

Entamoeba coli and *Entamoeba histolytica* infections as risk of allergic reactions: a case study of population of Bamenda, Cameroon

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Abstract

Gastrointestinal parasites remains one of the major health problem in low-income countries. Despite of the induction of IgE responses that results, the role of the various gastrointestinal parasites in allergic is not well known. This study aimed at determining the prevalence and relationship of allergic diseases and *Entamoeba coli* (*E. coli*) and *Entamoeba histolytica* (*E. histolytica*) infections. The study conducted from December 2018 to February 2019 in the Bamenda Regional hospital, Cameroon. A total of 301 patients were interviewed and systematically screened for *E. coli* and *E. histolytica* using formol ether method. The prevalence of *E. coli* and *E. histolytica* was 8.22%, and 3.95% respectively, and coinfection accounted for 2.63% of all participants. The prevalence of allergic diseases was 57.48%. Wheezing, asthma, rhinitis and eczema were present in the population for 3.62%, 40.53%, 13.30% and 15.61% respectively. IgE-response against *E. coli* and *E. histolytica* constitutes a risk for allergic diseases in parasites-infected patients. This is the first study assessing parasites infection and allergy relationship in patients suffering from gastrointestinal parasites in Cameroon. This study outlines a high prevalence of allergy infections and gastrointestinal parasites as associated factors. Further investigations of this relation could be a good complement to control strategies of allergic diseases.

Introduction

In high-income countries and in urbanizing populations in low-income countries, the hygiene hypothesis has been proposed to explain temporal trends of increasing of allergy prevalence. Otherwise, increased allergy prevalence has been also associated to exposures to infectious diseases through a failure to educate appropriately the developing immune system leading to inadequate regulation of allergic inflammation [1,2]. In low-income countries notably in poor populations, gastrointestinal parasite infections are particularly common and have been put forward to explain the low prevalence of allergy [3]. However, there is exact data based supporting this hypothesis. Parasites, are commonly dedicated to induce IgE response. This IgE is known to be associated with allergy, which studies commonly attributed to a Th1/Th2 imbalance [4,5].

Over recent years, the allergy prevalence is extremely in increment as well as the prevalence of diarrhoea due to gastrointestinal parasites notably protozoan in Bamenda, Cameroon. Therefore, the present study aimed to examine the interaction between allergic diseases and *Entamoeba coli* and *Entamoeba histolytica* infections. This research, examining the production of human IgE responses in parasitic and allergic diseases, is unique in having the potential to help understand two extremely common diseases, one being one of the most common diseases in developing countries and the other one of the most common diseases in developed countries.

Materials and methods

Study period and area

This study was conducted from December 2018 to February 2019 at the laboratory of the Bamenda regional hospital in the city of Bamenda (North West Region, Cameroon). The city has a population of about 2

million people, where frequent intestinal parasites and atopic diseases are diagnosed. It has a coordinate of 5056N10010E and its climate is a tropical savanna climate and very heavy rainfall, especially during the rainy season that extends from June to October [6,7].

Study design

This study was a cross-sectional survey of patients attending the Bamenda regional hospital. Before the study, the Director of the hospital was approached, the aims of the study were explained and dates were proposed for activities in their sites. After obtaining written approval of the hospital, the participants meeting the eligibility criteria were included in the study upon signing an informed consent form. A pre-tested and structured questionnaire was used to document information of interest of each participant such as sex, age and allergic diseases in the past 12 months.

Inclusion and exclusion criteria

The study included adult patients who were recommended for stool analysis in the Bamenda Reginal Hospital and who had given their consent and children recommended for stool analysis whose parents or legal guardians had given their consent. Any patient who did not give his/her consent or whose parent or legal guardian did not give his/her consent was excluded from the study. Also, cases of double allergic

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infections were not included in the association of the allergies, intestinal parasites and immunoglobulin levels.

Ethical statements

Ethical clearance was obtained from the University of Bamenda Institutional Review Board (CEI218 DU/268/05/2019/T) (S1 File). The study was carried out in accordance with guidelines for human experimental models in clinical research as stated by the Cameroon Ministry of Public Health. An information sheet was prepared and presented to the participants, so as to have a clue about the study. A code was attributed to each participant to guarantee the respect for anonymity. Participation was strictly voluntary, anonymous and without compensation. The objectives of the study were explained to patients in French or English depending on the language they understood best, and their questions were answered. Written informed consent was obtained from all participants prior to enrolment (S2 File).

