

Original article

The role of parasitic infections in atopic diseases in rural schoolchildren

Background: There is increasing evidence that the farming environment has a protective effect as regards allergic diseases. Exposure to animal parasites, particularly helminth infections, is common in the farming environment. However, the role of helminths in this environment is not well determined to date.

Methods: This analysis focuses on 613 children 6–13 years of age from rural areas of Austria, Germany and Switzerland, who took part in the Allergy and Endotoxin (ALEX) study. Allergic diseases and farming characteristics were assessed by a standardized questionnaire and as a crude measure of possible exposure to helminths, IgG antibodies to *Ascaris lumbricoides* were measured.

Results: Exposure to nematodes, as determined by the levels of antibody to *A. lumbricoides*, was more frequent among farmers' children than non-farmers' children (39.8% vs 31.1%, $P = 0.03$). This positive serology was found to be significantly associated with high total IgE levels [odds ratio (OR) = 3.05, 95% confidence interval (CI) = 1.81–5.12] and eosinophilia (OR = 2.84, 95% CI = 1.66–4.84). However, no association between anti-nematode serology and the prevalences of asthma, wheeze, hay fever or atopy was found. A weak association for atopy was observed after adjustment for total IgE.

Conclusion: Immunoglobulin G antibodies to *A. lumbricoides*, as a crude measure of possible exposure to helminths, did not indicate any protective effect against allergic diseases in this population. Although farmers' children had increased antibody levels reactive to helminth parasites indicating exposure, this did not explain the protective effect of farming against atopic diseases.

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The environment may influence the development of asthma and atopy in childhood (1–4). Several studies have shown that growing up on a farm has a protective effect as regards allergic diseases (4–8). Regular contact with farm animals and consumption of unpasteurized milk were found to have a significant protective effect in farm environments (4, 5, 8).

Children in farm environments are not only exposed to farm animals but may also experience infections more frequently (9). In a large study among adults living in a rural area, regular contact with animal stables and raw milk consumption were found to be the strongest risk factors for *Toxoplasma gondii* infection. Although the association between *T. gondii* infection and atopy was found to be weak in that study, seropositivity for *T. gondii* was suggested to be an intermediate factor in the association between farm environment and atopy (9).

One other potential source of infection related to farm environment is exposure to helminths. Studies have shown that helminthic infections are associated with a

lower risk of allergic disease and exposure to animal parasites, particularly helminth infections, will be inevitable in the farm environment (10–15). Helminth eggs and larvae present in stables may invade or hatch and at least undergo partial migration, or even occasionally reach adulthood (16–18). Until recently, studies investigating the association between helminths and allergic diseases have been performed mostly in tropical areas and in developing countries, where helminthic infections are endemic. The findings of these studies indicate that the intensity and the chronicity of helminthic infections might determine their effect on the prevalence of allergic disorders (10). In most of the studies, active and chronic helminthic infections were reported to be have a protective effect against atopy, but findings are less consistent for wheezing and asthma (11–15, 19–21).

It is still unclear whether the same association holds true in non-developed countries. Therefore, we aimed to investigate whether exposure to nematode infections has a protective role against allergic diseases in a farming environment in European countries.

Methods

Study population

This analysis focuses on 613 children 6–13 years of age from rural areas of Austria, Germany and Switzerland, who took part in the ALEX study as previously described (2, 8). Participating parents (2618 of 3504 potential participants; 74.7%) were asked to give consent to blood sampling for measurement of total and specific IgE [radioallergosorbent test (RAST)]. For these additional investigations, 1406 (53.7%) parents gave consent. Of these, all children from farming families ($n = 319$), all children from non-farming families who reported regular contact with a farm environment ($n = 111$), and a random sample of children from non-farming families who were not exposed to a farm environment from the same rural area ($n = 382$) were invited to participate in the study. In 613 of 812 children (196 farmers' children and 417 children from non-farming families), enough serum was available for nematode-specific IgG antibody analysis.

