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Association of helminth infestation with childhood asthma: a nested case-control study

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ABSTRACT

Objectives: The association between helminthiasis and asthma remains inconclusive but can only be investigated in counties where helminthiasis is transitioning from a high to low burden. We investigated this association using data from a childhood respiratory cohort in Sri Lanka.
Methods: A case-control study was nested within a population-based cohort of children aged 6-14 years in Sri Lanka. The stool samples of 190 children with asthma and 190 children without asthma were analyzed to assess the burden of helminth infestation. Logistic regression models were fitted to investigate the association of gastrointestinal helminth species with asthma.
Results: Helminthiasis in children with and without asthma was 23.3% (n = 44) and 15.3% (n = 23), respectively. Those with asthma were more likely to have helminthiasis (odds ratio 3.7; 95% confidence interval 1.7, 7.7; $P = 0.001$), particularly with *Trichiuris trichura* (odds ratio 4.5; 95% confidence interval 1.6, 12.3; $P = 0.004$). Helminth eggs per gram of feces were not associated with asthma ($P > 0.05$).
Conclusion: Our findings demonstrate a positive association between *T. trichura* infestation and asthma and point to the need to fully characterize this association to understand the likely immunological mechanism that drives it. This association highlights an important public health intervention in countries where these infestations are still prevalent, affecting 24% of the population worldwide.

Background

Asthma has a high global prevalence and disease burden [1]. Its prevalence has increased in high-income countries while remaining relatively low in low-income countries [2]. However, some increase in asthma prevalence has also been reported in low-income countries with improving socioeconomic conditions [2]. Attempts to explain this inverse relationship between better socioeconomic conditions and asthma and allergic diseases include the hygiene hypothesis [3], which helped broaden the understanding of the environmental origin of asthma and suggested that low early-life exposure to infective agents predisposed children to subsequent allergic diseases/immune-mediated disorders, including asthma [4]. However, it could not explain the high prevalence of asthma in the poor urban communities in high-income countries. The mi-

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crobiome hypothesis [5] and the theory of developmental origins of health and disease [6] attempted to provide other explanations. These hypothesized how humans, together with all cohabiting and parasitic organisms, collectively evolve under evolutionary pressures and how epigenetic mechanisms govern health and disease, including asthma.

Asthma results from airway hyper-responsiveness due to various triggers [7]. The mechanism by which these triggers induce asthma is helpful to understand asthma endotypes and better manage asthma [8]. Asthma mostly results from initial sensitization of airways to common aeroallergens that induces immune-mediated bronchial reaction when re-exposed [9]. The pathophysiology of respiratory reactions in asthma is complex and mediated by several interlinked inflammatory pathways and inflammatory chemical mediators [7]. Some of the triggers for asthma are infections that induce immune responses, but there are other mechanisms that modulate host immunity to minimize sensitization to allergens and thereby reduce asthma [10]. Some of this immune regulation was suggested to occur in the presence of helminth infestations because the regulation of immune reaction might have been of evolutionary benefit to both the helminths and the host [3]. This immune modulation by gastrointestinal helminth infestations is believed to reduce allergic conditions [11], likely due to the immune regulatory potential of helminths, which is used to suppress the host immune system to establish chronic infestation and have an indirect suppressive effect on host responses against allergens [12]. This was supported by the increase in host reactivity to allergens subsequent to antihelminth treatment for chronic helminthiasis [13] and by the increase in asthma seen in high-income countries as they transitioned from having high to low prevalence of helminthiasis [1]. This inverse association has recently been seen in some low-income countries as well, where the prevalence of allergic diseases was increasing, with a reduction in gastrointestinal helminthiasis [2,14]. However, there are some studies that showed no protective effect of helminth infestation on asthma or showed that such infestations increased the risk of asthma [15]. A recent systematic review also found the current evidence on this association to be inconclusive [16].

