

Original Article

***Toxocara* seropositivity in Sri Lankan children with asthma**

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Abstract *Background:* Toxocariasis occurs in humans due to infection with *Toxocara canis* or *T. cati*, the nematode parasites of dogs and cats, respectively. The relationship between toxocariasis and asthma is complex, with some studies demonstrating that children with asthma were more likely to be *Toxocara* seropositive as compared to non-asthmatic children, and other studies indicating no such significant relationship. The aim of the present study was to investigate *Toxocara* seropositivity and its association with asthma in a selected group of Sri Lankan children.

Methods: Two groups of children were studied: group 1 included 100 children with confirmed bronchial asthma who were on regular inhaler steroid treatment for asthma; group 2 included 96 children who did not have physician-diagnosed asthma or upper respiratory tract infections, attending the same hospital. Diagnosis of *Toxocara* seropositivity was based on IgG *Toxocara* Microwell Serum Elisa Kits. Enzyme-linked immunosorbent assay was regarded as positive for a reading of ≥ 0.3 optical density units. Stool samples were examined for helminth ova.

Results: *Toxocara* seropositivity in children with asthma was 29% and this was significantly more than *Toxocara* seropositivity among non-asthmatic children ($P < 0.001$). *Toxocara* seropositivity was identified as a significant risk factor of asthma in a univariate model. Eosinophilia was seen in a significantly higher proportion of non-asthmatic and asthmatic children who were *Toxocara* seropositive. *Toxocara* seropositivity, however, was not identified as a significant risk factor in a multivariate model.

Conclusions: The analysis confirmed previously identified risk factors for asthma but there was no association between the helminth parasitic infection, toxocariasis and bronchial asthma in children.

Key words asthma, children, risk factors for asthma, Sri Lanka, toxocariasis.

Human toxocariasis is a neglected zoonotic parasite disease caused by accidental ingestion of embryonated eggs of *Toxocara canis* and *T. cati*, the common round worms of dogs and cats, respectively. The natural hosts are usually infected before birth by the mother ingesting the infective eggs, but young puppies and kittens may also be infected with larvae through the milk or by ingesting eggs. In the human host the hatched larvae fail to develop into mature adult worms and perforate the intestinal wall, migrating throughout extra-intestinal tissues. Disease occurs mainly in young children who have close contact with infected household pets or who have been exposed to eggs in the environment and is mainly due to *T. canis*. In >80% of the cases there is a marked eosinophilia and hepatomegaly. In >50% of cases pulmonary symptoms such as coughing and wheezing with dyspnea and fever may be found.¹ There are three main clinical forms of toxocariasis, namely visceral larva migrans, ocular larva migrans, and covert toxocariasis.²

Asthma is a major, chronic airway disorder that is a serious health problem in countries throughout the world. It is considered to be one of the most common chronic diseases of childhood and its prevalence in many parts of the world is gradually increasing.³ The prevalence of asthma in children varies from 0% to 30% in different populations.⁴ Asthma causes a high morbidity in Sri Lankan children. A study carried out in the Gampaha district, in the Western Province of Sri Lanka, reported a prevalence of 30% among 5–11-year-old school children.⁵ The island wide prevalence among 6–7-year-old children is recorded as 28% (ISAAC study, unpubl. data, 2000/01).

The relation between toxocariasis and asthma is complex. It has been shown that asthmatic children are likely to be more *Toxocara* seropositive than non-asthmatic children.^{6–9} In contrast, there is controversial evidence to establish an unequivocal relationship between toxocariasis and asthma.¹⁰

Because asthma poses a significant burden for the parents and the national health-care system, identifying a significant treatable environmental factor that may lead to its particular immunological responses of the disease is of utmost importance. The aim of the present study was to investigate whether there is an association between childhood asthma and *Toxocara* seropositivity.

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Methods

Study site

The study was carried out at the University Paediatric Unit of Lady Ridgeway Hospital, the only children's hospital in Sri Lanka that serves as a national referral center for pediatric care.

Study design

A prospective study was conducted from April 2006 to February 2007. The study population consisted of 196 children aged 5–12 years presenting to the hospital. Two groups of children were studied: group 1 included children with confirmed bronchial asthma who were on regular inhaled steroid treatment; group 2 included children who were attending the same hospital for different ailments but who had never had bronchial asthma and were not suffering from upper respiratory tract infection at the time of the study. The controls were matched on age, sex and ethnic group. Children were enrolled after obtaining informed written consent from their parents or guardians.