Study population

The study population consisted of patients attending the Bamenda Regional hospital. The recruited population were of both sexes and of all age groups (>1 year) attending the Bamenda Regional hospital, who had been recommended for stool analysis by the physicians.

Collection of personal and assessment of allergy diseases

A structured questionnaire adapted from the questionnaire of the international symptoms for asthma and allergy committee (ISAAC) [8] was given to the participants. The questionnaire was written in English, one of the national language in Cameroon. The questionnaire was administered to each participant by the principal investigator with the assistance from associate investigators. The interviews lasted between 5–10 minutes and was strictly confidential in order to avoid response bias. The questionnaire was designed to obtain information on core allergic symptoms such as: wheezing, asthma, rhinitis and eczema (S3 file).

Validation of the questionnaire

The answers were validated according to the principle of ISAAC [8] A patient was declared as having wheezing when he/she had wheezing or whistling in the chest in the last 12 months, at the time of the study. Asthma was declared based on the presence of nocturnal cough in the last 12 months. A patient had rhinitis when he/she had a problem with sneezing, or a runny, or blocked nose in the last 12 months, at the time of the study when they did not have a cold or the flu. Eczema was declared if a patient had itchy rashes at any time in the last 12 months or at the time of the study.

Stool samples collection

A wide open mouth transparent container with a tight fitting lid, labelled with the initials of the patient's name, date of birth and the date of collection were handed to each participant or of participant parent. Instructions on how to collect and hand over the specimen were also given to them by the physician. The participants were also reminded to wash their hands thoroughly with soap and running water immediately after they had given their samples. Samples were observed about ten minutes after collection. Samples which could not be observed on the day of collection were preserved in a 10% formalin solution and then observed the next day.

Parasitological examination of stool samples

Stool samples were analyzed using the formol ether sedimentation concentration technique for the eggs/cysts of intestinal parasites as

described previously. Briefly, 1g of stool was put in one of the stool containers and 7 mls of 10% formalin were added to it. The stool was then dissolved with an applicator stick. A sieve with pore size 400-450 µm was used to filter the stool sample into a glass centrifuge tube. Three millimeter of ether (diethylether) were then added to the solution in the centrifuge tube, and the tube was shaken vigorously for 30 seconds. The mixture was then centrifuged at 3000 rpm for 5 minutes. After centrifuging, there was sediment at the bottom, then a fatty deposit at the middle separating the formalin and the ether. A sharp object was then used to loosen the fatty deposit at the middle after which the supernatant was decanted. A drop of Lugol's iodine was then added to the deposit and the deposit was then put on a slide, covered with a cover slip and then examined under the 10x objective of the microscope [9,10].

Blood collection and serum preparation

Blood samples were also collected by the laboratory technicians from each patient by venipuncture. The collected blood was then centrifuged at 3000 RPM for 5 minutes, after 5-10 minutes of collection. The serum was then collected into ependorf tubes for the measurement of immunoglobulin E (IgE).

Antibody IgE measurement

Serum total IgE was evaluated using ELISA technique using high-binding-level microassay plates (Costar, Cambridge) coated with 4 µg/ml of an anti-human IgE antibody overnight at 4°C [11,12]. In detail, after coating the plates, plates were then blocked with 200 µl of 0.15 M PBS, pH 7.2, containing 10% FCS and 0.05% Tween 20 (Sigma, St. Louis, MO) overnight at 4°C. Thereafter, 200 µl of samples, diluted 1:10 in PBS containing 5% FCS and 0.05% Tween 20, were incubated overnight at 4°C. Later, the plates were incubated with biotinylated anti-human IgE (Sigma Chemical Co., Germany) followed by streptavidin-peroxidase (Sigma Chemical Co., Germany) and H₂O₂-OPD substrate (Sigma Chemical Co., Germany). Positive control and negative control (Sigma Chemical Co., Germany) were used and the plates were read using a 480-nm filter.

Antibody IgE based determination of allergy

According to the AllergyScreen[®] system, serum total IgE was divided into 3 levels: grade 1 (100-300 IU/ml), grade 2 (300-1000 IU/ml) and grade 3 (>1000 IU/ml). A serum total IgE level >100 IU/ml was considered to be positive [13].

