

Atopy, asthma, and antibodies to *Ascaris* among rural and urban children in Kenya

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Objective: The purpose of this study was to evaluate differences in the relationship between asthma and immune responses to allergens in children living in rural and urban areas of Kenya.

Study design: Children (mean age, 11 years) from Kabati (n = 136), a rural village, and Thika (n = 129), a small town, were studied by skin testing and serum immunoglobulin E (IgE) and immunoglobulin G (IgG) antibody measurement. Asthma was evaluated by symptoms, as well as spirometry before and after vigorous exercise to test for exercised-induced bronchospasm (EIB). School children from a study performed in Atlanta, Georgia, were used for comparison of anthropometric and immunologic results.

Results: Compared with the urban area of Kenya, children living in the rural area had a lower percentage of body fat, smaller and fewer skin test responses to allergens, a higher prevalence of IgE antibodies to *Ascaris* (67% vs 26%) and 10-fold higher total IgE. In the urban area of Kenya, there was a strong correlation between EIB and atopy determined both by IgE antibodies ($P = .02$) and skin tests ($P = .002$). By contrast, in the rural area, none of the 13 children with EIB were skin-test positive (vs 13/109 of children without EIB).

Conclusions: Among the rural children, there was no association between immune responses to allergens and airway-related symptoms or reactivity. The association between asthma and atopy seen in the town of Thika may represent an important step in the increase in asthma seen both in urban Africa and in the West. (J Pediatr 2002;140:582-8)

An increased prevalence of asthma has been reported in many Western countries during the last 40 years.^{1,2} In addition, differences in the prevalence of

asthma have been found in different areas within developing countries. In Gambia, Ethiopia, Kenya, and South Africa, the prevalence of asthma is

higher in urban compared with rural communities;³⁻⁶ these differences have been associated with relative affluence.^{7,8} It has been assumed that understanding the causes of the changes in Africa would help explain the increase in asthma in the West. In studies on children in the West, there is a consistent strong association between asthma and immediate hypersensitivity to indoor allergens. Previous data from several African countries, including Kenya, reported that sensitization to dust mite allergens (Der f 1) was common among

See related article, p 576.

children with asthma.⁹ Conversely, a study from Ethiopia found that positive skin tests to mite antigens among a rural population were unrelated to airway symptoms.³ Several studies have assessed the possible relevance of helminth infections to asthma. Although it is clear that these parasites can enhance immunoglobulin E (IgE) antibody production, the evidence does not support a consistent positive or negative influence on asthma.¹⁰⁻¹²

ABA-I	Antigen I from the nematode <i>Ascaris lumbricoides</i>
CAP	Assay for measuring IgE
Der f 1	The major allergen of <i>Dermatophagoides farinae</i>
EIB	Exercise-induced bronchospasm
Fel d 1	The major allergen of the cat <i>Felis domesticus</i>
FEV ₁	Forced expiratory volume in one second
FVC	Forced vital capacity
IgE	Immunoglobulin E
IgG	Immunoglobulin G

It has been widely proposed that the increase in asthma can be explained by an increase in allergic sensitization sec-

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ondary to increased cleanliness. On the basis of previous results from Kenya and other African countries, we hypothesized that the lower prevalence of asthma in the villages was not simply because of decreased allergic sensitization. The purpose of the current study was to determine if there were immunologic differences between children living in urban and rural communities that could explain the differences in asthma prevalence. In addition, anthropometric and lung function differences were examined and compared with data from a concurrent study in Atlanta, Georgia.

MATERIAL AND METHODS

Anthropometric measurements, spirometry, skin tests, serum assays, and exercise challenges were carried out on children in the fourth grade of 2 elementary schools in Kenya. Both of the study locations are close to the equator, at approximately 6000 feet above sea level, where there is little seasonal variability in the weather (average high temperatures: 80°F in summer, 71°F in winter). Children in the fifth grade from 2 inner-city schools in Atlanta, Georgia were used for comparison of anthropometric, immunologic, and spirometry results. In Kenya, ethical permission was obtained through the Kenya Medical Research Institute, as well as the local school boards. In Atlanta, approval was obtained from the Human Investigation Committee of Emory University and the local school board.

