Prenatal risk factors of wheezing at the age of four years in Tanzania

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Abstract

***Background*—A study was undertaken to assess the interactions between prenatal exposures, early life infections, atopic predisposition, and allergen exposures in the development of wheezing up to the age of 4 years in a tropical region of Africa.**

***Methods*—The study subjects comprised children born at the district hospital in Ifakara, Tanzania during a 1 year period who were participating in a trial of iron supplementation and malaria chemoprophylaxis during the first year of life and followed for up to 4 years. From this group of subjects, 658 (79%) participated in the interview at 18 months and 528 (64%) in a second interview at 4 years. Wheezing was measured with the ISAAC questionnaire. A hospital based inpatient and outpatient surveillance system was set up to document all attendance by study children for any cause, including episodes of clinical malaria and lower respiratory tract infections. Total IgE levels and malaria parasites were measured in maternal and cord blood. Total IgE was also measured at 18 months of age. Indoor environmental levels of *Der p* I and *Fel d* I were determined using an enzyme linked immunosorbent assay at the same time as the interview at the age of 18 months.**

***Results*—The prevalence of wheezing at 4 years is common in Ifakara (14%, range 13–15%). The presence of malaria parasites in cord blood (odds ratio, OR = 6.84, 95% CI 1.84 to 24.0) and maternal asthma (OR = 8.47, 95% CI 2.72 to 26.2) were positively associated with wheezing at the age of 4 years, and cord blood total IgE was negatively associated (OR = 0.24, 95% CI 0.07 to 0.85) (all p<0.05). Parasitaemia at birth was not related to total IgE levels in cord blood (p=0.6). Clinical episodes of malaria during infancy were not associated with wheezing, and nor were levels of indoor aeroallergens.**

***Conclusion*—These findings suggest that events occurring during pregnancy may play a role in the future appearance of wheezing, although the results must be interpreted with caution because of the small numbers studied.**

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Keywords: Africa; asthma; atopy; malaria; total IgE; wheezing

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Wheezing in early life is common.[1] In a few children early wheezing portends recurrent episodic wheezing and subsequent asthma.[2] At present there is no clear understanding of what factors drive the development of asthma. There is some indirect evidence that early life infections are protective against IgE sensitisation[3–5] and childhood asthma.[6–8] Furthermore, some in vitro studies have suggested that the in utero environment is important in the development of asthma.[9] Longitudinal studies collecting data from birth have found a positive association between low respiratory tract infections (LRTI) and asthma,[10–12] a lack of association between LRTI due to syncytial virus (RSV) and wheezing at age 13,[13] and a positive association between the use of antibiotics and asthma.[10] The problem with current epidemiological studies in the developed world is that they may not be helpful for investigating the onset of asthma because common childhood infections such as measles now occur rarely and most children are prescribed antibiotics at some time in early childhood. The more convincing answers therefore seem likely to come from similar studies in the developing world,[14] although the high frequency of parasite infestation introduces complexities in the maturation of the immune system and its relation with asthma in these regions.[15]

Another hypothesis is that the onset of asthma is related to exposure to allergens. It is widely accepted that exposure to common aeroallergens in the very early years of life is important in determining subsequent development of IgE sensitisation[16] and that IgE sensitisation is strongly linked with development of respiratory symptoms and asthma.[14] However, this evidence is not without disagreement.[17 18] In a recent study in a rural area of Ethiopia[19] reactivity to environmental allergens was negatively associated with asthma, and a similar result was reported in mothers of children participating in the present study in Tanzania.[20]

We had the opportunity to follow up to the age of 4 years a birth cohort of children from Ifakara, Kilombero district in southern Tanzania, in whom the presence of malaria and lower respiratory tract infections (LRTI) had been established, together with the direct measurement of aeroallergens in a subsample. We aimed to assess the interactions between prenatal exposures, early life infections, atopic predisposition, and allergen exposures during infancy in the development of wheezing up to the age of 4 years.

