Atopy Is Inversely Related to Schistosome Infection Intensity: A Comparative Study in Zimbabwean Villages with Distinct Levels of *Schistosoma haematobium* Infection

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Abstract

Background: The hygiene hypothesis suggests that parasitic infections protect against allergic diseases by modulating the host's immune responses. Experimental studies indicate that this protection depends on the intensity of parasitic infection, but this observation has not been tested in human populations. The aim of this study is to investigate whether the intensity of *Schistosoma haematobium* infection is related to atopic responses and whether this relationship differs between populations with distinct parasite transmission dynamics. *Methods:* The study was conducted in two villages with different *Schistosoma haematobium* transmission dynamics, i.e. high (n = 365) and low (n = 307) transmission. Allergic reactivity to the common house dust mite *(Dermatophagoides pteronyssinus)* was measured by skin prick tests and allergen-specific IgE and IgG4 quantified by enzyme-linked immunosorbent assay. Atopic responses were related to current infection intensity and schistosome transmission levels. *Results:* Schistosome infection intensity was nega- tively associated with the skin prick reactivity, mite-specific IgE and the ratio IgE/IgG4 in the high-transmission village. However, when only low levels of infection were analyzed in the 2 villages, there was no correlation between mite-specific responses and infection intensity. *Conclusion:* The relationship between schistosome infection and atopic responses is dependent on the intensity of current schistosome infection. Thus, consistent with results from animal models, with an increasing parasite burden, the immunoregulation of immune responses to allergens appears to become more pronounced.

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Introduction

Atopy, i.e. the genetic predisposition to become IgE-sensitized to environmental allergens [1], is an important risk factor for allergic diseases but only a small proportion of atopic individuals develop clinical symptoms [2, 3]. The clinical manifestation of allergic symptoms in atopic individuals is influenced by environmental factors such as air pollution, diet and exposure to pathogens [4], leading to the heterogeneous distribution of atopic diseases worldwide [5]. The rise in atopy with urbanization

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has been attributed to reduced exposure to pathogens and their products in childhood as a result of improved hygiene [4]. In particular, helminth parasites are believed to induce regulatory immune responses that dampen atopic responses [6], but this remains controversial [7, 8]. Animal studies have shown that only heavy, chronic helminth infection provides protection against atopy [9], which would explain some of the conflicting results from human studies [8, 10]. To date, despite the several elegant mechanistic studies in the mouse models of the relationship between helminth infections and atopy [11], only a few of the hypotheses and mechanisms have been validated in human studies. In particular, there is a paucity of studies investigating the effects of heterogeneities in host and parasite genotype, parasite transmission dynamics and previous history of host infection.

A meta-analysis of human epidemiological studies has suggested that in areas of high helminth (geohelminth) prevalence, the risk of allergy is decreased, whereas in areas of low prevalence this risk is increased [12]. This highlights the importance of helminth infection levels in the epidemiological patterns of helminth and atopy in human populations.

We therefore set out to investigate the effect of different parasite transmission dynamics on atopic responses in human populations naturally exposed to *Schistosoma haematobium* which causes urogenital schistosomiasis. To capture the dynamics of the relationship between helminth infection and atopy, the study was conducted in two villages of differing schistosome transmission dynamics, i.e. low and high transmission areas, resulting in differences in current infection intensity as well as in the cumulative history of schistosome infection [13, 14]. Skin prick reactivity to the house dust mite (HDM) *(Dermatophagoides pteronyssinus)* – the most prevalent allergen in Zimbabwe [15, 16] – as well as levels of schistosome-specific and HDM-specific IgE and IgG4 antibody responses were related to schistosome infection intensity in the two villages.

Materials and Methods

Study Design
The study was comparative, contrasting the levels of atopic responses in high- and low-schistosome infection villages. This comparative approach, used by us and in other studies [14, 17–20], is very powerful for studying disease and immunology patterns where the history of exposure to the pathogen cannot be separated from age in lifelong residents of endemic areas. As previously published [21], Zimbabwe has low levels of geohelminths. In addition, *Schistosoma mansoni* levels are also low in most regions of Zimbabwe [22], while *S. haematobium* is the most prevalent helminth infection in Zimbabwe. In this study area, the prevalence of *S. mansoni* was 2%, thus the study focused on *S. haematobium* infection. Differences in infection levels reflect the differences in infection transmission rates and in the history of infection [13]. Subjects in the high-infection village accumulated infection more rapidly, acquiring higher infection intensities at a younger age than their counterparts in the low-infection village [13].

Study Area and Population
The study was conducted in two villages, Magaya and Chitate, in the Mashonaland East province of Zimbabwe where *S. haematobium* is endemic. The study villages are in close proximity within a 10-km range of each other and villagers are of similar ethnicity (Shona) and socioeconomic background (rural subsistence farmers). Safe water and sanitation coverage are equally poor in the villages (indicated by our questionnaire studies). The only obvious difference in the villages is the seasonality of the rivers which provide habitats for schistosome intermediate-host snails.

Magaya village is characterized by perennial rivers which lead to high transmission rates of schistosome parasites compared to Chitate village which is characterized by seasonal streams. In addition, households in Magaya are built along the rivers, whereas in Chitate they are more dispersed and are built further from the rivers. Human contact with water harboring cercariae, the infective stage of schistosomes, is frequent (assessed by questionnaire studies) in this area due to insufficient safe water and sanitation facilities.

In 2002, a revised stratification based on a national parasite prevalence survey, Health Management Information Systems data, entomological data and expert opinion classified our study area under the sporadic transmission regions with low transmission [23, 24] meaning that this is a mesoendemic area for *Plasmodium* transmission and malaria [25].

Ethical Statement
Permission to conduct the study was obtained from the Provincial Medical Director, institutional and ethical approval was received from the University of Zimbabwe and the Medical Research Council of Zimbabwe, respectively. All participants had the aims and procedures of the project explained and written consent was obtained before enrolment into the study, and all were offered treatment with the recommended dose (40 mg/kg of body weight) of the antihelminthic drug, praziquantel. For young children, written consent was obtained from parents/guardians.

