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## **Allergy, soil-transmitted helminths and their association**

van der Werff, S.D.

2016

### **document version**

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### **citation for published version (APA)**

van der Werff, S. D. (2016). *Allergy, soil-transmitted helminths and their association: Epidemiological studies in Cuban schoolchildren*. [PhD-Thesis - Research and graduation internal, Vrije Universiteit Amsterdam].

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## Abstract

**Background:** Soil-transmitted helminth (STH) infections have been suggested to protect from allergic sensitization and atopic diseases. Consequently, anthelmintic treatment would increase the prevalence of atopic disease in STH endemic populations.

**Objective:** To investigate the effect of deworming on allergic sensitization and atopic diseases in Cuban schoolchildren.

**Methods:** We followed up 108 STH positive schoolchildren aged 5-13 in six-monthly intervals for 24 months. Four consecutive groups of, respectively, 104, 56, 68, and 53 STH positive children were used as 'untreated' reference groups to assess general time trends. STH infections were diagnosed by stool examination. Asthma, allergic rhinoconjunctivitis, and atopic dermatitis were diagnosed by International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire and allergic sensitization by skin prick testing (SPT). At each time point, STH positive children were treated with one single dose of 500 mg mebendazole.

**Results:** After deworming, the frequency of asthma significantly decreased ( $P < 0.001$ ) while the frequency of allergic rhinoconjunctivitis and atopic dermatitis was not affected ( $P = 0.129$  and  $P = 0.751$ , respectively). The percentage of SPT positives temporarily increased ( $P < 0.001$ ) and subsequently returned to nearly baseline values ( $P = 0.093$ ). In the reference groups, no change over time was observed in the proportion of children with allergic sensitization and atopic diseases ( $P > 0.05$ ).

**Conclusion & Clinical Relevance:** Our results indicate that atopic diseases do not increase after anthelmintic treatment. Allergic sensitization on the other hand increases after deworming. As this increase appears only temporarily, deworming of schoolchildren does not seem to be a risk factor for the development of allergic sensitization, nor for atopic diseases.

## Introduction

Atopic diseases and soil-transmitted helminth (STH) infections are two important childhood health problems world-wide (1, 2). The observation that atopic diseases are very common and STH infections relatively uncommon in affluent and urbanized populations while the opposite is true in populations of developing countries and rural areas (2-4), has led to the speculation that the two phenomena may be inversely associated (4). 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' (5, 6). However, the relationship between atopic diseases and STH infection remains uncertain and controversial (7, 8). Yet if the hypothesis is true, periodic anthelmintic treatment, as has been endorsed by the WHO (9), may increase the prevalence of atopic disease in STH endemic populations.

So far, most studies on the relationship between STH infection and atopic diseases have been cross-sectional, and do not allow making strong temporal associations. Prospective intervention studies are more suitable to examine a causal association between STH infections and atopic diseases (10). With the latter approach only few studies have been performed and results vary. Anthelmintic treatment was shown to increase (11-14), decrease (15) or have no effect (13, 14, 16) on allergic sensitization and atopic diseases.