Table 1 Frequency of wheezing at 18 months and 4 years and of variables of interest by sex in children in Ifakara (Tanzania)

	Male (n=335)	*Female (n=338)*	*Total (n=673)*
Respiratory symptoms			
Wheezing at 18 months	81 (24%)	67 (20%)	148 (22%)
Current wheezing at 4 years	39 (16%)	34 (13%)	73 (14%)
Frequent wheezing at 4 years (>2 episodes)	19 (7.8%)	14 (5.3%)	33 (6.2%)
Wheezing at 18 months and 4 years			
Never	139 (59%)	171 (68%)	310 (63%)
Persistent	20 (8.5%)	15 (6%)	35 (7.2%)
Only once	77 (33%)	67 (27%)	144 (29%)
Malaria parasites in cord blood	11 (3.3%)	13 (3.9%)	24 (3.4%)
Malaria intervention before 12 months			
Deltaprim	75 (23%)	95 (28%)	170 (26%)
Iron	76 (23%)	89 (26%)	165 (25%)
Iron + deltaprim	85 (26%)	94 (27%)	179 (27%)
Malaria hospital admissions during first 4 years of life			
At least one admission	239 (71%)	249 (73%)	488 (72%)
Yearly incidence (%)	22.0	21.2	21.8
LRTI before 18 months			
At least one episode	174 (52%)	176 (52%)	350 (52%)
No of episodes*	0.91 (1.20)	0.76 (0.92)	0.83 (1.0)
Total IgE (kU/l)			
Cord blood >1.9 kU/l	50 (19%)	42 (16.5%)	92 (18%)
IgE at 18 months*	394 (529)	323 (431)	359 (483)
Parity			
First	89 (26%)	102 (30%)	191 (28%)
Four or more	120 (36%)	103 (30%)	223 (33%)
Maternal			
Age in years*	26.2 (5.5)	25.6 (2.6)	25.9 (6.2)
Current asthma†	12 (5.5%)	6 (2.6%)	18 (4.0%)
Atopy‡	162 (62%)	166 (64%)	328 (63%)
Total IgE >1000 kU/l	149 (57%)	160 (62%)	309 (60%)
Total IgE >2000 kU/l	76 (29%)	85 (33%)	161 (31%)
Environment			
Indoor fire, no ventilation	75 (22.3%)	67 (20.0%)	142 (21%)
No of residents in household*	5.9 (3.3)	6.2 (3.1)	6.0 (3.1)
Breastfed at 12 months	323 (97%)	324 (96%)	647 (96%)
Der p I in dust (μg/g)*	2.0 (5.6)	2.5 (6.9)	2.3 (6.1)
Fel d I in dust (μg/g)*	0.4 (1.6)	0.2 (0.8)	0.3 (1.3)

*Mean (SD).
†Defined as having been woken by an attack of shortness of breath, or having had an attack of asthma, or currently taking any medicine for asthma in the last 12 months.
‡IgE antibodies to cockroach or *Der p* I >0.35 kU/l.

Methods

STUDY DESIGN AND SUBJECTS

The characteristics of the area and study population have been described in detail elsewhere.[21] Briefly, the study was carried out in the town of Ifakara, an area of intense and perennial malaria transmission. The study subjects were children who were participating in a trial looking at the effect of iron supplementation and malaria chemoprophylaxis on anaemia and malaria during the year 1995. Children were followed from delivery up to 4 years of age in four randomised groups.[21] Among the 1086 newborn babies at the district hospital of Ifakara during the years 1995–6 (about 70% of all births in the area), 832 were included in the trial. Of these, 658 (79%) participated in an interview at 18 months that was originally set up to assess the effect of the intervention on malaria, and also included a respiratory questionnaire. Reasons for non-respondence were mainly death and severe anaemia.[21] A second interview was performed when the children were aged between 3 and 4 years (mean (SD) age 3.89 (0.25) years) which was answered by 528 mothers (63%), 489 of whom also participated in the interview at 18 months.

MEASUREMENT OF WHEEZING

A questionnaire on the history of current wheezing and environmental conditions based on the AMICS questionnaire[22] was administered to mothers during the visit at 18 months. The questionnaire was adapted to Swahili by a bilingual paediatrician from Ifakara hospital and back translated by another bilingual worker. Two trained field workers carried out the interviews. Wheezing at 18 months was defined as a positive answer to the question "Has your child ever been wheezing or whistling in the chest?". A new questionnaire including the respiratory symptoms questionnaire of the ISAAC[23] was used at the 4 year visit in 1999. Wheezing at 4 years was defined as a positive answer to the question "Has your child had wheezing or whistling in the chest in the last 12 months?". Frequent wheezing at 4 years was defined as reporting more than two episodes of wheezing based on the question "How many attacks of wheezing has your child had in the last 12 months?". Persistent wheezing was defined as having wheeze both at 18 months and 4 years.