Sample Collection
The gold standard method for detecting and quantifying schistosome infection is through microscopic enumeration of eggs excreted in stool or urine [26]. Stool samples for the diagnosis of *S. mansoni* and geohelminths and urine specimens for the diagnosis of *S. haematobium* infection were collected on 3 consecutive days and processed using standard procedures [23, 24]. For infants unable to produce stool or urine samples on demand, an overnight sample was collected by the parent/guardian and submitted on the following day. Up to 5 ml venous blood was collected from each participant in citrate tubes, which was used to obtain sera for antibody assays. Malaria status was determined by

blood smears and confirmed by a serological Paracheck dipstick (Orchid Biomedical systems).

Inclusion Criteria

To be included in the study, participants had to (1) be lifelong residents of the village (assessed by questionnaire), (2) be free from the geohelminth, *S. mansoni*, and *Plasmodium* infections (7 people were excluded on this criterion because they were *S. mansoni*-positive but none was positive for *Plasmodium* infection, which is consistent with this area being mesondemic for *Plasmodium* transmission [25]), (3) not have previously received antihelminthic treatment (no one was excluded on this criterion), (4) provide urine and stool samples for helminth diagnosis and a blood sample for serological assays (107 people were excluded on this criterion because they did not provide either of the samples, see table 1a). A total of 672 individuals fulfilled the inclusion criteria and formed the study populations of Magaya (n = 365, age range 3–86 years) and Chitate (n = 307, age range 1–86 years) described in table 1a.

Skin Prick Test

Skin prick tests (SPTs) were conducted (on a small consenting population, n = 332) using 4 allergens prevalent in Zimbabwe [15]: HDM, Grass mix, *Alternaria tenius* and *Aspergillus fumigatus*. Briefly, a drop of each allergen was placed on the upper side of the lower arm before the skin beneath the drop was pricked using a lancet. The skin was then wiped, and 15 min later, the size of the wheal diameter was measured whenever the skin reacted for a given allergen. Histamine and saline solutions (Diagenics Ltd) were used as positive and negative controls, respectively. Reactions were considered positive if the wheal diameter was equal to or greater than the positive control.

This procedure showed that allergy to the HDM was the most prevalent (67.8% of the positive reactions were due to HDM alone), consistent with previous studies from Zimbabwe [15, 16]. Therefore, serological assays focused on *D. pteronyssinus*-specific responses.

Serology and Laboratory Assays

Levels of antibodies directed against parasite antigens were measured by indirect enzyme-linked immunosorbent assay (ELISA) following published procedures [13] using soluble *S. haematobium* adult-worm antigen (SWAP) and soluble egg antigen (SEA) from the Theodor Bilharz Institute (Egypt). The parasite strain is one used in previous epidemiological studies [25, 26]. Mite-specific responses were measured by ELISA using natural affinity-purified *D. pteronyssinus* Der p 1 allergen from Indoor Biotechnologies. Microtiter plates were coated overnight at 4°C with 50 μl/well of Der p 1 allergen in 5 μg/ml carbonate-bicarbonate buffer. Serum samples were diluted at 1:10 and the secondary antibodies, anti-human horseradish peroxidase conjugated IgE (Sigma) and IgG4 (the binding site) at 1:1,000. The substrate 2, 2′-azino-bis-(3-benzthiazoline-6-sulfonic acid) (Southern Biotech) was used for the colorimetric reaction and the optical density read at 405 nm. All sera were assayed in duplicate and a pool of sera from 6 individuals presenting with the highest skin-prick reactivity was included on each plate to serve as a positive control. A blank control containing no sera was included on each plate (in duplicate) and the background absorbance of reagents in the absence of serum was subtracted from all readings.

Statistical Analysis

Schistosome infection prevalence and intensity were compared between the 2 villages by Pearson χ^2 test and analysis of variance (ANOVA), respectively. Age was categorized into 5 groups (table 1). The ANOVA used sequential sums of squares to calculate the mean squares to derive the test statistic. These allow the hierarchical decomposition of the sources of variation in the dependent variable, so that the effects of the preceding variable on the test variable are taken into account before testing for the variable of interest [19]. In this way, they allow for confounding variables before testing for the effects of the variable of interest. This approach has been used to test for the relationship between schistosome infection and immune responses or other markers of immunity or pathology, while allowing for the potential confounding effects of the sex and age of the host [27].

The effect of mite-specific antibodies on the SPT was assessed by ANOVA using sequential sums of squares to account for the effects of host age, sex, village of residence and infection intensity [28].

A binary stepwise logistic regression was used to determine the relationship between SPT status and antibody levels with age, sex, village of residence and infection intensity included in the model.

Following exploratory plots, the hypothesis that helminth infection is negatively associated with atopy was tested using a 1-tailed Pearson correlation analysis between the SPT results and infection intensity while controlling for the effects of host age, sex and village of residence. To test whether this association occurs at the immunological level, the same correlation analysis was conducted relating antibody levels to infection intensity in each village. The effect of infection intensity on antibody responses (Der p 1- and schistosome-specific) was determined by multivariate analysis using sequential sums of squares, entering the variable 'infection intensity' last in the model [28]. β-coefficients (for the direction of infection-antibody relationship) were calculated using linear regression analysis on residuals saved after accounting for the variations due to age, sex and village. All statistical analyses were conducted using PASW statistics (version 17; formerly called SPSS) and values of p < 0.05 were considered significant.

Results

S. haematobium *Infection Levels Differ Significantly between the Two Villages*

Schistosome infection levels were significantly higher in Magaya than Chitate with a prevalence of 45.4 versus 8.5% (χ^2 = 111.4, d.f. = 1, p < 0.001) and mean infection intensity of 26.3 eggs/10 ml versus 0.755 eggs/10 ml ($F_{1, 662}$ = 92.75, p < 0.001), respectively. When partitioned by age, there were significant differences in infection levels in the two areas in younger age groups (fig. 1). Infection peaked at a higher level and at an earlier age in Magaya (fig. 1), a phenomenon referred to as peak shift [29] and confirmed by a significant age and village interaction ($F_{4, 662}$ = 5.938, p < 0.001) [13].