Data analysis

Prevalence of intestinal parasites and allergies were determined as percentage. The allergy prevalence results were analyzed by descriptive statistics and the Chi-square test to determine the analyzed variables using the SPSS version 21. The association between the intestinal parasites and allergic infections was evaluated by determining the odds ratio. Serum total IgE concentration was expressed as mean ± SD and ANOVA one way followed by t test to compare the different groups. Calculations were done using the Graph Prism software v.3.

Results

Demographic characteristics and prevalence of intestinal infections and allergic diseases of the study population

A total of 301 patients were recruited. All participants gave the stool to assess the prevalence of parasites and examined for allergic diseases. Among them 178 (59.14%) were female and 123 (40.86 %) were male.

Participants were comprised of 20 participants (6.64%) of age < 10 years, 32 (10.63%) aged between 10 - 20 years and 249 (82.72%) have high than 20 years. A total of 45 (14.95%) patients had *Entamoeba coli* and *Entamoeba histolytica* alone or in co-infection (Table 1). The prevalence of *Entamoeba coli* alone was 8.22% and that of *Entamoeba histolytica* alone was 3.95% and 2.63% of patients were co-infected with *E. coli* and *E. histolytica*. Four types of allergic diseases (wheezing, asthma, rhinitis and eczema) were identified with 173 (57.48%) overall prevalence (Table 1).

Influence of sex and age groups on prevalence of allergic diseases in patients

The relation between the allergy prevalence and sex and age of patients is presented in Table 2. The overall prevalence of allergic diseases in male (52.03) were slightly lower compared to that in female (61.24). The four types of allergy are observed in both male and female. Asthma had the highest occurrence in males while in females it was rhinitis. With exception of wheezing in patients of 10 – 20 years, the various types of allergic were present in all the age groups. Asthma and rhinitis were more prevalent in patients with age > 20 years and less in 1 – 10 years. While, wheezing and eczema were more prevalent in patients with age 1 – 10 years and less age groups > 20 years.

Association of wheezing with *Entamoeba coli* and *Entamoeba histolytica* infections in patients

Of the 25 patients with *E. coli*, 12% (3) have declared had wheezing compared to 6.01% (16) of 266 patients without *E. coli* infection (Table 3). Analysis showed a high risk of association between *E. coli* and wheezing, but it was not significant (P = 0.20; OR: 2.21; 95%CI: [0.59–8.19]).

Table 1. Prevalence of parasites (*E. coli* and *E. histolytica*) and allergies (wheezing, asthma, rhinitis and eczema) in studied population

Total patients; N = 301	Positive patients	Prevalence (%)
Parasites species		
<i>E. coli</i>	25	8.22
<i>E. histolytica</i>	12	3.95
Co-infection	8	2.63
Overall prevalence	45	14.95
Allergy diseases		
	Positive patients	Prevalence (%)
Wheezing	41	13.62
Asthma	122	40.53
Rhinitis	40	13.30
Eczema	47	15.61
Total	173	57.48

Table 2. Comparison between prevalence of the various types of allergies and sex and age group in patients

Sex (N=301)	group	Total (%)	Allergy diseases			
			Wheezing (%)	Asthma (%)	Rhinitis (%)	Eczema (%)
Male	N = 123	64 (52.03)	6 (4.88)	48 (39.02)	15 (12.20)	18 (14.63)
female	N= 178	109 (61.24)	13 (7.30)	25 (14.04)	74 (41.57)	29 (16.29)
P value		P = 0.51	P = 0.09	P = 0.04	P = 0.15	P = 0.25
Age (N=301)	group	Total %	Allergy diseases			
			Wheezing (%)	Asthma (%)	Rhinitis (%)	Eczema (%)
< 10years;	N = 22	12 (54.55)	2 (9.09)	5 (22.73)	1 (4.55)	11 (50)
10-20years;	N=33	9 (27.27)	0 (0)	6 (18.18)	4 (12.12)	3 (9.09)
> 20years;	N=249	252 (61.04)	17 (6.83)	111 (44.58)	35 (14.06)	33 (13.25)
P value		P = 0.1	P = 0.26	P = 0.91	P = 0.88	P = 0.12
No statistically significant (p>0.05)						

Table 3. Multivariate analysis of the association between wheezing and *Entamoeba coli* or *Entamoeba histolytica* infection

Infection	Total	Patients with wheezing N = 19 (%)	OR (95% CI)	P value
<i>E. coli</i>				
Positive	25	3 (12.00)		
Negative	266	16 (6.01)	2.21 (0.59 - 8.19)	0.201
<i>E. histolytica</i>				
Positive	0	0 (0.00)		
Negative	12	12 (100)	OR = 0	P = 0
Multivariate logistic model was used to compute the values of odds ratio (OR). 95%CI: Confidence interval at 95%; statistically significant at p-value <0.05.				