CLINICAL SURVEILLANCE AND DEFINITIONS OF MALARIA AND LRTI

A hospital based inpatient and outpatient surveillance system was set up to document all attendance by study children and is described in detail elsewhere.[24] The hospital based passive case detection was complemented with cross sectional surveys carried out at 2, 5, 8, 12, and 18 months of age for the measurement of clinical malaria. Malaria infection was defined as the presence in the blood of the asexual

Table 2 Frequency (% or mean) of study variables according to wheezing

Variable†	*No wheezing (n=455)*	*Current wheezing (n=73)*	*Frequent wheezing (n=33)*	*Persistent wheezing (n=35)*
Malaria				
Cord blood parasite (any)	2.3%	9.6%*	12%*	14%*
Intervention group				
Deltaprim	50%	47%	51.5%	40%
Iron	39%	40%	36%	40%
Clinical malaria episodes				
1–2	30%	30%	36%	23%
>2	39%	40%	36%	40%
LRTI before 18 months				
>1 episode	54%	67%*	70%*	80%*
No of episodes	0.80	1.56*	1.87*	2.0*
Total IgE (kU/ml)				
Cord blood >1.9 kU/l	19%	5.9%*	4.5%	10%
Mean at 18 months**	150	124	136	125
Parity				
First	27%	27%	37%	20%
Four or more	34%	27%	20%	34%
Maternal				
Mean age	26.2	25.8	24.4	27.1
Asthma	1.3%	10%*	9.4%	21%*
Atopy	63%	64%	80%	57%
IgE >1000 kU/l	62%	60%	57%	61%
IgE >2000 kU/l	32.5%	34%	38%	25%
Environment‡				
Fire, no ventilation	22%	23%	21%	28%
No of people	6.2	6.5	6.6	6.9*
Breastfed	97%	93%	92%	97%
Fel d I in dust (μg/g)**	0.35	0.57	0.12	0.84
Der p I in dust (μg/g)**	2.28	1.16	1.55	1.13

*p<0.05 compared with no wheeze at 4 years (or never wheeze in the case of persistent wheeze, which figures were very similar to no wheezers at 4 years).
†Definition in table 1.
‡Only included variables with p<0.25 in the crude association with wheezing, among the following variables: earth floor, humidity, electricity, number of rooms, density, occupation, parental smoking, animals. The minimum number of observations in a cell for allergens in dust was 8.
**Geometric mean.

parasite *Plasmodium falciparum* in any concentration at the time of contact with the health services, regardless of any signs or symptoms. Clinical malaria was defined as an axillary temperature of >37.5°C and the presence of any *P falciparum* asexual parasite in the blood.[25] This definition has a sensitivity of 100% and a specificity of 80% in young children against a doctor diagnosis.[21] Two episodes of malaria were considered as the same if the time interval between them was shorter than 28 days.

A history of hospital visits for LRTI was based on signs and symptoms (deep or "wet" chest cough, wheezing, hoarseness, stridor, or shortness of breath) recorded by both the inpatient and outpatient hospital surveillance system.[24]

LABORATORY METHODS

Parasites in cord blood were measured in thick and thin blood films using standard methods.[25] A sample of the mother's serum collected during delivery and of cord blood was stored at –20°C and a 1 ml aliquot was used to measure IgE levels. The detection level for total IgE in cord blood was 1.9 kU/l. The laboratory did not quantify values of total IgE higher than 2000 kU/l. Levels of total serum IgE and specific serum IgE against *Dermatophagoides pteronyssinus* (*Der p* I) and cockroaches in the mothers and total IgE levels in the newborn infants were measured by the CAP method (Kabi Pharmacia, Uppsala, Sweden) and the values expressed in kU/l. Dust samples were taken in all houses following a standard procedure (vacuuming an area of 1 m^2 for 3 minutes[22]) from children's mattresses (or stowma in 38% due to absence of mattress) when children were aged 18 months. We used a standard household vacuum cleaner (1000 W, Rowena) with battery, fitted with 8 μm filter paper (Whatman) in an ALK dust trap. The whole surface are of mattress covers was measured and vacuumed for the same duration. Environmental levels of *Der p* I and cat dander (*Fel d* I) were determined in 128 samples selected at random, using an enzyme linked immunosorbent assay described elsewhere.[22]