Table 1. Study population description

a Demography and socioeconomic status in the study areas

	Magaya (HTA)	Chitate (LTA)
Number of participants (baseline)	442	344
Mean age, years (range)	11.4 (3–86)	18.5 (1.5–86)
Sex ratio (M/F)	0.825 (199/241)	0.57 (127/221)
Ethnic group	Shona	Shona
Socioeconomic status	rural subsistence farmers	rural subsistence farmers
S. haematobium prevalence	52.94% (95% CI 48–57.67)	8.6% (95% CI 5.9–12.1)
S. haematobium intensity, eggs/10 ml (range)	44.79 (0–859), SEM = 5.654	0.958 (0–70.3), SEM = 0.2723
S. mansoni cases (excluded)	6 cases (7–13 years old)	1 case (69 years old)
Malaria prevalence	0%	0%
STH prevalence	0%	0%
No urine sample provided (excluded)	16 cases (3–26 years old)	2 cases (unknown age)
No blood sample provided and/or no age record (excluded)	55	34
Total number of participants included	365	307
SPT sampled	26 (3–65 years old, mean infection = 2.69 eggs/10 ml, range 0–22 eggs/10 ml)	306 (1.5–86 years old, mean infection = 0.758 eggs/10 ml, range 0–30 eggs/10 ml)

b *S. haematobium* infection levels by age in the selected populations

Age group years	Magaya (HTA)			Chitate (LTA)			Comparison (statistics)	
	Infection intensity range (sample size)	Infection intensity mean (SEM)[a]	Infection prevalence, % (95% CI)[b]	Infection intensity range (sample size)	Infection intensity mean (SEM)	Infection prevalence, % (95% CI)	Prevalence χ^2 and (p value)	Infection intensity F and (p value)
1–5	0–458 (47)	28.3 (13.5)	32 (20–50)	0–0.3 (38)	0.009 (0.0088)	2.6 (0.07–14)	11.35 (**<0.001**)	15.95 (**<0.001**)
6–10	0–481 (180)	20.6 (5.1)	44.4 (40–50)	0–27.5 (103)	0.67 (0.3)	8.7 (4–20)	42.8 (**<0.001**)	39.39 (**<0.001**)
11–15	0–692.7 (85)	42.5 (12.1)	60 (50–70)	0–20 (55)	0.99 (0.49)	12.7 (5–20)	34.05 (**<0.001**)	46.5 (**<0.001**)
16–20	0–50.7 (13)	5.8 (4.1)	30.7 (10–60)	0–25.7 (16)	2.5 (1.63)	25 (7–50)	0.115 (0.734)	0.021 (0.885)
21 and above	0–3.5 (17)	0.3 (0.2)	17.7 (4–40)	0–30.3 (75)	0.91 (0.48)	6.7 (2–15)	4.04 (**0.044**)	0.002 (0.962)
Overall	0–692.7 (365)	26.3 (4.45)	45.2 (40–50.5)	0–30.3 (307)	0.755 (0.21)	8.5 (5.61–12.16)	111.4 (**<0.001**)	92.75 (**<0.001**)

Baseline variables (**a**) and infection levels by age groups in the selected populations (**b**) are shown for the 2 villages. Statistical results of χ^2 analysis and ANOVA on the prevalence and infection intensity, respectively, are presented for each age group and significant p values are highlighted in bold.

CI = Confidence interval; HTA = high-transmission area; LTA = low-transmission area; SEM = standard error of the mean; STH = soil-transmitted helminths.

Antibody Profiles for Schistosome- and Der p 1-Specific Responses

Antischistosome adult worm IgE and IgG4 (absorbencies) were significantly higher in the high-transmission area compared to the low-transmission area (table 2). Only IgG4 responses directed against the egg stage of the parasite differed between the two villages, being also higher in the high-transmission area. Neither of the parasite-specific IgE:IgG4 ratios differed between the two areas (table 2). Anti-adult worm IgE and IgG4 responses increased with age and rose faster in the high-transmission area compared to the low-transmission area (fig. 2)

and exhibited a peak shift in these responses. Amongst the Der p 1-specific responses, IgG4 levels were higher in the high-transmission area resulting in significantly lower IgE:IgG4 ratio in this village compared to the low-transmission village (table 2).

SPT Reactivity Is Related to Allergen-Specific IgE and IgG4 Levels

Overall, the prevalence of positive SPT was 17.7% (95% CI 3.96–40.1) but 67.8% (95% CI 13.4–147.2) of SPT-positive individuals were sensitized to the HDM. Initial analyses (univariate ANOVA) confirmed that the severity of
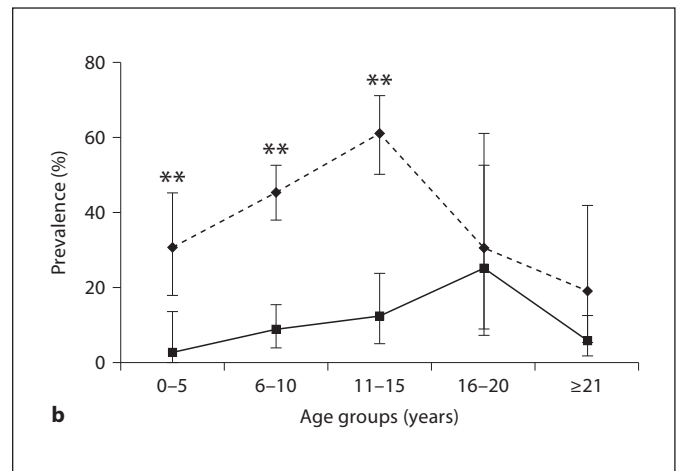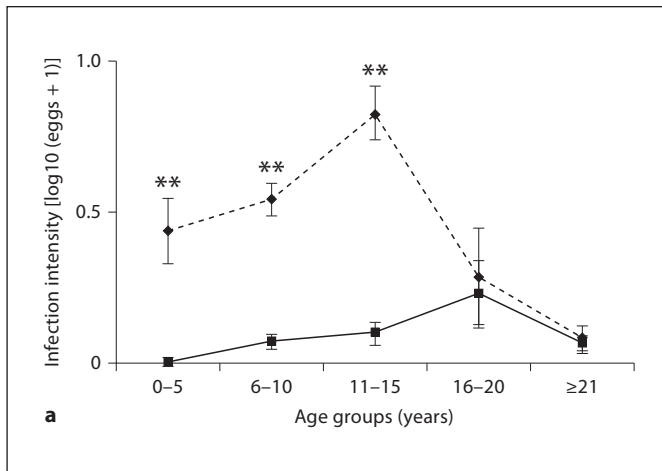
Fig. 1. Prevalence and infection intensity according to age in the study area. Infection intensity (**a**) and prevalence (**b**) are plotted against age groups in the high-transmission (– – –) and the low-transmission area (——). Bars represent standard error of the means for infection intensity and 95% CI for the prevalence. * p < 0.05 and ** p < 0.01 represent significant differences in each group between the two villages.

