wheeze only in the medium-dose exposure of *Der p1* (2-10 μg/g dust). In the higher exposure of *Der p1* (> 10 μg/g dust), the effect of hookworm infection on wheeze was not significant. However, multiple

Table 2 Univariate analysis of mite allergens, helminthic infections and wheeze symptoms

Variables	Levels	Wheeze symptoms					
		Parent reported		Physician diagnosed		Hospitalized	
		N	OR (95%CI)	N	OR (95%CI)	N	OR (95%CI)
Der p1 (μg/g dust)	<2	32/248	1.00	21/248	1.00	6/247	1.00
	2-10	21/111	1.58(0.86-2.88)	13/111	1.43(0.69-2.98)	9/111	3.54(1.23-10.22)*
	>10	30/178	1.37(0.80-2.35)	24/178	1.68(0.91-3.13)	11/178	2.65(0.96-7.29)
Ascariasis	Negative	110/597	1.00	118/631	1.00	127/673	1.00
	Positive	24/109	1.25(0.76-2.06)	16/75	1.18(0.66-2.12)	7/31	1.25(0.53-2.97)
Trichuriasis	Negative	55/597	1.00	59/631	1.00	61/673	1.00
	Positive	9/109	0.89(0.42-1.85)	5/75	0.69(0.27-1.78)	3/31	1.07(0.30-3.64)
Hookworm infection	Negative	23/597	1.00	25/631	1.00	29/673	1.00
	Positive	10/109	2.52(1.16-5.46)*	8/75	2.89(1.25-6.67)*	3/31	2.37(0.68-8.28)

* *P* value < 0.05

Table 3 Association between helminthic infection and the physician's diagnosis of wheeze stratified by *Der p1* levels

Helminths		***Der p1* level** (μg/g dust)								
		< 2			2-10			> 10		
		Wheeze	%	*P* value	Wheeze	%	*P* value	Wheeze	%	*P* value
Ascaris	Negative	18/201	8.9	0.73	7/78	8.9	0.17	17/133	12.7	0.82
	Positive	3/41	7.3		5/27	18.5		4/35	11.4	
Trichuris	Negative	20/222	9.0	0.54	11/93	11.8	0.72	20/147	13.6	0.25
	Positive	1/20	5.0		1/12	8.3		1/21	4.7	
Hookworm	Negative	18/227	7.9	0.10	10/101	9.9	0.01	20/161	12.4	0.88
	Positive	3/15	20.0		2/4	50.0		1/7	14.2	

logistic regression revealed no significant interaction effect between helminthic infections and mite allergens. After adjustment for confounders, hookworm infection and mite allergen were still the most significant predictors of wheeze (Table 4).

DISCUSSION

Our study subjects were low-education rural residents with a high rate of exposure to smoking, a high level of exposure to *Der p1*, and a high prevalence of helminthic infections, yet a relatively low prevalence of wheeze. Our survey confirms that hookworm infection and mite allergen exposure are independent risk factors for childhood wheeze and there is no evidence of important interaction between the two.

The effects of helminthic infection on the risk of wheeze found in our study are different from a nested case-control study in a survey of 7,155 children aged 1 to 4 years in Ethiopia where helminthic infection was endemic, predominantly with *T. tichiura* (54%), *A. lumbricoides* (38%), and hookworm (10%). In that study, wheeze in the previous year was associated with *Ascaris* infection (age, sex, and urban/rural adjusted OR 0.5, 95% CI 0.3-0.9), although no significant association was found with hookworm (adjusted OR 0.6, 95% CI 0.2-1.8).[7] A similar study in adult Ethiopians also reported that hookworm infection was associated with a reduced risk of wheeze (OR 0.48, 95% CI 0.24-0.93).[12] Aside from this, there have been no studies directed at hookworm infection and wheeze in young children. The difference of our results from the previous

Table 4 Multivariate analysis of helminthic infections on risk of physician diagnosed wheeze in past six months

Variables	Levels	Wheeze symptoms			
		Physician diagnosed		Hospitalized	
		OR*	95%CI	OR*	95%CI
Der p1 (μg/g dust)	<2	1.00		1.00	
	2-10	1.83	0.82-4.11	4.95	1.50-16.31
	>10	1.63	0.81-3.29	2.42	0.75-7.74
Ascariasis	Neg	1.00		1.00	
	Pos	1.09	0.50-2.37	0.42	0.10-1.70
Trichuriasis	Neg	1.00		1.00	
	Pos	0.52	0.14-1.91	1.07	0.20-5.59
Hookworm infection	Neg	1.00		1.00	
	Pos	4.20	1.45-12.10	5.40	1.26-23.01

*Adjusted for age, sex, family history and variables in the table

study may be due to different settings in study designs, prevalences of helminthic infections, age of subjects, nutritional status and other unknown immunologic factors. A related study in rural China, however, found a significant association between helminthic infection (*A. lumbricoides*) and childhood asthma (OR 1.85, 95%CI 1.37-2.49).[9] The effect of regular anti-helminthic treatment with albendazole for 1 year on a group of asthmatic patients in an endemic zone of ascariasis showed significant improvement in asthma status, not only for the period of anti-helminthic administration, but also for the following year. However, after 2 years without treatment, the asthma prevalence reverted to the initial state. No significant changes were observed in the control group over the entire period of evaluation.[13] At this stage, the accumulated evidence to date from these previous studies, plus our own, indicates that helminthic infections can contribute to the clinical symptoms of asthma in an endemic situation.

Our study also demonstrated an increased risk of hospitalization for wheeze in children exposed to mite allergens. Similarly, Custovic *et al*.[14] and Tunnicliffe *et al*.[15] demonstrated a positive relationship between severity of asthma and exposure to *Der p 1* in asthmatics sensitized to house dust mites. In our study, there was no linear relationship between the dose of mite allergen exposure and the risk of wheeze. Cullinan *et al*.[16] also reported that the risk of asthmatic attack increased at low levels of exposure, but attenuated thereafter among the birth cohort. One possible explanation is immunological, in that very high allergen exposures can exert a tolerance effect on sensitization in early childhood. Platts-Mills *et al*.[17] after observations from a cross-sectional study, suggested that high exposures to a cat allergen (*Fel d 1*) may produce a form of tolerance characterized by a modified Th2 response with specific IgG4 production.

After stratification by *Der p1* levels, we found a significant association between hookworm infection and physician diagnosis of wheeze only in the lowest dust mite exposure group. In the higher exposure group, the wheeze effect of hookworm might have been suppressed, however, as the prevalence of hookworm infection among our subjects was low, the sample size did not have enough power to detect any statistical significance of the interaction term.

Even though there was an evidence suggesting a protective effect resulting from helminthic infection against risk of wheeze or asthma,[18] the relationship between malnutrition and helminthic infections has been well established,[19] and studies of developmental quotients in children less than two years of age in Nicaragua have shown a correlation between slow development, stunting and helminthic infections.[20] Considering these studies, along with our results reported herein, we suggest that the program

for helminthic control in the study area should be enhanced. Also, the presence of wheeze and/or asthma should be incorporated as parts of outcome evaluations in the future.

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