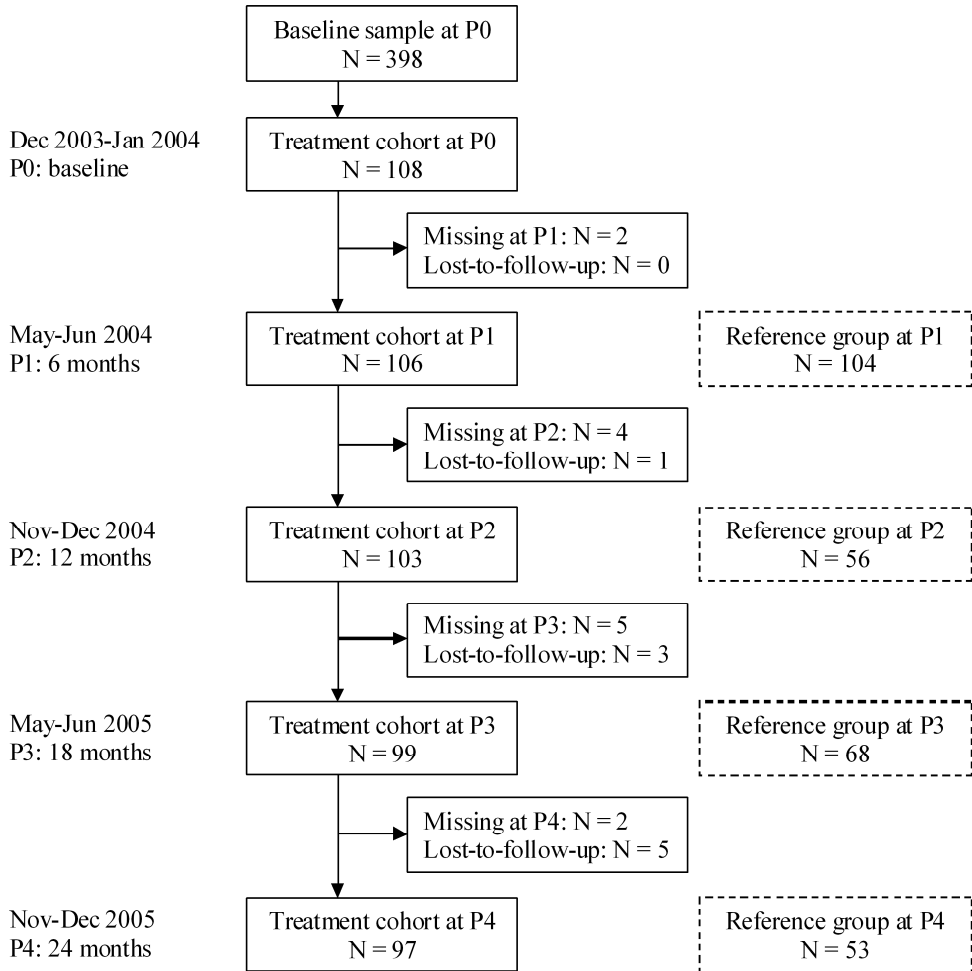
Here, we further investigate the apparent complex interactions of STH infections and anthelmintic treatment with atopic diseases and allergic sensitization. In a two-year follow up study in schoolchildren from Cuba, where helminth infections and asthma are prevalent, we determined the effects of anthelmintic treatment on allergic sensitization, asthma, allergic rhinoconjunctivitis, and atopic dermatitis.

## Methods

### *Study design and population*

A longitudinal study was performed between December 2003 and December 2005 in primary schoolchildren in San Juan y Martínez (SJM), a municipality in the west of Cuba with relatively high STH prevalences (17). From five randomly selected primary schools ( $N_{\text{children}}=398$ ) (18), all STH positive children ( $N=108$ ) were included in the study (measurement P0). This cohort was treated and followed up at six-monthly intervals for 24 months (measurements P1-P4). For ethical reasons inclusion of an STH positive control cohort without treatment was not possible. Alternatively, four consecutive groups of STH positive children from randomly selected primary schools

in the same municipality were used as reference groups to assess 'general time trends' in STH infection, allergic sensitization, and atopic diseases ( $N_{p1}$  after 6 months=104,  $N_{p2}$  after 12 months=56,  $N_{p3}$  after 18 months=68,  $N_{p4}$  after 24 months=53). The study outline is shown in Figure 1.



**Figure 1.** Outline of study

Informed written consent was obtained from the parents or guardians of each participating child. The study was approved by the Ethical Committees of the Institute of Tropical Medicine (ITM) in Antwerp, Belgium, the National Institute for Hygiene, Epidemiology and Microbiology (INHEM) and the Pedro Kourí Institute (IPK) of Tropical Medicine in Havana, Cuba.

### ***Infection and treatment***

From each child one fresh stool sample was collected and used for one direct smear and two 25 mg Kato Katz examinations (19, 20). Infection with STHs, i.e. *Ascaris lumbricoides*, *Trichuris trichiura*, and hookworm, was defined as the presence of species-specific eggs detected by either of the two methods. At each measurement period STH positive children received under supervision of a doctor or nurse one single dose of mebendazole (500 mg). Percentages of STH positive children having a light, moderate or heavy intensity infection were defined according to the WHO classification (21).

