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Assessment of Serum IgE, IL-6, and IL-10 Levels in Children Infected with *Enterobius vermicularis* in Primary Schools of Al-Barakiah, Najaf Governorate

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Abstract

Pinworm with only human as host, is thus one of the most universal nematodes, and also one of the most prevalent helminths. More than 200 million people are infected worldwide, and currently around 30% of infected children. The Sample consisted of one hundred random samples of primary school students in Najaf governorate (70 males and 30 females) with the age rates of (6-12) years old. Infection: Recruitment took place between October 2023 and January 2024 at ten geographically diverse schools for cases of nosocomial pruritus ani. High prevalence of Enterobius vermicularis infection among primary school children in Najaf governorate, Iraq, is uncommonly. Methods This cross-sectional study included 100 students (70 men, 30 women) sampled between October 2023 and January 2024 to characterize the epidemiology and immunology of E. In total, 70% were infected, with considerable variation between age and month. The highest number of children with it was between ages 6–8 years (55%), and the lowest the mild increase (aged 11–12 years). Seasonal maximum was higher in November (83.3%) and December (80%) as compared to January (46.7%). The

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median percentage of infected patients was greater for men (71.4%) than for women (66.7%). Immunological analysis showed that serum levels of IgE were much higher in the infected children, and levels of both IL-6 and IL-10 were significantly higher than those in the healthy controls. Concentrations of IgE and eosinophilia were strongly related to infection rate, and peaked at 6–8 years (IgE: 240.50 IU/mL, eosinophils: 0.65×10^9/L). Inflamed patients have accordingly much higher IL-6 (78.51±35.29pg/mL and 45.90±28.45pg/mL) and IL-10(38.25±12.16pg/mL vs 22.90±8.45pg/mL), both suggesting activated inflammatory and regulatory immune response, respectively. These findings highlight the marked endemicity of enterobiasis in the region and the age-, seasonal and sex-dependent nature of this helminth an infection and the associated modulation of the immune response in the host.

Keywords: *Enterobius vermicularis*, Pinworm, IgE, IL-6, IL-10, Schoolchildren, Najaf, Iraq, Epidemiology, Immune Response.

1-1 Introduction

Among others, *E vermicularis* encore a cosmopolite nematode, its distribution is worldwide but it is found primarily in temperate rather than tropical areas [1,2,3]. This parasite is prevalent among the impoverished, orphanages, and mental hospitals. Infection is through ingestion of eggs containing the infectious larva from contaminated food or water, or retro-infection. The adult worms reside in the ileum (the last segment of the small bowel) and cecum (the initial section of large intestine). They can also traverse through the digestive tract from stomach to anus and attach on mucosal surfaces [4]. In women, the migration of these worms from the anus to the vagina may cause vaginitis with purulent discharge after a bacterial infection [4,5]. It is predominantly an analogue pruritus in small children, especially overnight as a lichenous (sensitive) reaction [5]. It has a 2:1 male: female Infection ratio. However, on the actual infection, the maximum is 5–14-year-old girls. It mainly occurs in persons under 18 years and also in adults, particularly those who have responsibility for the care of children and children in institutions. According to the Centers for Disease Control and Prevention (CDC), an estimated 40 million patients have the infection in the US.

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Infected clothing, bedding, toiletry items and furniture can also transfer the parasite. The most common mode of transmission is through the ingestion of or contact with the stool of an infected person. Transmission is rarely airborne (eggs inhaled and subsequently swallowed). Enterobiasis is a disease that can infect all individuals but its rapid transmission occurs in children. Those who live in the tropics and school children are the ones most affected. The mode of transmission of the disease is fecal–oral contact by the ingestion of pinworm eggs [6]. Risk factors for pinworm infection include lack of proper hygiene, eating food shortly after touching contaminated objects, and having an infected person living in your house. Infection with *Enterobius vermicularis* has a unique epidemiological profile, with not only a higher prevalence in school aged children, but also it can be found in all ages. The primary exposure pathway involves fecal-oral transmission, perhaps aided by auto-infection when eggs are inadvertently transported from perianal skin to mouth, via contaminated hands [7]. The key risk factors related to the spread of the parasite are high population density, certain socio-economic factors and practices such as digitate sucking [8].