Resolving this uncertainty in the association between helminthiasis and asthma has significant implications for asthma-related health policies and targeted population-based interventions in low- and middle-income countries where helminthiasis is still prevalent. A demonstrated consistent positive association would inform improvements to existing policy and interventions for mitigation of asthma that these countries already have in place and/or introduction of new policy/interventions. Implications of such resolution is enormous, given that 1.5 billion individuals, that is 24% of the global population, are currently affected by helminthiasis [17]. However, the nature of this association between helminthiasis and asthma can no longer be sufficiently characterized in high-income countries because helminthiasis is already low in those countries. On the other hand, countries that are currently transitioning from high to low burden of helminthiasis are likely to provide an ideal platform to investigate this association further. The evidence for this association from such countries is limited [14] but likely to help resolve the ambivalent nature of current evidence [18]. Investigating this association in Sri Lanka, which is currently undergoing this transition from high to low burden of helminthiasis [14] and has reported increasing prevalence of childhood asthma [19–21], is likely to provide additional evidence for the link between gastrointestinal helminthiasis and asthma. Given this backdrop, we conducted a population-based study to determine the association between gastrointestinal helminthiasis and asthma in a subsample of a large cohort of children in Sri Lanka.

Materials and methods

Study design and the sample

This study was nested in a subsample of a larger study aimed at establishing a cohort to study risk factors and consequences of chronic sleep and respiratory diseases in children in Sri Lanka. A community-based stratified cluster sampling method was used to recruit 1500 children aged 2-14 years from the Eastern Province of Sri Lanka. Clusters were randomly selected from a list of field health areas that catered to about 500 families. A total of 20 families with eligible children were randomly selected from a list maintained by the field health workers of these areas for provision of child and adolescent health care. When there was more than one eligible child in a family, only one child was selected randomly.

From the children aged 6-14 years in this sample, a subsample of 190 children with current asthma and 190 children without current asthma were randomly selected to study any gastrointestinal helminthiasis that they may have. Asthma status was determined using parents' responses to validated questions [22] (see subsequent section for details). The children in the subsample represented all field health areas included in the larger study. The children with severe mental disability, severe physical disability, and on immunocompromising systemic treatment were excluded. Ethical approval for this study was granted by the ethics review committee of the District General Hospital/Trincomalee of the Provincial Department of Health/Eastern Province (approval number ERC/2018/06).

Study instruments and data collection

Parents of all children aged 6-14 years provided responses to cross-culturally adapted questions on asthma that have been validated to be used in the local context. These were based on the International Study of Asthma and Allergies in Childhood questionnaire [22]. Current asthma was defined as (i) ever having asthma/wheezing or whistling in the chest and having wheezing or whistling in the chest at least four times during the past 12 months or (ii) being on an inhaler for diagnosed asthma. Information on sociodemographics and other likely risk factors were additionally collected using pretested questions validated using modified Delphi technique.

Stool sample collection and microscopic analysis

Each child provided a fresh stool sample for parasitological analyses. Stool samples were transported maintaining the cold chain from the homes of children to the laboratory and stored at 4°C until processed. The presence of helminth eggs in each sample was assessed using a modified sucrose flotation method [23,24]. From each stool sample, approximately 2 g were measured and mixed with distilled water in a capped centrifuge tube to a final volume of 15 ml. The mixtures were stirred thoroughly and centrifuged at 2045 g for 20 minutes at room temperature (~27°C). Subsequently, the supernatants were discarded and the pellets at the bottom of the tubes were resuspended in distilled water and centrifuged (twice) until clear supernatants were obtained. The pellets were then emulsified using saturated sucrose solution, mixed thoroughly, and centrifuged for 20 minutes at 2045 g. Approximately 5 ml of the top meniscus of the resulting suspensions were collected in a centrifuge tube and mixed with distilled water up to a final volume of 15 ml and centrifuged for 10 minutes at 1370 g. This procedure was repeated and 1 ml of each suspension with the pellet was transferred to 1.5-ml Eppendorf® tubes

Table 1
Sociodemographic information of the sample.