Table 2. Results from a multivariate analysis of the effects of age, sex, village and infection intensity on antibody levels and ratio

Dependent variable	Sex	Age	Village (comparison)	Infection intensity F and (β) values	Age and village interaction	Infection and village interaction
Antischistosome responses						
SWAP-IgE	**17.769 (M>F)****	**6.446****	**22.648 (H>L)****	**20.782 (0.155)****	**3.061***	0.778
SWAP-IgG4	**28.019 (M>F)****	**9.478****	**20.055 (H>L)****	**49.113 (0.233)****	**3.54****	0.139
SWAP-IgE/IgG4	2.450 (F>M)	1.895	1.014 (L>H)	**4.948 (−0.076)***	**2.558***	0.001
SEA-IgE	**16.520 (M>F)****	**14.299****	3.185 (L>H)	**10.749 (0.112)****	1.804	**5.177***
SEA-IgG4	**21.685 (M>F)****	**15.090****	**42.185 (H>L)****	**181.472 (0.416)****	1.889	**8.324****
SEA-IgE/IgG4	0.026 (M>F)	2.303	**22.963 (L>H)****	**21.026 (−0.157)****	1.27	0.096
Antiallergen responses						
Der p 1-IgE	**10.769 (F>M)****	**5.270****	1.013 (L>H)	**5.673 (-0.079)***	**3.81****	0.354
Der p 1-IgG4	**6.178 (M>F)***	**4.711****	**15.219 (H>L)****	0.527 (−0.024)	1.195	0.025
Der p 1-IgE/IgG4	**11.474 (F>M)***	0.806	**11.460 (L>H)****	**4.405 (−0.072)***	1.856	0.853

F values from a multivariate analysis determining the effects of different variables on the levels of parasite and allergen-specific antibody responses. The effects of infection intensity were determined after allowing for the effects of a host's age, sex and village of residence by sequential sums of squares. The β-coefficients from a linear regression are also shown for the direction of the relationship 'infection-antibody'. Degrees of freedom: 1 for sex, village and infection; 4 for age; the total for the error was 660. F values with significant p values (i.e. * p < 0.05 and ** p < 0.01) are highlighted in bold.

F = Female; H = high-transmission village; L = low-transmission village; M = male; SEA= soluble egg antigen; SWAP = soluble worm antigen preparation.