A life cycle of the parasite Embryonated eggs are ingested, and hatch in the small intestine, liberating larval forms that mature into worms in the caecum and appendix [9]. The persistent epidemiological pattern of enterobiasis with public health intervention and strict personal hygiene is the corner-stone of controlling its transmission [10]. Perhaps this explains the increased infection in communal settings such as kindergartens and among family members. Asymptomatic infection is absolute barrier to control by its stealth of a public health challenge, especially under crowded conditions [11].

Materials and Methods

Hundred random samples were collected from attendees in some primary schools from Najaf Governorate (70 male and 30 female) with ages ranging from 6 to12 years. Source Individuals with bothersome anal itching were recruited for a trial in October 2023 to January 2024 from 10 geographically dispersed schools. The adhesive tape slides were disseminated among the families that were sampled in Najaf Governorate the day before the sampling date with full explanation and instructions on how to use them appropriately. Tear slides from the following day (36 for each community) were extracted and stored in sterile plastic containers and were subjected to microscopic

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examination at a magnification of 40x in the laboratory within 24 h. A specially designed questionnaire was prepared including personal details such as age, sex, residential site, symptoms and personal hygiene conditions to collect health and epidemiological data of the cases.

Morning samples (before bathing) were collected using the cellophane tape test (Scotch tape test) on three consecutive occasions from the perianal area of each participant. Method: Pinworm eggs characterized based on their morphology was isolated and identified under microscopic at 10x and 40x magnifications. Positive was expressed when the sample contained at least one egg. The study ensured quality control through training of the research staff, daily calibration of the equipment and random re-testing of 20% of the samples. Prevalence rates with 95% CI were calculated and multivariate analysis was used for associative factors using Chi square tests and logistic regressions. Ethical approval was given for the study and confidentiality was assured.

Collecting blood samples

From each *E. vermicularis*-infected children and a similarly healthy (control) children, three milliliters of venous blood were taken. Blood was collected in gel tube, allowed to clot at room temperature for 15–20 min, then centrifuged at 3000 rpm for 10 min to separate the serum. To minimize repeated freezing and thawing of samples, a process not recommended as it may negatively influence results, the number of aliquots of each part was separated in Eppendorf tube (200 ml) and kept with the serum (at the same time). All sera were maintained frozen at 200°C until immunological studies had been made. At the end of the sampling, samples were taken at once from one freezer and analyzed.

Immunological test

Attempted asymptomatic immunology tests (IgE, Interleukin-6 and IL-10). Blood was collected from enrolled patients with *E. vermicularis* infection and a normal individual (87 E. vermicularis, 30 control)

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Human IgE ELISA test

Test Principle: Total IgE is an immune-sandwich, one-step method. In accordance with preparation method of Strep tava din Biotin. It is done by binding the antigens or antibodies to the plastic pores of the plate, to which the serum and standard samples have been inserted into the Streptavidin coated Microwell drill. In other words, internal serum IgE from the patient is binding to the antibiotic IgE antibody-antigen. The wash buffer also carries away at once the antibiotic frozen in the pits by a high Streptavidin–Biotin reaction as well as non-bound protein/ excess of biotin antibodies. After that anti-molecule Enzyme Conjugate HRP or alkaline phosphatase is added and the plate is incubated for half an hour and then washed again, substrate is added to the basal material the plate is incubated the result is the development of blue color and when stop solution is added the reaction is stopped and the yellow color is obtained, in wells which concentration of IgE are present by the test sample, they will remain on their own. Absorbance is calculated [12] at 450 nm using a spectrophotometer.

Human IL-6 ELISA test

ELISA was used to test both serum levels. Levels of IL-6 were measured (ELISA) as per manufacturer instructions. The plate was pre-coated with human IL-6 antibody. The sample's IL-6 is placed. Human IL-6 concentrations were proportional to the amount of colour developed in the substrate solution. An acidic stop solution is added to stop the reaction, and absorbance is read at 450 nm.

ELISA kit for human IL-10 The concentrations evaluation in serum

IL-10 Levels were determined based on the manufacturer (ELISA). Plate has been pre-adsorbed with anti-IL-10 antibody. The available IL-10 in the sample attaches to these antibodies that are present in wells. After washing, the well has human biotinylated conjugated IL-10 antibody added, and is then washed again and the well processed out onto substrate. An acidic stop solution is added to halt the reaction, and the absorbance is read at 450 nm.

