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DEPARTMENT OF MEDICAL LABORATORY SCIENCES

ASSOCIATION OF INTESTINAL HELMINTH INFECTION WITH ATOPY AND ALLERGIC SYMPTOMS IN YOUNG CHILDREN IN BATU, ETHIOPIA

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This is to certify that the thesis prepared by Sosina Walelign, entitled: **Association of Intestinal Helminth Infection with Atopy and Allergic Symptoms in Young Children in Batu, Ethiopia** and submitted in fulfillment of the requirements for the Degree in Master of Science (Clinical Laboratory Sciences) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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Table of Contents

List of Tables

List of Figures

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List of abbreviations

CBC	Complete Blood Count
CD	Cluster of Differentiation
Der p	*Dermatophagoides pteronyssinus*
EDTA	Ethylene diamine tetraacetic acid
ELISA	Enzyme Linked Immunosorbent Assay
EPHI	Ethiopian Public Health Institute
HDM	House Dust Mite
HIV	Human Immunodeficiency Virus
IFNγ	Interferon-γ
IgE	Immunoglobulin E
IgG	Immunoglobulin G
IL	Interleukin
ISAAC	International Study of Asthma and Allergies in Childhood
PBMCs	Peripheral Blood Mononuclear Cells
SAF	Sodium acetate-Acetic acid-Formalin
SOP	Standard operating procedure
SPT	Skin Prick Test
TGF β	Transforming Growth Factor-β
T_H 1	T helper 1
T_H 2	T helper 2

Operational Definitions

- **Atopy**: A skin prick test result with a wheal size of 3mm and above for the house dust mite (*Dermatophagoides pteronyssinus)* and German cockroach (*Blattella germanica*) allergens.
- **Allergic participants**: Participants who report one of the following; wheeze, asthma, eczema or hay fever within the last 12 months.
- **Formal education vs. Illiterate**: Those who can read and write versus those who cannot read and write.
- **Helminth infected**: Any helminth infection detected either by direct wet mount or formol ether concentration technique.
- **Young children:** refers to children aged 2-14 years

Abstract

Background: Helminths are potent immunomodulators and chronic infections may protect against allergy-related disease and atopy. They are also known for inducing allergic conditions. This study was aimed to assess the association between helminths and atopy and allergic conditions.

Objective: To assess the association between intestinal helminth infection and atopy/allergic outcomes in young children in Batu, Ethiopia.

Methods: Questionnaire data on allergic symptoms and a range of confounding variables was gathered in a cross-sectional study of 461 children aged from 2 to 14 years from Batu. Allergic skin sensitization to house dust mite and cockroach was measured, and a stool sample collected for qualitative and quantitative geohelminth analysis. Serum IgE using ELISA and Manual eosinophil count were measured. Data was entered and analyzed using SPSS version 20.

Results: Overall sensitivity to both allergens was 2.6 %. Self - reported allergic outcomes in the last 12 months for the 461 participants had been; wheeze (3.7%), asthma (2.2%), eczema (13.2%), and hay fever (6.9%). A burden of 8.1% (36/444) was recorded for helminths. A borderline significant association was found between atopy and any allergy symptoms [OR 3.32 (95% CI: 0.99, 11.1), P = 0.052]. There was no significant association between helminths and atopy [OR 0.64 (95% CI: 0.29, 1.41) p = 0.268] and also between helminths and allergic symptoms [OR 0.64 (95% CI: 0.29, 1.41) p = 0.268]. Bivariate analysis showed keeping animas in the house associated with atopy while maternal and paternal history of allergy associated with allergic symptoms in the children.

Conclusion: Contrary to the majority of related studies that reported a positive or negative association between helminths and allergy-related outcomes, we found no relation between the two. Further longitudinal studies are warranted to further elucidate the controversy.

Keywords: Atopy, helminths, Allergy, IgE

1. INTRODUCTION

1.1. Background

Helminth infections and allergy disorders are common causes of morbidity around the globe though their distribution shows an inverse association. In developed and industrialized countries, helminth infections are not a problem while allergy is a common causes of chronic morbidity [1]. On the other hand, in developing countries, intestinal helminth infections are major health concerns [2] while allergic conditions are less prevalent though they are increasing steadily along with urbanization [3]. A potential explanation for this inverse global distribution of allergic diseases and helminth infections, was given by Strachan, who first proposed the hygiene hypothesis [4].

The 'hygiene hypothesis' explains the increase of allergic conditions through a hygienic environment and reduction in the frequency of childhood infections causing a failure to program the immune system for adequate immune regulation. This hypothesis encompasses a list of causative agents that might account for the dramatic rise in allergies in recent years in industrialized societies. Factors that have been implicated include improved public health, the use of antibiotics or vaccines and the subsequent reduction in childhood infections, the exposure to endotoxins, domestic-animal ownership, farm habitation and alterations in gut flora. Of the factors studied, the most striking associations with allergy (hay fever) were those for family size and position in household in childhood [4, 5].

The hypothesis have proposed that early childhood infections might reduce the risk of developing atopic disorders by deviating the immune response from a T helper 2(T_H2) response to an anti-allergic T helper 1 (T_H1) phenotypic response (elevated interferon-γ (IFNγ) and interleukin (IL)12 production and suppression of T_H2 responses) [5].

The human immune response to a geohelminth infection and allergic disease have a close resemblance. Both cases are associated with tissue mastocytosis, increased numbers of peripheral-blood eosinophils, high levels of polyclonal and specific immunoglobulin E (IgE), and CD_4^+ T cells that preferentially secrete the T_H2 cytokines [6]. (Figure 1). In allergic conditions the T_H2 immune response is only associated with pathology, while in helminth infection it is usually associated with protection (sometimes it can associate with pathology) [7]. Many believe that the IgE axis evolved to counter metazoan parasites (worms and parasitic arthropods) which are too large to be phagocytosed, and that allergy is a misdirected anti-parasite response in hypersensitive people (atopic individuals) [8].

In healthy individuals, IgE antibodies are generated only in response to parasitic infections. However, some people, referred to as atopic, are predisposed to generate IgE antibodies against common environmental antigens. This type of reactions encompass the most common allergic reactions, including hay fever, asthma, atopic dermatitis, and food allergies. IgE antibodies cause allergic reactions by binding variety of innate immune cells that express Fc receptors (FcεRs) specific for their constant regions. These are expressed by a variety of innate immune cells, including mast cells, basophils, and eosinophils. The binding of IgE antibodies to FcεRs activates these granulocytes, inducing a signaling cascade that causes cells to release the contents of intracellular granules into the blood, a process called degranulation. The contents of granules vary from cell to cell, but typically include histamine, heparin, and proteases. Together with other mediators that are synthesized by activated granulocytes (leukotrienes, prostaglandins, chemokines, and cytokines), these mediators act on surrounding tissues and other immune cells, causing allergy symptoms [9].

Parasitic helminths, characteristically, are capable of infecting humans for years or decades. To achieve such chronic infections, the host's immune system is tolerant to the presence of the parasite through the stimulation of selective immune suppression [5]. They are capable of evoking a "modified type-2 immune response" that might not lead to allergic inflammation following exposure to allergens. Dendritic cells, alternatively activated macrophages, regulatory T and B Cells are the major cells targeted by parasitic immune modulation [10]. Features of helminth infection that might suppress allergies are the state of T-cell hyporesponsiveness, increased frequency of T_{Reg} and other regulatory T cells and elevated production of IL 10 and transforming growth factor-β (TGFβ). These changes in T-cell response, which are characterized by the increase in total and parasite-specific IgG and IgG4 antibody levels, and alterations in the T_H2- cytokine profile collectively are part of the helminth modified T_H2 response [5].

Chronic helminth infections have been associated with an IL 10 dominated regulatory state that impairs both responses to parasite specific and bystander antigens. Unlike chronic infections, acute parasite infections induce a strong T_H2 like polarization that has been associated with the development of allergic diseases and the production of polyclonal IgE. In addition, parasites encode and secrete proteins that have a high degree of identity (or similarity) with known allergens so that following helminth infection the host develops an IgE response to the parasite that can cross-react with aero-allergens [11].

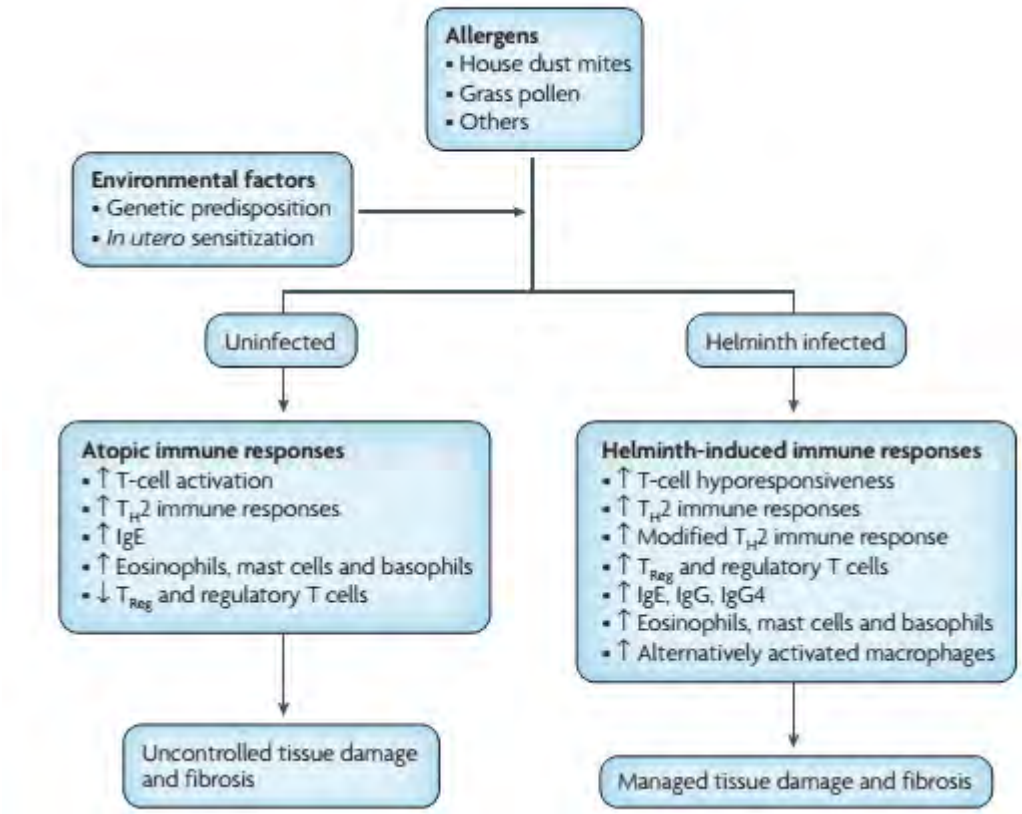

Figure 1: Influence of helminths on the immune response to allergens [Taken from: Fallon PG and Mangan NE. Suppression of T_H2 -type allergic reactions by helminth infection. *Nature reviews*. 2007].

The interest that rose in 1970's to investigate the effect of helminthes on allergy still goes on today without reaching a consensus on their protective or inducing role. Regardless of the conflicting results from studies, the current science has shown advancement as far as working on trials that aim to treat immune-mediated diseases using helminth-derived molecules. Such helminth-derived molecules could be used to selectively mimic the desirable regulatory effects of parasitic helminths, without the side-effects of infection, in new therapeutic approaches for immune-mediated diseases [12,13].

1.2. Statement of the problem

There is no general consensus regarding the association between helminthes and allergy disorders. Many studies that explored the association between atopic diseases and helminth infections revealed conflicting results. Some studies report that children infected with some helminths have lower prevalence and milder atopic symptoms [16,17,18]. Other studies have suggested that helminth infections induce or increase the severity of atopic diseases [19,20, 21].

There are also studies that claim there is no association at all [22]. An animal model study revealed an evidence that helminthic colonization appears to protect against asthma and atopic disorders [23]**.**

Low income countries are highly burdened with intestinal helminth infections [24] making them an ideal place to conduct studies on interaction between atopic disorders and intestinal helminth infections. On the other hand, there is an increase in allergy [3] in some urban areas of developing countries which might be linked with the improved treatment in urban than rural areas. Hence understanding the relationship between helminthes and allergy could gear the future treatment strategies and provide an insight about risks and benefits of eradicating helminth infections in endemic areas.

1.3. Significance of the study

Currently the government is working on accelerating deworming programs especially in schools; although unintended effect could be inevitable. This study provided latest and current information regarding the interaction between helminth infections and atopy or allergic diseases in Ethiopian context. In addition, it provided information on atopic sensitization and allergic symptom status of Batu area which is a first information to our knowledge.

2. LITERATURE REVIEW

The literatures relating allergy and helimnths infection are diverse and complex, however this literature review section provides a summary of those topics considered to be most relevant to the research problem. It begins with discussing the potential protective effect of hemlnths infection on allergic disorder followed by a positive and no association.