Rural Kenyan School

Mukerengu Primary School is located in Kabati, an area approximately 80 km north of Nairobi. The major sources of income in Kabati are farming, raising livestock, and working for large pineapple and coffee farms. Most of the children live in thatched-roof homes. Three of 4 classes from the fourth grade were randomly selected. The exercise challenge, skin tests, and venipuncture were

conducted on separate days. Of the 136 children enrolled, 129 were exercise-tested, 128 were skin-tested, and blood was drawn from 131.

Industrial Kenyan School

Kenyatta Primary School is located in Thika, a small industrial town 50 km north of Nairobi and only 30 km from Kabati. The many factories in Thika include pineapple processing, battery manufacturing, and bread making. Most of the children from the school live in housing built by the companies in the town, 1-room dirt-floor brick houses arranged as 8 units around a courtyard (one family per room). Of the 129 children in the 3 classes of the fourth grade, 127 were exercise-tested, 127 were skin-tested, and blood was drawn from 126.

Atlanta School

Children in the fifth grade at Boyd and Woodson elementary schools in Atlanta, Georgia, were enrolled as part of an ongoing study on asthma education. These schools serve an area where >50% of the families are below the poverty level, and 90% of the children qualify for subsidized lunches. All of the children enrolled were African American. Anthropometric measurements and spirometry were performed. Neither skin testing nor venipuncture was possible in the schools in Atlanta, but they were carried out subsequently on 50 of the children at the Northwest Grady Clinic.

Body Fat Measurement

In addition to measurements of height and weight, subscapular and tricep skin-fold thicknesses were measured in triplicate by using calipers (Lafayette Instrument Co, Lafayette, Ind). These measurements were used to calculate body fat.¹³

Skin Test

Skin testing was done on the inner aspect of the forearm by lancet (Hollister-Stier, Spokane, Wash) by using histamine (1 mg/mL, Center Laboratories, Port Washington, NY), dilu-

ent, *Dermatophagoide*s *pteronysinus*, *Dermatophagoide*s *farinae*, cat hair, dog epithelium, mixed cockroach, mixed feathers, mosquito, cattle epithelium, goat epithelium, *Aspergillus* mix, and *Alternaria tenuis* (Greer Laboratories, Lenoir, NC).

Serum IgE and IgG

Total and specific serum IgE antibodies were measured by the CAP system (Pharmacia, Uppsala, Sweden). Specific IgE antibodies were measured to *D pteronyssinus*, *D farinae*, cat, dog, cockroach, *Aspergillus*, *Alternaria*, and *Ascaris*. Specific immunoglobulin G (IgG) antibodies were measured by an antigen-binding assay by using radio-labeled cat allergen (Fel d 1), dust mite allergen (Der f 1), *Ascaris* (ABA 1) (University of Glasgow, Scotland), and *Tetanus toxoid*.¹⁴ The proteins, radio-labeled with ¹²⁵I, a radioactive isotope of iodine, were incubated with the serum. A goat antihuman IgG was used to precipitate the IgG. Counts were measured in a γ counter after 3 washes of the precipitate.¹⁵ Values for each IgG antibody assay were obtained from a control curve carried out in parallel with the assays.

Inhibition of IgE Assays

Because of the high prevalence of IgE antibodies to Der f 1 and Fel d 1 among children in Kenya who did not have a positive skin test to those allergens, studies on the specificity of the IgE assay were performed. The children with the highest levels of IgE antibodies to cat and *D pteronyssinus* were selected for inhibition studies. Only one child from the Atlanta study had measurable IgE to cat; therefore, sera from 13 children with IgE to cat from a previous study of asthmatics in the same area of Atlanta were used.¹⁶ Serum was tested diluted 1:1 with a protein diluent, as well as serum incubated for 3 hours with 25 μ L of 1:5, 1:15, or 1:45 dilution of skin test extracts.