### ***Atopic diseases and allergic sensitization***

Atopic disease occurrence, i.e. asthma, allergic rhinoconjunctivitis, and atopic dermatitis, was determined by means of the standard Spanish version of the International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire (22), whereby a parent or guardian of each child was interviewed by a trained local team member. ISAAC definitions of atopic diseases were used: current asthma or wheeze, shortened to 'asthma' throughout the text, was defined as an affirmative answer to the second ISAAC core question on current wheeze (23); allergic rhinoconjunctivitis was defined as an affirmative answer to the second and third core questions of the ISAAC modules on rhinitis (24); and atopic dermatitis was defined as an affirmative answer to the second and third core questions of the ISAAC modules on eczema (25).

Skin prick testing for allergic sensitization (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 to the diluent control as a positive reaction. Allergic sensitization was defined as a positive reaction to at least one of the seven applied allergens in the presence of a positive reaction to histamine.

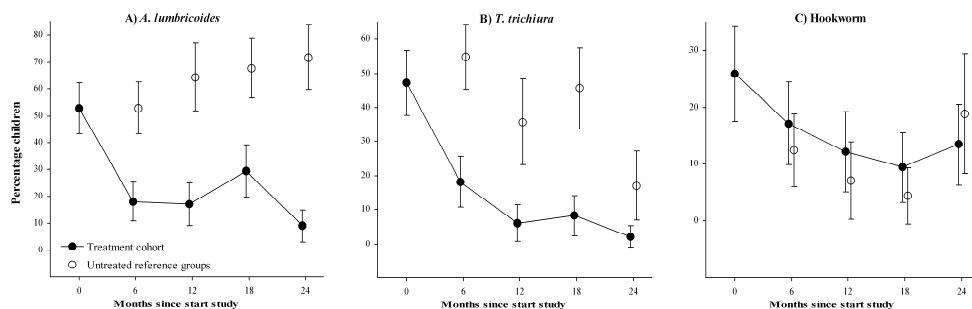
## Statistical analysis

All statistical analyses were performed with SPSS Statistics 17.0 for Windows (SPSS Inc., Chicago, IL, USA) and a  $P$ -value of  $\leq 0.05$  was considered as statistically significant. Within the treatment cohort the time trends for STH infections, atopic diseases, and allergic sensitization after treatment were assessed by logistic Generalized Estimating Equations (GEE) analysis with an exchangeable correlation structure. Time trends within the reference groups were assessed using simple logistic regression.

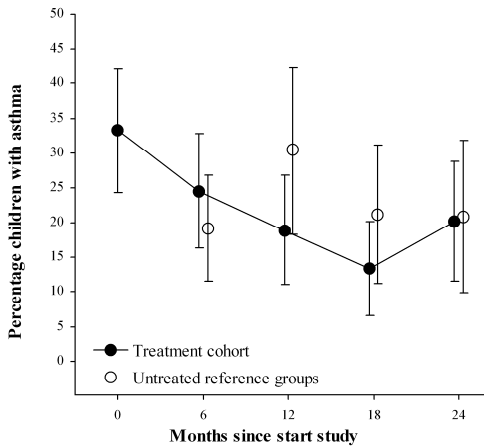
## Results

In total 108 children aged 5-13 years (mean: 8 years) were included in the treatment cohort; 61 boys (56.5%) and 47 girls (43.5%). Ninety percent (97/108) of the children still attended the study after two years. Losses of follow-up were mainly due to migration to another municipality.

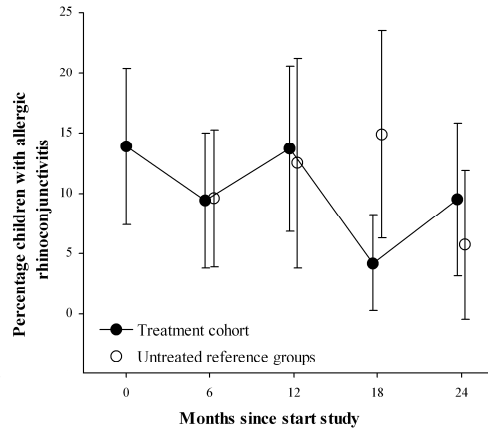
Deworming significantly reduced the prevalence of STH infections over 24 months. The percentage of infection decreased from 52.8% to 9.0% for *A. lumbricoides* ( $P < 0.001$ ), from 47.2% to 2.2% for *T. trichiura* ( $P < 0.001$ ), and from 25.9% to 13.5% for hookworm ( $P = 0.002$ ) (Figure 2). At all measurement points more than 94% had a low intensity infection for *T. trichiura* and hookworm and more than 63% for *A. lumbricoides*.