2.1.1. Parasites are negatively associated with atopy or allergy symptoms

A systematic review and meta-analysis by Feary *et al.* that included 21 studies showed that any current geohelminth infection was associated with a reduced risk of allergen skin sensitization. In species-specific analysis, a consistent protective effect was found for infection with *Ascaris lumbricoides, Tricuris trichuria*, hookworm and Schistosomiasis. And they concluded that intestinal parasite infection appears to protect against allergic sensitization [14].

Leonardi-Bee *et al*. did another systematic review and meta-analysis that included 33 studies revealed infection with any parasite was associated with a small, non-significant increase in asthma risk. In species-specific analysis, *Ascaris lumbricoides* was associated with significantly increased odds of asthma, while hookworm infection was associated with a significantly strong reduction that was directly and significantly related to infection intensity. Other species had no significant effects on asthma [15].

A cross-sectional study conducted in Vietnam by Flohr *et al.,* on 1601 children that studied effect of poor sanitation and helminth infection on skin sensitization showed the risk of sensitization to dust mites was reduced in those with higher hookworm burden and with Ascaris infection, and increased in those using flush toilets. In contrast, sensitization to cockroach was not independently related to geohelminth infection but was increased in those regularly drinking piped or well water rather than from a stream [16].

A prospective study done on 126 newly arrived Ethiopian immigrants in Israel in the years 1997–2001 by Stein *et al*., showed that helminth infection is significantly associated with low allergy and low SPT reactivity. One year after immigration to Israel, allergy and SPT reactivity increased significantly in all immigrants and also higher increases in positive SPT and allergy were observed after a year in the group that remained infected with helminths, even though they had a lowered helminth load; the study concluded that the reasons for the increased allergy one year after immigration needs further

investigation but probably reflects the combined influence of the decreased helminth load and novel environmental factors [17].

A nested case control study conducted in Jimma in 1996 by Scrivener *et al*. revealed that the risk of wheeze was independently reduced by hookworm infection, increased in relation to Der p 1 level. In the urban population, *D pteronyssinus* skin sensitization was more strongly related to wheeze than in the rural areas, where *D pteronyssinus* sensitization was common, but unrelated to wheeze in the presence of high-intensity parasite infection. The study concluded that high degrees of parasite infection might prevent asthma symptoms in atopic individuals [18].

2.1.2. Parasites are positively associated with atopy or allergy symptoms

Buendía *et al.* conducted a cross-sectional study in Colombia to analyze the relationship between Ascaris sensitization and asthma severity in 313 patients. The result showed after adjustment for HDM sensitization, Ascaris sensitization remained associated with asthma severity (severe dyspnea and > 4 ER visits). This study concluded that in that tropical population, IgE sensitization to Ascaris and the cross-reactive tropomyosins was frequent and associated with clinical indicators of asthma severity. The significant relationship between sensitization to the nematode-specific marker Asc s 1 and ER attendance supports these findings. Moreover, ascariasis increases the human IgE responses to HDM specific allergens [19].

Another cross-sectional study conducted in Uganda on 2316 individuals by Webb *et al.,* showed *S. mansoni* was positively associated with *Dermatophagoides*-specific IgE, *T. trichiura* with SPT, *Mansonella perstans* with cockroach-specific IgE, *A. lumbricoides* with wheeze in participants' ≥5 years and with *Dermatophagoides*-specific IgE. So this study provided strong evidence that individuals with certain helminths were more prone to atopy in this setting [20].

A nested case-control study conducted in urban and rural South African children by Calvert *et al.* to investigate the effect of Ascaris infection on bronchial hyper reactivity, skin testing, and specific IgE levels showed geometric mean total IgE levels were higher in Ascaris infected subjects versus uninfected subjects, and high levels of total IgE were positively associated with detection of specific IgE to the aeroallergens tested, but there was no significant association between Ascaris infection and titers of specific IgE. Ascaris infection was associated with a decreased risk of a positive skin test response but an increased risk of Exercise Induced Bronchospasm [23].

2.1.3. Parasites do not have effect on atopy or allergy symptoms

A study was conducted by Amberbir *et al.* in Butajira to determine the independent effects of *Helicobacter pylori*, intestinal microflora (commensal bacteria) and geohelminths infections on allergic disease symptoms and sensitization in an Ethiopian birth cohort, in 2008. Regarding geohelminths they have concluded that there was no significant association with any of the outcomes measured [22].

Like we have seen from the above literatures, there is no generalized consensus regarding helminth and allergy association. Possible reasons for the variation in results might be due to a lack of consistency between human population cohorts (for example, because of genetic and environmental differences), and variations between species of helminths that are endemic or the intensity of infection, different study designs; therefore, drawing generalized worldwide epidemiological conclusions is difficult [5]. Thus, generating more data which will contribute to better understanding of the interaction between helminthes infection and allergic symptoms or atopy is still needed.

3. OBJECTIVES

3.1. General objective

To assess the association of intestinal helminth infection with atopy and allergic outcomes in young children in Batu, Ethiopia.

3.2. Specific objectives

- To determine the association between intestinal helminthes with atopy
- To determine the association between intestinal helminthes with allergic outcomes
- To compare serum total IgE and peripheral eosinophil counts among different groups of study participants
- To assess the association between atopy and allergy
- To determine the associated risk factors for atopy and allergic outcomes

4. MATERIALS AND METHODS

4.1. Study area

The research project was done at Batu (Ziway), Ethiopia. The town is located in Oromia National Regional State, in East Shoa zone, Adami Tulu Jiddo Woreda, at a distance of 160 Km from Addis Ababa. Its astronomical location is 7º 56' North Latitude and 38º 43' East Longitude with an elevation of 1643 meters above sea level. Batu town was founded in 1961 [25]. Adjacent to Lake Ziway (Lake Dambal), the economy of the town is based on fishing and horticulture [26]. Study participants were recruited from five sites; Batu Hospital, Sher Hospital, Batu 1 Health Center, Sher Elementary School and Batu Elementary School.

4.2. Study design

A Hospital and school based cross sectional study was conducted.

4.3. Study period

The study was conducted from October 2015 – May 2017. Actual data collection period was from May-June 2016.

4.4. Population

4.4.1.Source population

Young children visiting the selected facilities during the study period were the source population.

4.4.2. Study population

Young children (2-14 years of age) who were present at the five sites during the study period and who qualified our inclusion criteria included in the study.

4.5. Eligibility

4.5.1. Inclusion criteria

- Young children (2-14) years who visited the five sites during the study period whose parents volunteered to take part.

4.5.2. Exclusion criteria

- Children who received anti- helminthic drugs for the last one month
- Children who received drugs that interfere with the skin prick test response
    - Second generation antihistamines

- Antidepressants such as doxepin, other tricyclics, and tetracyclics have antihistamine activity and may need to be withheld for 1-2 weeks or more.
- Phenothiazines also have antihistamine activity
- Over the counter cold and flu remedies, "sinus" analgesics, antitussives; antiemetics, sedatives, relaxants, migraine prophylactics (cyproheptadine, pizotifen).
- Prolonged topical corticosteroids have been shown to reduce skin reactivity

4.6. Study variables

4.6.1. Dependent variables

- Atopic status (SPT) and allergy symptoms
- Total IgE profile
- Eosinophil count level

4.6.2. Independent variable

- Socio demographic characteristics
- Intestinal helminth infection
- Other associated risk factors
  - Maternal education
  - Maternal allergic history
  - Paternal allergic history
  - Breast fed till age 3
  - Number of older siblings
  - De-worming medication
  - Proper latrine
  - Animals kept in the house
  - Presence of smokers in the house
  - Charcoal fuel use
  - Insecticide use
  - Vaccination history
  - Protozoan infection

4.7. Sample size calculation and sampling technique

4.7.1. Sample size calculation

The minimum required sample size to determine a single population proportion was calculated using the following formula.

$$n = \frac{(Z_{1-\alpha/2})^2 \times p \times (1-p)}{d^2}$$

Where,

n= the sample size estimate, P= 50%, $Za/_2$ is 1.96

$$n = \frac{(1.96)^2(0.05)(0.05)}{(0.0225)^2}$$

n= 384

A contingency of 38.4 (422) participants was planed to be added. We enrolled a total of 461 participants surpassing the contingency plan.

4.7.2. Sampling technique

Convenient sampling technique was used.

4.8. Study Procedure and Data collection

Consent and assent forms were filled by the study participant's guardians and the participants themselves. Questionnaires were completed regarding household features and individual social-demographic characteristics by asking the guardians of the study participants. Information regarding asthma, eczema and allergy symptoms was obtained using questions from the International Study on Allergy and Asthma in Children (ISAAC) questionnaire. Questionnaires and skin prick tests were done at the sample collection sites. In addition blood was drawn into two tubes one containing EDTA (3 ml) for CBC and plane tube for ELISA tests (2 ml); and stool sample was also collected on site.

Thin blood smears for manual differential count (For estimation of Eosinophil percentage) and direct stool examinations were done on site at hospitals and health centers. Among the five sites Sher Hospital was chosen as a station site. At this hospital direct stool exam and thin smears were done for those samples that came from the schools. In addition CBC, preserving stool with sodium acetate-acetic acid-formalin (SAF) and separating serum were done for all samples at this site.

Methanol fixed thin smears, preserved stool with SAF and separated serum were transported to Addis Ababa where further analysis was done.

ELISA for serum total IgE was carried out at EPHI, molecular biology laboratory while stool concentration and manual differential eosinophil counts were done at department of medical laboratory sciences of AAU, Addis Ababa.

4.8.1. Skin prick test

Skin prick test provides information about the presence of specific IgE to protein and peptide antigens (allergens). Small amounts of allergen are introduced into the epidermis and non-vascular superficial dermis and interact with specific IgE bound to cutaneous mast cells. Histamine and other mediators are released, leading to a visible "wheal-and-flare" reaction peaking after about 15 minutes [27]. Allergen skin sensitization to house dust mite (*Dermatophagoides pteronyssinus)* and German cockroach (*Blattella germanica*) allergens (*inmunotek inc.*) were measured.

Generally the most convenient and frequently used skin sites for testing are either the volar surface of the forearm or outer upper arm, and the back. Place one drop of each allergen solution on the skin. Push the lancet through the drop of allergen and apply the lancet at 90° to the skin without drawing blood. Repeat the procedure for each allergen and the controls using a new lancet for each allergen. To prevent contamination between the allergens carefully remove the surplus fluid from all sites simultaneously by placing a paper tissue over the drops. Take care not to cross contaminate the sites with other allergen solutions. If the child is moving it may be preferable to complete each allergen separately. The results should be read 15 minutes after the positive was completed. A positive test was defined as an average of two perpendicular wheal diameters, one of which was the maximum measurable diameter, of at least 3 mm greater than the saline control response.

4.8.2. Stool examination

4.8.2.1. Direct wet mount

The direct wet mount is used primarily to detect motile protozoan trophozoites. These organisms are very pale and transparent, two characteristics that require the use of low light intensity. The direct wet smear is prepared by mixing a small amount of stool (about 2gm) with a drop of 0.85% NaCl. This mixture provides a uniform suspension under a 22 by 22 cover slip .The entire 22 by 22 mm cover slip

was systematically examined with the low power objective (10X) and low intensity; any suspicious objects was then examined with the high dry objective (40X).

4.8.2.2. Formol ether concentration

In this method, faeces are emulsified in formol water, the suspension was strained to remove large faecal particles, ether or ethyl acetate was added, and the mixed suspension was centrifuged. Cysts, oocysts, eggs, and larvae are fixed and sedimented and the faecal debris is separated in a layer between the ether and the formol water. Faecal fat is dissolved in the ether [28].

4.8.3. Enzyme linked immunosorbent assay (Total IgE)

This IgE quantitative test is a solid phase enzyme linked immunosorbent assay based on the sandwich principle. (*Diagnostic Automation, Cortez Diagnostics Inc., USA*). The test specimen (serum) is added to the IgE monoclonal antibodies immobilized on polystyrene microtiter wells (solid phase) and incubated with the Zero Buffer. If human IgE is present in the specimen, it will combine with the antibodies on the well. The well is then washed to remove any residual test specimen, and goat anti-IgE in the antibody-enzyme (horseradish peroxidase) conjugate reagent is added. The conjugate reagent will bind immunologically to the IgE on the well, resulting in the IgE molecules being sandwiched between the solid phase and the enzyme-linked antibodies. After an incubation at room temperature, the solid phase is washed with water to remove unbound labeled antibody. A solution of 3,3',5,5'-Tetramethylbenzidine (TMB) is added and incubated for 20 minutes, resulting in the development of a blue color. The color development is stopped and the resulting yellow color is measured spectrophotometrically at 450 nm. The concentration of IgE is directly proportional to the color intensity of the test sample [29].