Table 4. Multivariate analysis of the association between asthma and *Entamoeba coli* or *Entamoeba histolytica* infection

	Total	Patients with asthma N= 122 (%)	OR (95% CI)	P value
<i>E. coli</i>				
Positive	25	7 (28.00)		
Negative	276	115 (41.66)	0.54 (0.22 - 1.34)	0.207
<i>E. histolytica</i>				
Positive	12	8 (66.66)		
Negative	289	118 (40.83)	0.72 (0.21 - 2.46)	0.767
<i>E.coli/E. histolytica</i>				
Positive	8	3 (37.50)		
Negative	293	119 (40.61)	0.87 (0.20 - 3.74)	0.859
Multivariate logistic model was used to compute the values of odds ratio (OR). 95%CI: Confidence interval at 95%; Statistically significant at p-value <0.05.				

Association of asthma with *Entamoeba coli* and *Entamoeba histolytica* infections in patients

The prevalence of asthma was 28% (7) in patients with *E. coli* compared to 41.66% (115) non-infected *E. coli* patients (P = 0.21; OR: 0.54; 95%CI: [0.22-1.34]). In patients infected with *E. histolytica*, the prevalence of asthma was 66.66% (8) compared to 40.83% (118) in those non-infected with *E. histolytica* (P = 0.77, OR = 0.72, 95%CI: [0.21-2.46]). In patients co-infected, 37.5% (3) of patients had asthma (P = 0.86 and OR = 0.87; 95%CI: [0.20-3.74]) (Table 4).

Association of rhinitis with *Entamoeba coli* or *Entamoeba histolytica* infections in patients

The prevalence of rhinitis was higher in *E. coli* - infected patients than those non-infected with *E. coli* and there was a significant difference (P = 0.01; OR = 3.58, 95%CI: [1.43-8.98]). None of the patients with *E. histolytica* had rhinitis. In co-infected patients 12.50% (1) had rhinitis compared to 13.31% (39) in patients non-coinfected with *E. coli* and *E. histolytica* (P = 0.94; OR = 0.93; 95%CI: [0.11-7.77]) (Table 5).

The Association of eczema with *Entamoeba coli* or *Entamoeba histolytica* infections in patients

The proportion of eczema in patients with *Entamoeba coli* or *Entamoeba histolytica* infection is presented in Table 6. The prevalence of eczema in patients with *E. coli* infection was 16% for 25 patients similar to that in 276 non-infected patients (P = 0.96; OR = 1.03; 95%CI: [0.33-3.15]). In people with *E. histolytica*, the prevalence of eczema was higher than in non-infected patients (P = 0.19; OR = 2.86; 95%CI: [0.82-9.91]). Similarly, the prevalence eczema in coinfectd patients was higher than in the non-infected controls (P = 0.80; OR = 1.83; 95%CI: [0.35-9.39]).

Effect of *Entamoeba coli* or *Entamoeba histolytica* infection on the total serum level of IgE of patients

Serum total IgE was higher in patients with *Entamoeba coli* or *Entamoeba histolytica* infection than patients without parasitic. Patients with coinfection (*Entamoeba coli* and *Entamoeba histolytica* infections) had high serum total IgE than those infected with one type of parasite (Table 7).

Serum total IgE-based prevalence of allergy in patients with *Entamoeba coli* or *Entamoeba histolytica* infection

In patients free of parasites, the serum total IgE level was low than 100 UI/ml, while none of the patients having *E. coli*, *E. histolytica* or co-infected had less than 100 UI/ml. The distribution of patients according the allergy classes varied in each, but with high percentage of patients in grade 3 allergy (Table 8).

Serum Total IgE level in *E. coli*-infected patients, *E. histolytica*-infected patients, co-infected patients with respect to allergic diseases

The serum total IgE levels were observed to be significantly higher in patients with parasites and patients positively diagnosed for allergy than those who were free from parasites and allergic diseases. In patients with allergy associated with parasites, results showed that there were significant increase in serum total IgE compared to those diagnosed positive for asthma, rhinitis and wheezing but did not have any of parasites (Table 9).