		No asthma (n = 190) N (%[a]) or mean (SD)	Asthma (n = 189) N (%[a]) or mean (SD)	*P*-value
Age		8.2 (3.0)	8.1 (3.2)	0.869
Sex	Male	88 (46.3)	109 (57.7)	0.027
	Female	102 (53.7)	80 (42.3)	
Ethnicity	Tamil	115 (60.5)	120 (63.5)	0.271
	Moor	68 (35.8)	66 (34.9)	
	Sinhala	0 (0.0)	1 (0.5)	
	Burgher	7 (3.7)	2 (1.1)	
Religion	Hindu	107 (56.3)	109 (58.0)	0.850
	Islam	67 (35.3)	66 (35.1)	
	Christianity	16 (8.4)	13 (6.9)	
Maternal educational level	Grades 1-5	15 (9.9)	17 (10.7)	0.001
	Grades 6-11	97 (63.8)	79 (49.7)	
	Passed GCE/OL	32 (21.0)	40 (25.2)	
	Grades 12-13	2 (1.3)	18 (11.3)	
	Graduate	6 (4.0)	2 (1.3)	
	Postgraduate	0 (0.0)	3 (1.9)	
Paternal educational level	Grades 1-5	34 (22.3)	24 (15.5)	0.047
	Grades 6-11	81 (54.3)	82 (52.9)	
	Passed GCE/OL	22 (14.9)	38 (24.5)	
	Grades 12-13	6 (4.0)	5 (3.2)	
	Graduate	5 (3.4)	2 (1.3)	
	Postgraduate	0 (0.0)	4 (2.6)	
Maternal occupation	No occupation or homemaker	159 (89.8)	171 (94.5)	0.339
	Elementary occupations/ Agriculture/ Fisheries	6 (3.4)	4 (2.2)	
	Clerical / Sales/ Technical	10 (5.6)	4 (2.2)	
	Professional or similar	2 (1.1)	2 (1.1)	
Paternal occupation	No occupation or homemaker	65 (39.4)	76 (44.4)	0.332
	Elementary occupations/ Agriculture/ Fisheries	67 (39.4)	61 (35.1)	
	Clerical / Sales/ Technical	27 (15.8)	32 (18.1)	
	Professional or similar	9 (5.4)	4 (2.3)	
Monthly household income (Sri Lanka Rupees)		[b]24000 (3000, 85000)	[b]24000 (4500, 90000)	[c]0.177

[a] Out of the valid responses.
[b] Median (range).
[c] For Wilcoxon rank-sum test.GCE/OL, General Certificate of Education/Ordinary Level.

using a Pasteur pipette. Distilled water was added to a final volume of 1.5 ml and the tubes were centrifuged at 1150 g for 10 minutes. The clear supernatants were discarded, and microscope slides were prepared using the remaining 0.5 ml pellets and examined under a light microscope. Helminth eggs were identified using both morphology and morphometry [25]. The egg count in 0.50 ml was assessed as eggs per gram (EPG) of feces, assuming that the Modified Sheather sucrose flotation method has concentrated all the eggs/cysts in the whole 2 g of feces to the 0.5 ml that was used for direct counting [23,24].

Statistical analysis

The distributions of variables were described using numbers and proportions or means and standard deviations. Student's *t*-test and chi-square test were used appropriately to check differential distribution of these variables among those with and without asthma. Logistic regression models were used to determine the association of gastrointestinal helminthiasis with asthma. Adjustments were made for the effects of relevant confounding variables. The associations were presented as unadjusted and adjusted odds ratios (ORs) and 95% confidence intervals (CIs).

Role of the funding source

The funders had no role in study design, in the collection, analysis, or interpretation of data, in the writing of the manuscript, and/or in the decision to submit the paper for publication.

Results

The final sample that was used for analysis consisted of 189 children with asthma and 190 children without asthma. The stool sample of one child with asthma could not be used. Those with asthma in this sample were more likely to be male and have better educated mothers (Table 1).

Gastrointestinal helminthiasis was present in 23.3% of children with asthma and 15.3% of children without asthma (Table 2). Only three children (0.8%) had co-infestations by more than one gastrointestinal helminth: two children with *Ascaris lumbricoides* and *Trichiuris trichura* and one child with *T. trichura* and hookworm spp. The intensity of the infestations was low [26], ranging from 0.03 (± 0.02) EPG for hookworm spp. to 0.34 (± 0.13) EPG for any helminth species.

The odds of having gastrointestinal helminths were significantly higher among children with asthma than those without asthma (OR 3.7; 95% CI 1.7, 7.7; Table 2). The children with asthma had a higher odds of harboring *T. trichura* (OR 4.5; 95% CI 1.6, 12.3), but asthma was not associated with harboring other helminths or the total or species-specific intensity of infestation as measured by EPG ($P < 0.05$; Table 2).

Discussion

We investigated the association between gastrointestinal helminthiasis and asthma in children and found that those with gastrointestinal helminths, particularly *T. trichura,* were at higher odds of having asthma. The socioeconomic environment of our

Table 2
Association of asthma with the type of gastrointestinal helminths and the burden of helminths (eggs per gram of feces).