Children whose parents/guardians did not give consent, those who had any other known medical condition, or children with hepatosplenomegaly, generalized lymphadenopathy and ocular symptoms were excluded from the study.

Measurement of seropositivity for toxocariasis and other hematological investigations

An aliquot of 2 mL of blood was collected under aseptic conditions by trained nurses and divided equally into ethylenediamine tetraacetic acid (EDTA)-containing tubes and plain tubes. The samples in the plain tubes were centrifuged at 800g for 5 min, after which the sera were collected and stored at -20°C until use. Diagnosis of *Toxocara* seropositivity was based on IgG *Toxocara* Microwell Serum ELISA Kits (Cat No. 8206-3; Diagnostic Automation, Inc., Calabasas, CA, USA). The enzyme-linked immunosorbent assay was regarded as positive for a reading of ≥ 0.3 optical density units.

The sample collected into the EDTA tube was used to perform a white blood cell count using the hemocytometer and a differential count using a counting chamber. A high eosinophil count was indicated for absolute eosinophil count $>400/\text{mm}^3$.

The technical officers performing the investigations were blind to the clinical status, age and sex of the child.

Stool examination for intestinal helminthes

A sample of feces collected from each child was examined for helminth ova and protozoan cysts on saline and iodine smears.

Previously identified risk factors for asthma

The risk factors for developing asthma such as family history of asthma, exposure to cigarette smoke at home, exposure to mosquito coils, presence of low birthweight as estimated from birth records or history of recurrent respiratory infections from childhood were recorded. Exposure to pesticides was assessed on history of using pesticides in crop fields surrounding the house.

Sociodemographic family status

The sociodemographic status of the family was assessed for mother's and father's educational status, monthly family income

and house type. The house was classified as 'good' or 'poor' depending on the materials used for roof, floor and walls as previously defined.¹¹ The area of residence was classified as urban or rural based on the Census and Statistics Report, 2001.¹²

Data analysis

Data were analyzed using SPSS version 15.0 for Windows (SPSS, Chicago, IL, USA). Effect of risk factors on presence of asthma was determined using χ^2 test. Fisher's exact test was used when number of expected count was <5 . Unadjusted odds ratio (OR) with 95% confidence intervals (CI) was used to determine the associations between asthma and risk factors. The significant risk factors were included in the final multivariate model; backward logistic regression was performed to identify independent risk factors and adjusted OR were calculated.

Ethics considerations

Ethics clearance was obtained from the Ethical Review Board of the Faculty of Medicine, University of Colombo, Sri Lanka. To protect the identity of the children, blood samples and questionnaires were assigned a number that was kept separate from patient identification information.

Results

Subject characteristics

A total of 200 children were enrolled in the study, of which 100 children suffered from bronchial asthma, and 96 children were age-, sex- and ethnicity-matched controls. Investigations could not be completed in controls for four of the subjects and were hence excluded from the study. The asthma and control groups did not differ significantly in terms of age, sex, ethnicity or area of residence (rural or urban; Table 1).

The socioeconomic variables of the two groups of children were similar (Table 1). The educational status of the parents who were deceased was not recorded. More than 65% of the parents of the children with asthma had completed the first National examination (O-Level examination) held in Sri Lanka. Approximately 65% of fathers and 55% of mothers of the control children had also completed this examination. Approximately 50% of parents of both groups of children received a monthly family income $>$ Sri Lankan rupees 10 000.00, and $>80\%$ of children lived in households classified as 'good'.

Clinical status of asthmatic children

Eighty-seven percent of children were on inhaler therapy for asthma at the time of presentation, while the remaining 13% indicated that they were on inhaler treatment periodically. None of the control children had a history of asthma or had been on inhaled steroid or other asthma prevention treatment for asthma during their lifetime. Approximately 60% of the children with asthma suffered from at least one attack per month. Forty percent of parents indicated that their child's sleep was disturbed due to episodes of asthma.