The mean absorbance value (OD450) was calculated for each set of reference standards. From that a standard curve was constructed by plotting the mean absorbance obtained for each reference standard against its concentration in IU/mL on graph paper, with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis. The absorbance value for each sample, was determined from the corresponding concentration of IgE in IU/mL from the standard curve.

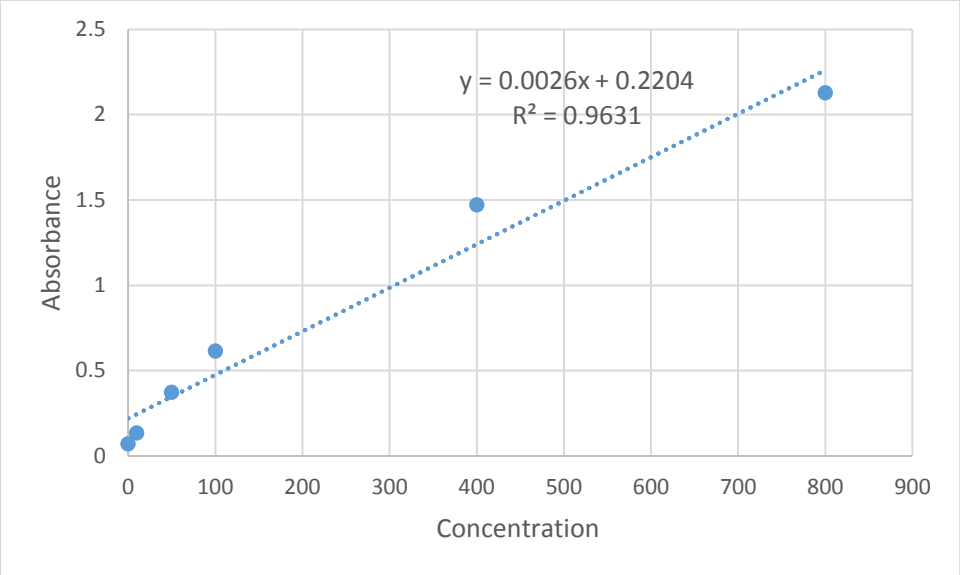

Figure 2: Standard curve for total IgE

4.8.4. Manual differential count

A Wright stained blood film was systematically examined for the different white cells seen in each field, until a total of 100 cells have been counted. The absolute number of each white cell type was calculated by multiplying the number of each cell counted (expressed as a decimal fraction) by the total WBC count [30].

4.8.5. Hematological analysis using Mindray BC-3000 Plus hematology analyzer

The Mindray BC-3000 Plus auto hematology analyzer is a 3 part differential, quantitative, automated hematology analyzer and leukocyte differential counter for In Vitro Diagnostic Use in clinical laboratories. The two independent measurement principles are used in this analyzer: the Impedance method for determining the WBC, RBC, and PLT data and the colorimetric method for determining the HGB [31].

4.9. Data quality assurance

4.9.1. Pre analytical

- Venous blood and stool specimens collected from participants were properly labeled and transported
- Reagents were kept according to the manufacturer's instructions.
- Appropriate protocols or SOPs were written and/or used according to the manufacturer's instructions for all the laboratory tests

- All reagents were checked for their expiry date and prepared and handled according to manufacturer's instructions

4.9.2. Analytical

- Protocols for each laboratory tests were strictly followed
- Quality control materials were used to check for accuracy and precision of hematological analyzer (three level controls) and skin prick test (Glycerol saline as negative control and histamine dihydrochloride as positive controls)
- Reagents were used before their expiry date.

4.9.3. Post analytical

- The data were rechecked on daily basis.
- All clinical and laboratory result of each test were recorded and documented properly
- Randomly selected samples were reanalyzed and checked by the principal investigator.

4.10. Statistical analysis

Data was entered into IBM SPSS version 20 (SPSS INC, Chicago, IL, USA) for analysis. The consistency of data was checked by tabulating variables and making simple frequencies using SPSS. Descriptive statistics were used to summarize continuous variables and simple frequencies were done to show the distribution of the socio-demographic and clinical characteristics of the patients. Crosstabs and binary logistic regression tests were used to show association between categorical variables. Kruskal wallis test was used to compare the means of serum total IgE /peripheral eosinophil counts among the different participants. Bivariate analysis and multivariate analysis were performed to calculate unadjusted odds ratio/ adjusted odds ratio with 95% confidence interval to quantify the strength of association between atopy/allergy symptoms and helminth infections and other possible predictors of atopy/allergy symptoms. P- Value < 0.05 was considered as statistically significant.

4.11. Ethical consideration

Ethical clearance was obtained from departmental research and ethics review committee (DRERC) of the department of medical laboratory science. Permission was obtained from the hospitals, health centers and schools authorities to conduct the study. Written informed consent (signed or thumb print) and assent was gained from each participant. Confidential identifiers were used to code participant's

identities. Participants with parasites were provided with the appropriate anti-helminth medication. Results and any information regarding patients are kept confidential during and after the completion of the research project by password protected electronic programs and locking hard copy files.

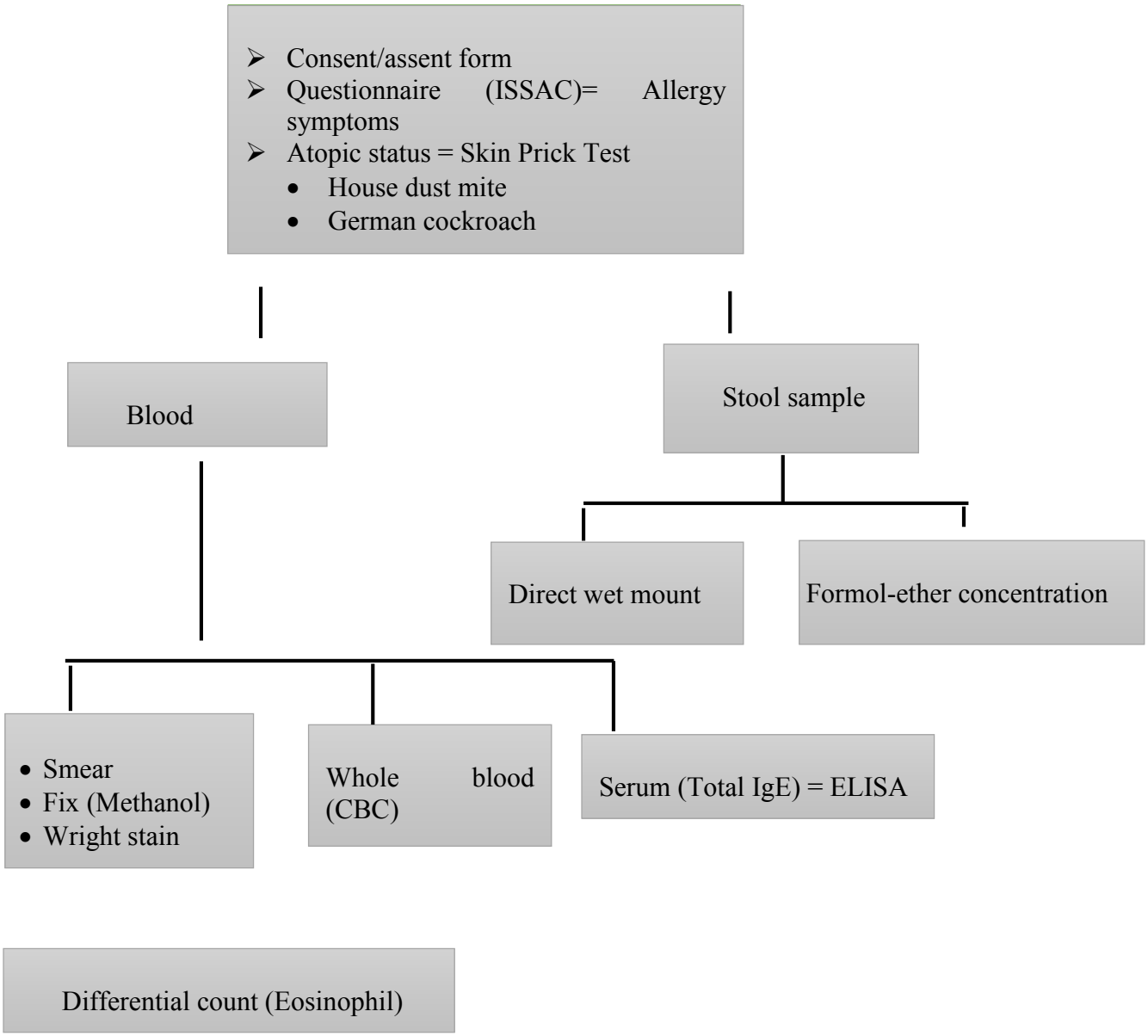

Figure 3: Study Procedure data collection and analysis

5. RESULTS

5.1. Demographic and socioeconomic characteristics of Study participants

A total of 461 eligible study participants were recruited from five sites: Batu Hospital (3, 0.7%), Sher Hospital (103, 22.3%), Batu 1 Health Center (7, 1.5%), Sher Elementary School (160, 34.7%) and Batu Elementary School (188, 40.8%). Just over half were female (50.8%) and majority (96.1%) were from urban areas. The mean age was 8.8 years (the age range being between 2 and 14) for those 458 participants. Majority of the participants (97.9%) had a proper (any) vaccination. 57.9% of the participants' mothers had a formal education and 43.8% were housewives. Table 1 describes the detailed characteristics of the study participants.

Table1: Socio demographic characteristics of the study participants, Batu, Ethiopia 2016.

Variables		Number	Percent
Sex (N=461)	Male	227	49.2
	Female	234	50.8
Age* (N=458)	2-4	37	8.1
	5-9	229	50
	10-14	192	41.9
Residency (N=461)	Rural	18	3.9
	Urban	443	96.1
Maternal education(N=461)	Formal	267	57.9
	Cannot read and write	194	42.1
Maternal occupation (N=461)	Farming and related	88	19.1
	Trading and related	73	15.8
	Government employee	12	2.6
	Housewife	202	43.8
	Others	86	18.7
Vaccination history (N=461)	Never vaccinated	10	2.2
	Vaccinated	451	97.8

*Age classified based on "Ethiopia Mini Demographic and Health Survey 2014". Central Statistical Agency. Addis Ababa, Ethiopia. 2014.

5.2. Allergic sensitization and self - reported allergic symptoms

Skin Prick Test was performed for 454 participants for the two allergens, house dust mites (*Dermatophagoides pteronyssinus)* and German cockroach (*Blattella germanica*), with rate of 1.1% and 1.5 % sensitivity, respectively. Overall sensitivity or atopic status was 2.4 %. Only one participant was sensitive to both allergens. Self - reported allergic outcomes in the last 12 months for the 461 participants had been; wheeze (3.7%), asthma (2.2%), eczema (13.2%), and hay fever (6.9%). Only 5 participant's asthmatic status was confirmed by a doctor. Table 2 describes the frequency of each type of self - reported allergic symptoms and allergic sensitization.

Table 2: Frequency of allergic sensitizations and self - reported allergic symptoms of young children, Batu, Ethiopia 2016.

Variables	Number	Percent
Self - reported allergic symptoms (N= 461)		
Wheeze	17	3.7
Asthma	10	2.2
Hay fever	32	6.9
Eczema	61	13.2
Any allergy symptom	94	20.4
Skin Prick Test (N= 454)		
HDM * (*Dermatophagoides pteronyssinus)*	5	1.1
German cockroach (*Blattella germanica*)	7	1.5
Sensitization to both allergens	1	0.22
Any sensitization §	11	2.4
Both atopic and allergic	5	1.1

*HDM – house dust mite

§ Sensitization either *to Dermatophagoides pteronyssinus* or German cockroach (*Blattella germanica)*

5.3. Burden of Helminth parasites

Stool sample collected from the participants was tested with direct microscopic examination (Wet mount) and Formol-Ether concentration techniques. Merged results from the two tests were used for the analysis. A burden of protozoa 22.7% (101/444), and helminthes 8.1% (36/444) was recorded. Among the helminth only infected groups *Hymenolepis nana* was found to be the most frequent. Different sorts of mixed infections were observed; protozoa and helminth 3.4% (15/444), mixed protozoan infection 2% (9/444), mixed helminth infection 0.5% (2/444). Based on egg load determination, all infections were light infections. The maximum number of eggs reported was 22 eggs/ gram of feces.