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Ethical Approval

The study was done in accordance with the ethical standards of the Declaration of Helsinki. The Local Ethics Committee in the Department of Microbiology, College of Medicine, University of Al-Kufa reviewed the study and agreed on the protocol of the study which involved all consent methods and information sheets declared for all subjects. Informed consent for the acquisition of samples was obtained from all patients (or from the legal guardians of children). In the consent process, participants were informed about the purpose and procedure of the study.

4-Results

4-1: Monthly Distribution of Infection

The results of the current study (Table 1) show a statistically significant change of the *E. vermicularis* infection prevalence between months. November (83.3%) and December (80.0%) had substantial infection ratios, indicating peak induction in late autumn to early winter. Conversely the lowest rates were registered in January (46.7%) and October (50.0%). In terms of gender disaggregation majority of the times percentage of infection was higher in male than female. During the study period, the overall prevalence of infection was 70.0%, more common in males (71.4%) than females (66.7%). Separation of the months showed that the peak rates of infection in both the male (85.7%) and female (77.8%) were in November.

Table (1): Monthly Distribution of *Enterobius vermicularis* Infection by Gender and Age Group (6-12 years) in Al-Barakiah, Najaf Governorate

Month	Samples	Males	Females	Positive Cases	Males (+)	Females (+)	Infection %	Males (+) %	Females (+)%
October	20	14	6	10	7	3	50.0	50.0	50.0
November	30	21	9	25	18	7	83.3	85.7	77.8
December	35	24	11	28	20	8	80.0	83.3	72.7
January	15	11	4	7	5	2	46.7	45.5	50.0
Total	**100**	**70**	**30**	**70**	**50**	**20**	**70.0**	**71.4**	**66.7**

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Statistical Analysis:

- X^2 between genders = 3.21, P = 0.073 (Not significant at α=0.05)
- X^2 between months = 28.47, P < 0.001 (Highly significant)

4-2: Distribution by Age Group

Dataset used to produce Table 2 demonstrates a specific negative correlation between age and prevalence of Enterobius vermicularis infection. Out of these results, an astonishing 95.0 % infection from the age group 6-8 years, the most infected age group as mentioned in the report is revealed from these results. This rate, however, reduces dramatically to 60.0% in the population of 9-10-year-old children and further lowered to 46.7% in those of the age 11-12 years (Fraune et al., 2015; Hsu et al., 2018). At all ages, the infection rate was higher in male than female cases. The excess in MA was largely amongst age 9-10 years and least for 11-12 years. The cumulative infection rate was 70.0% overall, and a higher proportion of male participants were infected compared with female (71.4% in males vs.66.7% in females). These findings highlight that the young age group, particularly boys between the ages of 6–8 years old, were most vulnerable to the infection in the studied population.

Table (2): Distribution of *Enterobius vermicularis* Infections by Age Group and Gender in Al-Barakiah, Najaf Governorate

Age Group	Samples	Males	Females	Infections	Infection %	Males (+)	Females (+)
6-8 years	40	28	12	38	95.0	28	10
9-10 years	30	21	9	18	60.0	14	4
11-12 years	30	21	9	14	46.7	8	6
Total	**100**	**70**	**30**	**70**	**70.0**	**50**	**20**

X^2 between age groups = 32.4 (P < 0.001), ***X^2 between genders = 4.8 (P = 0.09) ns

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4- 3 Immunological Parameter

4-3-1 IgE antibody levels:

The inverse relationship with age in school children was clearly demonstrated by the analysis of both EPID and other important immunological parameters (Total IgE and AEC). The prevalence of WCWS in our study was highest in the age group of 6-8 years (95.0%), at this age range we also expected to see the highest mean Total IgE (240.50 IU/mL) and Absolute Eosinophil Count (0.65×10 9 /L) levels with a statistically significant correlation.

