Effect of deworming on atopy, asthma, allergic rhinoconjunctivitis and atopic dermatitis in Cuban children.

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Abstract

Background: Although helminth infections have been suggested to protect from atopy and atopic diseases, there is still no consensus on their relationship.

Objective: We investigated the effect of deworming and intestinal helminth (re)infections on atopy, asthma, allergic rhinoconjunctivitis and atopic dermatitis.

Methods: We examined 824 4-14 year-old children from two Cuban municipalities in six-monthly intervals for 24 months. Intestinal helminth infections were diagnosed by stool examination. Atopic diseases were defined as asthma (current wheeze), allergic rhinoconjunctivitis and atopic dermatitis by ISAAC (International Study of Asthma and Allergies in Childhood) questionnaire, asthma (bronchial hyperresponsiveness) additionally by spirometry, and atopy by skin prick testing (SPT). Helminth positive children treated with anthelminthics were compared with untreated and with helminth negative ones, respectively.

Results: After deworming the frequency of current wheeze (p=0.0006) and allergic rhinoconjunctivitis (p=0.0151) significantly decreased in one municipality. (Re)infection with A. lumbricoides and T. trichuria was positively associated with the development or retention of these two atopic diseases, while (re)infection with hookworm showed a negative association. The percentage of SPT positives on the other hand temporarily increased from 9.7% (95% CI: 5.5 - 16.6%) to 32.7% (95% CI: 24.7 - 42.9%) (p<0.0001 in one municipality) after the first treatment and subsequently returned to baseline values (11.9%, 95% CI: 6.9-19.6%). Associations between intestinal helminth (re)infections and atopy were not significant, but a trend opposite to that observed for atopic diseases was seen.

Conclusion: Our data demonstrate an improvement of atopic diseases after anthelminthic treatment and suggest that certain helminth infections, in particular Ascaris lumbricoides and Trichuris trichuria, could contribute to the development of atopic diseases. Atopy on the other hand increases after deworming and seems to be negatively associated with these helminth species. As this increase appears only temporarily, deworming of schoolchildren is unlikely to promote atopy or atopic disease.

Keywords: anthelminthic treatment, atopy, asthma, allergic rhinoconjunctivitis, atopic dermatitis

Introduction

The observation that atopic diseases are very common and helminthic infections relatively uncommon in developed countries, while the inverse is true in many developing countries, has led to the speculation that the two phenomena may be inversely associated. The idea is part of a broader hypothesis suggesting that exposure to infections in early childhood reduces the risk of developing allergies, the so-called 'hygiene hypothesis'. However, the relationship between atopic diseases and helminthic infection remains uncertain and controversial. So far, most studies on this topic have been cross sectional, and do not allow making any strong temporal associations. Prospective intervention studies are more suitable to examine the causal association between helminth infections and atopic diseases. With the latter approach, anthelminthic treatment was shown to increase atopy incidence, to increase atopy prevalence, or to decrease asthmatic symptoms in children from helminth endemic areas. Recently, Cooper et al showed that a deworming programme in Ecuadorian schoolchildren reduced helminthiases without promoting atopy or atopic diseases. No other follow up studies have been performed so far to further investigate these apparent complex interactions of helminth infections and anthelminthic treatment with atopic diseases and atopy.

In a two-year follow up study in Cuban schoolchildren, we determined the effects of anthelminthic treatment on atopy and the development of atopic diseases, and investigated the specific interrelationships between general and species-specific helminth (re)infections and atopy, asthma, allergic rhinoconjunctivitis and atopic dermatitis.

Materials and Methods

Study group and study design

The studies were performed between December 2003 and May 2006 in two Cuban municipalities, Fomento and San Juan y Martínez, both rural mountainous areas with relatively high intestinal helminth prevalences. In each municipality, a cohort of initially helminth positive children was treated and followed up at six-monthly intervals for 24 months. In San Juan y Martínez these were all helminth positive children (n=113) from five randomly selected primary schools (n=398), in Fomento all helminth positive children (n=191) from fourteen randomly selected primary schools out of all schools in the municipality (n=922). For ethical reasons we did not follow up helminth-positives without treatment. Instead, in San Juan y Martínez four groups of helminth positive children from randomly selected primary schools in the same municipality were used as untreated controls at the respective time points of follow-up (p1 after six months: n=112, p2 after 12 months: n=71, p3 after 18 months: n=75, p4 after 24 months: n=63). In Fomento, a cohort of initially helminth negative children (n=199) was randomly selected, treated if positive and followed up.