Discussion

Intestinal parasites infections have been reported to be prevalent in Bamenda, Cameroon [14] The present study showed that *Entamoeba coli* was prevalent with 8.22% suggesting that, this parasite species is the

Table 5. Multivariate analysis of the association between rhinitis and *Entamoeba coli* or *Entamoeba histolytica* infection

<i>Entamoeba histolytica</i> infection				
Infections	Total	Patients with rhinitis N = 40 (%)	OR (95%CI)	P value
<i>E. coli</i>				
Positive	25	8 (32.00)		
Negative	276	32 (11.59)	3.58 (1.43-8.98)	0.01
<i>E. histolytica</i>				
Positive	12	0 (0.00)		
Negative	276	40 (14.49)	OR = 0	P = 0
<i>E. coli/E. histolytica</i>				
Positive	8	1 (12.50)		
Negative	293	39 (13.31)	0.93 (0.11-7.77)	0.94

Multivariate logistic model was used to compute the values of odds ratio (OR). 95%CI: Confidence interval at 95%; Statistically significant at p-value <0.05.

Table 6. Multivariate analysis of the association between eczema and *Entamoeba coli* or *Entamoeba histolytica* infection

Infection	Total	Eczema positive N = 47 (%)	OR (95%CI)	P value
<i>E. coli</i>				
Positive	25	4 (16.00)		
Negative	276	43 (15.57)	1.03 (0.33 - 3.15)	0.955
<i>E. histolytica</i>				
Positive	12	4 (33.33)		
Negative	289	43 (14.87)	2.86 (0.82 - 9.91)	0.186
<i>E. coli/E. histolytica</i>				
Positive	8	2 (25.00)		
Negative	293	45 (15.35)	1.83 (0.35 - 9.39)	0.804

Multivariate logistic model was used to compute the values of odds ratio (OR). 95%CI: Confidence interval at 95%; Statistically significant at p-value <0.05.

Table 7. Serum total IgE level in *E. coli*-infected patients, *E. histolytica*-infected patients, co-infected patients and uninfected patients

	Number of patients/infection	Serum total IgE (UI/ml)
Control	10	86.6 ± 43.1
<i>E. coli</i> infection	25	1989.02 ± 1568.4*a
<i>E. histolytica</i> infection	12	2318.2 ± 2419.1*ab
Co-infection	8	6919.03 ± 2949.2**b

Table 8. IgE-based prevalence of allergy with respect to *E. coli*-infected patients, *E. histolytica*-infected patients, co-infected patients and uninfected patients

	Total	No allergy (%)	Grade 1 (%)	Grade 2 (%)	Grade 3 (%)
Control	10	100	-	-	-
<i>E. coli</i> infection	25	-	20.00	16.00	64.00
<i>E. histolytica</i> infection	12	-	33.33	25.00	41.66
Co-infection	8	-	37.50	12.50	50.00

Table 9. Variation of serum total IgE of patients with respect to *E. coli* and *E. histolytica* infections and associated allergic diseases

Allergic diseases Parasites species	Patients free of allergic diseases	Patients without allergic diseases			
		Asthma	Rhinitis	Wheezing	Eczema
Control	86.6 ± 43.13a	195.7 ± 9.54b	450.7 ± 350.7b	803.0 ± 497.8b	395.8 ± 205.8a
<i>E. coli</i> infection	875.0 ± 475.4a**	1921.9 ± 950.1a***	3418.2 ± 1723.1b**	4586.1 ± 621.9b**	1851.2 ± 857.2a**
<i>E. histolytica</i> infection	1404.3 ± 1384.2a**	2188.4 ± 1226.9a***	-	-	836.50 ± 651.69a
Co-infection	8006.2 ± 3024.5a***	7111.3 ± 4166.6a***	-	-	6870.8 ± 3561.4a***

*p<0.05, **P<0.01; ***p<0.001: statistically significant compared to control at P-value <0.05. In the same column, values with different letters are statistically different at P-value <0.05.

more prevalent. The prevalence of intestinal parasites based on gender as found in this study observed a bias with more infected females than males. Adolescents were the most infected with *E. coli* of all the age groups. This may be due to the fact that they engage in many activities with inadequate environmental sanitation, insufficient water supply, as reported by Shah and Shahidullah [15].