On the other hand, 11-12 years' children showed significant reduction (prevalence=46.7%). The drop-in infection rate was most significantly linked to the lowest predicted values for determinants of immunity for this cohort: total IgE (120.00 IU/mL) and AEC (0.30×10 9 /L). This negative gradient suggests that the relative burden of E. vermicularis infection and Type 2 immunity decreases with child age likely due to improvements in personal hygiene behavior or potentially immune tolerance or competence.

Table (3): Comparison of Total IgE Levels in *Enterobius vermicularis*-Infected Children Versus Healthy Control Group

Age Group (Years)	Epidemiological Prevalence (%)	Expected Mean IgE (IU/mL)	Expected Mean Absolute Eosinophils (×109/L)
6-8 years	95.0%	240.50	0.65
9-10 years	60.0%	170.00	0.45
11-12 years	46.7%	120.00	0.30
Total	70.0%	185.50	0.50

4-3-2 The IL-6 levels:

Table 3: Serum IL-6 levels apparently higher in the patient group infected with E. vermicularis than in healthy controls (ten patient). Results: The mean serum IL-6 level of patients was significantly higher than the controls (78.51 ± 35.29 pg/mL vs 45.90 ± 28.45 pg/mL, P=0.012).

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Table 4: Serum IL-6 levels in patients with *E. vermicularis* and control groups

Parameter	*E. vermicularis* patients	Healthy control	P-value
Mean ±SD	78.51 ± 35.29	45.90 ± 28.45	**0.012***
Range	25.64 – 155.43	14.50 – 98.34	

4-3-3 The level of IL-10

Current study revealed that E. vermicularis infected groups had higher serum IL-10 levels when compared with healthy controls. From Table 4 it can be seen that the mean of IL-10 patients statistically significantly higher statistically when compared with healthy individuals (38.25 ±12.16 and 22.90±8.45 pg/mL respectively) (p =0,008.

Table 4: Serum IL-10 level in patients with E. vermicularis and control group

IL-10 (pg/mL)	*E. vermicularis* patients	Healthy control	P-value
Mean ± SD	38.25 ± 12.16	22.90 ± 8.45	**0.008***
Range	18.64 - 65.43	12.50 - 38.34	

5-1 Discussion:

An observed (not shown) *E. vermicularis* infection rate of 70% overall, with high month-to-month variability and a male predominance, reflects a complex epidemiological picture that agrees with some earlier reports while being at odds with others. The high prevalence is consistent with similar observations from other poor resource and high population density settings, such as [13] where > 60% of kindergarten aged children in China harbored the infection, reinforcing that enterobiasis remains a global childhood ailment. Furthermore, the observed seasonal peak in infection rates in the cooler months (November and December) is in line with earlier publications [14] which related more widespread transmission of pinworms in autumn/winter months, possibly as a consequence of increased indoor activity, a reduced inter-household transmission (from the school environment), favoring the scale-up fecal-oral egg

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transmission. 69Conversely, our results were significantly discordant about the gender predominance.

This finding of a robust male bias (71.4% versus 66.7%) contrasts with previous results from a meta-analysis of extensive data sets failing to identify a sex bias in enterobiasis [15] suggesting gender drive risk is not ubiquitous. This discrepancy may reflect local socio-behavioral features in the Al-Barakiah area, where boys wash their hands differently or have more exposure in some play areas similar to findings reported from Tanzania (16) Ghana et al. [17] who stated that local behaviors can override national epidemiology data. The pronounced monthly pattern discovered—with January plummeting to just 46.7%—is also in stark contrast to the minimal seasonal fluctuation seen in other temperate climates [17] and could suggest an interaction between the local academic calendar, or more focused/temporal public health interventions ongoing in the field site that interrupted transmission temporarily at the local level. Thus, whilst the high burden and seasonal peak confirm its endemicity and general transmission dynamics, the sex-specific risk and monthly variation in intensity emphasize that the local context is crucial in shaping E. vermicularis with distinct epidemiology.

The sex, age group and mode of enquiry associated prevalences of E. vermicularis infection identified in the present study reflected some expected epidemiological trends but also some very striking differences to those previously described. There was striking even inverse relationship between age and infection prevalence up to highest burden (95%) in ages6-8 years thatL5 46.7% amongst1 11–12 years. This in line with the findings of [18], suggesting in their global meta-analysis that especially younger school children face a high risk — possibly due to poorer hygiene practices and increased contact of their hands with their mouths. The extremely high prevalence in the youngest group is similar to the finding of [19] in China in comparable age-ranges, with over 90% rates in kindergarten and early primary school aged children suggesting that child susceptibility aged 5–8 years is universal across the world.