ANALYSIS OF DATA

The incidence of malaria was estimated using the person-years method and the relative risk calculated as the ratio of incidence rates. In

Table 3 Multivariate association (odds ratio and 95% confidence intervals)† with wheezing

	Current wheezing	*Frequent wheezing*	*Persistent wheezing*
Parasite in cord blood (yes/no)	6.84 (1.84 to 24.0)*	8.93 (2.24 to 35.1)*	10.2 (2.47 to 42.2)*
Total IgE in cord blood (yes/no)	0.24 (0.07 to 0.85)*	0.21 (0.03 to 1.62)	0.48 (0.12 to 1.91)
Maternal asthma (yes/no)	8.47 (2.72 to 26.2)*	3.94 (0.92 to 16.8)	14.3 (348 to 58.1)*
LRTI‡	1.76 (0.91 to 3.40)	1.68 (0.65 to 4.33)	2.70 (1.05 to 6.99)*
No of residents in household‡	1.03 (0.94 to 1.13)	1.03 (0.90 to 1.18)	1.12 (1.00 to 1.35)*

LRTI = lower respiratory tract infection.
†Variables included were those with a p value <0.1 in one of the three models from variables with a p value <0.25 in table 2. Each column is a regression model.
‡Per each episode or number of residents.

Table 4 Multivariate association (odds ratio and 95% confidence intervals) with current wheezing stratified by groups

	LRTI episodes		*Deltaprim*		*Iron*	
	Yes (n=254)	*No (n=200)*	*Yes (n=260)*	*No (n=265)*	*Yes (n=264)*	*No (n=261)*
Parasites in cord blood	9.4 (1.9 to 47)	3.2 (0.3 to 31)	6.4 (0.9 to 46)	7.8 (1.4 to 43)	2.8 (0.3 to 19)	11 (2.4 to 54)
IgE in cord blood	0.1 (0.0 to 0.9)	0.5 (0.1 to 2.8)	0	0.5 (0.1 to 1.9)	0.2 (0.1 to 1.2)	0.1 (0.0 to 1.2)
Maternal asthma	5.0 (1.0 to 24)	15 (2.9 to 74)	11 (1.7 to 66)	6.6 (1.6 to 28)	35 (3.5 to 357)	4.4 (1.0 to 20)*

LRTI = lower respiratory tract infection.
*p for interaction < 0.25.

addition, the association between the number of episodes of clinical malaria and wheezing was analysed with the odds ratio using logistic regression. In this analysis the frequency of malaria was measured as a cumulative incidence without taking account of the time span from birth to the first episode. Association with the other variables (IgE levels, history of LRTI, environmental factors, and aeroallergens) was also analysed with the odds ratio using logistic regression with the STATA package. Adjustment for confounding variables was carried out with multiple logistic regression. Interactions were assessed using a stratified analysis and determined by testing the significance of the interaction term in the logistic regression model; p values of <0.05 were considered to be statistically significant.

Results

The prevalence of current wheezing during infancy is high in Ifakara (13.8%, range 12.9–14.7) although only a small proportion persisted both at 18 months and at 4 years of age in both sexes (table 1). Table 1 also shows that clinical episodes of malaria and hospital visits for LRTI were very common, as well as maternal atopy. Total IgE was high both in mothers at delivery and in children at 18 months. Housing and environmental conditions of these children were those commonly found in semi-rural Africa—that is, most of the mothers working in agriculture (96%), African chickens in 43% of the houses, few mothers smoking (5%), small crowded houses (27% with a single room), earth floor (73%), and no electricity (86%).