Risk factors for developing asthma

Table 2 lists the unadjusted OR determined through univariate analysis for asthma and risk factors previously identified in the

Table 1 Subject characteristics

Variable	Asthma group (<i>n</i> = 100)		Non-asthma group (<i>n</i> = 96)		χ^2	<i>P</i>
	<i>n</i>	%	<i>n</i>	%		
Sociodemographics						
Sex						
Male	57	57	51	53.1	0.297	0.586
Female	43	43	45	46.9		
Age (years)						
4–6	38	38	34	35.4	0.277	0.871
7–9	39	39	37	38.5		
10–12	23	23	25	26.0		
Ethnicity [†]						
Sinhalese	82	82	77	81.1	0.116	0.944
Tamil	06	06	06	6.3		
Muslim	12	12	13	13.5		
Place of residence						
Urban	39	39	40	41.7	0.145	0.704
Rural	61	61	56	58.3		
Fathers' literacy [‡]						
Completed O-levels	67	69.1	62	65.3	0.316	0.574
Primary and Secondary	30	30.9	33	34.7		
Mothers' literacy [‡]						
Completed O-levels	64	65.3	52	54.2	2.503	0.114
Primary and Secondary	34	34.7	44	45.8		
Monthly family income (SLR)						
>10 000.00	46	46	51	53.1	0.995	0.319
<10 000.00	54	54	45	46.9		
House type						
Good	84	84	88	91.7	2.679	0.102
Poor	16	16	98	8.3		
Clinical characteristics						
Eosinophilia						
Present	71	71	24	25	41.495	<0.001
Absent	29	29	72	75		
<i>Toxocara</i> seropositivity						
Yes	29	29	10	10.4	10.612	0.001
No	71	71	86	89.6		

[†]Ethnic groups Tamil and Muslim were amalgamated to derive the χ^2 . [‡]Total number of subjects who responded to maternal and paternal education status differs according to questionnaire response for each variable. SLR, Sri Lankan rupee.

published literature. Children were at a greater risk of developing asthma if they were exposed to cigarette smoke at home (OR, 4.98; 95% CI: 2.11–12.06); had at least one parent with asthma (OR, 15.61; 95%CI: 5.89–43.73), gave a history of recurrent respiratory tract infections from birth (OR, 16.16; 95%CI: 4.64–71.24) or had a low birthweight (OR, 6.41; 95%CI: 1.3–42.73). Children who were positive for *Toxocara* serology were at a threefold greater risk of having asthma compared to the control group (OR, 3.51; 95%CI: 1.51–8.32). No significant association was found with the other risk factors.

Laboratory Investigations

Twenty-nine percent of children with asthma were positive for *Toxocara* serology (29/100; Table 1). *Toxocara* seropositivity was significantly lower among non-asthmatics (10.4%; $P = 0.001$). A significantly higher number of children with asthma (71%) had an eosinophil count >400 cells mm^3 as compared to children without asthma (25%; $P < 0.001$; Table 1). When the children with asthma were considered, the presence of eosinophilia was significantly higher ($P = 0.032$) in the *Toxocara*-seropositive

group compared to the negative group (data not shown). Of the 29 children with bronchial asthma and positive *Toxocara* serology, 25 had an absolute eosinophilia (86%). Of the 71 children with bronchial asthma and negative *Toxocara* serology, 46 had an absolute eosinophilia (64.8%). Similarly when the non-asthmatic group was considered, of the 10 children who were positive for *Toxocara* serology, eight had an absolute eosinophilia (80%). This was significantly higher than the non-asthmatic children who were seronegative for Toxocariasis with eosinophilia (20%; $P < 0.001$).

The fecal samples examined for helminth ova and protozoa cysts were negative in all children.

Multivariate analysis

A multivariate model was constructed using backward logistic regression. Of the five variables tested (low birthweight, recurrent lower respiratory tract infections from birth, exposure to smokers at home, presence of a parent with asthma and *Toxocara* seropositivity), *Toxocara* seropositivity did not meet the significance level to enter the final model. The adjusted OR for low