		No asthma (n = 190) N (%[a])/ mean (SD)	Asthma (n = 189) N (%[a])/ mean (SD)	Unadjusted OR (95% CI); *P*-value	Adjusted OR[b] (95% CI); *P*-value
Gastrointestinal helminths		29 (15.3)	44 (23.3)	1.7 (1.01, 2.8); 0.049	3.7 (1.7, 7.7); 0.001
Gastrointestinal helminth species					
	Ascaris lumbricoides	11 (5.8)	16 (8.5)	1.5 (0.7, 3.3); 0.314	2.9 (0.9, 9.6); 0.079
	Trichiuris trichura	17 (9.0)	28 (14.8)	1.8 (0.9, 3.4); 0.080	4.5 (1.6, 12.3); 0.004
	Hookworm spp.	3 (1.6)	1 (0.5)	0.3 (0.03, 3.2); 0.341	0.4 (0.03, 4.6); 0.454
Number of helminth eggs in 1 g of feces					
	A. lumbricoides	0.17 (0.11)	0.10 (0.03)	0.9 (0.8, 1.2); 0.552	4.4 (0.9, 22.0); 0.069
	T. trichura	0.14 (0.04)	0.13 (0.06)	1.0 (0.7, 1.4); 0.861	1.4 (0.8, 2.6); 0.198
	Hookworm spp.	0.03 (0.02)	0.00 (0.00)	0.2 (0.01, 5.4); 0.364	0.2 (0.01, 4.8); 0.307
	Any helminth species	0.34 (0.13)	0.25 (0.05)	0.9 (0.8, 1.1); 0.458	1.7 (1.0, 2.9); 0.070

[a] Column percentages.
[b] Adjusted for age, gender, maternal educational level, paternal educational level, maternal occupation, and paternal occupation.CI, confidence interval; OR, odds ratio.

study area likely represents that of low- and lower-middle-income countries that are transitioning from high to low prevalence of gastrointestinal helminthiasis. Investigating the helminth-asthma association in this setting enabled us to provide evidence that adds to the current limited knowledge on how this association evolves together with the socioeconomic environment.

The high prevalence of asthma in regions in the world where there are better hygienic practices has long been tied to reduced exposure to infections in early life that would increase the later propensity for allergy sensitization. However, other evidence shows no definite relationship between asthma and hygiene, which challenges this hypothesis [27]. The presence of gastrointestinal helminths in children often suggests poor hygiene and is a proxy for early exposure to infections in general but also are known to induce immunoglobulin (Ig)E producing Th2 immune responses and change the host microbiome [28]. In our study, we found that the proportion of gastrointestinal helminth infestations to be 23% in those with asthma and 15% in those without. This compares with the prevalence of helminthiasis reported in recent studies in Sri Lanka that ranges from 0% to 29% [29–31]. The significantly higher proportion of children with asthma harboring gastrointestinal helminths does not support the hygiene hypothesis proposed for asthma and adds to the growing body of evidence against it [15,32,33]. On the other hand, the induction of IgE producing Th2 immune responses [28] by helminths may have strong potential to cause asthma [34] and may explain our findings, at least in part. Although the potential of helminthiasis leading to asthma through changes in the host microbiome could not be characterized within our study, there is evidence that gastrointestinal helminths influence the composition of intestinal microbiota [35,36], which has a profound effect on human health, including development of immune-mediated diseases [37].

The significant association that we saw between asthma and current infestation with *T. trichura* is also unusual. *A. lumbricoides* and hookworms, such as *Ancylostoma duodenale* and *Necator americanus*, migrate through the respiratory tract during their lifecycle and potentially give rise to respiratory symptoms that may be misconstrued as symptoms of asthma [32], but *T. trichura* has no pulmonary migration in its life cycle. In contrast to our findings, there are reports of children with a heavy burden of *T. trichura* in early childhood having a significantly reduced sensitization to many different types of aeroallergens as evidenced by subsequent skin prick tests, even in the absence of current infestations [38,39]. However, there is another evidence that *T. trichura* infestation is strongly associated with sensitization to house dust mite (*Dermatophagoides pteronyssinus*) allergens in rural areas similar to those in our study [40], which may explain our finding but could not be tested with the data that we have. Another proposed explanatory model is that of mutual immunomodulation between *T. trichura* infestation and asthma with positive modulation of both pro- and anti-inflammatory cytokine levels [41] that could lead to chronic sustenance of both conditions in children. However, this hypothesis needs more direct evidence to support it.