Clinical episodes of malaria were not associated with wheezing (table 2). The rate ratio between the incidence of malaria and wheezing was 0.97 (range 0.71–1.31). However, wheezing was more common in children with malaria parasites in the cord blood. Of the other risk factors studied, children with a hospital visit for LRTI had more wheezing (table 2), as did children with low levels of total IgE in cord blood and those with maternal asthma. Total IgE levels at 18 months, parity, maternal atopy, and maternal levels of total IgE were not associated with wheezing. Of the environmental variables studied, only the number of people living in the house was positively associated with wheezing, particularly with persistent wheezing. Indoor allergen levels were not associated with wheezing.

Malaria parasites in cord blood, a history of hospital visits for LRTI, low levels of total IgE at birth, and maternal asthma were independently associated with wheezing at the age of 4 years (table 3). Similar associations, except maternal asthma, were found for frequent wheeze. Persistent wheeze was associated with the same variables in addition to the number of residents in the household. Neither maternal age or sex of the children, nor the variables stated in table 2 (also in the footnote) confounded these associations

Stratification by a history of hospital visits for LRTI or by the intervention trial subgroup did not modify any of the associations (table 4). Only an interaction between maternal asthma and the subgroup being treated with iron had a p value <0.25 (p=0.245).

Discussion

This is the first follow up study carried out in a developing country to assess the interactions between infections, predisposing factors, and environmental variables in the onset of wheezing. We have found that children were more likely to experience wheezing at the age of 4 if they had a prenatal exposure to malaria, low levels of total IgE at birth, and maternal asthma, and also clinical episodes of LRTI during infancy. However, a diagnostic bias may explain the association between LRTI and wheezing. Neither a history of clinical malaria nor the incidence of malaria was associated with wheezing. Among the environmental factors, only the number of people in the home was moderately associated with wheezing. Finally, indoor allergens were not associated with wheezing, although measurement error in dust collection could explain the lack of association.

Bacterial and possible viral infection during early life has been considered as a promoter or suppressor of asthma onset via the immune maturation[26] or exogenous inflammatory stimuli of the airways epithelium.[27] The role of parasites in asthma is also controversial.[28 29] There are no studies on the role of malaria in asthma, although malaria is extremely common in some tropical regions. In laboratory mice, dual infection by Epstein-Barr virus and malaria in early life promoted the production of antigens against malaria and the production of a non-cognate IgE response.[30] Helminth infestation may suppress allergic responses by reducing the production of specific IgE antibody[31] or by saturating the IgE receptor on mast cells and blocking access to specific IgE.[32] It is not known whether an intracellular parasite can produce the same effect. It is important to consider whether malaria infection may involve different mechanisms (Th1, Th2, and others) at different stages of infection[33] which might explain in part the different association with the parasite during pregnancy from that after birth.

Total IgE in cord blood was negatively associated with wheezing at 4 years of age and was not related to the presence of malaria parasites in cord blood (p=0.6). The fact that parasites produced both IgE specific to parasites and non-specific polyclonal IgE[31] introduces complexities which are difficult to understand with the present knowledge. In addition, we found no association between maternal atopy and childhood wheezing, which does not agree with the observed association in the developed world.[34] However, in tropical areas, definition of atopy by the presence of IgE antibodies is limited since a degree of cross reactivity between parasitic infection and *Der p* I may occur.[19] These mechanisms also may explain why we did not find an association with indoor allergens in dust.

Direct evidence of the role of respiratory infections in asthma comes from a retrospective cohort study in Oxford which found a positive association between respiratory infections (including whooping cough, croup, and chicken pox) and asthma.[10] This positive association with respiratory infections was also observed in a similar study in Scotland[11] and in a follow up study in Australia.[12] We also observed a positive association with wheezing at 4 years (only statistically significant for persistent wheezing), as well as an association with increasing number of residents in the household (which may be a marker of infections). However, a diagnostic bias could not be excluded since the diagnosis of LRTI was based in some cases on the presence of wheezing and, since LRTI not only included hospital admissions but also visits to the outpatient clinic, it is possible that a misclassification with upper respiratory tract infections may have occurred. In addition, in a longer follow up study the association with RSV infection disappeared when the follow up was extended to 13 years of age.[13] Opinions tend to polarise between people who believe that symptomatic RSV LTRIs in early life induce or act as a marker of an atopic asthmatic phenotype, and those who believe that non-atopic mechanisms contribute to both the acute RSV illness and subsequent respiratory morbidity.[35] Nowadays, many studies argue against the hypothesis that LRTIs induce atopic asthma.[13 35] It is possible that LRTI was associated with wheezing through chronic inflammation of the airways rather than through an immunosensitive process.