Informed written consent was obtained from the parents of each child. The study was approved by the ethical committees of the Institute of Tropical Medicine in Antwerp, Belgium, the Pedro Kouri Institute of Tropical Medicine (IPK) and the National Institute for Hygiene, Epidemiology and Microbiology (INHEM) in Havana, Cuba.

Study methods

At each time point children were submitted to a parental questionnaire, spirometry before and after exercise, skin prick testing, and faeces examination.

A parent or guardian of each child was interviewed by using an adjusted version of the ISAAC questionnaire on symptoms of atopic diseases during the previous 6 (or 12 at baseline) months. Asthma (current wheeze) was defined as an affirmative answer to the second ISAAC core asthma question on current wheeze, allergic rhinoconjunctivitis was diagnosed as defined by Strachan, and atopic dermatitis as defined by Williams (an affirmative answer to the second and third question of the ISAAC rhinitis or dermatitis questionnaire, respectively).

To demonstrate bronchial hyperresponsiveness (BHR) as another measure for asthma, spirometry was performed before and 5 and 10 minutes after exercise (6 minutes free running) as previously reported. Erreur ! Signet non défini.

Skin prick testing for atopy was performed using extracts of seven allergens (Dermatophagoides pteronyssinus, D. farinae, cat dander, mixed tree, mixed grass, Alternaria alternata, and cockroach) produced by ALK (Nieuwegein, The Netherlands). Histamine (10 mg/ml) was used as a positive and allergen diluent as a negative control. The extracts and controls were placed on the volar side of the left forearm using separate ALK lancets. Skin response was measured after 15 minutes, considering a wheal of 3 mm or larger in the absence of significant reactivity of the diluent control as a positive reaction. Atopy was defined as a positive reaction to any of the allergens tested on skin prick test.

From each child one fresh stool sample was collected, and subsequently one direct smear and two 25 mg Kato Katz examinations were performed for the detection of eggs of A. lumbricoides, T. trichuria, hookworm or E. vermicularis. Intestinal helminth infection was defined by the presence of any helminth eggs. For A. lumbricoides, T. trichuria and hookworm also species-specific effects were assessed. As Kato Katz examination is not a sensitive method for the detection of E. vermicularis, we did not assess the species-specific effects of this particular helminth. At each time point of follow up each helminth positive child received one dose of Mebendazole (500 mg).

All statistical analyses were performed with Stata 8.0 (Statacorp LP, College Station TX, USA), SAS 9.1 (SAS Institute Inc., Cary NC, USA), and R 2.2 (R Foundation for Statistical Computing, Vienna, Austria). Confidence intervals were calculated using Wilson's score method for disease and infection prevalences.

For the two helminth positive and one helminth negative cohort time trends were assessed by repeated measures logistic regression (using Generalized Estimating Equations) for proportions; the time trend within the untreated control groups in San Juan y Martínez was assessed using simple logistic regression. First, the overall time effect was tested in the respective regression models. If a significant time effect ($p<0.05$) was observed, the individual timepoints were compared with the baseline visit. In San Juan y Martínez the first visit for the cohort was used as baseline for both the cohort and the untreated control groups.

For both helminth positive cohorts after treatment, a first-order transition logistic regression model was used to assess the effect of intestinal helminth infection (i.e. after treatment failure or helminth reinfection) on atopic conditions. In this model, the presence or absence of the atopic disease or atopy (outcome variable) was modeled based on the outcome at the previous time point, the helminth infection status (yes/no) or helminth infection by species (A. lumbricoides, T. trichuria, hookworm) at the current time point and additional covariates: time point (p1 to p4), municipality (Fomento vs San Juan y Martínez), municipality-by-time point interaction, area (rural versus urban, as defined by population size and infrastructural facilities in the area where the schools are located), gender and age. Models were fitted for each municipality and for the two municipalities combined (i.e. pooled analysis). The coefficients of the model are presented as odds-ratio's for the development of an outcome or 1/odds-ratio for the disappearance of an outcome.