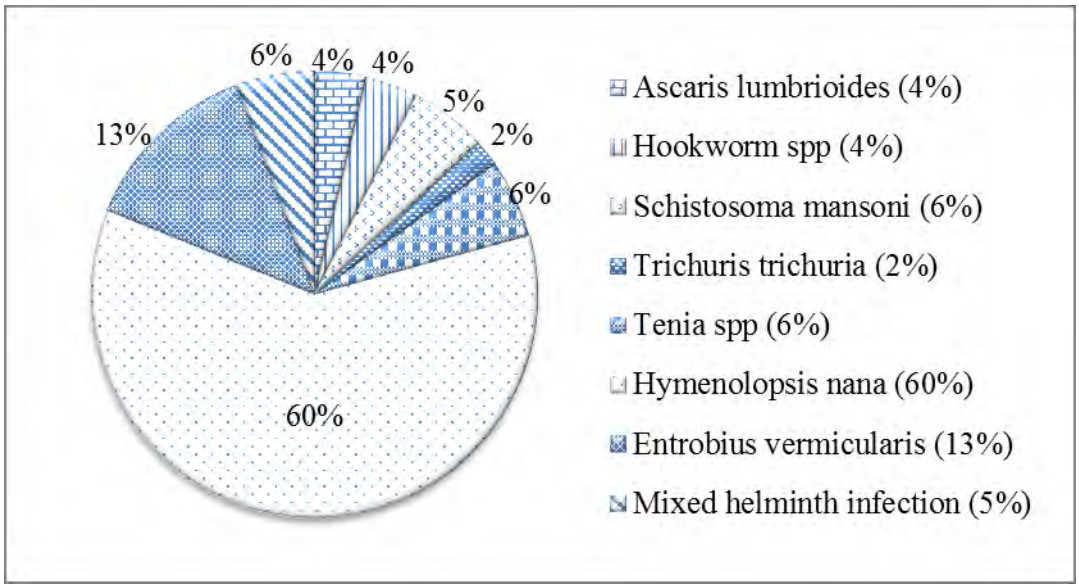

Figure 4: Frequency of individual helminth parasites among helminth positive participants, Batu, Ethiopia 2016.

5.4. Association of helminthes and protozoa infection with allergic symptoms and Atopy

Overall helminth and protozoa infections were used to see the association with allergic symptoms. Individual helminth was not used for analysis since the prevalence of each helminth was small to yield an appropriate valid association. Neither helminth [OR 0.64 (95% CI: 0.29, 1.41) p = 0.268] nor protozoa [OR 0.93 (0.56, 1.58), P=0.796] were significantly associated with allergy symptoms (Table 3).

Table 3. Associations between allergic conditions and intestinal parasites infection among young children, Batu, Ethiopia, 2016.

		Any allergic conditions			
Variables	**Overall N**	**Yes n(%)**	**No n(%)**	**Crude OR (95 % CI)**	**P-value**
Helminthes					
No	391	85(21.7%)	306(783%)	0.64 (0.29, 1.41)	0.268
Yes	53	8 (15.1%)	45(84.9%)	1	
Protozoa					
No	334	69(20.7%)	265(79.3%)	0.93 (0.56, 1.58)	0.796
Yes	110	24(21.8%)	86(78.2)	1	

Though not being infected with protozoa seemed to increase the risk of atopy by 3.08 times, as Table 4 shows the association was not statistically significant. The association between helminth infections and atopy was not statistically significant as well.

Table 4. Associations between atopy and helminth & protozoan infection among school children of five selected facilities, Batu, Ethiopia, 2016.

		Atopy			
Variables	**Overall N**	**Yes n(%)**	**No n(%)**	**Crude OR (95 % CI)**	**P-value**
Helminthes					
No	387	8(2.1%)	379 (97.9%)	0.52(0.11, 2.51)	0.413
Yes	51	2(3.9%)	49(96.1%)	1	
Protozoa					
No	328	9(2.7%)	319 (97.3%)	3.08(0.39, 24.55)	0.289
Yes	110	1 (0.9%)	109 (99.1%)	1	

5.5. Distribution of total IgE and Eosinophils among the different groups of the study participants

Independent sample Kruskal Wallis test was used to check if there was a significant mean rank difference of total IgE concentrations distribution among the different helminth/atopy (Figure 5) and helminth/allergy (Figure 6) groups of the study participants. The analysis showed the distribution of total IgE did not differ significantly across the categories of both helminth/allergy (p=0.136) and

helminth/atopy (p=0.147) groups. As shown in Figure 6, there is an increasing pattern in the total IgE level from no helminthes no atopy to those with both helminthes and atopy groups.

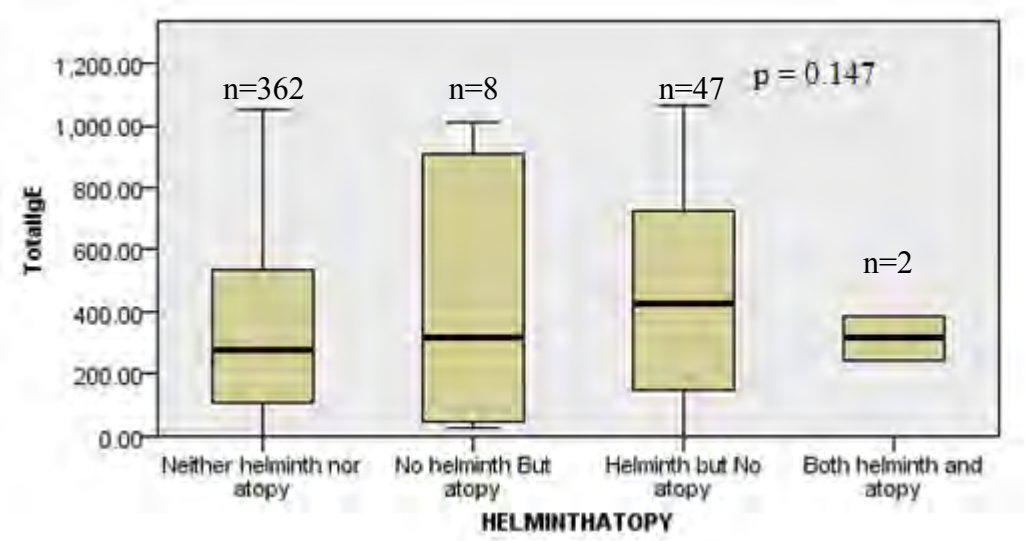

Figure 5: Distribution of total IgE among the different helminth/atopy groups Batu, Ethiopia, 2016.

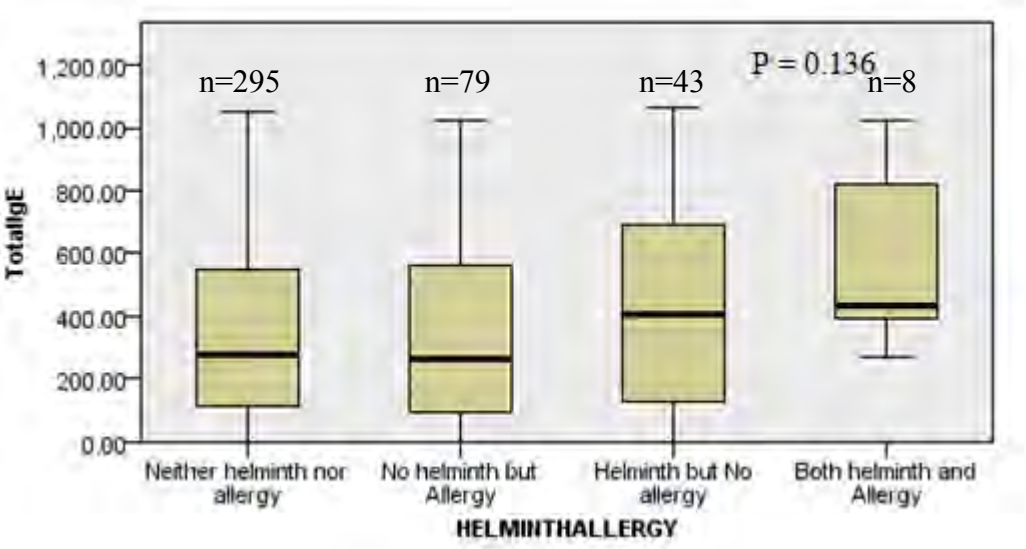

Figure 6: Distribution of total IgE among the different helminth/allergy groups Batu, Ethiopia, 2016.

Since there were outliers in eosinophil distribution, independent sample median test was used to check if there was a significant median difference of among the different helminth/allergy (Figure 7) and helminth/atopy groups (Figure 8). Both revealed a non-significant association.

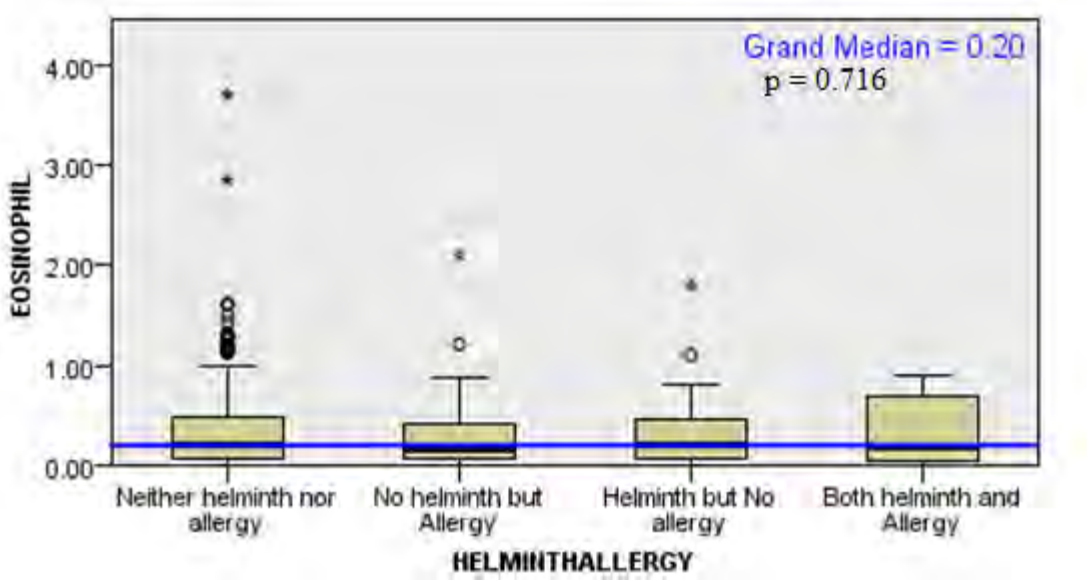

Figure 7: Distribution of Eosinophil among the different helminth/allergy groups Batu, Ethiopia, 2016.

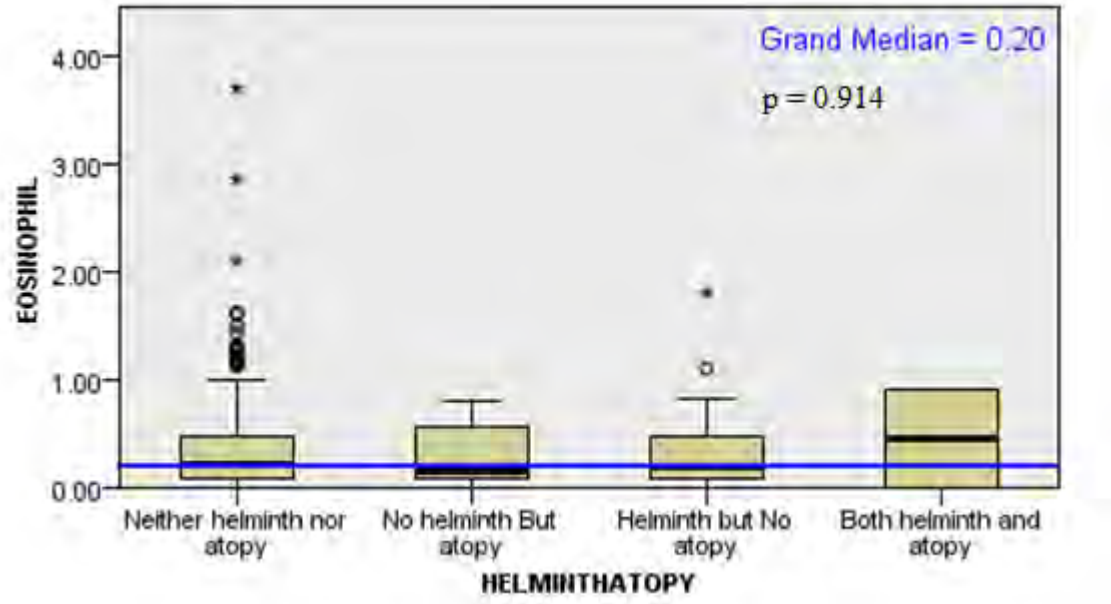

Figure 8: Distribution of Eosinophil among the different helminth/atopy groups Batu, Ethiopia, 2016.

5.6. Association between skin sensitization (atopy) and allergy symptoms

A borderline significant association was found between atopy and any allergy symptoms. Individuals with a positive SPT response to any allergen were 3.32 times more likely to report any allergy symptom [OR 3.32 (95% CI: 0.99, 11.1), P = 0.052]. Table 5 describes the association between atopy and allergy symptoms.