**Figure 2.** Percentages (95% confidence interval) of children with *A. lumbricoides* (A), *T. trichiura* (B), and hookworm (C) in treatment cohort and untreated reference groups in San Juan y Martínez (Absolute numbers of children infected with *A. lumbricoides*/*T. trichiura*/hookworm in the treatment cohort were 57/51/28 per 108 children at P0, 19/19/18 per 105 children at P1, 14/5/10 per 82 children at P2, 25/7/8 per 85 children at P3, and 8/2/12 per 89 children at P4; in the untreated reference groups these numbers were 55/57/13 per 104 children at P1, 36/20/4 per 56 children at P2, 46/31/3 per 68 children at P3, and 38/9/10 per 53 children at P4)



**Figure 3.** Percentages (95% confidence interval) of children with asthma in treatment cohort and untreated reference groups of STH-positive children in San Juan y Martínez (Absolute numbers of children with asthma in the treatment cohort were 36/108 at P0, 26/106 at P1, 18/95 at P2, 13/97 at P3, and 17/84 at P4; in the untreated reference groups these numbers were 20/104 at P1, 17/56 at P2, 14/68 at P3, and 11/53 at P4)

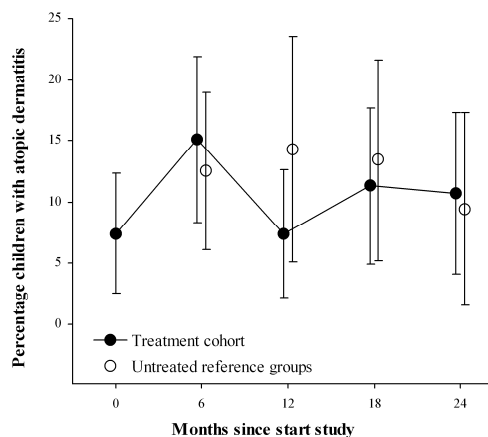


**Figure 4.** Percentages (95% confidence interval) of children with allergic rhinoconjunctivitis in treatment cohort and untreated reference groups of STH-positive children in San Juan y Martínez (Absolute numbers of children with allergic rhinoconjunctivitis in the treatment cohort were 15/108 at P0, 10/106 at P1, 13/95 at P2, 4/97 at P3, and 8/84 at P4; in the untreated reference groups these numbers were 10/104 at P1, 7/56 at P2, 10/68 at P3, and 3/53 at P4)

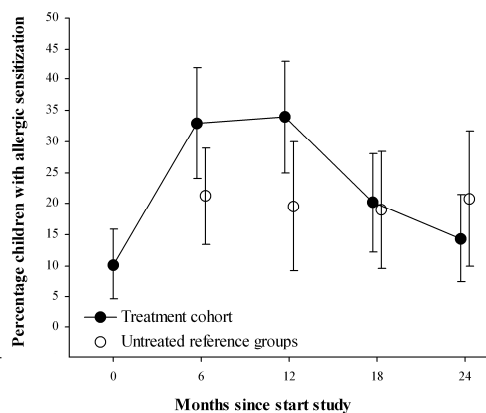
After deworming, a significant decrease over time was observed in the proportion of children with asthma ( $P < 0.001$ ), but not for allergic rhinoconjunctivitis and atopic dermatitis ( $P = 0.129$  and  $P = 0.751$ , respectively) (Figure 3-5). For allergic sensitization a significant change over time ( $P < 0.001$ ) was observed after deworming. The percentage of skin prick test positives temporarily increased after the first and second treatment ( $P < 0.001$ ) and then gradually returned to nearly baseline level ( $P = 0.093$ ) (Figure 6).