For most children (30/33 sera), the mite CAP assay could be inhibited with

Table I. Grade school children in Kabati and Thika, Kenya, and Atlanta, Georgia

	Kabati (rural)	Thika (urban)	Atlanta
No. enrolled	136	129	112
Ages (y)	11.5 (9-15)	10.0 (8-13)	10.8 (10-12)
Height (cm) [†]	137 (120-159)	135 (123-154)	150 (112-168)
Weight (kg) [†]	31 (20-51)	30 (20-45)	46 (22-112)
Body fat (%) [°]	15.5 (6.0-23)	17.4 (12-31)	19.8 (14-32)
Initial lung function test			
FVC (L) [†]	1.99 (1.27-3.08)	1.93 (1.06-2.58)	2.49 (1.41-5.92)
FEV ₁ (L) [†]	1.66 (0.97-2.53)	1.60 (0.92-2.19)	2.01 (1.19-4.16)
% Predicted ^{†‡}	95.6	96.4	89.2
PEF (L/m) [†]	242 (91-426)	228 (102-358)	292 (174-692)

Mean (range).
[°]*P* < .01 between all 3 locations (Mann-Whitney *U* test).
[†]*P* < .01 between Kenyan and Atlanta children.
[‡]Percent predicted FEV₁ values were race-adjusted.

the skin test extract (30% reduction in binding considered inhibition). However, with the cat CAP and Kenyan sera, IgE binding was not effectively inhibited by the skin test extract (1/24 Kenyan sera inhibited, 10/12 in Atlanta). In addition, IgE binding to the cat CAP among the Kenyan sera was blocked with nonspecific proteins in the diluent, a result not seen with dust mite. It is important to note that many of the same sera that were inhibited with mite would not inhibit with cat.

Lung Function and Exercise Challenge Test

Forced vital capacity (FVC), forced expiratory volume at one second (FEV₁), and peak expiratory flow were measured by using a portable spirometer (Vitalograph, Lenexa, Tex). The tests were performed in a standing position with no nose clip. In accordance with the American Thoracic Society guidelines, a minimum of 3 and a maximum of 8 trials were allowed for each child, and the best of 3 reproducible trials was used in the analysis of lung function. Predicted FVC and FEV₁ values were calculated by the Crapo-Hsu equation adjusted for race by multiplying the predicted value by 0.85.^{17,18} After initial spirometry, the children ran vigorously for 6 minutes in a field. The approximate average distance run,

which varied because of running speed, was 0.5 km. Spirometry was repeated at 5 and 10 minutes after running. EIB was defined as a decrease in FEV₁ by ≥15% from the prerun test at either the 5- or the 10-minute postrun test.

The children were questioned about asthma symptoms before they ran and were asked to describe any medications they were taking. A "yes" answer to either the asthma symptoms questions or the use of asthma medication was considered a positive history for asthma.

Statistics

For comparing means, the nonparametric Mann-Whitney *U* test was used. The Pearson's χ^2 test was used for the comparison of sensitization and asthma prevalences. Logarithmic regression was used to analyze the correlation between IgE antibodies to *Ascaris* and total IgE.

RESULTS

Similarities in anthropometrics and initial lung function were seen between the rural and industrial areas of Kenya; however, the children in Kabati had a significantly lower percentage of body fat than children in Thika (Table I). Of the 112 children weighed in Atlanta, 31 were heavier than the heaviest child

weighed in Africa. The children in Atlanta had higher absolute values for FEV₁ and FVC; however, the FEV₁ values expressed as a percent predicted were lower than in both of the communities in Africa (*P* < .01).

Skin Tests

In all 3 groups, the most common skin test responses were to dust mite (Table II), with 8.6%, 17%, and 35% of the children in Kabati, Thika, and Atlanta, respectively, having a positive skin test to *D pteronyssinus* (≥3 mm). In Kabati, only 4% of the children responded to any allergen with a ≥4 mm wheal. In Thika and Atlanta, skin tests of this size were found in 14% and 32%, respectively. Although there were children who reacted to goat, cattle, mixed feathers, or mosquito, very few of them were allergic only to these. Despite the differences in reactivity to allergens, the mean wheal diameters for histamine, 3.2 mm, 3.4 mm, and 3.3 mm (for Kabati, Thika, and Atlanta, respectively), were not significantly different between the groups.