Results

In total 824 4-14 year old children were included in the study; 447 boys (54%) and 377 girls (46%). 89% (101/113) of the children in the cohort from San Juan y Martinez, and 92% (176/191) and 95% (190/199) of those in Fomento, still attended the study after 2 years. Losses of follow-up were mainly due to migration to another municipality (n=34).

Treatment reduced the prevalence of intestinal helminth infections in both municipalities (p≤0.0010) over 24 months. In San Juan y Martínez, the percentage of infection in the helminth positive children decreased from 46% to 5% for A. lumbricoides, from 45% to 2% for T. trichuria, from 25% to 11% for hookworm; in Fomento A. lumbricoides from 13% to 2%, T. trichuria from 45% to 15%, and hookworm from 48% to 10%.

In San Juan y Martínez, a significant decrease over time was observed in the percentage of children with wheeze for the treated helminth positive cohort (p=0.0006), but not for the untreated control groups (Figure 1). This trend was observed across genders, age classes and rural and urban schools. Also in Fomento current wheeze declined after treatment, but this effect was less clear and not statistically significant. The transition logistic regression model for current wheeze indicated that children who were helminth free after treatment were less likely to develop or retain current wheeze than children who remained infected or were reinfected (p=0.0080 in the pooled analysis, p=0.0334 in San Juan y Martínez, p=0.1226 in Fomento). The odds-ratio for the development or retention of current wheeze in helminth infected compared to helminth free children was 1.78 (95% CI: 1.16 - 2.72). Previous outcome was significantly related to current outcome (p<0.001); other covariates did not show a significant effect on current wheeze. No significant interaction between helminth infection status and previous outcome was observed. The analysis by helminth species indicated that for A. lumbricoides and T. trichuria, but not for hookworm, children who remained infected or were (re)infected after treatment were more likely to develop or retain wheeze than children who cleared their infection (Table 1).

After anthelminthic treatment, a reduction in the frequency of exercise induced bronchial hyperresponsiveness (BHR) was observed in San Juan y Martínez, but not in Fomento. However, this time trend was not significant.

Like for current wheeze, a significant reduction over time was observed in the percentage of children with allergic rhinoconjunctivitis after anthelminth treatment (p=0.0151), but not for the untreated ones. However, this time trend was only seen in San Juan y Martinez and was less clear than for current wheeze. The transition model showed significant differences in the development or retention of allergic rhinoconjunctivitis between children who had cleared their infection and those that remained infected or were reinfected with A. lumbricoides after treatment. (Re)infection with hookworm on the other hand was negatively associated with symptoms of allergic rhinoconjunctivitis (Table 1). The percentage of children with atopic dermatitis was not significantly decreased after deworming in either municipality.

Also for atopy a significant change over time was observed for the treated helminth positive cohort (p<0.0001) but not for the untreated control groups in San Juan y Martinez; the percentage of skin prick test positives temporarily increased after the first treatment and then gradually returned to baseline level (Figure 2). This trend was not observed in Fomento. Although not significant, the transition logistic regression model for atopy indicated a trend opposite to that observed for atopic diseases; children who remained infected or were reinfected with A. lumbricoides or T. trichuria after treatment were less likely to show a positive skin prick test than children who cleared their infection, whereas hookworm was positively associated with the development or retention of atopy (Table 1).

Discussion

Despite a body of suggestive observations, an inverse association between helminth infections and atopic diseases has so far not been conclusively established.[3] Prospective intervention studies that could show whether helminth infections, or anthelminthic treatment, promote or suppress atopy and atopic diseases are scarce[6,7,8,9,10] and results varying. Depending on the study, deworming resulted either in an increase of atopy incidence in Gabon,6 an increase of atopy prevalence in Venezuela,[7] a decrease of asthmatic symptoms in Venezuela,[8 9] or no effect on atopy or atopic diseases in Ecuador.[10] Our study indicates that these contradictory results may not be due to country differences or methodological variations, but that different associations of helminth (re)infections and anthelminthic treatment with atopy and atopic diseases can indeed coexist.