Table 5: Association between atopy and allergy symptoms, Batu, Ethiopia 2016.

		Allergy symptoms				
Variable		Overall N	Yes n(%)	No n(%)	Crude OR (95 % CI)	P-value
Atopy	Yes	11	5 (45.5%)	6 (54.5%)	3.32 (0.99, 11.1)	0.052
	No	443	89 (20.1%)	354 (79.9%)	1	

5.7. Potential risk factors for allergy and skin sensitization

Different factors were observed to be associated with having allergic symptoms and atopy (Table 7). Bivariate analysis showed keeping animals in the house increased the odds of atopy by 3.72 times [OR 3.72, (95% CI: 1.06, 13.1) p = 0.041]. In addition, parental history of allergy was significantly associated with developing allergic symptoms in the children; Paternal history of allergy [OR 4.15 (95% CI: 2.10, 8.23) p = 0.000] was shown to increase the risk of developing allergic symptoms, similarly maternal history of allergy [OR 3.25 (95% CI: 1.63, 6.48) p = 0.001] was associated with increased risk. Surprisingly, our result showed using charcoal everyday as a source of fuel is associated with decreased risk of developing allergic symptoms [OR 0.5 (95% CI: 0.30, 0.84) p = 0.009].

Table 6: Potential risk factors for allergy and skin sensitization in young children, Batu, Ethiopia 2016.

Variables	n%	Any sensitization	P-value	Any allergy symptoms	P-value
Maternal education (formal vs. **none**)	267 (57.9%)	1.27 (0.37, 4.39)	0.709	1.29 (0.81, 2.10)	0.287
Maternal allergic history (yes vs **no**)	38 (8.3%)	1.09 (0.14, 8.77)	0.93	**3.25 (1.63, 6.48)**	**0.001***
Paternal allergic history (yes vs **no**)	38 (8.3%)	2.5 (0.52, 12.01)	0.253	**4.15 (2.10, 8.23)**	**0***
Breast fed till age 3 (**Fed** vs not fed)	262 (64.5%)	0.56 (0.39,5.70)	0.562	1.24 (0.73, 2.09)	0.429
Older siblings					
0	174 (38.2%)	1.23 (0.14, 10.79)	0.853	1.9 (0.75, 4.82)	0.176
1 to 3	238 (52.3%)	0.90 (0.10, 7.86)	0.92	1.36 (0.51, 3.43)	0.514
4 to 10	43 (9.5%)	1		1	
De-worming medication (yes vs **no**)	331 (72.6%)	1.01 (0.27, 3.90	0.979	0.91 (0.55, 1.52)	0.726
Proper latrine					
None, bush, field	6 (1.3%)	0	0	1	
Traditional pit	430 (93.5%)	0	0	3.00 (0.30, 29.94)	0.349
Flush toilet	24 (5.2%)	0	0	1.21 (0.14, 10.53)	0.86
Animals kept in the house (**no** vs yes)	63 (13.7%)	**3.72 (1.06, 13.1)**	**0.041***	1.26 (0.67, 2.37)	0.469
Smoking in the residential (**no** vs yes)	20 (4.4%)	0	0	1.37 (0.48, 3.87)	0.554
Charcoal fuel use					
Never	95 (20.6%)	1		1	
Sometimes	50 (10.8%)	1.23 (0.21, 7.90)	0.793	0.43 (0.18, 1.04)	0.061
Everyday	316 (68.5%)	0.59 (0.14, 2.39)	0.459	**0.50 (0.30, 0.84)**	**0.009***
Insecticide use (yes vs **no**)	145 (31.5%)	1.22 (0.36, 4.39)	0.713	1.47 (0.92, 2.36)	0.11
Vaccination (yes vs **no**)	451(97.8%)	0	0	0.43 (0.05, 3.42)	0.428
Any protozoan infection (**yes** vs no)	334(75.2%)	0.36 (0.04, 2.60)	0.289	1.07 (0.63, 1.81)	0.796
Atopy (yes vs **no**)	11 (2.4%)	0	0	3.32 (0.99, 11.1)	0.052

***significant association at a=0.05 level**

Bold group – taken as referent groups

0 = Available number too small to calculate estimate.

6. DISCUSSION

In this cross sectional study of young children, association between helminth and atopy/allergy and also the distribution of total IgE was assessed. The result showed that there was no significant association between helminths and atopy and also between helminths and allergy. Bivariate analysis showed keeping animals in the house associated with atopy while maternal and paternal history of allergy associated with allergic symptoms in the children.

Since there was no previous similar study or survey done in this particular study area it was difficult to estimate the burden of both atopic status and allergic conditions of this particular study population. In the end, this cross sectional study showed the prevalence of allergic conditions were comparable to previous studies from different parts of Ethiopia except with eczema. Prevalence of various self-reported allergic conditions were; asthma 2.2%, eczema 13.2%, and hay fever 6.9%. Other studies carried out in Gondar, Addis Ababa, and Jimma showed a prevalence of asthma 2.2%, 2.8%, and 2.2%, eczema 2.7%, 11.2%, and 5.6% and hay fever 5.1%, 7.5%, and 4.5%, respectively. In addition, the magnitude of the atopic dermatitis (eczema) was found to be 9.6 % in a research carried out in Mekelle. No obvious reason was found to explain the relatively elevated frequency of eczema in our study. However, some unidentified environmental and socioeconomic factors may have played a role [18, 32, 33, 34].

This study shows skin sensitization to *D. pteronyssinus* and cockroach (1.1% and 1.5% respectively) was low when compared with a study in Butajira (10.8% and 8.2% respectively) [35]. These could be explained by the very large number of study participants they used (N=7649). Or the differences in location can be a possible reason [36].

Like that of skin sensitization, the burden of helminths (8.1%) in our study was relatively low when compared to studies in southwest Ethiopia (43.7%), Babile (27.2 %) and Gamo area (24.2%) [37, 38, 39]. Possible explanation for this noticeable difference include the deworming program that is recently launched in the country and provided to our study population, differences in geographic locations, study population's age, latrine coverage and coverage of prevention control programs. Different sensitivities of the techniques used for stool concentrations can provide a possible explanation.

Our result demonstrate a borderline significant association between positive Skin Prick Test and presentation of any allergy symptom. Individuals with a positive Skin Prick Test were 3.32 times more

likely to develop one of the three allergic conditions (asthma, eczema or hay fever). This agrees well with a study in Gondar [18] and Butajira [35].

Results of this study revealed no significant association between helminth and atopy/allergy. This is consistent with findings presented by Amberbir *et al* that reported no association [22] and Davey *et al.,* [35]. However, our results go against Scrivener *et al* in Gondar, which reported an association of hookworm infection with a reduced risk of wheeze [18]. Another study by Webb *et al.,* conducted in Uganda provided strong evidence that individuals with certain helminths were more prone to atopy, also contradicting our findings [20]. Calvert *et al.,* in South Africa reported Ascaris infection was associated with a decreased risk of a positive skin test response but an increased risk of exercise-induced bronchospasm [21]. Possible explanations for the difference in associations could be from variations in the study designs, sample population size and the different types of helminths and also the intensity of infection.

In addition to assessing the association between helminths and allergic condition, this study tried to determine the total IgE concentration in this study population. The result showed, the median total IgE concentration in the "only helminth infected" and "both allergic and helminth infected" groups of our study were found to be elevated even though the overall difference among the four groups was not statistically significant. This elevation is expected since helminths and allergic conditions are both associated with elevated levels of total IgE. A similar non-significant difference among the groups was reported in Gondar [41]. The median total IgE (437 IU/ml) in "both allergic and helminthic" groups in this study's result was somehow close to those found in a Brazilian study (660 IU/ml) [40] but different from a report from a study in Gondar (1411 kU/L) [41].

When compared with other studies the overall mean total IgE concentration of this study population was quite low (418 IU/ml). A study in Wondo Genet area, Ethiopia showed "only helminth infected" groups to have around a mean total IgE concentration of 1400 IU/ml [42] and 1044 IU/ml in Ethio-Israeli groups [43] dissimilar to our result.

Possible reasons for the general lower total IgE concentration could be due to the low prevalence of both helminths and allergic conditions, which have proved to elevate total IgE. The cross-sectional method used in this study instead of a case control studies could have also impacted the result.

Additionally, the low egg burden of the helminth infections (heavy infections are associated with high IgE) could also be a possible reason.

A similar, non-significant total IgE difference was found among the four helminth/atopy groups. Relatively lower median total IgE was observed in the "both helminth and atopy group" could be explained by the small number of participants that fall in that group.

Eosinophil distribution among the groups also showed no significant difference. As expected the helminth/atopy group showed elevated amount of eosinophil when compared to the other groups since atopy and helminth infections are both associated elevated number of eosinophils.

This study supports that both genetics and environmental fact or affect the development of allergic conditions or skin sensitization. Parental history of allergy was shown to increase allergic symptoms in the children was shown to increases skin sensitization in our finding. A study by Amberbir *et al,* agrees that parental history of wheeze increase the risk of allergy for the child [22]. Keeping animals (Cats) in the house was shown to decrease skin sensitization in our study which goes against a study by Platts-Mills *et al*., [44]. This difference could be due to their focus was only on cats allergen while we searched any kind of animals.

Surprisingly, our result showed using charcoal everyday as a source of fuel decreases the risk of developing allergic symptoms though literatures say the reverse. We could not come up with a possible explanation for this. This could reflect the limitation of cross-sectional studies which do not show the cause and effect relationships.

In conclusion, our study ultimately showed no significant association between helminth and atopy/allergy.

7. Strength and limitation of the study

7.1 Strength of the study

- Batu was a new site to explore this topic as to our knowledge (Previous studies were carried out in Addis Ababa, Jimma, Gondar)
- Provided a current information on Atopic and allergic status for the area

7.2. Limitation of the study

- A cross sectional design was used
- Number of participants atopy and helminth infections were low in order to investigate their relationship
- Allergic symptoms are self - reported by the study participants and their guardians.
- We defined atopy with only two dominant aeroallergens, house dust mite and cockroach

8. CONCLUSION AND RECOMMENDATION

8.1 Conclusion

- Contrary to the majority of related studies that reported a positive or negative association between helminths and allergy-related outcomes, we found no relationship.
- Environmental factors and genetic factors (Familial history) were shown to be associated with atopy and allergy-related outcomes.

8.2 Recommendation

- We strongly recommend the same study with different design like cohort, case control designs
- It is fair to expand the study using additional allergens to understand the roles of other allergens
- Immunological studies integrated with implementation of helminth control measures may elucidate how helminth elimination contributes to ongoing epidemics of inflammatory diseases.

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Annex I: Information Sheet (For Participants, English Version)

Title of the Research Project: Effect of intestinal helminth infection on atopy in young children at Zeway, Ethiopia

Name of Investigator: Sosina Walelign (BSc, Msc candidate)

Name of the Organization: Addis Ababa University, College of Health Science, Department of Clinical Laboratory Science.

Introduction

You are invited to participate in a study to be conducted by MSc student at Addis Ababa University, College of health sciences, School of Allied Health Science, Department of Medical Laboratory Sciences. It is aimed at studying the effect of intestinal helminth infection on atopy. The result will be useful to narrow the conflicting results found in studies carried out on this topic. It will also be an input for researches that hope to use helminths as therapuetic options for atopy. Please read the following statements and ask any unclear points before you agree to participate.

Participation in the study is exclusively voluntary. If you are not willing to participate in the study or if you want to withdraw even after deciding to participate, there will be no consequences. If you decide to participate, you have to sign an informed consent form and you can get a copy of this information sheet.

What is expected from you as a participant of the study?

As a participant of this study your child is expected to give 3-4 ml blood and stool specimen and get check for atopic status using skin prick test. In addition you are expected to give answers for some questions about your family and child's health, atopic history in the family and socio demographic conditions. You need to know that the results might be discussed with appropriate individuals. But your name or your child's name, address and phone number will not be disclosed to anyone and to be more precise, identification code will be used in such conditions.

How long participation will take you?

You will spend 20-40 minutes until the specimen is collected, the questionnaire is filled and the consent form is signed.

What are the risks of participating in this study?

There are no anticipated risks to your child's participation except minor discomfort during venipuncture and skin prick test because well experienced professionals will carry out the procedures.

How the information is to be kept confidential?

All information that you give and the results from your child's specimen will be used for this study only. Only limited number of professionals will have access to the information. All the information will be encoded in a computer and will be password protected.

What are the benefits from participation?

Since this study is MSc student research, there will not be payment for participants. But your participation is important for studying the association of geohelminths and atopy and will be useful in to narrow the conflicting results found in studies carried out on this topic. It will also be an input for researches that hope to use helminths as therapuetic options for atopy.

What are your rights as a participant of this study?

You can ask any question questions for further explanation. The principal investigator and the data collectors are responsible to clear any doubt you may have during participation. You have the right to get the results of the analysis.