The four reference groups together comprised 281 children aged 4-13 (mean: 8 years) of which 143 boys (50.9%) and 138 girls (49.1%). In the reference groups the percentage of infection increased for *A. lumbricoides* ( $P = 0.003$ ), and decreased for *T. trichiura* ( $P = 0.001$ ) and hookworm ( $P = 0.015$ ) (Figure 2). At all measurement points more than 75% had a low intensity infection for *T. trichiura* and hookworm and more than 70% for *A. lumbricoides*. Prevalences of asthma, allergic rhinoconjunctivitis, atopic dermatitis, or allergic sensitization did not change over time in the untreated reference groups ( $P = 0.123$ ,  $P = 0.931$ ,  $P = 0.468$ , and  $P = 0.162$ , respectively) (Figure 3-6).





**Figure 5.** Percentages (95% confidence interval) of children with atopic dermatitis in treatment cohort and untreated reference groups of STH-positive children in San Juan y Martínez (Absolute numbers of children with atopic dermatitis in the treatment cohort were 8/108 at P0, 16/106 at P1, 7/95 at P2, 11/97 at P3, and 9/84 at P4; in the untreated reference groups these numbers were 13/104 at P1, 8/56 at P2, 9/68 at P3, and 5/53 at P4)



**Figure 6.** Percentages (95% confidence interval) of skin prick test positive children in treatment cohort and untreated reference groups of STH-positive children in San Juan y Martínez (Absolute numbers of children with allergic sensitization in the treatment cohort were 11/108 at P0, 35/106 at P1, 35/95 at P2, 20/97 at P3, and 14/84 at P4; in the untreated reference groups these numbers were 22/104 at P1, 11/56 at P2, 13/68 at P3, and 11/53 at P4)

## Discussion

Despite a body of suggestive observations, an inverse association between STH infections and atopic diseases has so far not been conclusively established (7, 8). Prospective intervention studies that could show whether STH infections, or anthelmintic treatment, promote or suppress allergic sensitization and atopic diseases are scarce and results vary between countries (11-16). The present study shows that in one population deworming promotes allergic sensitization, suppresses asthma and does not affect allergic rhinoconjunctivitis and atopic dermatitis. This indicates that contradictory results may not be due to country differences or methodological variations, but that different associations of anthelmintic treatment with allergic sensitization and atopic diseases can indeed coexist.

Some limitations exist with respect to the interpretation of the study findings. For ethical reasons, we could not include a 'control cohort' with untreated STH positive children. However, by using the alternative of four reference groups of STH positive children, which are each representative of the primary schoolchildren in the same municipality, we were able to assess general time trends in STH infection, allergic sensitization, and atopic disease. Nevertheless, we cannot totally exclude any independent effects over time in the treatment cohort. Our atopic disease data are based on the ISAAC questionnaire. Although this has become the standard diagnostic method in childhood epidemiology of atopic diseases world-wide, questionnaires have important inherent limitations which should be kept in mind when interpreting the data (22). Also, it cannot be excluded that the reported wheeze diagnosed by ISAAC questionnaire is due to parasite infection, e.g. Loeffler's syndrome related to parasite larvae migrating through the lung, rather than asthma.

Anthelmintic treatment led to significantly lower percentages of STH infections, with highest prevalence reduction rates for *T. trichiura*. Unexpectedly, we also found significant time trends in the reference groups, for which we do not have a straightforward explanation. Nevertheless, these time trends were clearly different from those in the treatment cohort, except for hookworm, where the decrease in the proportion of treated children was almost similar to that of the reference groups. This might be due to the fact that mebendazole is less effective against hookworm as recently shown in a meta-analysis (26).

Deworming did not result in an increase of any of the atopic diseases. Asthma prevalences even significantly decreased over time, a trend which was not observed in the reference groups. Lynch *et al.* (15) also observed an improvement in clinical asthma after anthelmintic treatment, while others found that anthelmintic treatment had no effect on asthma (13, 14, 16). The latter studies also supported our findings regarding allergic rhinoconjunctivitis and/or atopic dermatitis. Overall, we can conclude that anthelmintic treatment does not seem to be a risk factor for the development of atopic diseases.