Interestingly, co-infestation by more than one gastrointestinal helminth species was almost non-existent in our sample, which likely suggests low community reservoirs for gastrointestinal helminths and infestations through sporadic sources. However, the most common gastrointestinal helminths that are found elsewhere were all observed in these children but with very low [26] worm load, as shown by the EPG values. We could not find a significant association between the worm load as indicated by the EPG and asthma, which might partly be due to this low worm load seen in both groups. Similarly low worm load has also been reported recently by others for Sri Lanka [23], which is likely to be due to mass anthelmintic programs that particularly targeted socioeconomically challenged communities.

Because this study was nested in another cohort study, there were both advantages and limitations. As already consented participants of a cohort study, the invitees were more likely to consent to the substudy and provide responses and stool samples, unlike in the case of a separate independent study. The cohort had near-zero nonresponse rate, which helped to minimize any selection bias due to selective nonresponse and therefore is representative of the general child population. This advantage was also seen in the nested study, which had no refusals. The data collectors and other staff were already familiar with the cohort and how it functioned, which made data collection and sample collection easy. Due to the same reason, the logistics were better managed, allowing us to maintain strict cold chain without difficulty and leading to a minimal loss of stool samples. However, because the respondents had already responded to lengthy questionnaires within the existing cohort, the questionnaire in the nested study had to be made as short as possible to minimize undue burden on the cohort members, and the data that we collected had to be limited. As a result, data collection was on a minimum number of variables, which in turn prevented us from performing more complex analyses to explain our findings. Given this, the possibility of unmeasured variables influencing our findings remains. The detection of helminths was done using a flotation technique, followed by microscopic analysis on a single stool sample due to resource and time limitations. This method has a low sensitivity and can additionally be influenced by the skills and experience of the mi-

croscopist [42]. Similarly, the sucrose concentration method that we used may not concentrate eggs of trematodes (*e.g.,* schistosome eggs) and Strongyloides larvae. Any resulting misclassifications are likely to have affected the prevalence estimates nondifferentially and therefore underestimated the strengths of the associations that we detected. Finally, given the nature of this epidemiological study, asthma was defined using validated clinical questions. Therefore, we did not have measures of sputum or peripheral blood eosinophils nor IgE levels, which would have assisted in phenotyping asthma in the participants. Such information would also have allowed a more detailed consideration of potential mechanisms underpinning the relationship between helminth infection and asthma.

Despite these limitations, our findings confirm and add to the existing evidence on a positive association between helminthiasis and asthma to help resolve the inconclusive nature of this association. Given that 1.5 billion individuals amounting to 24% of the global population are affected by helminthiasis, which can be significantly reduced by low-cost interventions [17], the resolution of this association to inform policy and population-based interventions to mitigate the burden of asthma has immense health and financial benefits.

In conclusion, using a case-control study nested in a cohort study of children, we found that *T. trichura* infestation is strongly associated with current asthma but that other gastrointestinal helminthiasis or the helminth load are not associated with asthma. Our findings add to the limited evidence on the positive association between *T. trichura* and asthma that needs to be fully characterized in future research.

Declaration of competing interest

The authors have no competing interests to declare.

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Ethical approval

This study conforms to the amended Declaration of Helsinki. It was approved by the ethics review committee of the District General Hospital/Trincomalee of the Provincial Department of Health/Eastern Province (approval number ERC/2018/06).

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Author contributions

CS conceptualized, designed, and conducted the study, analyzed the data, and drafted successive versions of the manuscript. PKP provided inputs to the planning of the study and managed and oversaw the full laboratory component. SA managed and oversaw the administrative and field data collection processes. NA conducted the laboratory analyses of stool samples. PP managed data entry and cleaning. GH, GN, and RR provided inputs for the planning of the study and revised successive drafts of the manuscript. SD supervised and guided all steps of the study process. All authors contributed to critical interpretation of the data and revised and approved the final manuscript.

Data availability

All manuscript related data are available in the tables. Further information can be obtained from the corresponding author.

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