Some potential limitations exist with respect to the interpretation of the study findings. For ethical reasons, we could not include a 'control cohort' with untreated helminth-positive children. However, by using the alternative of four consecutive groups of helminth positive children, which are each representative of the primary school children in the same municipality, a longitudinal study has been approximated as far as possible. Our atopic disease data are mainly based on ISAAC questionnaires. Although this has become the standard diagnostic method in childhood epidemiology of atopic diseases worldwide, questionnaires have important inherent limitations which should be kept in mind when interpreting the data. Erreur ! Signet non défini. Due to the non-experimental design of our study, it is not possible to exclude confounding effects on the relation between intestinal helminth (re)infection and atopy or atopic diseases. We corrected for apparent potential confounders, but (re)infection with helminths as well as the development or retention of atopic diseases or atopy may be affected by unknown or unmeasured factors.

In our study population we found current wheeze to improve significantly after deworming. A similar trend was observed in exercise-induced bronchial hyperresponsiveness, but not significant due to the very small percentage (3%) of children with an abnormal spirometry. Children who were helminth free after treatment were less likely to develop or retain current wheeze than children who remained infected or were reinfected. This effect appeared to be helminth species-specific and was only significant for A. lumbricoides and T. trichuria, not for hookworm.

A positive association between A. lumbricoides and asthma was recently confirmed in a meta-analysis of comparative epidemiological studies on this topic. Our findings were also supported by the results of a study of the effects of deworming on asthma by Lynch et al, who observed an improvement in clinical asthma and peak flow responses after anthelminthic treatment of children mainly infected with A. lumbricoides and T. trichiura.[8 9] Cooper et al on the other hand found that anthelminthic treatment had no effect on asthma in children with intestinal helminth infections, among which A. lumbricoides, T. trichuria, hookworm and S. stercoralis.[10] They did not analyse species specific effects, however. As for current wheeze, they did not observe any effects on atopic dermatitis and allergic rhinoconjunctivitis. In our study population, deworming did not significantly change the occurrence of atopic dermatitis either, but in one municipality a significant decrease of allergic rhinoconjunctivitis was found. Differences in the effect of deworming on current wheeze and allergic rhinoconjunctivitis were also found within our own study, with much stronger effects observed in San Juan y Martinez as compared to Fomento. Helminth species-specific effects on atopic diseases could have played a role in the latter observation as well. A. lumbricoides was considerably more prevalent in San Juan y Martínez and was associated with an increase of wheeze and allergic rhinoconjunctivitis, whereas hookworm which was more prevalent in Fomento tended to protect from atopic diseases (Table 1). Helminth species-specific effects on atopic diseases have been reported before.

In our study population, prevalences of atopy significantly increased 6 months after deworming, followed by a gradual decrease over time to baseline level. Again, helminth species specific trends were seen, but less clear and opposite to those found for current wheeze. A transient effect of deworming on atopy was also reported by Van den Biggelaar and colleagues in a 30-months follow up study in Gabonese children.[6] The use of different methodologies (e.g. incidence vs prevalence) does not allow further detailed comparison, however.

The atopic prevalence peak that we observed after 6 months, might be due to an early strong reaction of the immune system shortly after treatment, which may have been missed by others, who did not monitor effects before 12 months after treatment.[7 10] Possibly, this reaction happened even earlier and we only measured the aftermath at 6 months.

The subsequent gradual decrease in atopy over time as observed in our study might have been the consequence of repeated treatments. We have no explanation for the underlying mechanisms, however. Reinfection is likely in our study population due to the large treatment intervals, and may also account for the decrease of skin prick test reactivity in between two treatment periods.[6]

The overall effect of deworming on atopy in Gabonese children was seen mostly after the first year of follow up.[6] Lynch et al measured an increase of positive skin prick test prevalence at 22 months after treatment in Venezuelan children.[7] Cooper on the other hand did not find any increase in atopic reactivity at 12 months after treatment in Ecuador. Lau & Matricardi therefore put forward that the study done by Cooper et al had been too short to record variations.[5] Their suggestion is however not supported by our findings, however, as we observed atopic prevalences in Cuban children to be significantly different from baseline levels both at 6 and 12 months after treatment (Figure 2). We propose that the effect of deworming on atopy should not necessarily be monitored longer but rather at much shorter time intervals to allow recording of apparent early and transient variations.