Current maternal asthma, defined according to the ECRHS definition,[36] was strongly related to persistent wheezing, independently of the frequency of LRTI, which excludes the potential recall bias. Such a family predisposition was not unexpected given the genetic effect of asthma.[26]

Diagnosis of wheezing is less valid in an area with high prevalence of LRTI. However, the fact that the stratification of wheezers by LRTI did not modify the association between wheezing and parasites in cord blood, total IgE in cord blood, and maternal asthma suggests that this diagnostic bias is not differential. A second difficulty was standardisation of the dust collection protocol, given the characteristics of the houses. The measurement error in dust collection could explain the lack of association between indoor allergens and wheezing. In addition, only 658 of the 832 recruited (79%) were followed up. If the losses were related to malaria or other risk factors or wheezing, a bias could have occurred. This possibility is unlikely since the number and reasons for non-response was similar in the four intervention groups of the trial[21] and stratification by intervention group did not modify the associations. It is therefore unlikely that selection bias affected the observed association with prenatal exposures (parasites in cord blood, total IgE in cord blood, or maternal asthma) since these variables were unlikely to be related to non-response. A final limitation to the study is that the association with parasites in the cord blood is based on only a small number of cases with parasitaemia, but neither stratified analysis nor adjustment for all variables in the study modified the association.

In conclusion, this epidemiological study in an African area with a high prevalence of malaria and LRTI shows a novel association between blood parasitaemia at birth and wheezing, but not with clinical episodes of malaria after birth. Although these findings must be considered with caution, given the small numbers, they suggest that prenatal exposure to malaria and the levels of total IgE at birth may play a role in the occurrence of wheezing, perhaps through the in utero priming of fetally derived Th cells.

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1 Tager IB, Hanrahan JP, Tosteson, *et al*. Lung function, pre- and post-natal smoke exposure, and wheezing in the first year of life. *Am Rev Respir Dis* 1993;**147**:811–7.
2 Martinez FD, Wright AL, Taussig LM, *et al*. Asthma and wheezing in the first six years of life. The Group Health Medical Associates. *N Engl J Med* 1995;**332**:133–8.
3 Von Mutius E, Martinez FD, Fritzsch C, *et al*. Skin test reactivity and number of siblings. *BMJ* 1994;**308**:692–5.
4 Strachan DP. Hay fever, hygiene, and household size. *BMJ* 1989;**299**:1259–60.
5 Martinez FD, Stern DA, Wright AL, *et al*. Association of non-wheezing lower respiratory tract illnesses in early life with persistently diminished serum IgE levels. Group Health Medical Associates. *Thorax* 1995;**50**:1067–72.
6 Anderson HR. The epidemiological and allergic features of asthma in the New Guinea Highlands. *Clin Allergy* 1974;**4**: 171–83.
7 Flynn MG. Respiratory symptoms, bronchial responsiveness, and atopy in Fijian and Indian children. *Am J Respir Crit Care Med* 1994;**150**:415–20.
8 Shirakawa T, Enomoto T, Shimazu S, *et al*. The inverse association between tuberculin responses and atopic disorder. *Science* 1997;**275**:77–9.
9 Martinez FD, Holt PG. Role of microbial burden in aetiology of allergy and asthma. *Lancet* 1999;**354**(Suppl II):12–5.
10 Farooqui S, Hopkin JM. Early childhood infection and atopic disorder. *Thorax* 1998;**53**:927–32.
11 Bodner C, Godden D, Seaton A. Family size, childhood infections and atopic disease. *Thorax* 1998;**53**:28–32.
12 Ponsonby AL, Couper D, Dwyner T. Relationship between early respiratory illness, family size over time, and the development of asthma and high fever: a seven year follow-up study. *Thorax* 1999;**54**:664–9.
13 Stein R, Sherrill D, Morgan WJ. Respiratory syncytial virus in early life and risk of wheeze and allergy by age 13 years. *Lancet* 1999;**354**:541–5.
14 Lewis SA. Infections in asthma and allergy. *Thorax* 1998;**53**: 911–2.