Serum IgE and IgG Antibodies

Among the children in Africa, serum IgE antibody test results revealed more positives than had been found with skin tests, especially in the rural area. As with the skin tests, the most common IgE antibodies were to mite and cock-

Table II. Percentage of children with a positive skin test

	Kabati (rural) (n = 128)				Thika (urban) (n = 127)				Atlanta (n = 47)			
	+/-	+	++	+++	+/-	+	++	+++	+/-	+	++	+++
Histamine	24	52	20	2.3	18	46	29	3.9	15	49	34	0
Control*	0	0	0	0	2.4	0	0.8	0	0	0	0	0
<i>D pteronyssinus</i>	16	4.7	1.6	2.3	14	7.1	5.5	4.7	6.4	8.5	6.4	20
<i>D farinae</i>	3.9	2.3	0.8	0.8	7.1	2.4	5.5	2.4	0	2.1	4.3	11
Cat	12	0	0	0	6.3	1.6	0	0	0	4.3	2.1	0
Dog	7.8	0.8	0	0	3.2	1.6	0.8	0	2.1	0	0	0
Cockroach	15	0	0	0	25	0.8	2.4	0	2.1	11	6.4	4.3
Aspergillus	2.3	0	0	0	5.5	0	0	0	2.1	0	0	0
Alternaria	2.3	0	0	0	4.7	0.8	0	0	2.1	2.1	4.3	0
Any allergen†	30	7.0	1.6	2.3	32	11	8.7	5.5	11	15	11	21

+/-, ≥ 2 mm to < 3 mm, +, ≥ 3 mm to < 4 mm, ++, ≥ 4 mm to < 6 mm, +++, ≥ 6 mm mean wheal sizes.
 *All of the negative controls were smaller than 2 mm except for 3 children in Thika.
 †Skin test positive to any allergen tested, including mixed feathers, goat, cattle, and mosquito, except for the Atlanta children, who were not tested for these allergens. Only 6 of the 255 children who were sensitized to these allergens were not also sensitized to other of the more common allergens.

roach. When the serum assays were compared with skin test results, the correlation was very poor in Kabati, reasonable in Thika, and excellent among the children in Atlanta, with 5.2%, 29.6%, and 62% of the children with IgE antibodies (≥ 0.7 IU IgE/mL) having a positive skin test in Kabati, Thika, and Atlanta, respectively. In Kabati, of the children who had ≥ 0.7 IU IgE/mL to cat or cockroach, none had a positive skin test (≥ 3 mm) to the same allergen, and only 39% of those with IgE to *D pteronyssinus* were skin-test positive. In Thika, a similar pattern was observed for cat and cockroach allergens (ie, 0% and 12%, respectively); however, in that town, a majority of the children (81%) who had IgE antibodies to *D pteronyssinus* also had a positive skin test. In Atlanta, with the exception of the results for *Aspergillus*, most of the children with a positive serum test also had a positive skin test to the same allergen.

The prevalence of an immune response to the parasite *Ascaris* was assessed in these populations by measuring IgE antibodies (Figure). In Kabati, 67% of the children had ≥ 0.7 IU IgE antibodies/mL (26% in Thika and 12% in Atlanta). Total IgE levels were significantly higher in Kabati ($P < .001$), but there was not a significant difference between Atlanta and Thika ($P = .67$).

The antigen-binding radio precipitation assays for IgG antibodies provide additional evidence about immune responses in the children. Although many of the children in Africa had a positive test for IgE antibodies, the presence of IgG antibodies to mite (1.6%) or cat (2.7%) allergens was not common compared with the children in Atlanta, of whom 42% had IgG antibodies to mite and 8% to cat. IgG antibodies to ABA-1 were common in the sera from Kabati (Figure). A majority (94%) of the children in all locations had made an IgG response to tetanus.