the SPT reactivity to HDM was significantly associated with the levels of Der p 1 IgE antibodies ($F_{1, 321}$ = 37.26, p < 0.001), and the interaction between Der p 1-specific IgE and IgG4 ($F_{1, 321}$ = 5.1, p = 0.025). Furthermore, SPT-positive people had significantly higher Der p 1-IgE and slightly higher Der p 1-IgG4 antibody titers (absorbencies) compared to SPT-negative people (fig. 3), as determined by stepwise logistic regression analysis (Wald χ^2 = 11.484, d.f. = 1, p = 0.001 and Wald χ^2 = 1.573, d.f. = 1, p = 0.21, respectively, for IgE and IgG4 mean absorbencies).
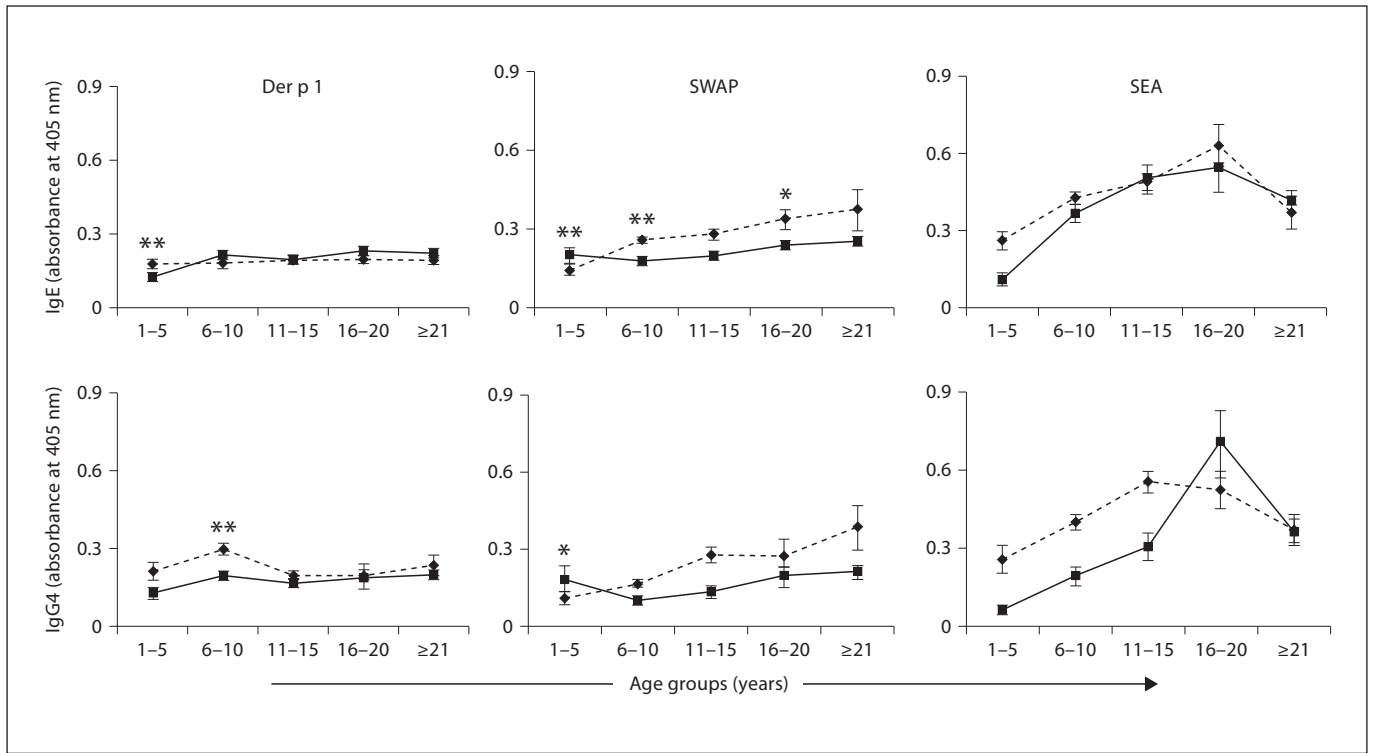
Fig. 2. Antibody levels according to age. Der p 1-specific, SWAP-specific and SEA-specific responses in the high-transmission (– – –) and the low-transmission area (——). Bars represent standard error of the means. * $p < 0.05$ and ** $p < 0.01$ represent significant differences in each group between the two villages.
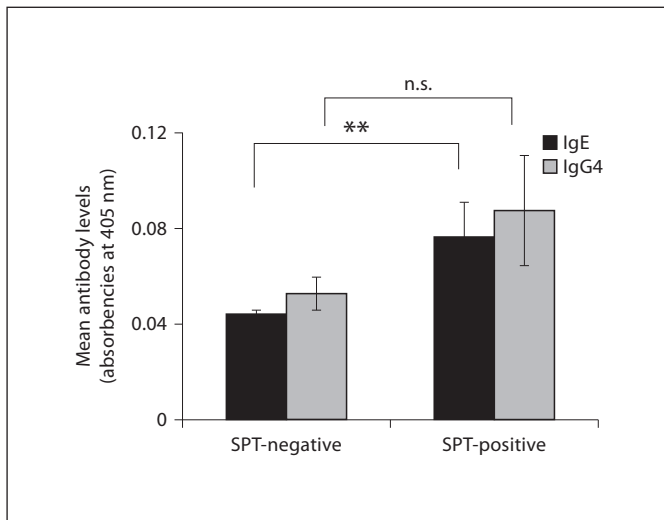

Fig. 3. Der p 1-specific antibody levels in SPT-positive versus SPT-negative individuals. Levels (absorbencies) of anti-Der p 1 IgE and IgG4 are plotted for SPT-negative and SPT-positive people. Bars represent standard error of the means. n.s. = Difference not significant, versus ** $p < 0.01$ = a significant difference.
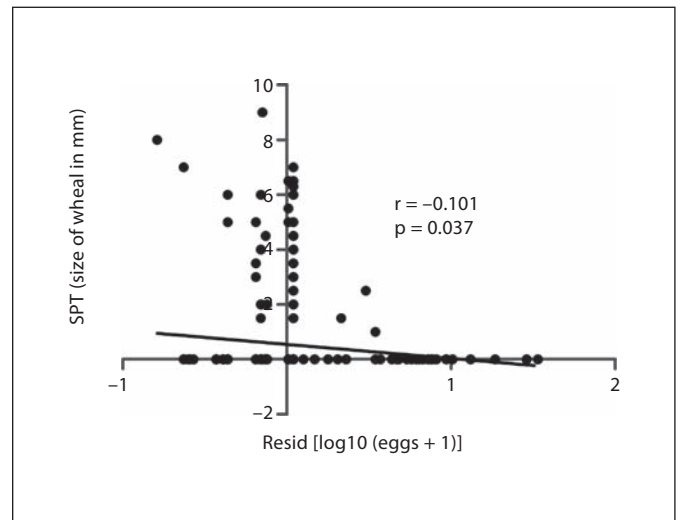
Fig. 4. Correlation between SPT results and infection intensity. Residuals of infection intensity after controlling for age, sex and village of residence are plotted against the size of the wheal in millimeters.