What can I do if I have a problem or a question?

Please forward any question or problems you may encounter during this study to

Sosina Walelign

Department of medical laboratory science

School of Allied health sciences

College of health sciences

Addis Ababa University

Mob: +251-913-89-91-73, office of DMLS : +251 112 75 51 70

Email: arkisos@gmail.com or Sosina.walelign@aau.edu.et

Agree to participate?

- Yes
- No

Annex II- Subject information sheet (for participants, Amharic version)

አዲስ አበባ ዩኒቨርሲቲ,የጤና ሣይንስ ኮሌጅ,የአላይድ ጤና ሣይንስ ት/ቤት,የሕክምና ላቦራቶሪ ሣይንስ ክፍል

እድሜያቸው ከ 14 አመት በታች ከሆኑ ህጻናት ላይ የደምና የሠገራ ናሙና ተወስዶ ለሚሰራው የሆድ ጥገኛ ትላትሎች በአለርጂ ላይ ያላቸውን ተጽዕኖ ለማጥናት ታስቦ ለተሳታፊዎች የተዘጋጀ መረጃ

በአዲስ አበባ ዩኒቨርሲቲ, የጤና ሣይንስ ኮሌጅ ,የአላይድ ጤና ሣይንስ ት/ቤት ,የሕክምና ላቦራቶሪ ሣይንስ ክፍል በአዲስ አበባ ዩኒቨርሲቲ ጤና ሳይንስ ኮሌጅ የሕክምና ላቦራቶሪ ሳይንስ ት/ክፍል የማስተርስ ድግሪ ተማሪ የመመረቂያ ጥናት ላይ እንድትሳተፍ/እዲሳተፍ ተጋብዘዋል፡፡ እባክዎ በዚህ ጥናት ለመሳተፍ ከመስማማትዎ በፊት ከዚህ ቀጥሎ የሚገኘዉን ምንባብ በጥሞና ያንብቡና ግልጽ ያልሆነዉን /ኑትን ማንኛዉም ሃሳብ ይጠይቁ፡፡

መግቢያ

የጥናቱ ርዕስ፡ “የሆድ ጥገኛ ትላትሎች በአለርጂ ላይ ያላቸው ተጽዕኖ በልጅነት የዕድሜ ክልል በሚገኙ ህጻናት ” የሚል ሲሆን እርስዎ በዚህ ጥናት ላይ የሚኖሮት ተሳትፎ ሙሉ በሙሉ በበጎፈቃደኝነት ላይ የተመሰረተ ነዉ፡፡በዚህ ጥናት ዉስጥ ላለመሳተፍ ወይም ለመሳተፍ ከወሰኑ በኋላ ለማቋረጥ የሚወስኑ ቢሆንም እንኩዋ ምንም የሚደርስቦት ችግር የለም፡፡በጥናቱ ለመሳተፍ የሚስማሙ ከሆነ የስምምነት ቅጹ ላይ በጹሁፍ ወይም በጣት ፊርማ ማስቀመጥ ይጠበቅዎታል ፡፡ከፈለጉ ይህንን መረጃ አንድ ቅጅ ለራስዎ ሊያስቀሩ ይችላሉ፡፡

የጥናቱ ተሳታፊ በመሆኖ የሚጠበቅቦት ምንድን ነው?

በዚህ ጥናት ለመሳተፍ የሚስማሙ ከሆነ ከልጅዎ የደምና የሠገራ ናሙና ለመስጠት እንዲሁም የልጅዎት የአለርጂ ሁኔታ በ ስኪን ፕሪክ ቴስት ለማረጋገጥ መስማማት ይጠበቅብዎታል፡፡ ይሁን እንጅ ይህ አይነቱ መረጃ የርስዎንም ሆነ የልጅዎን ማንነት የሚገልጡ መረጃዎችን ማለትም ስም፤ አድራሻና የስልክ ቁጥር የመሳሰሉትን መረጃዎን አይጨምርም፡፡ ይልቁንም ለዚህ አገልግሎት ብቻ የሚዉልና ለማወቅ የሚያስችል መለያ ቁጥር ጥቅም ላይ እንዲዉል ይደረጋል፡፡ በተጨማሪም ስለልጅዎና ቤተሰቦት አጠቃላይ የጤና ሁኔታ ለሚቀርቡ አንዳንድ ተጨማሪ ጥያቄዎች መልስ መስጠት ይጠበቅብዎታል፡፡

በዚህ ጥናት መሳተፍ ምን ያህል ጊዜ ይፈጃል?

የተዘጋጀዉን መጠይቅ ለመሙላት፤ የስምምነት ቅጹ ላይ ለመፈረምና ናሙና ለመስጠት ከ20-40 ደቂቃ ያስፈልጋል፡፡

በዚህ ጥናት መሳተፍ የሚያስከትላቸዉ ቸግሮች ምንድን ናቸዉ?

የደም ናሙናበሚሰበሰብበትና የስኪን ፕሪክ ቴስት በሚሰራበት ወቅት ልጅዎ ምንም አይነት የከፋ ችግር አያጋጥመውም ምክንያቱም ናሙናው የሚወሰደው ልምድ ባላቸው የጤና ባለሙያዎች በመሆኑ ነው፡፡

የእኔ የህክምና መረጃ በሚስጥር ተጠብቆ መቆየት የሚችለዉ እንዴት ነዉ?

የሰጡት ማንኛውም መረጃና ከተወሰደው ናሙና ላይ የተገኘው የላቦራቶሪ ውጤት የሚውለው ለጥናቱ አላማ ብቻ ነው፡፡ ይህንን ማህደር ሊያገኙ የሚችሉት የተወሰኑ የጥናቱ ተባባሪ ሰራተኞች ብቻ ናቸው፡፡ ከዚህም በላይ ስለእርሶ ያለውን ማንኛውም መረጃ የተለየ የይለፍ ቃል ባላው የኮምፒውተር የመረጃ ማህደር ውስጥ እንዲቀመጥ ይደረገል፡፡

በዚህ ጥናት መሳተፍ የሚያስገኛቸው ጥቅሞች ምንድንናቸው ?

ይህ ጥናት የማስተርስ ዲግሪ መመረቂያ ፅሁፍ እንደመሆኑ መጠን ለተሳታፊዎች ገንዘብ አይሰጥም፡፡

የዚህ ጥናት ተሳታፊ መብቱ ምንድን ነው ?

ከዚህም በተጨማሪ ጥናቱን በተመለከተ ማንኛውንም አይነት ጥያቄ የመጠየቅና ገለጻ የማግኘት መብት አለዎት፡፡ የላብራቶሪ ምርመራ ውጤቱንም በነጻ ማግኘት ይችላሉ፡፡

ጥያቄ ካለኝ ወይም ችግር ቢያጋጥመኝ ምን ማድረግ ይገባል?

ይህንን ጥናት በተመለከተ ወይም ከዚህ ጥናት ጋር በተዛመደ መልኩ ስለሚያጋጥሙ ድንገተኛ አደጋዎች ወይም ጥያቄ ካለዎት በሚመለከተው አድራሻ ይጠቀሙ፡፡

ሶስና ዋለልኝ

የህክምና ላባራቶሪ ሳይንስ ት/ክፍል

የአላይድ ጤና ሳይንስ ት/ቤት

የጤና ሳይንስ ኮሌጅ

አዲስ አበባ ዩኒቨርሲቲ

ሞባይል: +251-913-89-91-73

ኢሜይል: arkisos@gmail.com or Sosina.walelign@aau.edu.et

ለመሳተፍ ይስማማሉ?

እስማማለሁ □ አልስማማም □

Annex-III- Consent Form (for participants, English version)

Code number----------

Name of the participant----------

I have been informed about the study which is aimed at studying the effect of intestinal helminth infection on atopy. For this study blood and stool samples are required and skin prick test will be carried out. The aims of the study and possible risks were explained to me as well.

I am also informed that all the information contained within the questionnaire is to be kept confidential. Moreover I have been well informed of my right to keep hold of information, decline to cooperate and make withdrawal from the study.

It is therefore with full understanding of the situation that I gave the informed consent voluntarily to the researcher to use my child's blood and stool sample for the investigation. In addition, I have had the opportunity to ask questions about it and received clarification to my satisfaction. I have also been informed that the benefit of participation is to get the results of analysis from my child's sample measured for free via the health personnel.

Participant's signature /finger print ----------

Name of Data collectors ---------- signature---------- Date----------

Please direct any questions or problems you may encounter during this study to:

Sosina Walelign

Department of medical laboratory science

School of Allied health sciences

College of health sciences

Addis Ababa University

Mob: +251-913-89-91-73

Email: arkisos@gmail.com or Sosina.walelign@aau.edu.et

For additional information, please contact Addis Ababa University, College of Health Science institutional review board (IRB) office at:

Tell. +251-11-8-96-13-96

Fax +251-11-5-51-1-51-30-99

P.O.Box. 9086, Addis Ababa, Ethiopia

Email: aau.mf.irb@yahoo.com

Annex-IV - Consent form (for participants, Amharic Version)

<u>የተሳታፊዎች ስምምነት ማረጋገጫ ቅጽ</u>

የሚስጥር ቁጥር ----------

የተሳታፊዉ ስም ----------

እኔ ስሜ ከላይ የተጠቀሰው ተሳታፊ የሆድ ጥገኛ ትላትሎች በአለርጂ ላይ ያላቸው ተጽዕኖ በልጅነት የዕድሜ ክልል በሚገኙ ህጻናት ስለሚደረገው ጥናት በቂ ገለጻ ተደርጎልኛል፡፡ ለጥናቱም ከልጄ የተወሰደ የደምና የሰገራ ናሙና እንደሚያስፈልና ስኪን ፕሪክ ቴስት እንደሚከናወን ተገልጾልኛል፡፡ የጥናቱንም አላማዎች በሚገባ ተረድቻለሁ፡፡

በመጠይቁ ላይ የገለጽኳቸው መረጃዎች በሙሉ በሚስጥር የተጠበቁ እንደሚሆኑ ተነግሮኛል፡፡ በጥናቱ ላይ ያለመሳተፍና ማንኛውንም መረጃ ያለመስጠት እንዲሁም በማንኛውም ጊዜ ከጥናቱ **ልጄን** የማግለል መብቴ የተጠበቀ እንደሆነ ተገልጾልኛል፡፡

ስለዚህ ለዚህ ጥናት መረጃና የስምምነት ቃሌን የሰጠሁት በአጠቃላይ ሁኔታውን በመረዳትና በፍጹም ፍቃደኝነት ነው፡፡ የምሰጠውም ናሙና ለምርምር ብቻ እንደሚውልም ተረድቻለው፡፡ በተጨማሪም ጥያቄ ለመጠየቅ ተፈቅዶልኝ ለማወቅ የፈለኩትን ያህል ማብራሪያ አግኝቻለሁ፡፡ የዚህ ጥናት ተሳታፊ በመሆኔ የማገኘው ጥቅም የሁሉንም ምርመራ ውጤት በነጻ ማግኘት እንደሆነ ተረድቻለሁ፡፡

የተሳታፊዉ ፊርማ /የጣት አሻራ ----------

የምስክር ሙሉ ስም ፊርማ

1. ---------- ----------
2. ---------- ----------
3. ---------- ----------

(የስምምነት ቅጹን ማንበብ ለማይችሉ ተሳታፊዎች)

የመረጃ ሰብሳቢው ስም ---------- ፊርማ ----------ቀን----------

<u>ጥናቱን የሚያካሂደዉ ሰዉ ማረጋገጫ</u>

ይህን ጥናት በተመለከተ ወይም ከዚህ ጥናት ጋር በተዛመደ መልኩ ስለሚያጋጥሙ ድንገተኛ አደጋዎች ወይም ጥያቄ ካሎዎት በሚከተለው አድራሻ ይጠቁሙን፡፡

ሶስና ዋለልኝ

የህክምና ላባራቶሪ ሳይንስ ት/ክፍል

የአላይድ ጤና ሳይንስ ት/ቤት

የጤና ሳይንስ ኮሌጅ

አዲስ አበባ ዩኒቨርሲቲ

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ANNEX V: Assent form (for participants, Amharic Version)

<u>የተሳታፊዎች ስምምነት ማረጋገጫ ቅጽ</u>

የሚስጥር ቁጥር ----------

የተሳታፊዉ ስም ----------

እኔ ስሜ ከላይ የተጠቀሰው ተሳታፊ የሆድ ጥገኛ ትላትሎች በአለርጂ ላይ ያላቸው ተጽዕኖ በልጅነት የዕድሜ ክልል በሚገኙ ህጻናት ስለሚደረገው ጥናት በቂ ገለጻ ተደርጎልኛል፡፡ ለጥናቱም ከእኔ የተወሰደ የደምና የሰገራ ናሙና እንደሚያስፈል ተገልጾልኛል፡፡ የጥናቱንም አላማዎች በሚገባ ተረድቻለሁ፡፡