Approval to conduct the survey was obtained from the three local ethics committees for human studies and from the principals of the schools involved. Written informed consent was obtained from all parents.

Questionnaire

Demographic factors, history of anti-helminthic treatment, information about the health of parents and information about the child's activities on farms (contact with stable animals, presence in stables and barns, helping with harvesting) and the prevalence of diseases and symptoms were assessed by a questionnaire. It comprised the questions of the International Study of Asthma and Allergies in Childhood (22) as described previously (2, 8).

Asthma diagnosis was defined as a physician's diagnosis of asthma, or recurrent asthmatic, obstructive, or spastic bronchitis. Current wheezing was considered positive, if parents reported at least one episode of wheeze during the previous 12 months. Atopic dermatitis and hay fever were defined as parents' report of a doctor's diagnosis of atopic dermatitis and hay fever. Current symptoms of hay fever were defined as a positive response to the question: 'In the last 12 months, has your child had problems with sneezing or a runny or blocked nose without a cold accompanied by itchy-watery eyes?' Current atopic eczema symptoms were considered positive if the child ever had an itchy rash in the previous 12 months. Farmers' children were defined as children whose parents answered 'yes' to the question: 'Does your child live on a farm?' The children's activities on farms were categorized into rarely (not at all, rarely) and frequently (now and then, or frequently) with respect to questions on frequency of visits to the barn and the stable and providing help during hay harvesting.

Measurement of IgE

Total IgE and the concentration of specific IgE against a panel of aeroallergens and food allergens was measured in all serum samples by fluorescence enzyme immunoassay (SX1 and FX5 CAP; Pharmacia, Uppsala, Sweden) in a central laboratory (University Children's Hospital Charité, Berlin, Germany). Differential blood counts were also performed. Atopy was defined as at least one positive specific IgE result of ≥ 0.35 kU/l for the six aeroallergens (house dust mites, storage mites, mixed-grass pollen, birch pollen, cat dander and cow epithelium). We also considered a higher cut-off level of 3.5 kU/l in the analyses.

ELISA to measure antibody responses to nematodes

Nematodes are the most common helminths infecting cattle. We therefore used a phosphate-buffered saline (PBS) extract of *Ascaris lumbricoides* to detect IgG antibodies present in the ALEX sera. Briefly, plates were coated with 5 µg/ml of a PBS extract of adult female worms, and sera tested at a dilution of 1:200. As nematodes are extremely cross-reactive, the presence of antibody will signify exposure to any nematodes (23). A British control population with no history of helminthic infections ($n = 12$) was used to define normal levels. Specific IgG antibody levels three standard deviations above the mean of the controls were defined as positive.

Statistical analyses

Data analysis was performed with SAS 9.1.3 (The SAS Institute, Cary, NC, USA). IgG levels for exposure to nematode infections, eosinophil count, eosinophil percent, and total IgE levels were log-transformed, resulting in normal distributions. Eosinophil count, percentage of eosinophils and total IgE levels were divided into quintiles. Age was categorized into groups of 6–7, 8, 9, 10 and 11–13 years. The prevalence of symptoms, diagnoses and allergic sensitization was compared by chi-squared tests between children living on farms and those from non-farming families; children having positive and negative serology to nematode infections; and children having been treated and not having been treated for helminthic infections. The power of the study to detect an odds ratio of 0.5 for atopic diseases dependent on exposure to nematodes as reported previously (12, 20) was 0.95 at an α level of 0.05.

Adjusted odds ratios (ORs) with 95% confidence intervals (CIs) were calculated by fitting logistic regression models. All variables with univariate association ($P \leq 0.25$) were subsequently tested for inclusion into multivariable logistic regression models. The included co-variables were: age (categorical), sex, study area (categorical), maternal and paternal history of allergic diseases, parental education, number of older siblings (in three categories), having contact with cattle, pigs and barn visits. In a final step, nematode serology was dichotomized and modelled in logistic regression. Exposure to farm milk in the first year of life had a significant association with the serology of exposure to nematodes and was therefore included in the logistic model for serology. Finally, we adjusted the model also for total IgE.