a
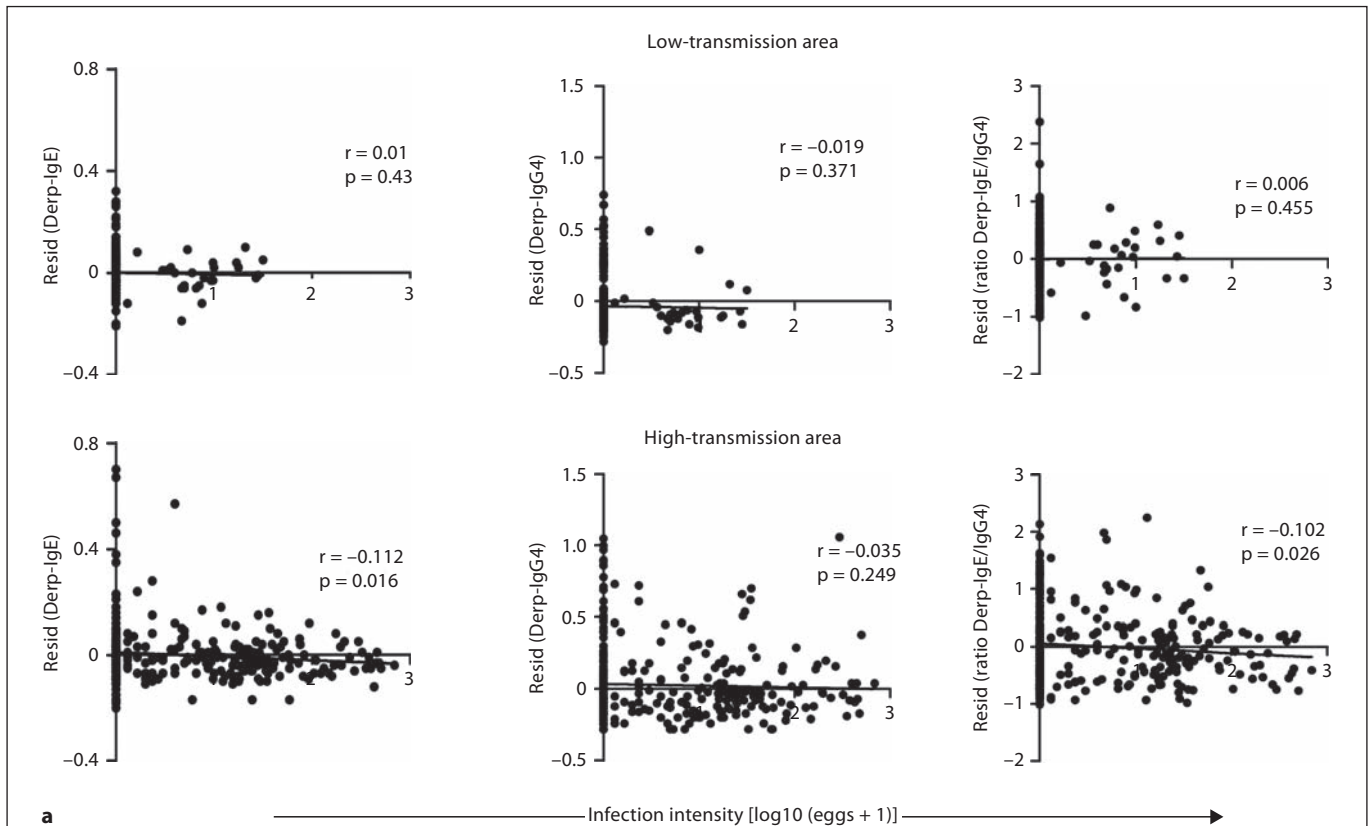
Infection intensity [log10 (eggs + 1)]