በመጠይቁ ላይ የገለጽኳቸው መረጃዎች በሙሉ በሚስጥር የተጠበቁ እንደሚሆኑ ተነግሮኛል፡፡ በጥናቱ ላይ ያለመሳተፍና ማንኛውንም መረጃ ያለመስጠት እንዲሁም በማንኛውም ጊዜ ከጥናቱ **ራሴን** የማግለል መብቴ የተጠበቀ እንደሆነ ተገልጾልኛል፡፡

ስለዚህ ለዚህ ጥናት መረጃና የስምምነት ቃሌን የሰጠሁት በአጠቃላይ ሁኔታውን በመረዳትና በፍጹም ፍቃደኝነት ነው፡፡ የምሰጠውም ናሙና ለምርምር ብቻ እንደሚውልም ተረድቻለው፡፡ በተጨማሪም ጥያቄ ለመጠየቅ ተፈቅዶልኝ ለማወቅ የፈለኩትን ያህል ማብራሪያ አግኝቻለሁ፡፡ የዚህ ጥናት ተሳታፊ በመሆኔ የማገኘው ጥቅም የሁሉንም ምርመራ ውጤት በነጻ ማግኘት እንደሆነ ተረድቻለሁ፡፡

የተሳታፊዉ ፊርማ /የጣት አሻራ ----------

የምስክር ሙሉ ስም ፊርማ

1. ---------- ----------
2. ---------- ----------
3. ---------- ----------

(የስምምነት ቅጹን ማንበብ ለማይችሉ ተሳታፊዎች)

የመረጃ ሰብሳቢው ስም ---------- ፊርማ ----------ቀን----------

<u>ጥናቱን የሚያካሂደዉ ሰዉ ማረጋገጫ</u>

ይህን ጥናት በተመለከተ ወይም ከዚህ ጥናት ጋር በተዛመደ መልኩ ስለሚያጋጥሙ ድንገተኛ አደጋዎች ወይም ጥያቄ ካሎዎት በሚከተለው አድራሻ ይጠቁሙን፡፡

ሶስና ዋለልኝ

የህክምና ላባራቶሪ ሳይንስ ት/ክፍል

የአላይድ ጤና ሳይንስ ት/ቤት

የጤና ሳይንስ ኮሌጅ

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ANNEX VI: QUESTIONNAIRE ENGLISH VERSION

Section 1_ Child Characteristics

G01	Has your child ever had wheezing or whistling in their chest?	Yes	1		WHZL6A
		No	2		
G02	In the last 2 years, has your child had wheezing or whistling in their chest?	Yes	1		WHZT6A
		No	2		
G03	In the last 1 year, has your child had wheezing or whistling in their chest?	Yes	1	→ G04	WHZ6A
		No	2	→ G05	
G04	How many times in the last year has your child had an attack of wheezing?	0	1		WHZFRQ6A
		1-3	2		
		4-12	3		
		>12	4		
G05	Has your child ever had Asthma?	Yes	1		ASTL6A
		No	2		
G06	In the last 2 years, has your child had Asthma?	Yes	1		ASTT6A
		No	2		
G07	Has your child had Asthma in the last year?	Yes	1	→ G08	AST6A
		No	2	→ G09	

G08	Has this been confirmed by a doctor?	Yes	1		ASTHDR6A
		No	2		
G09	Has your child ever had an itchy skin rash which has affected the skin creases (eg, the folds of the elbow or behind the knees)?	Yes	1		RASHL6A
		No	2		
G10	In the last 2 years, has your child had an itchy skin condition affecting the skin creases (front of the elbow, behind the knees, the front of the ankles, around the neck, or around the eyes?	Yes	1		RASHT6A
		No	2		
G11	In the last 1 year, has your child had an itchy skin condition affecting the skin creases (front of the elbow, behind the knees, the front of the ankles, around the neck, or around the eyes?	Yes	1	→G11A	RASH6A
		No	2	→ G12	

G11A	IF YES, has this rash affected any of the following places? **(Multiple Answers possible)**	The elbow folds	1	RASHL6AA
			2	
		Behind the knees	1	RASHL6AB
			2	
		In front of the ankles	1	RASHL6AC
			2	
		Under the buttocks	1	RASHL6AD
			2	
		Around the neck	1	RASHL6AE
			2	
		Around the eyes/ears	1	RASHL6AF
			2	

G12	Has your child ever had hay fever or persistent sneezing attacks?	Yes	1		HAYFL6A
		No	2		
G13	In the last 2 years, has your child had hay fever or persistent sneezing with sneezing or running nose (excluding colds or flu), or problems with itchy watery eyes?	Yes	1		HAYFT6A
		No	2		
G14	In the last year, has your child had hay fever or persistent sneezing with sneezing or running nose (excluding colds or flu), or problems with itchy watery eyes?	Yes	1		HAYF6A
		No	2		
G15	How many people are living in your home now? [ ] [ ]	[ ] [ ]			PEOP6A
G16	How many older brothers/sisters does your child have who are alive now?	[ ] [ ]			SIBS6A

G17	Is paracetamol the same as aspirin?	Yes	1	→ G18	PARASP6A
		No	2	→ G19	
G18	Can you tell me which one of these is paracetamol and which aspirin? **(show medication strip)**	Correct identification	1		PARADIF6A
		Incorrect identification	2		
G19	Has your child taken any paracetamol/panadol in the last year?	Yes	1	→ G20	PARA6A
		No	2	→ G21	
G20	How many tablets of paracetamol/panadol has your child taken in the last month?	[ ] [ ]			PARAFR6A
G21	Can you name any symptoms for which you have given your child paracetamol? **(Multiple answers possible)**	Headache	Yes	1	PAHED6A
			No	2	
		Fever	Yes	1	PAFEV6A
			No	2	
		Malaria	Yes	1	PAMAL6A

			No	2	
		Common cold	Yes	1	PACOLD6A
			No	2	
		Birrd	Yes	1	PABIRD6A
			No	2	
		Wheeze	Yes	1	PAWHEZ6A
			No	2	
		Cough	Yes	1	PACOU6A
			No	2	
		Shortness of breath	Yes	1	PASOB6A
			No	2	
		Sneezing/running nose/itchy eyes	Yes	1	PASNEZ6A
			No	2	
		Skin rash in the creases	Yes	1	PARASH6A
			No	2	
		Other (specify)			PAOTHE6A
G22	Is paracetamol available close to where you live?	Yes	1		PAVAIL6A
		No	2		
G23	Is paracetamol affordable to you? ???????	Yes	1		PAFORD6A
		No	2		
G24	Do you avoid giving your child aspirin? ????	Yes	1		ASAVOD6A
		No	2		

G25	Should any people **NOT** take aspirin? **(PW – people with....)**	Children	Yes	1	ASCHIL6A
			No	2	
		PW gastritis	Yes	1	ASGAS6A
			No	2	
		PW asthma	Yes	1	ASASTH6A
			No	2	
		PW hay fever	Yes	1	ASHAY6A
			No	2	
		Don't know		9	ASAVDK6A
G26	Do you prefer to give aspirin or paracetamol for your child? ????	Aspirin	1		ASPREF6A
		Paracetamol	2		
		Depends	3		
		Don't mind	4		
G27	Has your child taken any drug prescribed by the health institution for any illnesses currently? **(Other than paracetamol/panadol/aspirin)**	Yes	1	→ G27A	ANTIB6A
		No	2	→ G28	
G27A	If Yes, please observe the drug and write the name and type the child currently taking.	----------			ANTIBM6A
G28	Has your child taken any de-worming medication in the last 6 months? **(De-worming refers to antihelmintics treatment given by the heath office free of charge without stool examination)**	Yes	1		DEWOR6A
		No	2		

G29	Is there anyone who smokes cigarettes in your home?	Yes	1	→ G29A	HCIGR6A
		No	2	→ G30	
G29A	If yes, please write the total number of people who smoke cigarettes in the home where the child living?	[ ]			HCIGRN6A
G30	What does your child sleep on?	Bed		1	CHSLP6A
		Medeb		2	
		Floor		3	
		'Jibba'		4	
		'Sigaja'		5	
		Other (Specify)		9	
G31	What is your child's bed made of?	Iron metal		1	CHBED6A
		Wood		2	
		Flat metal		3	
		Rope		4	
		leather		5	
		No bed		6	
		Other (Specify)		9	
G32	What is your child's mattress made of?	Cotton		1	CHMAT6A
		Sponge		2	
		Greass		3	
		Kapoak		4	
		No matress		5	
		Other (Specify)		9	

G33	What is your child's pillow made of? ///???	Cotton	1	CHPIL6A
		Sponge	2	
		Grass	3	
		kapoak	4	
		Cloth	5	
		No pillow	6	
		Other (Specify)	9	

Section 2_Maternal Characteristics

G34	Have you had wheezing or whistling in your chest in the last 1 year?	Yes	1	→ G35	MOWHZ6A
		No	2	→ G36	
G35	How many times in the last year have you had an attack of wheezing?	0	1		MOWHFR6A
		1-3	2		
		4-12	3		
		>12	4		
G36	Have you had asthma in the last 1 year?	Yes	1	→ G37	MOAS6A
		No	2	→ G38	
G37	Was this confirmed by a doctor?	Yes	1		MOASSDR6A
		No	2		
G38	Has the baby's father had wheezing or whistling in the chest in the last 1 year?	Yes	1		FAWHEZ6A
		No	2		
G39	Has the baby's father had asthma in the last 1 year?	Yes	1	→ G40	FAAS6A
		No	2	→ G41	
		NA	9		
G40	Was this confirmed by a doctor?	Yes	1		FAASDR6A
		No	2		
G41	In the last 1 year have you had hay fever?	Yes	1		MOHAY6A
		No	2		
G42	In the last 1 year has the baby's father had hay fever?	Yes	1		FAHAY6A
		No	2		
		NA	9		
		Yes	1		

G43	Have you had eczema in the last 1 year?	No	2	MOEZC6A
G44	Has the baby's father had eczema in the last 1 year?	Yes	1	FAEZC6A
		No	2	
		NA	9	
G45	Have you taken paracetamol/Panadol in the last year?	Yes	1	MOPAR6A
		No	2	
G46	How many tablets of paracetamol/Panadol have you taken in the last month?	[ ] [ ]	1	MOPAF6A
G47	Have you taken any drug prescribed by the health institution for any problem currently? (Other than Paracetamol/Panadol)	Yes	1	GANTIB6A
		No	2	
G48	If Yes, please observe the drug and write the name and type the mother currently taking.	----------		GANBTY6A

Section 3_Housing characteristics

G49	What type of roof does your house have? ??	Thatched	1	GROOF6A
		Corrugated iron	2	
		Other (specify)	9	
G50	What are the walls of your house made of? ???	Wood	1	GWALL6A
		Wood and grass	2	
		Cement	3	
		Brocket	4	
		Bricks	5	
		Corrugated iron	6	
		Other (specify)_____	9	
G51	How many rooms does your house have? **(observe and fill the no of rooms) ????**	[ ]		GROOM6A
G52	What type of floor does your house have?	Cement	1	GFLOOR6A
		Wood	2	
		Bricks	3	
		Mud	4	
		Other (specify)_____	9	
G53	Is the floor covered by any material?	Yes	1	GCOVER6A
		No	2	

G54		Inside the house in the main living area	1	

	Where do you do most of your cooking? (tick one that applies)	Inside the house in a room other than the main living area	2	GCOOK6A
		Outside the house in a separate building	3	
		Outside the house in the open air	4	

G55 How often do you use the following for cooking?

Fuel	Never	Sometimes	Every day
1. Charcoal	1	2	3
2. Wood	1	2	3
3. Leaves	1	2	3
4. Dung	1	2	3
5. Nafta/Lanba	1	2	3
6. Gas	1	2	3
7. Electricity	1	2	3
9. Other	1	2	3

GFUEL6A

G56 ¾ How often do you use the following inside the house for purposes other than cooking (eg heating, lighting)?