Results

The study population consisted of 613 children: 196 farmers' children with mean age 9.43 years [standard deviation (SD) 1.59], and 417 children from non-farming families, mean age 9.49 years (SD 1.59). Of all children, 319 (52%) were boys.

Subject characteristics of those 613 children with anti-nematode serology did not differ in the prevalence of asthma, hay fever and atopy from the total group of 2618 children with questionnaire information. However, the prevalence of wheeze (10.2% vs 6.8%, $P = 0.004$), doctor-diagnosed dermatitis (17.1% vs 13.6%, $P = 0.02$), and the proportion of children living on a farm (31.9% vs 26.6%, $P = 0.008$) was higher in the study subjects than in the source population. The OR (95% CI) for the association between farming and wheeze in children participating in

this study on nematode exposure was 0.45 (0.25–0.80) and 0.40 (0.21–0.76) for non-participating children. The Breslow–Day test for homogeneity of effects revealed a *P*-value of 0.79, ultimately not proving heterogeneity of ORs.

In the study population, 14.1% had at least one previous treatment for helminthic infections. Of all children, 8.2% had one, 4.7% had two and 1.1% had more than two previous treatments for helminthic infections. In turn, a positive serology indicating exposure to nematodes was found in 33.9% of the children. Concordance of previous treatment and a positive serology for nematode infections was low (kappa coefficient = 0.03). But the prevalence of a positive serology for exposure to nematodes was higher in the group having more than one previous treatment for helminthic infections (55.5%) than the groups having one previous treatment (26%, *P* = 0.005) and none (33.2%, *P* = 0.006).

The number of older siblings was significantly higher among seropositive children than among seronegative

subjects (*P* = 0.001) (Table 1). Furthermore, a positive serology was found to be more frequent in girls. Farming as well as farm milk consumption was also significantly associated with a positive anti-nematode serology (Table 1). In turn, treatment for helminthic infections was only associated with other farming characteristics namely regular contact with pigs (22% vs 13.3%, *P* = 0.04) and with poultry (36.3% vs 24%, *P* = 0.02).

Although the prevalence of atopic symptoms and diseases except a doctor-diagnosed hay fever and atopy was higher in the previously treated group, this effect did not reach statistical significance in crude and adjusted analyses (data not shown). Moreover, a previous treatment for helminthic infections was not associated with median total IgE levels (treated: 92.9 kU/l vs untreated: 64.6 kU/l, *P* = 0.13) and eosinophil counts (treated: $300 \times 10^6/l$ vs untreated: $200 \times 10^6/l$, *P* = 0.48).

In contrast, the median of total IgE levels was higher among the children with a positive anti-nematode serology than those with negative tests (108.5 kU/l vs 49 kU/l, *P* < 0.0001). Furthermore, the median of eosinophil counts ($300 \times 10^6/l$ vs $200 \times 10^6/l$, *P* < 0.0001) was found to be significantly higher among seropositive children than their seronegative peers. Although the prevalence of doctor-diagnosed atopic dermatitis was lower in children with a positive anti-nematode serology (13.2% vs 19.1%, *P* = 0.06), other allergic outcomes were not different from children with a negative anti-nematode serology.

In multivariable analysis, crude and adjusted ORs associated with serology for nematode infections are presented in Table 2. Additional adjustment for passive smoke exposure did not significantly change the results. Positive anti-nematode serology was neither a protective nor a risk factor for atopy, asthma, eczema and hay fever. After adjustment, anti-nematode serology was still found to be associated with total IgE levels and eosinophil counts (Table 2). This association did not depend on the cut-off levels because using specific IgG antibody levels for nematodes as a continuous variable did not change the results. However, exposure to nematodes as a continuous