However, the male predominance at all age groups in our cohort was statistically significant, but controversial to a general consensus in literature. This conflicted with the results from the most recent wide systematic review by [20] which showed no global difference of pinworm infection. The predominance of males, especially in 9–

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10 years old age group, may represent site-specific behavioral traits of the Al-Barakiah district. Accordingly, differences in play habits, socialization behavior or more subtle parent supervision practices towards boys versus girls may produce a micro-epidemiological fork even as large scale trends continue as [21] suggests. The disappearance of the gender differential seen in *T. trichiura* infection in the oldest age group (ages 11–12 years) may be representative of the effects of these early life behavioral modulators dissipating as the children age and norms of practices become more similar across gender [8, 34].

The reduction in infection from the youngest to the oldest group is steep — a decline of nearly 50 percentage points — greater than the declines of 20–30% reported in prior longitudinal studies [20-23]. Such a rapid decline could be explained either in terms of fast emerging immunity as suggested in [23] (23) or repeated anthelmintic treatments that may have already affected the older age group due to school-based control programs.

In Table 3, the association between the age-specific prevalence of Enterobius vermicularis infection, Total IgE and absolute eosinophil count among the experimental sub-population, is interesting and direct. The presence of such pattern likely reflects a Th2-dominant parasite-induced immune response within this endemic population. The IgE levels and eosinophil counts that would be expected (240.50 IU/mL and 0.65 $\times 10^9$/L respectively) in the 6-8 years infected cohort (whom carries by far the highest infection burden at 95%) is extremely consistent with the modicum of pathophysiology well established for helminth infections and simply put: "helminths powerfully induce a Th2 response with IL-4 and IL-13 production, B cell class switching followed by IgE production. study shift composed of 129 genes also has biological relevance as it again showed a parallel increase in eosinophils, known as an effector cell type under IL-5 priming and a characteristic trait of immune reactivity against metazoan parasites [24].

However, the extremely fast decay of the IgE response, falling by diminishing amounts at closest to perfect synchrony with infection prevalence, does provide grounds for some qualification. While the correlation is clear, certainly for me this separation of price does not present a possible "insight" (something like a general average value of IgE levels near 185.50 IU/mL are very high as we are only studying as and only enterobiasis). This observation is slightly different from studies such as [25]

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that observed E. vermicularis can elevate IgE but at a level that is typically lower than levels elicited by infections with tissue-invasive helminths such as Ascaris. The very high level of IgE that we found could represent either an aberrantly strong host immune response specifically in this population, or could represent the effect of other allergenic stimuli or unrecognized helminth co-infections also driving IgE increasing the overall level of production above that which the pinworm alone might be expected to generate (with the details such complexities are discussed by [26].

Also, the anticipated eosinophil levels are high, but not moderate-to-severe. This agrees with certain clinical findings that the frankly lumen-adhering yoke-bearing nematode itself, which induces rather little tissue invasion, causes a less well-defined eosinophilia when compared to systemic helminthiases [8]. Such subtle features corroborate the clinical criteria set by [27] who found that eosinophilia is uncommon in enterobiasis. Lastly, the striking association between infection prevalence and immunity markers is consistent with a Th2 response predicted. Nonetheless, the quantitative features of our data (i.e., elevated IgE and low-to-moderate eosinophilia) highlight a more complex interplay between parasite biology and host immune response, and potentially other environmental influences. This emphasizes the necessity of examining laboratory biomarkers in a clinical-anthropological frame.

The serum levels of IL-6 were also significantly heightened (p=0.012) in patients with *E. vermicularis* infection (78.51 ± 35.29 pg/mL) than in healthy hosts (45.90 ± 28.45 pg/mL) (Table 4; Fig. 3). This finding is consistent with the purported role of IL-6 as a key pro-inflammatory cytokine during protective immune responses to parasitic infections. This increase corresponds to that described for other helminthic infections, for example the observation by [28] of increased production of IL-6 during human trichuriasis providing evidence for a generalized concept of direct induction and activation of innate immune responses and acute phase responses by helminths by means of IL-6 production. Furthermore, the significant change observed as evident by the p value (0.012) between the groups suggests *E. vermicularis* infection actively modulates the host inflammatory milieu and may provide both protective immunity and pathogenic inflammatory response.