Table 2 Risk factors for asthma

Risk factor	Children with asthma (<i>n</i> = 100)		Children without asthma (<i>n</i> = 96)		OR	95%CI
	<i>n</i>	%	<i>n</i>	%		
Ethnicity						
Minority groups	18	18	19	19.8		—
Sinhalese	82	82	77	81.1	0.89	0.41–1.93
Fathers' literacy						
Completed O-levels	67	69.1	62	65.3		—
Primary and Secondary	30	30.9	33	34.7	0.84	0.44–1.61
Mothers' literacy						
Completed O-levels	64	65.3	52	54.2		—
Primary and Secondary	34	34.7	44	45.8	0.63	0.34–1.17
Monthly family income (SLR)						
>10 000.00	46	46	51	53.1		—
<10 000.00	54	54	45	46.9	1.33	0.73–2.43
House type						
Good	84	84	88	91.7		—
Poor	16	16	08	8.3	2.10	0.79–5.67
Exposure to smoking at home						
No	66	66	87	90.6		—
Yes	34	34	09	9.4	4.98	2.11–12.06
Allergy to soft toys						
No	76	76	76	79.2		—
Yes	24	24	20	20.8	1.20	0.58–2.48
Use of mosquito coils						
No	66	66	72	75		—
Yes	34	34	24	25	1.55	0.79–3.01
Parents with asthma						
No	49	49	90	93.8		—
Yes	51	51	6	6.3	15.61	5.89–43.73
Respiratory tract infections from birth						
No	65	65	93	96.9		—
Yes	35	35	3	3.1	16.16	4.64–71.24
Low birthweight						
No	88	88	94	97.9		—
Yes	12	12	02	21	6.41	1.3–42.73
Presence of pets at home						
No	71	71	50	52.1		—
Yes	29	29	46	47.9	0.51	0.28–0.95
Exposure to pesticides						
No	86	86	86	89.6		—
Yes	14	14	10	10.4	1.40	0.55–3.62
<i>Toxocara</i> seropositivity						
No	71	71	86	89.6		—
Yes	29	29	10	10.4	3.51	1.51–8.32
Eosinophilia						
No	29	29	72	75		—
Yes	71	71	24	25	7.34	3.73–14.58

CI, confidence interval; OR, odds ratio; SLR, Sri Lankan rupee.

birthweight, recurrent lower respiratory tract infections from birth, exposure to smokers at home, and presence of a parent with asthma were 5.1, 25.1, 5.4, 21.9, respectively.

Discussion

This hospital-based study determined the importance of *Toxocara* exposure in causing childhood asthma by examining the proportion of asthmatic and non-asthmatic Sri Lankan children who were *Toxocara* seropositive. *Toxocara* seropositivity was identified as a significant risk factor in the development of asthma in a univariate model but did not meet the significance level to

enter into the final multivariate model, indicating that *Toxocara* seropositivity was not an independent risk factor for developing asthma in this pediatric population in Sri Lanka. This study indicates that 29% of children who presented to the Lady Ridgeway Children's Hospital, Colombo, Sri Lanka with bronchial asthma were *Toxocara* seropositive. The overall proportion of *Toxocara* seropositivity recorded in the study population was 20%. The presence of a significantly higher eosinophilia in the non-asthmatic children who were *Toxocara* seropositive indicates that the eosinophilia is probably due to an early active disease or latent infection of toxocariasis. Similarly a high eosinophilia of 86%

was also observed in the children with asthma who were *Toxocara* seropositive.

These findings counter those of Bujis *et al.* and Chan *et al.*, who found a relationship between exposure to toxocariasis and bronchial asthma.^{7,8} The present findings, however, are similar to those reported by Sharghi *et al.* and Taylor, whose clinic-based case control studies failed to demonstrate an association between toxocariasis and asthma.^{10,13}

Previously identified risk factors of asthma such as exposure to smoking at home, lower respiratory tract infections, parents with asthma, and low birthweight remained significant risk factors in the multivariate model. Studies indicate that parental smoking in the presence of children increases the risk for asthma.^{14,15} There is also a clear association between respiratory infections early in life and the development of asthma in childhood.^{16–18} Evidence indicates that asthma is a heritable disease and a number of studies have shown an increased prevalence of asthma and the phenotype associated with asthma among the offspring of subjects with asthma compared to offspring of subjects without asthma.^{19–22}

The genetic and environmental risk factors of childhood asthma were studied in a case–control study in Sri Lanka,⁵ but toxocariasis as an environmental risk factor of asthma has not been previously investigated in Sri Lanka. *Toxocara* seropositivity was significant in univariate analysis but it was not significant in multivariate analysis. This may be due to an association between toxocariasis and other risk factors of asthma, rather than a direct association between toxocariasis and asthma. The proportion of individuals seropositive for toxocariasis does not reflect the seroprevalence in the general population because this is a hospital-based study. Further studies should be carried out in Sri Lanka, using a larger number of children with asthma, which will allow for a more in-depth investigation of the possible role of toxocariasis as a trigger for asthma.

It was important to note that of the 196 children, 20% were seropositive for *Toxocara* serology. Although the study did not find a significant relationship to bronchial asthma, toxocariasis per se is a serious threat to children. Therefore it is necessary to establish programs aimed to prevent this neglected tropical disease. We recommend control measures for canine toxocariasis by means of health education regarding preventing young children from lying in and ingesting contaminated soil, proper disposal of dog and cat feces and treatment of domestic dogs and cats with anti-helminthics.

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