Table 1. Determinants of serology for exposure to nematodes

	Serology for exposure to nematodes		<i>P</i> -value
	Positive (<i>n</i> = 208)	Negative (<i>n</i> = 405)	
Median age (IQR)	10 (3)	9 (2)	0.08
Male gender, % (<i>n</i>)	46.1 (96)	55 (223)	0.03
Number of older siblings, % (<i>n</i>)	50.9 (105)	50.9 (206)	0.001
1–2			
>2	14 (29)	5.2 (21)	
Farming, % (<i>n</i>)	37.5 (78)	29.1 (118)	0.03
Regular stay in stable (more than once a week), % (<i>n</i>)	49.5 (103)	44.6 (181)	0.25
Regular stay in barn (more than once a week), % (<i>n</i>)	37 (77)	32 (129)	0.21
Regular contact with pig, % (<i>n</i>)	15.7 (29)	13.9 (47)	0.57
Regular contact with cattle, % (<i>n</i>)	58.1 (107)	53.7 (181)	0.32
Regular contact with poultry, % (<i>n</i>)	21.2 (39)	28.1 (95)	0.08
Farm milk consumption in the first year of life, % (<i>n</i>)	58.6 (122)	45.9 (186)	0.002

Table 2. Multivariable analysis of atopic diseases in children according to serology for exposure to nematodes: crude and adjusted odds ratios

	Serology for exposure to nematodes		
	Crude OR (95% CI)	Adjusted* OR (95% CI)	<i>P</i> -value for aOR
Itchy rash in the past 12 months	1.33 (0.81–2.19)	1.33 (0.72–2.43)	0.35
Doctor's diagnosis of eczema	0.64 (0.40–1.03)	0.65 (0.38–1.10)	0.11
Rhinoconjunctivitis symptoms in the past 12 months	1.07 (0.62–1.84)	1.59 (0.76–3.32)	0.21
Doctor's diagnosis of hay fever	0.86 (0.47–1.57)	1.27 (0.59–2.74)	0.53
Wheeze in the past 12 months	1.15 (0.66–1.99)	1.51 (0.74–3.08)	0.25
Doctor's diagnosis of asthma	1.01 (0.52–1.98)	1.78 (0.71–4.08)	0.17
Atopy (allergen-specific IgE ≥ 3.5 kU/l)	0.83 (0.55–1.26)	1.16 (0.70–1.93)	0.54
Atopy (allergen-specific IgE ≥ 0.35 kU/l)	0.80 (0.56–1.15)	0.89 (0.58–1.38)	0.62
Total IgE (categorical, 5th quintile vs others)	2.08 (1.39–3.13)	3.05 (1.81–5.12)	<0.0001
Eosinophil count (categorical, 5th quintile vs others)	2.15 (1.40–3.31)	2.84 (1.66–4.84)	0.0001

*Adjusted for age, sex, study area, family history of atopy, number of older siblings, parental education, farm milk consumption in the first year of life, exposure to pig, cattle and barn.

Table 3. The association between farming and allergic diseases

Outcome	cOR (95% CI)	aOR (95% CI)*	aOR (95% CI) * (additionally adjusted for anti-nematode serology)
Atopy (allergen-specific IgE ≥ 3.5 kU/l)	0.71 (0.46–1.09)	0.74 (0.46–1.21)	0.74 (0.46–1.20)
Wheeze in the past 12 months	0.58 (0.31–1.08)	0.43 (0.21–0.89)	0.43 (0.21–0.88)
Doctor's diagnosis of asthma	0.57 (0.26–1.21)	0.74 (0.32–1.70)	0.73 (0.31–1.68)
Rhinoconjunctivitis symptoms in the past 12 months	0.44 (0.23–0.84)	0.36 (0.16–0.80)	0.36 (0.16–0.79)
Doctor's diagnosis of hay fever	0.38 (0.18–0.80)	0.33 (0.14–0.76)	0.33 (0.14–0.76)

*Adjusted for age, sex, study area, family history of atopy, number of older siblings and parental education.

variable was positively associated with hay fever symptoms [OR = 1.58, 95% confidence interval (CI) = 1.01–2.46, $P = 0.04$]. In stratified analysis for serology for nematode exposure, atopy was found to be associated with wheeze in both groups [cOR = 2.47 (1.03–5.94), $P = 0.04$ and cOR = 2.35 (1.20–4.59), $P = 0.01$ for children having a positive and negative serology, respectively]. In multivariate analyses, atopy was found to be a risk factor for wheeze (OR = 9.75, 95% CI = 1.62–58.60, $P = 0.01$) only in children with positive serology but not in children with negative serology [OR = 1.93 (0.78–4.81) $P = 0.15$].