Intensity and chronicity of helminth infections have been described to be an important determinant of the effect of helminth infection on atopic diseases and atopy, with high intensities and chronic infections protecting from allergic symptoms.[5] Cooper et al[10] did not find an effect of deworming on atopy or atopic diseases which was suggested to be due to the relatively low intensity and chronicity of helminth infections in his study population as compared to others.[5] In our study population, however, helminth intensities were much lower (data not shown) while we observed a significant increase in skin prick test reactivity after anthelminthic treatment. In addition, differences in intensity and chronicity could not explain the simultaneous decrease in current wheeze, as both results are based on data from the same study population.

In conclusion, our results demonstrate an improvement of atopic diseases after treatment of helminth infections and suggest a positive association of certain helminths – in particular A. lumbricoides and T. trichuria - with atopic symptoms. Atopy on the other hand increases after deworming and seems to be negatively associated with these helminth species. As the increase in skin prick test reactivity after anthelminthic treatment appears to be temporal, deworming does not seem to be a risk factor for the development of atopy, nor for atopic diseases.

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Conflict of interest statement

We declare that we have no conflict of interest.

Table 1

Frequencies of atopy/atopic disease by helminth infection status and results from transition logistic regression model for atopy/atopic disease by helminth infection.

	Frequencies of atopy/atopic disease		Odds-Ratio†		
Helminth species	in non-infected children‡	in infected children‡	Estimate	95% Confidence Interval	P-value
Current wheeze					
A. lumbricoides	13.0%	27.5%	2.59	(1.28, 5.16)	0.0087
T. trichuria	12.8%	22.6%	2.33	(1.35, 3.98)	0.0028
hookworm	14.8%	10.2%	0.75	(0.39, 1.37)	0.3523
Skin Prick Test					
A. lumbricoides	22.6%	16.3%	0.70	(0.34, 1.32)	0.2764
T. trichuria	22.1%	22.5%	0.93	(0.58, 1.44)	0.7427
hookworm	21.1%	27.5%	1.38	(0.92, 2.06)	0.1203
Allergic Rhinoconjunctivitis					
A. lumbricoides	8.6%	15.0%	2.76	(1.23, 5.89)	0.0149
T. trichuria	9.0%	10.2%	1.12	(0.58, 2.06)	0.7185
hookworm	9.9%	5.1%	0.49	(0.22, 0.98)	0.0420
Atopic Dermatitis					
lumbricoides	7.1%	12.5%	0.93	(0.40, 2.00)	0.8639
T. trichuria	7.0%	11.0%	1.76	(0.90, 3.27)	0.0943
hookworm	8.1%	4.5%	0.79	(0.33, 1.69)	0.5547

† Odds-ratio of developing atopy/atopic disease (for children who did not have atopy/atopic disease at previous visit) or retaining atopy/atopic disease (for children who had atopy/atopic disease at previous visit) in helminth infected versus not helminth infected children. Odds-ratio, 95% confidence interval, and p-value obtained from transition logistic regression model with adjustment for time, municipality, time-by-municipality interaction, age, sex, and rural/urban location of the school.

‡ After anthelminthic treatment, i.e. helminth-free children versus children with remaining infection or reinfection.