In our study population, the prevalence of allergic sensitization increased 6-12 months after deworming, followed by a gradual decrease over time to nearly baseline level. This trend was not seen in the reference groups. A transient effect of deworming on allergic sensitization was also reported by Van den Biggelaar and colleagues in a 30-month follow-up study in Gabonese children (12). However, the use of different methodologies (e.g. incidence vs. prevalence) does not allow further detailed comparison.

The atopic prevalence peak that we observed after 6 and 12 months might be due to an early strong reaction of the immune system to the clearance of STH infections shortly after treatment (13), which may have been missed by others, who did not monitor effects before 12 months after treatment (11, 16). The subsequent gradual decrease in allergic sensitization over time as observed in our study might have been the consequence of repeated treatments. Repeated treatment and subsequent reinfection (i.e. repeated exposure to worm antigens) may have an effect similar to allergen immunotherapy (i.e. repeated exposure to small amounts of allergens) and thus have the ability to suppress the immune response against allergies, e.g. by inducing IL-10 producing regulatory cells (12, 27, 28). This hypothesis merits further investigation.

Endara *et al.* (14) observed more allergic sensitization in communities that had been treated for 15-17 years as compared to non-treated communities. Van den Biggelaar *et al.* (12) and Flohr *et al.* (13) observed an increase in skin sensitization after deworming after one year of follow-up. Lynch *et al.* (11) measured an increase of positive skin prick test prevalence at 22 months after treatment in Venezuelan children. Cooper *et al.* (16) on the other hand did not find any increase in atopic reactivity 12 months after treatment in Ecuador. Lau & Matricardi (10) therefore put forward that the study done by Cooper *et al.* (16) had been too short to record variations. However, their suggestion is not supported by our findings as we observed atopic prevalences in Cuban children to be significantly different from baseline levels at 6 and 12 months after treatment. We propose that the effect of deworming on allergic sensitization should not only be monitored longer but also at much shorter time intervals to allow recording of apparent early and transient variations.

Intensity and chronicity of STH infections have been described to be important determinants of the effect of STH infection on atopic diseases and allergic sensitization, with high intensities and chronic infections protecting from allergic symptoms (10). Cooper *et al.* (16) did not find an effect of deworming on allergic sensitization or atopic diseases which was suggested to be due to the relatively low intensity and chronicity of STH infections in his study population as compared to others (10). However, in our study population STH intensities were much lower while we observed a significant increase in skin prick test reactivity after anthelmintic treatment. In addition, differences in intensity and chronicity could not explain the simultaneous decrease in asthma, as both results are based on data from the same study population.

So far, most prospective intervention studies on the association between anthelmintic treatment and atopic diseases have been performed in schoolchildren (11-14, 16). The effect of deworming in younger children, i.e. aged 0-5 years, is still largely unknown. Regarding a potential protective effect on allergic sensitization and atopic diseases, it is generally assumed that infections in early childhood are more important than those later in childhood (29). Therefore, the effect of deworming in pre-schoolchildren might be different from that in schoolchildren. Rodrigues *et al.* (30) showed that *T. trichiura* infection in early childhood was strongly associated with less allergic sensitization later in childhood. Consequently, deworming of pre-schoolchildren could lead to more allergic sensitization and atopic diseases later in childhood. Prospective interventions studies in pre-schoolchildren are needed to further investigate this important matter.

In conclusion, our results indicate that atopic diseases do not increase and asthma might even decrease after treatment of STH infections. Allergic sensitization on the other hand increases after deworming. As this increase in skin prick test reactivity after anthelmintic treatment appears to be temporal, deworming of school-aged children does not seem to be a risk factor for the development of allergic sensitization, nor for atopic diseases.

## Acknowledgements

We especially thank Lenina Menocal Heredia, Ana María Collado Madurga, Aniran Ruiz Espinosa, Raúl A. Cordovi Prado, Irais Atencio Millan, and Angel Escobedo for their valuable help during the study. We also thank Anita van den Biggelaar for her useful suggestion for the manuscript. Furthermore, we thank all children, parents, teachers, school staff, as well as the staff in the policlinics, the health authorities and all field workers in SJM who participated in this study.

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