Atopy and EIB

At each of the schools in Kenya, 15 children had a 15% decrease in FEV₁ after exercise (EIB). In Kabati, only 5 children (3.9%) gave a history of asthma and only one of those children also had EIB (0.8%). In Thika, 13 children (10.2%) gave a positive history for asthma and 5 (3.9%) of those children also had EIB.

Both skin tests and serum IgE were used to assess allergy as a risk factor for EIB. In the rural village, positive skin tests were more common among the group without EIB. In fact, none of the 12 children with EIB who were skin-tested had a positive skin test (≥ 3 mm). The children without EIB

also had a higher prevalence of IgE antibodies. Total IgE and the presence of IgE to *Ascaris* were not significantly different between the 2 groups.

In Thika, the prevalence of atopy was significantly higher for the group with EIB, and this association was highly significant for skin tests to dust mite ($P < .005$) (Table III). In addition, total IgE levels and the presence of IgE to *Ascaris* were significantly higher among the EIB group.

DISCUSSION

In keeping with findings in other areas of Africa, asthma is not a significant problem in the villages of Kenya.³⁻⁶ In the village we studied, although 15 of the children had a documented fall in FEV₁ after exercise, only one of these reported symptoms of asthma. The most striking finding was the complete absence of an association between bronchial reactivity and any aspect of allergy. This situation was reversed in the town, where asthma was present and was significantly associated with skin tests, IgE antibodies, and total IgE. The objective of our study was to investigate immunologic differences between these 2 sites.

Table III. Percentage of atopy as judged by skin test and serum IgE antibodies for children with and without EIB

	Kabati (rural)				Thika (industrial)			
	$\geq 3\text{ mm skin test}^{\S}$		$\geq 0.7\text{ IU IgE/mL}$		$\geq 3\text{ mm skin test}$		$\geq 0.7\text{ IU IgE/mL}$	
	EIB (n = 12)	non-EIB (n = 110)	EIB (n = 15)	non-EIB (n = 106)	EIB (n = 15)	non-EIB (n = 111)	EIB (n = 14)	non-EIB (n = 110)
<i>D pteronyssinus</i>	0	9.1	6.7	16	40 [†]	14	36 [†]	9.1
<i>D farinae</i>	0	3.6	13	25	33 [†]	5.4	36 [†]	7.3
Cat	0	0	20	51 [*]	0	1.8	21	8.2
Dog	0	0.9	20	50 [*]	6.7	1.8	21	9.1
Cockroach	0	0	47	49	13	5.4	50 [†]	17
Goat	0	1.8	—	—	6.7	0	—	—
<i>Aspergillus</i>	0	0	0	3.7	0	0	0	0.9
<i>Alternaria</i>	0	0	6.7	5.7	0	0.9	0	0
Any allergen [‡]	0	12	47	74 [*]	53 [†]	20	57 [*]	27
<i>Ascaris</i>	—	—	60	70	—	—	57 [†]	22
Total IgE (geomean IU/mL)			775	1034			257 [*]	93

* $P < .05$ Pearson's χ^2 difference between EIB and non-EIB groups.† $P < .01$.‡Positive to any allergen tested (not including *Ascaris*).

§There was a poor correlation between serum positives and skin test positives in Kabati, where only 5.2% of the children who were identified as positive by serum IgE also had a positive skin test. The correlation was better in Thika (30%) and much better among the children in Atlanta (62%).

||There were significantly more children with a positive skin test among those with EIB in Thika compared with those with EIB in Kabati ($P = .003$).