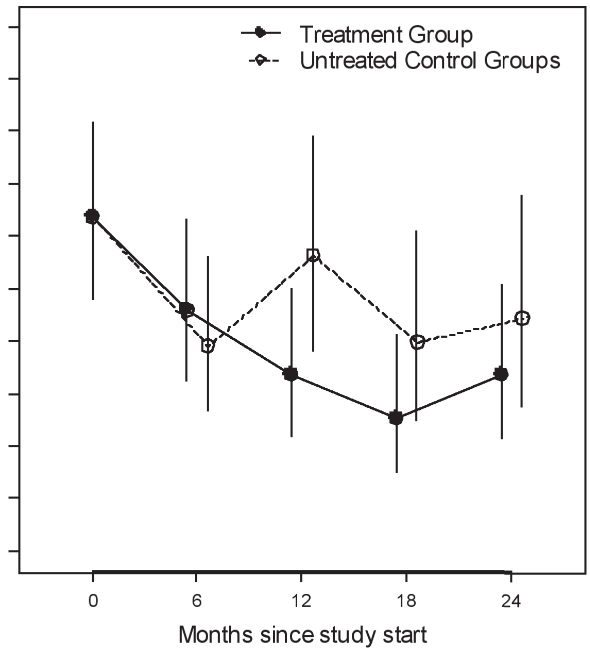

Figure 1: Percentages (95% confidence interval) of children with current wheeze in treated and untreated helminth-positive children in San Juan y Martínez. The percentage of children with wheeze in the treated group decreased over time from 31.9% (95% CI: 24.0 - 40.9%) at the baseline visit to 23.0% (16.2 - 31.6%) at 6 months (borderline significant, p=0.0661), 16.8% (10.9 - 25.0%) at 12 months (p=0.0010), 12.7% (7.6 - 20.6%) at 18 months (p<0.0001), and 16.8% (10.8 -25.3%) at 24 months (p=0.0023) as compared to p0.

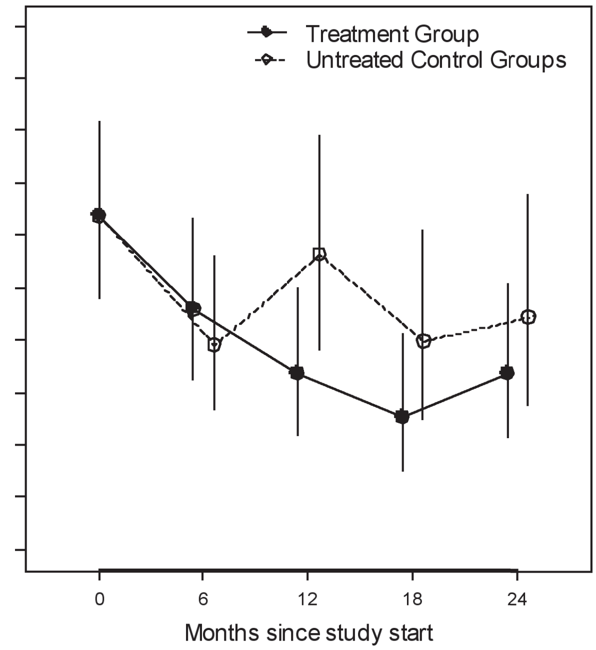

Figure 2: Percentages (95% confidence interval) of skin prick test positive children in treated and untreated helminth-positive children in San Juan y Martínez. The percentage of skin prick test positives increased from 9.7% (95% CI: 5.5 - 16.6%) to 32.7% (CI: 24.7 - 42.9%) after the first treatment (p<0.0001) and then returned to baseline levels over time. At 12 months, the percentage of skin prick test positives (23.4%; CI:16.4 - 32.2%) was still significantly higher than at baseline (p=0.0004); at 18 months 18.4% (CI: 12.1 - 27.0%) the difference was bordeline significant (p=0.0540) and at 24 months 11.9% (CI: 6.9 - 19.6%) there was no difference with baseline (p=0.5465).

References

1 Weiss ST. Parasites and asthma/allergy: what is the relationship? J Allergy Clin Immunol 2000; 105:205-10.

2 Strachan DP. Hay fever, hygiene, and household size. BMJ 1989; 299:1259-60

3 Palmer LJ, Celedon JC, Weiss ST, Wang B, Fang Z, Xu X. Ascaris lumbricoides infection is associated with increased risk of childhood asthma and atopy in rural China. Am J Respir Crit Care Med 2002; 165:1489-93

4 Schafer T, Meyer T, Ring J, Wichmann HE, Heinrich J. Worm infestation and the negative association with eczema (atopic/nonatopic) and allergic sensitization. Allergy 2005; 60:1014-20

5 Lau S, Matricardi PM. Worms, asthma, and the hygiene hypothesis. Lancet 2006; 367:1556-8.

6 Van den Biggelaar AH, Rodrigues LC, van Ree R, van der Zee JS, Hoeksma-Kruize YC, Souverijn JH et al. Long-term treatment of intestinal helminths increases mite skin-test reactivity in Gabonese schoolchildren. J Infect Dis 2004;189: 892-900

7 Lynch NR, Hagel I, Perez M, Di Prisco MC, Lopez R, Alvarez N. Effect of anthelmintic treatment on the allergic reactivity of children in a tropical slum. J Allergy Clin Immunol 1993; 92: 404-11