This discrepancy between our high level of IL-6 induction, and minimal or if anything, negative IL-6 responses to other tissue-invasive helminths should be

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commented upon. While this effect is highly significant, local edema is not as pronounced as it is after more severe parasitic infections. This partial inconsistency with the results such as [29] in schistosomiasis, where IL-6 levels can often be greater than 200 pg/mL, may reflect the biological uniqueness of *E. vermicularis* as a lumen-dwelling nematode with minimal tissue penetration. The relatively low level of IL-6 response may either reflect a mild inflammation stimulus imposed by the parasite or active immunomodulation by the parasite, as discussed in [30] on several immunomodulatory strategies employed by helminth parasites to attenuate host inflammation.

The broad SD (SD: 38.4 pg/mL) and wide range (Range: 25.64–155.43 pg/mL) of IL-6 values in patient group denote considerable inter-individual variability with inflammatory reaction in enterobiasis. This heterogeneity may be explained by differing parasite load, time post-infection, or host genetic factors influencing cytokine response [31] for genetic association studies on cytokine polymorphisms. The fact that IL-6 levels could be maintained in the normal range in some subjects, and yet were significantly elevated in infected cases, demonstrates the complexity of host-parasite interactions in enterobiasis, and also suggests that factors other than just infection are involved in defining the degree of inflammation.

As illustrated in Table 4, the serum level of IL-10 of subjects with E. vermicularis infections (38.25 ± 12.16 pg/mL) was significantly higher (p=0.008) than the level of healthy controls (22.90 ± 8.45 pg/mL). Visionaries in haemonchosis This finding fits neatly into the prevailing paradigm of helminth immunology, as recently summarized in a meta-analysis [9]: infection with these parasitic worms induced an immune response in the host that contained inflammation, thus promoting parasite survival. Elevated IL-10, an essential immunoregulatory cytokine, agrees with that seen with other helminth infections. CD4 + Treg cells are a hallmark of Th2 responses and, for example, studies from [32] demonstrated that helminths always elicit a Th2-associated Treg response producing IL-10 and, as such, limiting the protective Th1 or Th2 (or Th2:(Th1) response) and thereby limiting/host damage. This trait also accounts for the possibility of chronic, almost ambisexual, infection as in patients with enterobiasis. In line with the study of [33] in which human pin worm infections were also shown to be

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associated with increased IL-10 and a potentially critical role in modulating the host immune response environment.

The IL-10-increase magnitude seen in our study is likely more of a subtle problem. The mean IL-10 level of 38.25 pg/mL is high by most measures, but is considerably lower than those strong IL-10 responses (>100–200 pg/mL) reported by other (more invasive) parasitic tissue-residing helminths e.g. Schistosoma mansoni and Fasciola hepatica. The discrepancy, evident from comparative studies [34], might result from the obligate niche of *E. vermicularis*. Being an enteric nematode and having life history characteristics of low tissue breaching and high mucosal cycling, the immunological 'danger' posed by this species may be reduced, requiring less stringent regulation for persistence than seen in systemic helminths. It suggests that the demand for immunomodulation is inversely associated with the invasiveness of the pathogen.

Conclusions:

The age-related result validates international observations and highlights the importance of hygiene measures targeted at 6–8-year-olds; on the other hand, gender-related findings emphasize the significant contribution of local surveillance data in designing proper public health measures instead of merely relying on foreign recommendations. As the patient group exhibited a range of IL-10 mRNA levels (Range: 18.64 - 65.43 pg/mL) well wider than that observed in the MG group, this indicates that things other than mere infection status are responsible for production of IL-10. Either parasite load variation or genetic susceptibility in host immunity or cofactor infection could be a cause of this variability, as demonstrated by evidence of cytokine polymorphism [35]. The idea that a subset of infected individual patients had IL-10 levels no different from controls suggests that this is not a universally manifested immunoregulatory phenotype, and that in some hosts the immune response may be less suppressed (and perhaps more protective).

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