b
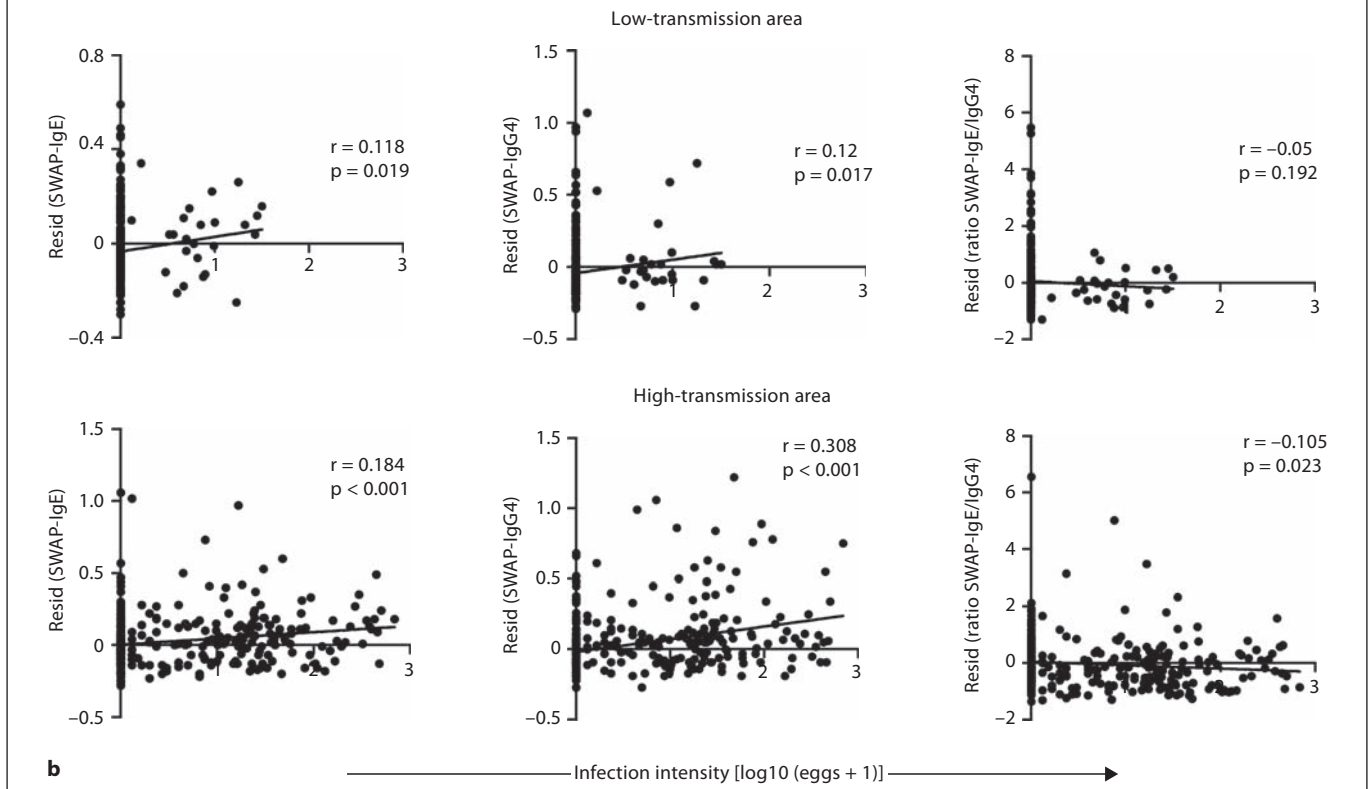
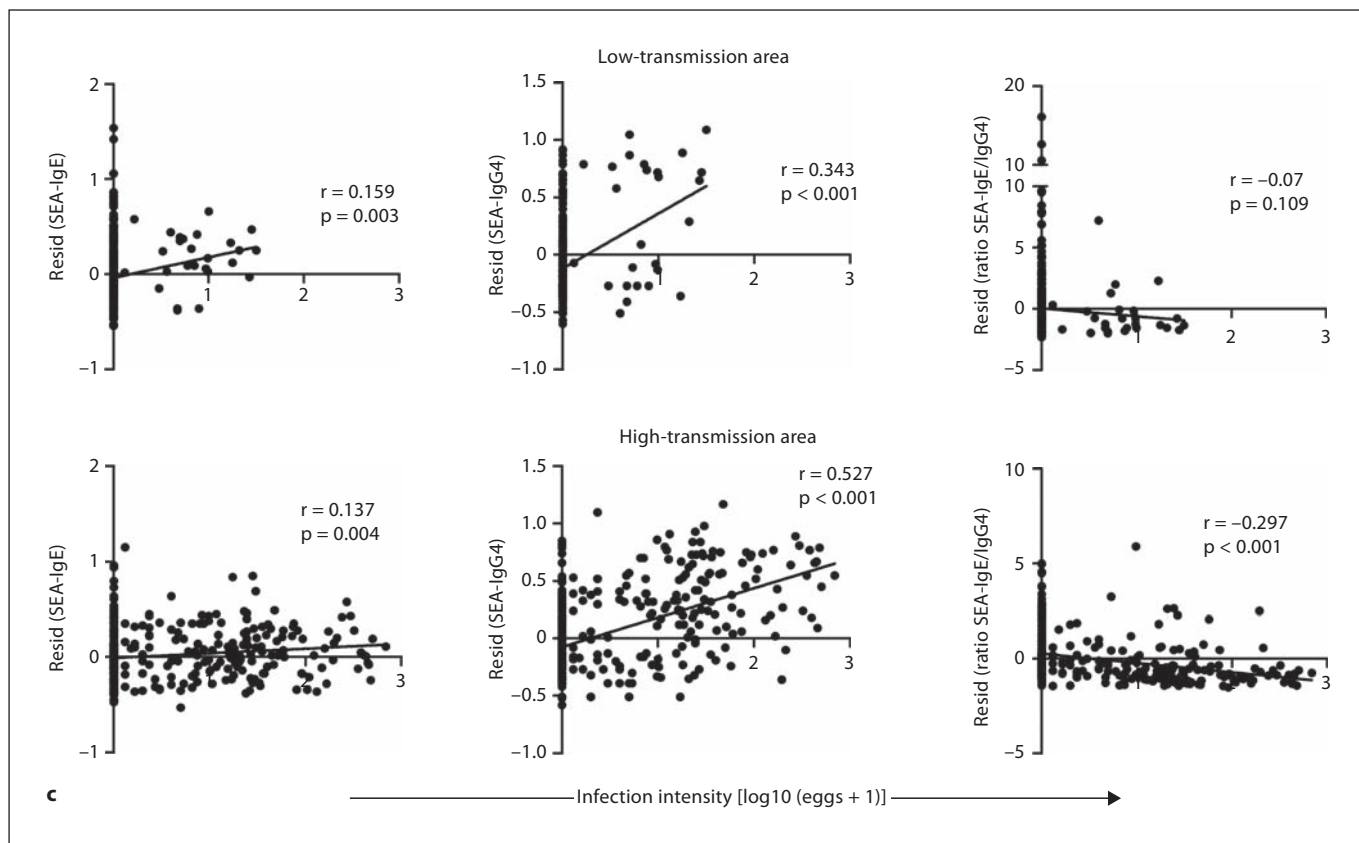Infection intensity [log10 (eggs + 1)]

5

Fig. 5. Correlations between schistosome-specific:mite-specific antibody ratio and infection intensity. Residuals (after controlling for age and sex) of IgE, IgG4 and IgE/IgG4 against Der p 1 (**a**), SWAPs (**b**) and SEAs (**c**) are plotted against infection intensity (log-transformed) for Magaya (high-transmission area) and Chitate (low-transmission area). One-tailed Pearson correlations were performed. Correlations significant at p < 0.05 are highlighted in bold. Resid = Residual.

Atopic Responses Are Negatively Correlated with Schistosome Infection Intensity

The prevalence of sensitization to HDM (% of people positive for the SPT test) was higher in the low-infection area (12.4%, 95% CI 3.48–29.1) than in the high-infection area (7.7%, 95% CI 6.74–32.8) and SPT-negative people had higher levels of schistosome infection, though these differences were not statistically significant. However, SPT wheal size was significantly inversely related to *S. haematobium* infection intensity after allowing for the variations due to age, sex and village of residence (fig. 4). An analysis to determine factors affecting levels of Der p 1-specific antibodies showed that after allowing for sex, age and village of residence, schistosome infection intensity negatively affected levels (absorbencies for all individuals) of Der p 1-specific IgE, but not IgG4, resulting in a significant effect on the ratio Der p 1-IgE:IgG4 (table 2). This analysis also showed that levels of these two variables differed between the two villages. Therefore, correlation analyses were conducted to determine the relationship between Der p 1 responses (absorbencies for all individuals) and infection intensity in each of the villages (after allowing for the confounding effects of sex and age). This showed that the correlation between infection intensity and the levels of Der p 1-specific IgE and the ratio Der p 1-specific IgE:IgG4 was significant only in the high-transmission village but not in the low-transmission village (fig. 5a). This contrasted with schistosome-specific responses, where although there were differences in the magnitude of some of the responses between the two villages (table 2), the relationship between the antibodies and infection intensity was similar in the two villages (fig. 5b, c).