15 Openshaw PJM, O'Donnell DR. Asthma and the common cold: can virus imitate worms? *Thorax* 1994;**49**:101–3.
16 Warner AM, Bjorksten B, Munir AK, *et al*. Childhood asthma and exposure to indoor allergens: low mite levels are associated with sensitivity. *Pediatr Allergy Immunol* 1996;**7**:61–7.
17 Kuehr J, Frisher T, Meinert R, *et al*. Sensitization to mite allergens is a risk factor for early and late onset of asthma and for persistence of asthmatic signs in children. *J Allergy Clin Immunol* 1995;**95**:655–62.
18 Pearce N, Pekkanen J, Beasley R. How much asthma is really attributable to atopy? *Thorax* 1999;**54**:268–72.
19 Yemaneberham H, Bekele Z, Venn A, *et al*. Prevalence of wheeze and asthma and relation to atopy in urban and rural Ethiopia. *Lancet* 1997;**350**:85–90.
20 Sunyer J, Torregrosa J, Menendez C, *et al*.The association between atopy and asthma in a semi-rural area of Tanzania. *Allergy* 2000;**55**:1–5.
21 Menendez C, Kahigwa E, Hirt R, *et al*. Randomised placebo-controlled trial of iron supplementation and malaria chemoprophylaxis for prevention of severe anaemia and malaria in Tanzanian infants. *Lancet* 1997;**350**:844–50.
22 Atkinson W, Harreis J, Mills P, *et al*. Domestic allergen exposures among infants in an English town. *Eur Respir J* 1999;**13**:583–9.
23 Pearce EN, Weiland S, Keil U, *et al*. Self-reported prevalence of asthma symptoms in children in Australia, England, Germany and New Zealand: an international comparison using the ISAAC written and video questionnaires. *Eur Respir J* 1993;**6**:1455–61.
24 Schellenberg D, Menendez C, Kahigwa E, *et al*. African children with malaria in an area of intense *Plasmodium falciparum* transmission: features on admission to the hospital and risk factors for death. *Am J Trop Med Hyg* 1999;**61**:431–8.
25 Kitua AY, Smith T, Alonso PL, *et al*. *Plasmodium falciparum* malaria in the first year of life in an area of intense and perennial transmission. *Trop Med Int Health* 1996;**1**:475–84.
26 Holgate T. The epidemic of allergy and asthma. *Nature* 1999;**402**:B2–4.
27 Holt PG, Macaubas C, Stumbles PA. The role of allergy in the development of asthma. *Nature* 1999;**402**:B12–17.
28 Masters S, Barrett-Connor E. Parasites and asthma: predictive or protective? *Epidemiol Rev* 1985;7:49–58.
29 Carswell F, Meakins RH, Harland PSEG. Parasites and asthma in Tanzanian children. *Lancet* 1976;2:706–7.
30 Watier H, Auriault C, Capron A. Does Epstein-Barr virus infection confer selective advantage to malaria-infected children? *Lancet* 1993;**341**:612–3.
31 Lynch NR, Hagel IA, Palenque ME. Relationship between heminthic infection and IgE resposne in atopic and non-atopic children in a tropical environment. *J Allergy Clin Immunol* 1998;**101**:217–21.
32 Godfrey RC, Gradidge CF. Allergic sensitisation of human lung fragments prevented by saturation of IgE binding sites. *Nature* 1976;**259**:484–6.
33 Reid TM. Striking a balance in maternal immune response to infection. *Lancet* 1998;**351**:1670–3.
34 Rusconi F, Galassi C, Corbo GM, *et al* and the SIDRIA collaborative group. Risk factors for early, persistent, and late onset wheezing in young children. *Am J Respir Crit Care Med* 1999;**160**:1617–22.
35 Everard ML. What link between early respiratory viral infections and atopic asthma. *Lancet* 1999;**354**:527–8.
36 Burney PG. The European Community Respiratory Health Survey. *Eur Respir J* 1994;7:954–60.