The polyclonal expansion of total IgE induced by parasitic infections may have confounded our results. Therefore, we additionally adjusted our model for total IgE levels. This adjustment decreased the ORs for wheeze and asthma, and the direction of the effect was changed for hay fever diagnosis and symptoms. A positive anti-nematode serology was then inversely associated with atopy (OR = 0.39, 95% CI = 0.22–0.68; $P = 0.001$). Although there was an inverse effect of a positive serology for helminthic infections on doctor-diagnosed hay fever (OR = 0.53, 95% CI = 0.20–1.35; $P = 0.18$) and hay fever symptoms (OR = 0.70, 95% CI = 0.29–1.71; $P = 0.44$), it did not reach statistical significance. When using a continuous variable for anti-nematode serology, results remained basically unchanged. The numbers of subjects in subgroups of farmers' and non-farmers' families were too small to analyse potential effect modification by farming status.

As in the larger population sample (8) farming was inversely associated with all allergic outcomes after adjusting for the same potential confounding variables (Table 3). Additional adjustment for anti-nematode serology did not significantly alter the risk estimates, thereby demonstrating that a positive serology to nematodes did not explain the farming effect.

Discussion

In this study, we used crude measures of exposure to helminths by: (i) recording treatment cycles and (ii) measuring IgG to *A. lumbricoides* and showed that this is not protective for allergy and asthma in a rural, partially farming population in Central Europe. Only a

weak association between a positive anti-nematode serology and atopy was observed after adjustment for total IgE. Although farmers' children seem to be more exposed to helminths, the exposure did not explain the protective 'farming effect' for atopic diseases.

The limitations of this study may be the cross-sectional design and a potential selection bias relating to the subgroup analysis of the original ALEX study subjects (2). The anti-nematode serological analysis was only performed in 613 of 812 subjects in whom blood was drawn because not enough serum was left. This raises the question of whether selection bias occurred. However, general characteristics such as age, gender, family history of atopy and number of older siblings were similar compared with the original study group. While the prevalence of wheeze, doctor-diagnosed dermatitis and the proportion of children living on a farm was higher in the subgroup with serological findings, the prevalence of asthma, atopy and hay fever was not significantly different from the original study group. Therefore, the subgroups may not be completely representative for the source population.

In the study population, only a tentative association between a previous treatment for helminthic infections and a positive anti-nematode serology was observed. Although there were some discrepancies between the effects of a previous treatment and a positive anti-nematode serology, the effect on allergic diseases was in the same direction. The findings relating to serology were seen for both definitions, either using a somewhat arbitrary cut-off or a continuous variable. The anti-nematode assay is sensitive to all human and animal nematodes, but has limited specificity. However, exposure to nematodes and other parasites need not result in clinically overt disease. In fact, exposures only occasionally proceed to manifest infection and need treatment. Therefore, the discrepancy between treatment cycles and nematode exposure assessed by serology is not surprising.

There is no gold standard test for diagnosing parasitic infections (24). Stool examinations, serology, history and clinical examination all contribute to a clinical diagnosis. Stool examinations are more likely to detect acute infections, whereas serology is more likely to also reflect long-term exposure which is of greater interest in this epidemiological study. Previous studies in endemic areas

have shown that the specificity of a nematode serology is somewhat limited (64–68% for *Strongyloides* compared with stool examinations) (24). Yet, the sensitivity has been shown to be higher (80–97%). Because exposure to helminths is not endemic in this farming population, a positive serology is more likely to more accurately reflect potential helminth exposures (compared with a helminth endemic population where exposures are universal). We therefore believe that we measured one marker of chronic exposure to nematodes by assessing IgG anti-nematode antibodies. This notion is also supported by the fact that serology was found to be positively correlated with eosinophilia and total serum IgE in this population.