In the highlands of Kenya, the most common tropical diseases are helminth infections, predominantly *Ascaris* and hookworm. These infections can increase total IgE, possibly as a result of carbohydrates on soluble antigens acting as adjuvants.¹⁹ An inverse relationship has been observed between asthma and parasitic infection in rural African communities; however, rather than cause and effect, these two are more likely to be independent features of rural living.^{11,12} In some countries, helminth infection has been associated with asthma.²⁰ In South Africa, a series of studies showed that both asthma and helminth infection were more common in the townships of Capetown than in rural areas.⁵ By contrast, in Venezuela, treatment of *Ascaris* infection reduced total IgE and specific IgE to allergens.²¹ Taking the studies together, it seems unlikely that our results can be explained simply by a decreased helminth load in the urban area.^{10-12,21,22}

Our finding of serum IgE antibodies to cat in the village without positive skin

tests is similar to the report for dust mite in Gabon. Although it has been suggested that the decrease in atopy associated with parasitic infection could be because of increased production of interleukin-10 suppressing skin-test reactivity,²² we do not believe that the results can be ascribed simply to suppression of skin tests. The children we studied did not have high exposure to cats by history; they had not made IgG antibodies to cat, and the apparent IgE antibodies to cat could not be inhibited by cat extract (data not shown). Studies in other African countries have shown very low levels of cat allergen in houses.⁸ Positive IgE antibodies for mite allergen could be inhibited in 80% of the sera in the village. Taken together, the results suggest that the IgE antibody results for cat and dog in the village should be regarded as false positives. The children in Kabati had normal levels of IgG antibodies to *Tetanus toxoid* and many of them had made both IgE antibodies and IgG antibodies to antigens derived from *Ascaris*. However,

very few of them had detectable IgG antibodies to dust mite or cat allergens. Thus, there were no findings to suggest that the differences between the village and the town could be ascribed to an overall shift from a Th₁ to Th₂ immune response. Similarly, the results do not suggest that any of the children had made a modified Th₂ response (ie, IgG and IgG4 without IgE antibodies) to cat allergen, which has been found in up to 20% of asymptomatic white children in the United States.²³

The "cleanliness hypothesis" suggests that increases in allergy and asthma in the Western world can be related to decreased early life infections or decreased exposure to endotoxin.²⁴⁻²⁶ In each case, it has been implied that these changes have led to a general decrease in Th₁ responses and an increase in Th₂. Although conditions in some areas of the developed world could be regarded as excessively clean, that would not be true for the small towns of Kenya, Ethiopia, or South Africa.^{3,5,27} Furthermore, it is very unlikely that children

living in the towns are exposed to less viral infections in childhood. It could be argued that the differences between Kabati and Thika are the result of improved nutrition, less exposure to livestock, and/or treatment of helminth infection. However, changes of this kind were complete in the Northern cities of the United States by 1920. Thus, they may relate historically to increases in allergic rhinitis but not to the increase in asthma, which did not start until the 1960s.

The children in Atlanta were taller and heavier than children in Africa but, despite having larger lungs, their FEV₁ as a percentage of predicted values was lower (ie, 89% compared with 96% in Africa). In preliminary results, EIB was more common in Atlanta (~20%) than the 2 Kenyan populations (11.7%). Among the children in the United States, there was a good correlation between skin tests and serum IgE antibodies. In addition, the prevalence of IgG antibodies to cat was high in this area where exposure to dust mite is common. The possible explanations for the increased severity of asthma in the United States compared with the towns in Africa include housing conditions, antibiotic use, and diet. However, the objective finding in our study is the increased weight and body fat of the children in Atlanta. We believe that changes in diet, body fat, and physical activity should all be considered in evaluating the increase of asthma in the cities.

In conclusion, children living in a rural Kenyan village had a very low prevalence of asthma, with only one child of 129 having both symptoms and evidence of bronchial hyperreactivity. Furthermore in this village, there was no relationship between markers of atopy (total IgE, skin tests, or IgE antibodies) and either symptoms of asthma or bronchial hyperreactivity. By contrast, in a town only 30 km away, the association between atopy and asthma, which is so obvious in the West, was present. Changes in body habitus that