Data showed that about 57.48%, out of 301 patients, had allergy diseases comprising of asthma, eczema, rhinitis and wheezing. This suggest that allergic reactions are recurrent in population in the North West of Cameroon. The “microbial diversity” hypothesis that suggests that environments rich in microbial diversity in the gut mucosa and respiratory tract are the key factors in priming and regulating the immune system might can be a justification of this high prevalence of allergic reactions. With regards to the specific allergic diseases, the prevalence of asthma was the highest (40.53%) suggesting that asthma might represent the high types of allergic reactions in population in the North West region of Cameroon.

In the present study, women had high prevalence of allergic reactions than men suggesting that they were more sensitive for allergic reactions. In fact, women were described to have more pronounced symptoms, which seem to change with the various life stages such as menstruation, pregnancy and menopause and in association with female sex hormone levels. These hormones might cause differences in the clinical manifestation of asthma. Thus, oestrogen promotes bronchial hyperreactivity, and both FEV1 and exhaled nitric oxide (NO) show a cycle-dependent course [16]

Parasitic infections are known to induce a Th2 response which is typically associated with allergic diseases and an inverse association between infection with different parasites species and allergic conditions [17]. In the present study, it was found that some patients who presented wheezing had *E. coli* infections, but this association was not significant suggesting *E. coli* might not have a direct impact on wheezing. Asthma was found to be insignificantly associated with *E. coli* and *E. histolytica*. Patients that declared rhinitis, also had *E. coli* and co-infections *E. coli* - *E. histolytica* showing significant association with *E. coli*. This suggest that *E. coli* infection might lead to the development of rhinitis infection. Numerous patients who declared eczema in the last 12 months, also had *E. histolytica* and associated *E. coli*/ *E. histolytica*. But the relation of eczema with these parasites was not significant. This might suggest that intestinal parasites might not have relation with the development of eczema.

Common environmental allergens stimulate IgE responses and produce allergic disease, but the allergens that produce the most potent IgE responses in nature originate from helminthic parasites [18, 19]. There is a general consensus that IgE antibody is an important component of the immune resistance to parasite infections [20,21], although some conflicting results have been obtained. It was demonstrated that the primary function of the allergic response may be a part of an anti-parasitic protective mechanism, and allergic disease may be the undesirable reaction towards otherwise inoffensive

environmental substances. In the present study, the total serum IgE levels were observed to be significantly higher in patients with *E. histolytica*, *E. coli* and *E. histolytica* and co-infected than those who were not infected with the parasites. This might suggest a possible implication of these parasites in allergic reactions even though the above data show a non-significant relationship.

Recent immunological studies demonstrated that there are two different IgE responses to helminthic infections. The first of these is the host's defensive response to produce IgE specific to parasite antigens. The second response is that the host also exhibits a strong non-specific Th2/interleukin 4 dependent polyclonal synthesis of IgE [22,23] which results in highly elevated total serum IgE levels in parasitised populations. This polyclonal synthesis of IgE may be the helminth's defence mechanism against the effects of anti-parasite IgE. The polyclonal stimulus might suppress allergic responses by reducing the production of specific IgE antibody, resulting in an inverse relationship between total and specific serum IgE levels [24]. The polyclonal IgE also saturates the IgE receptors on mast cells and blocks access to specific IgE, which further inhibits allergic reactions [24,25]. This suppressive activity may be the reason for the diminished prevalence of some allergic diseases reported in populations, subsequently the absence of significant relationship.

Of great significance is the likelihood that parasites evade the immune response by stimulating excess IgE production. For example, in populations endemically exposed to helminths, individuals with the highest total serum IgE levels are more quickly reinfected by the parasites after anthelmintic treatment than those with lower levels [26]. In addition, atopic individuals within such populations have significantly lower total IgE levels, higher specific anti-parasite IgE concentrations, and less intense helminth infections than their non-atopic counterparts [27] These observations suggest that atopic hosts may develop more effective specific responses against parasites through evolution, and that helminths, also through evolution, countered this by developing allergens that provoke a polyclonal IgE response [28,29]. The atopic state of patients therefore appears to favour a specific over a polyclonal IgE response, what might be the case in some of the population in the present study.

In conclusion, the data obtained in this study support the hypothesis that *E. coli* and *E. histolytica*-induced IgE in atopic subjects is risk factor of allergic reactions. Epidemiologic studies of atopic diseases in subjects with and without parasitic diseases may further clarify the relationship between atopic diseases and these protozoan infections.

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