Helminths are potent inducers of IgE production and are associated with high circulating IgE levels (25). Moreover, in our study, total IgE levels were found to be higher among children with positive serology than those with negative serology. But in contrast to other recent publications, no clear association between helminthic infection and asthma or allergic diseases was observed in the study reported herein.

One of the potential explanation for discrepancies in the literature reporting associations between helminths and allergic diseases may be the type of helminths in a particular area. In Central Europe, the most common helminths are *Enterobius vermicularis*, *Taenia saginata*, *A. lumbricoides* and *Trichuris trichura* (26). In African and Latin American countries, where the helminthic infections are reported to have protective effects on atopy, hookworms, *Schistosoma mansoni*, *Schistosoma haematobium* and *Strongyloides stercoralis* are also common (26). It may also be that exposure to animal parasites which rarely result in long-term or patent infections as part of the farming environment may result in different types of immune response to infection with the human parasites. In animal models, different parasites have been shown to have different effects on immunological responses. *Nippostrongylus brasiliensis* infection is an excellent model for examining the acute Th2 response, whereas *Trichuris muris* is shown to trigger either a Th1 or Th2 response (27, 28).

Furthermore, the intensity and chronicity of helminthic infection may play a role. In most of the studies from developing countries, both active and chronic helminthic infections were reported to be protective against allergy, but there are also some studies showing helminthic infections to be a risk for allergic diseases (11–15, 19–21). For example, in a large cross-sectional study conducted in a rural area of China with a low prevalence of parasite infestation, infection with *A. lumbricoides* was shown to be associated with an increased number of positive skin tests and increased risk of asthma in children (29). In contrast, infection with *Ascaris* was shown to be a protective factor for wheeze in some studies in Africa (12, 20). These findings may indicate that the parasite burden in different regions may be responsible for different effects. Using specific IgG antibody levels to nematodes as a continuous variable may therefore better

reflect the intensity and chronicity of nematode infections than the use of a cut-off value in our population. However, the findings remained basically unchanged suggesting that even at high levels of nematode serology we did not see a protective effect in this rural European population. In fact in areas with a low infestation rate and prevalence, an acute allergy-enhancing phenotype may even predominate while in areas with a high prevalence and continuous infestation, more chronic and allergy-suppressing phenotypes might be expected (19, 30, 31).

As the study presented herein is cross-sectional like most of the epidemiological studies, the direction of the relationship between atopy and infection with helminth parasites is not clear. It is also conceivable that an atopic phenotype might provide protection against geohelminth infection and could have afforded an evolutionary advantage to our ancestors, who may have been exposed to high levels of geohelminth infections (32).

As there is a close association between a positive serology for nematode infections and total IgE, this may mask an underlying protective effect of exposure to nematodes on atopy and allergic diseases. Therefore, we also investigated the effects of nematode infections on allergic disorders independent of total IgE levels. The effect of seropositivity for nematode infections on current wheeze and asthma remained unchanged. However a weak protective effect for atopy and to a lesser extent for the development of a doctor's diagnosis of hay fever after controlling for total IgE was shown. Therefore, overall no major protective effect on allergic diseases was seen even after controlling for total IgE.

The farming environment was shown to be protective for the development of allergic diseases, but most probably there is no single factor preventing children from developing such diseases, but the effects rather result from a complex interaction between various environmental and lifestyle factors. However, parasitic infections do not seem to contribute much to this protection.

In conclusion, we were unable to show any protective effect of exposure to nematode infections on atopic diseases on schoolchildren in rural, partially farming areas in Central Europe. In contrast, positive serology for nematode infections was found to be a risk factor for asthma in atopic children, and this effect persisted after adjusting for total IgE. Based on the present analyses we may conclude that the association between helminthic infections and allergic diseases may differ in a farming environment in industrialized countries from the setting in developing countries.

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