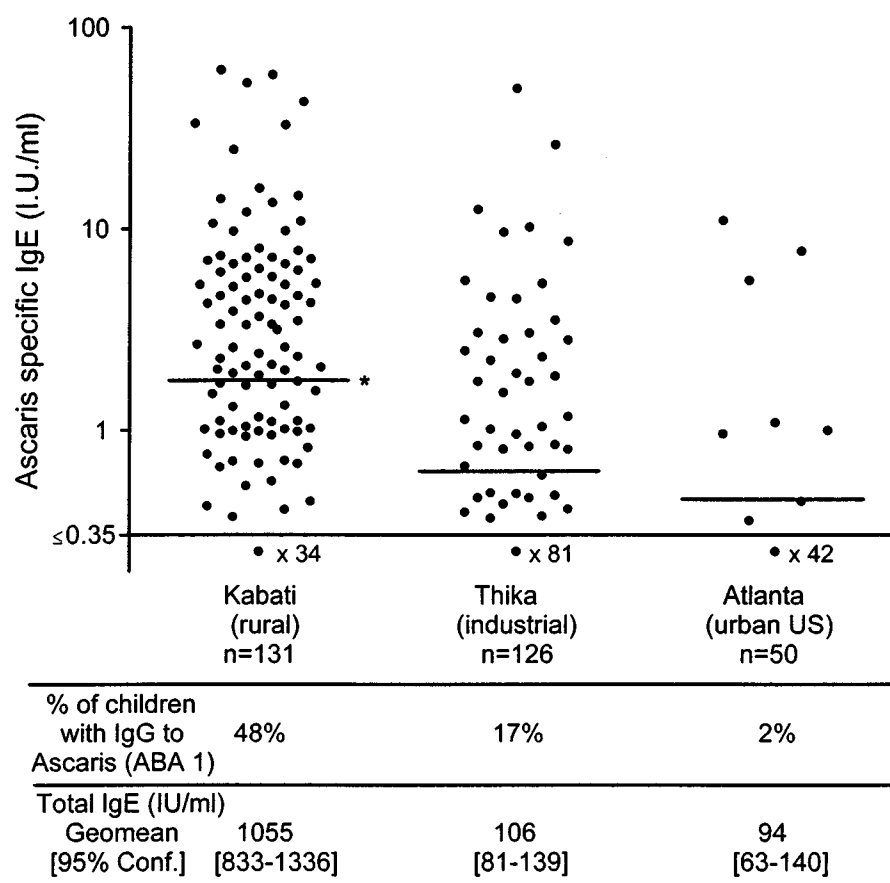


Figure. *Ascaris*-specific IgE in the serum of children in Kabati and Thika, Kenya, and Atlanta, Georgia, with the geometric means and 95% confidence intervals of total IgE of the same groups. There were 34, 81, and 42 children with <0.35 IU IgE/mL to *Ascaris* in Kabati, Thika, and Atlanta, respectively. IgG antibodies to the *Ascaris* protein ABA-1 were measured by precipitation assay (>200 U/mL was considered positive). The total IgE in Kabati was significantly higher than in Thika ($P < .001$) or Atlanta ($P < .001$). Bars represent the geometric means of the specific IgE to *Ascaris*. In calculating the geometric mean values, the negative results were allotted the lowest detectable value (ie, 0.35 IU/mL). The prevalence of IgE antibodies to *Ascaris* was significantly different between each of the sites ($P \leq .01$).

are increasingly recognized as an important aspect of poverty in the United States and also as a risk factor for asthma, were modest between Thika and Kabati.^{28,29} The results suggest that significant immunologic changes can occur with moderate changes in lifestyle in Africa. Although asthma was more common in the urban area, it was not comparable to the severity or prevalence of asthma, either in the inner cities of the United States or in other Western countries.^{1,2,6,9,30} The results suggest that there are several elements involved in the progressive increases in asthma. First, there is the appearance of the association between atopy and asthma

seen clearly in the comparison of Kabati and Thika. Second, there is evidence of an increase in the prevalence of positive skin tests, although when this occurred in Western societies is not clear. Third, there is the progressive increase in the severity of asthma among allergic children that has occurred in many countries during the last 30 years.

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