8 Lynch NR, Palenque M, Hagel I, DiPrisco MC. Clinical improvement of asthma after anthelminthic treatment in a tropical situation.Am J Respir Crit Care Med 1997; 56: 50-4

9 Lynch NR, Hagel I, Perez M, Di Prisco M, Alvarez N, Rojas E. Bronchoconstriction in helminthic infection. Int Arch Allergy Immunol 1992; 98: 77-9

10 Cooper PJ, Chico ME, Vaca MG, Moncayo AL, Bland JM, Mafla E et al. Effect of albendazole treatments on the prevalence of atopy in children living in communities endemic for geohelminth parasites: a cluster-randomised trial. Lancet 2006; 367:1598-1603

11 Wordemann M, Polman K, Menocal Heredia LT, Junco Diaz R, Collado Madurga AM, Nunez Fernandez F et al. Prevalences and risk factors of intestinal parasites in Cuban children. Trop.Med.Int.Health 2006; 11:1813-20

12 Wordemann M, Polman K, Diaz RJ, Menocal Heredia LT, Madurga AM, Sague KA et al. The challenge of diagnosing atopic diseases: outcomes in Cuban children depend on definition and methodology. Allergy 2006; 61:1125-31

13 Asher MI, Keil U, Anderson HR, Beasley R, Crane J, Martinez F et al. International Study of Asthma and Allergies in Childhood (ISAAC): rationale and methods. Eur Respir J 1995; 8:483-91

14 The ISAAC Steering Committee. Wordlwide variation in prevalence of symptoms of asthma, allergic rhinicinjunctivitis, and atopic eczema: ISAAC. The Lancet 1998; 351, 1225-32

15 Strachan D, Sibbald B, Weiland S, Ait-Khaled N, Anabwani G, Anderson HR et al. Worldwide variations in prevalence of symptoms of allergic rhinoconjunctivitis in children: the International Study of Asthma and Allergies in Childhood (ISAAC). Pediatr Allergy Immunol 1997; 8 161-76

16 Williams H, Robertson C, Stewart A, Ait-Khaled N, Anabwani G, Anderson R et al. Worldwide variations in the prevalence of symptoms of atopic eczema in the International Study of Asthma and Allergies in Childhood. J Allergy Clin Immunol 1999; 103:125-38

17 Katz N, Chaves A, Pellegrino J. A simple device for quantitative stoll thick-smear technique in schistosomiasis mansoni. Rev Med Trop Sao Paolo 1972; 14: 397-400

18 Tsibouris P, Galeas T, Moussia M, Sotiropoulou M, Michopoulos S, Kralios N. Two cases of eosinophilic gastroenteritis and malabsorption due to Enterobious vermicularis. Dig Dis Sci 2005; 50:2389-92

19 Renganathan E, Ercole E, Albonico M, De Gregorio G, Alawi KS, Kisumku UM et al. Evolution of operational research studies and development of a national control strategy against intestinal helminths in Pemba island, 1988-92. Bull.World Health Organ 1995; 73: 183-90

20 Newcombe RG. Two-sided confidence intervals for the single proportion: comparison of seven methods. Statistics in Medicine 1998; 17: 857-72

21 Comite Estatal de Estadisticas. Censo de poblacion y viviendas 1981;15: 35. La Habana 1983

22 Molenberghs G, Verbeke G. Models for discrete longitudinal data. Page 683 Springer New York 2005

23 LindenSmith J, Morrison D, Deveau C, Hernandez P. Overdiagnosis of asthma in the community. Can Respir J 2004; 11:111-16

24 Leonardi-Bee J, Pritchard D, Britton J. Asthma and Current Intestinal Parasite Infection: Systematic Review and Meta-Analysis. Am J Respir Crit Care Med 2006;174 : 514-23

25 Scrivener S, Yemaneberhan H, Zebenigus M, Tilahun D, Girma S, Ali S et al. Independent effects of intestinal parasite infection and domestic allergen exposure on risk of wheeze in Ethiopia: a nested case-control study. Lancet 2001; 358:1493-9

26 Dagoye D, Bekele Z, Woldemichael K, Nida H, Yimam M, Hall A et al. Wheezing, allergy, and parasite infection in children in urban and rural Ethiopia. Am J Respir Crit Care Med 2003; 167:1369-73