The analyses suggested that the lack of a relationship between schistosome infection intensity and Der p 1 antibodies and their ratio may be due to the overall lower

levels of infection in the low-infection area (interaction between infection and village is not significant in table 2). Therefore, the relationship between schistosome infection intensity and anti-mite antibody responses (absorbencies for all individuals) was compared in people with similar levels of infection (0–30 eggs/10 ml urine) in the high-transmission village, controlling for variations due to host age and sex. This showed that at these low levels of infection, there was no significant correlation between infection intensity and Der p 1-specific antibodies (r = −0.089, p = 0.062 for IgE and r = −0.074, p = 0.095 for IgE:IgG4).

Discussion

This study compared the effect of current schistosome infection and that of the cumulative history of infection on atopic responses in two villages of different schistosome transmission dynamics. Differences in these dynamics were confirmed by the differences in schistosome infection intensity and the existence of peak shifts [29]. Moreover, we demonstrated the previously described inverse relationship between infection intensity and the ratio IgE:IgG4 against the SEA and SWAP in both villages. This ratio has been associated with the development of protective immunity against schistosome infection/reinfection [30, 31]. In this study, the relationship was significant in the high but not in the low-transmission village. This supports previous comparative studies showing that protective immunity develops as a function of cumulative history of infection and will thus develop faster in areas of higher transmission compared to areas of lower transmission [19].

The majority of SPT-positive participants were sensitized to HDM compared to the other allergens (molds and grass) and the overall prevalence of the HDM sensitization (12%) was comparable to that previously reported for Zimbabwean populations, and is consistent with documented levels of atopy from other African countries [16, 27, 32]. The low-infection area was overrepresented in the participants surveyed for the SPTs. However, by allowing for the potential confounding effects of residential village in the statistical analyses, it meant that the relationship between schistosome infection intensity and SPT status could be analyzed regardless of village of residence. Furthermore, the SPT participants had comparable infection ranges in the two villages, of 0–22 versus 0–30, i.e. within the mild infection range as classified by the World Health Organisation, which meant the analy-

ses compared people with similar infection levels across the same infection range for the SPT versus infection intensity aspect of the study.

In this study, mite-specific IgE levels (absorbencies for all individuals) were positively correlated with the SPT reactivity to mite allergens, consistent with published reports [33]. Mean IgG4 levels (absorbencies) were higher, though not significantly so, in SPT-positive compared to SPT-negative people. The role of IgG4 in allergic conditions is still poorly understood, but the high levels of both IgE and IgG4 in the SPT-positive participants could be related to the fact that IgE-inducing allergens are also efficient IgG4 inducers [34]. SPT reactivity was negatively associated with *S. haematobium* infection as reported in the Gabon [10]. A comparison of Der p 1-specific antibody responses showed significant differences in levels of IgG4 but not IgE in the two villages together, with a significant difference in the IgE:IgG4 ratio. Nonetheless, IgE but not IgG4 was significantly inversely associated with schistosome infection intensity, and consequently, the ratio IgE:IgG4 was also inversely associated with schistosome infection. It seems as though the IgG4 response in this instance was modulating the pathological effects of the IgE, a concept which is important in allergy immunotherapy [35].

Differences were observed in the schistosome-anti-Der p 1 antibody relations between the two villages: there was no association between infection intensity and Der p 1-specific IgE (and the ratio IgE:IgG4) antibody responses (absorbencies) in the low-transmission area but a significant negative association in the high-transmission area. This finding that areas of different schistosome endemicity exhibit differences in the relationship between antimite antibody responses and infection intensity may explain findings from a recent meta-analysis which showed that results on the relationship between schistosome infection and atopy were heterogeneous [36]. The fact that people in the low-infection area mounted similar antischistosome IgE and IgG4 responses (albeit at a lower level) indicates that there was no impairment in their ability to mount IgE and IgG4 responses. One potential reason for the lack of an association between their allergen-specific antibody responses (absorbencies) and schistosome infection was the overall low level of schistosome infection. To investigate this, the relationship between schistosome infection intensity and antimite antibody responses (absorbencies) was compared in people with similar infection levels in both villages. This showed that at such low levels of infection (0–30 eggs/10 ml of urine), there was no significant correlation between infection in-

tensity and Der p 1-specific antibody responses in either village. This is consistent with findings from an experimental study relating *S. mansoni* infection and allergic responses [9]. This result also means that a cumulative experience of schistosome infection did not significantly affect antimite antibody responses since egg-negative older people (with a longer history of exposure [13]) did not differ in their antimite antibody responses when compared to younger egg-negative people with a shorter history of exposure to schistosome parasites, suggesting that immunomodulatory effects are only observed in viable worm infection [37].

To our knowledge, this study is the first comparative investigation conducted in two populations with the same ethnic group and socioeconomic status but different levels of schistosome infection, determining the effects of infection intensity on HDM sensitization. Overall, the study shows that regardless of the underlying biological mechanisms, the relationship between schistosome infection and HDM sensitization differs in areas of distinct schistosome infection levels, and that this difference is dependent on current infection levels and not on a history of infection. Furthermore, at low levels of infection, even in a high-transmission area, there was no relationship between infection intensity and antimite antibody responses.

Understanding the relationship between helminth infection and atopy at different infection intensities, as well as in areas with different transmission dynamics, is important not only for evaluating the long-term consequences of the current large-scale global antihelminthic treatment programs but may also influence the management of atopic diseases in people with concomitant helminth infections. In Zimbabwe, the majority of people showing the clinical signs of allergy, i.e. allergic rhinitis, conjunctivitis, asthma, dermatitis and urticaria, are sensitized to the HDM (measured by SPT or allergen-specific IgE) [16]. Nonetheless, differences in the relationship between helminth infection and skin sensitization compared to those between helminth infection and allergic disease [38] have been reported. Thus, further studies on the role of different parasite transmission dynamics in the clinical manifestation of atopy are required to inform therapeutic interventions [39] and the management of clinical allergy in helminth-endemic areas. Finally, the heterogeneities in the relationship between different helminth species and atopy also need to be studied in the light of the evidence that the effects of nematodes may vary from those of trematodes and between nematode species [36].

Disclosure Statement

The authors declare no financial or personal conflict of interest which could interfere with the study outcome.

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