Fuel	Never	Sometimes	Every day
1. Charcoal	1	2	3
2. Wood	1	2	3
3. Leaves	1	2	3
4. Dung	1	2	3
5. Nafta/Lanba	1	2	3

GFUELA6A

		6. Gas	1	2	3	
		7. Electricity	1	2	3	
		8. A locally made battery	1	2	3	
		9. Other	1	2	3	

G57 Which of the following animals do you or your household keep? (Multiple answers possible)

Animal	Not available	Inside	Outside	
1. Cat	1	2	3	
2. Dog	1	2	3	
3. Hen	1	2	3	
4. Cow/ox	1	2	3	GANIM6A
5. Sheep	1	2	3	
6. Horse	1	2	3	
7. Pig	1	2	3	
8. Goat	1	2	3	
10. mule/donkey	1	2	3	
9 Other	1	2	3	

G58	What is your main source of drinking water? **(Tick one which applies)**	Piped into compound	1	GWATER6A
		Piped outside compound	2	

		Open well or spring		3	
		Covered well or spring		4	
		River, pond or dam		5	
		Rainwater		6	
G59	What type of toilet facility do you use? **(Tick one which applies)**	Flush toilet		1	GTOILET6A
		Ventilated improved pit		2	
		Traditionnel pit toilet		3	
		None/bush/field		4	
G60	How do you dispose your waste?	Pit		1	GSAND6A
		Open field		2	
		Burning		3	
		Garbage bin		4	
		Other(Specify)_____		9	
G61	Do you use any of the following insecticides in your house? **(Multiple answers possible)**	DDT	Yes	1	GINSE6AA
			No	2	
		Malathion	Yes	1	GINSE6AB
			No	2	
		Flit	Yes	1	GINSE6AC
			No	2	
		Application of dung	Yes	1	GINSE6AD
			No	2	

		Other(specify)	Yes	1	GINSE6AE
			No	2	
G61	Where do you place insecticides in your house? (observe)	Out of reach of children		1	PROT6A
		Within reach of children		2	

ANNEX VII. QUESTIONNAIRE – AMHARIC VERSION

አሁን ልጅዎ ስላጋጠመው የደረት ህመምና ተመሳሳይነት ያላቸውን ጉዳዮች □□□ቅ□ታ-ስሁ። አባክዎን ባለፉት ጊዜያቶች ውስጥ ያጋጠሙዋን ከዚህ ጋር ተያያጅነት ያላቸው ችግሮች በማስታ-ወስ ይንገረኝ።

1.1 ህፃኑን/ኗን የተመለከተ

G01	**ህፃኑ/ኗ ከተወለደ/ች ጀምሮ በየትኛውም ጊዜ ቢሆን በ□ረቱ/ትዋ ውስጥ ሲጥ ሲጥ የሚል ወይም የፉጨት ድምፅ ኖሮት ያውቃል/ታውቃለች?**	**አዎን**	**1**		WHZL6A
		□ለም	**0**		
G02	**ባለፉት ሁለት አመ□ት በየትኛውም ጊዜ ቢሆን በ□ረቱ/ትዋ ውስጥ ሲጥ ሲጥ የሚል ወይም የፉጨት ድምፅ ኖሮት ያውቃል/ታውቃለች?**	**አዎን**	**1**		WHZT6A
		□ለም	**0**		
G03	**ባለፉት 12 ወራት ዉስጥ በሕፃኑ/ኗ ደረት ውስጥ ሲጥ ሲጥ የሚል ወይም የፉጨት ድምፅ ተሰምቶ ጸዉ፧ቃል/□ዉ፧ቃለች?**	**አዎን**	**1**	**→ G04** **→ G05**	WHZ6A
		□ለም	**0**		
G04	**ባለፉት 12 ወራት ህፃኑ/ኗ ደረት ውስጥ ሲጥ ሲጥ የሚል ወይም የፉጨት ድምፅ ተሰምቶ የነበረዉ ስንት ጊዜ ነበር?**	**0**	**0**		WHZFRQ6A
		1-3	**1**		
		4-12	**2**		
		ከ13 በላይ	**3**		
G05	**ህፃኑ/ኗ ከተወለደ/ች ጀምሮ በየትኛውም ጊዜ ቢሆን አስም ኖሮት ያውቃል/□ዉ፧ቃለች?**	**አዎን**	**1**		ASTL6A
		□ለም	**2**		
G06	**ባለፉት ሁለት አመ□ት በየትኛውም ጊዜ ቢሆን ህፃኑ/ኗ አስም ኖሮት/ሯት ያውቃል/□ዉ፧ቃለች?**	**አዎን**	**1**		ASTT6A
		□ለም	**0**		
G07	**ባለፉት 12 ወራት ዉስጥ ህፃኑ/ኗ አስም ኖሮት ጸዉ፧ቃል/□ዉ፧ቃለች?**	**አዎን**	**1**	**→G08**	AST6A
		□ለም	**0**		
G08	**ህፃኑ/ኗ አስም እንዳለበት/ባት በሐኪም ተረጋግጧል?**	**አዎን**	**1**		ASTHDR6A
		□ለም	**0**		
G09	**ህፃኑ/ኗ ከተወለደ/ች ጀምሮ በየትኛውም ጊዜ ቢሆን በአጥንት መታጠፊያ ቦታዎቹ(ጀ) (በክርን መታጠፍያ፧ከጉልበቱ ኋላ ባለዉ**	**አዎን**	**1**		RASHL6A
		□ለም	**0**		

	መታጠፍያ፤በቁርጭምጭሚት ፊት ለፊት፤ በአንገት □ሪጸ፤ እና በአይን አካባቢ) የሚያሳክክ ሽፍታ ወጥቶበት (ወጥቶባት) ነበር?				
G10	ባለፉት ሁለት አመ□ት በየትኛውም ጊዜ ቢሆን ህፃኑ/ኗ በአጥንት መታጠፊያ ቦታዎቹ(ቿ) (በክርን መታጠፍያ፤ከጉልበቱ ኋላ ባለዉ መ□ቷአጸ፤በቁርጭምጭሚት ፊት ለፊት፤ በአንገት □ሪጸ፤ እና በአይን አካባቢ) የሚያሳክክ ሽፍታ ወጥቶበት (ወጥቶባት) ነበር?	አዎን □ለም	1 0		RASHT6A
G11	ባለፉት 12 ወራት ዉስጥ ልጁ(ልጅቷ) በአጥንት መ□ቷኝጸ ቦታዎቹ(□) (በክርን መታጠፍያ፤ከጉልበቱ ኋላ ባለ□ መታጠፍያ፤በቁርጭምጭሚት ፊት ለፊት፤ በአንገት ዙሪያ፤ እና በአይን አካባቢ) የሚያሳክክ ሽፍታ ወጥቶበት (ወጥቶባት) ነበር?	አዎን □ለም	1 0	→G11A	RASH36
G11A	**መልሱ አዎን ከሆነ**፡ ሽአታዉ የነበረዉ በየትኛዎቹ ቦታዎች ላይ ነዉ? (መልሱ ይነበብ፤ ከአንድ በላይ መልሰ መስጠት ይቻላል)	በክርን መታቷኝጸ	1 0		RASH6AA
		ከጉልበትዎ ኋላ	1 0		RASH6AB
		በቁሮ□ ም□ ሚት ፊትለፊት	1 0		RASH6AC
		ከመቀመጫ በታች	1 0		RASH6AD
		በአንገትዎ ዙሪያ	1 0		RASH6AE
		በአይንና በጆሮዎች ዙሪያ	1 0		RASH6AF
G12	ህፃኑ/ኗ ከተወለደ/ች ጀምሮ በየትኛውም ጊዜ ቢሆን ንፍጥ የሚያበዛ ጉንፋን፤የማያቋርጥ ማስነጠስ፤አፍንጫ ወይም ዐይንን የሚያቃጥል ሕመም ነበረበት(ባት)? **(□ከኚህ ምልክቶች የ□ዩት ህፃኑ በጉንፋን ሳጁጸ□ መሆን አለበት)::**	አዎን □ለም	1 0		HAYFL6A
G13	ባለፉት ሁለት አመ□ት በየትኛውም ጊዜ ቢሆን ህፃኑ/ኗ ንፍጥ የሚያበዛ ጉንፋን፤የማያቋርጥ ማስነጠስ፤አፍንጫ ወይም ዐይንን የሚያቃጥል ሕመም ነበረበት(ባት)? **(□ከኚህ ምልክቶች የ□ዩት ህፃኑ በጉንፋን ሳይያዝ መሆን አለበት)::**	አዎን □ለም	1 0		HAYFT6A
G14	ባለፉት 12 ወራት ዉስጥ ልጅዎ ንፍጥ የሚያበዛ ጉንፋን፤የማያቋርጥ ማስነጠስ፤አፍንጫ ወይም ዐይንን የሚያቃጥል ሕመም ነበረበት(ባት)? **(□ከኚህ ምልክቶች □□ዩት ህፃኑ በጉንፋን ሳይያዝ መሆን አለበት)::**	አዎን □ለም	1 0		HAYF6A
አሁን ህፃኑን/ኗ በታመሙ ጊዜ ፓራሴታሞልና የመሳሰሉትን የህመም ማስታገሻ እንደወሰዱ □ቷጁቅ- □ለሁ::					
G15	□ራሴ□ሞል ከአስፕሪን ጋር አንድ ነው?	አዎን □ለም	1 0	 →G17	PADIF6A
G16	ከነዚህ ከሁለቱ መካከል ፓራሴታሞልና አስፕሪንን ልትለይልን ትችያለሽ ?	ትክክለኛ መለጸ □ተሳሳተ መለጸ	1 0		PADFA6A
G17	ሕጻኑ(ኗ) ባለፈዉ ዓመት ፓራሴታሞል/ፓናዶል ወስ□(□) ጸ□ ቃል(ታዉቃለች)?	አዎን □ለም	1 0	→G18 →G20	PARA6A

G18	**ህፃኑ/ኗ በላፈው ወር ስንት የፓራሴ□ሞል ወጁም ፓናዶል ኪኒኖች ወሰዶዋል? (በቁዓር ጁዞሽን)**	**[ ][ ] ወስዶዋል/ስች::**			PARAFR6A
G19	**□ራሴ□ሞል ለሕጻኑ(ኗ) የሰጡበት □ሕመም ምልክቶች/በሽታዎች ከሚከተሉት የትኞቹ ናቸው?** **(መልሱ ይነበብ፤ ከአንድ በላይ መልስ መስጠት ይቻላል)**	**ራስ ምታት**	**አዎን**	***1***	PAHED6A
			□ስም	***0***	
		ትኩሳት	**አዎን**	***1***	PAFEV6A
			□ስም	***0***	
		ወባ	**አዎን**	***1***	PAMAL6A
			□ስም	***0***	
		ጉንፋን	**አዎን**	***1***	PACOLD6A
			□ስም	***0***	
		ብርት	**አዎን**	***1***	PABIRD6A
			□ስም	***0***	
		ሳል	**አዎን**	***1***	PACOU6A
			□ስም	***0***	
		ሲዓ ሲዓ ሲልበት/ባት	**አዎን**	***1***	PAWHEZ6A
			□ስም	***0***	
		የትንፋሽ ማጠር	**አዎን**	***1***	PASOB6A
			□ስም	***0***	
		ማስነጠስ/ እንደ ንፍጥ ያለ በአፍንጫ ሲወርት/የአይን ማሳከክ ሲኖር	**አዎን**	***1***	PASNEZ6A
			□ስም	***0***	
		□ቆ□ ሽአ□ በመ□ ታኝጸ አካባቢዎች ሲኖር	**አዎን**	***1***	PARASH6A
			□ስም	***0***	
		ሌላ (ጁዞሽን) ----------			PAOTHE6A
G**20**	**በሚኖሩበት አካባቢ □ራሴታሞልን በቅርበት ያገኘ ·□ል?**	**አዎን**	***1***		PARAV6A
		□ስም	***0***		
G**21**	**□ርስ- □ራሴታሞልን ለመግዛት ዋጋውን ይችሉታል?**	**አዎን**	***1***		PAFORD6A
		□ስም	***0***		
G**22**	**አስፕሪን ለልጅዎ ላለመስጠት ይሞክራሉ?**	**አዎን**	***1***		ASAVOD6A
		□ስም	***0***		
		ሕፃናት	**አዎን**	***1***	

G23	አስፕሪን መውሰድ የሌለባቸው ሰዎች አሉ? **(መልሱ ይነበብ፤ ከአንድ በላይ መልስ መስጠት ይቻላል)**		☐ሉም	0	ASCHIL6A
		ፊ ጃራ ያለባቸው	አዎን	1	ASGAS6A
			☐ሉም	0	
		አስም ያለባቸው	አዎን	1	ASASTH6A
			☐ሉም	0	
		የአፍንጫ አስም ያለባቸው	አዎን	1	ASHAY6A
			☐ሉም	0	
		አላውቅም	አዎን	1	ASAVDK6A
			☐ሉም	0	
		ሌላ (ጁፆለን)_____			
G24	☐ርስዎ ለልጅዎ ለመስጠት የሚመርጡት አስፕሪንን ነው ወይንስ ☐ራሴታሞልን?	አስፕሪን	1		ASPREF6A
		☐ራሴ☐ሞል	2		
		እንደ ሁኔ☐ ዉ፡	3		
		ምንም ምርጫ የለኝም	4		
G25	ህፃኑ/ኗ ለማንኛውም አይነት መትጎኒት ለየትኛውም አይነት በሽ☐ በሕክምና በቅርብ ጊዜ ታዞለት/ላት ያውቃል(ለች)? **(ይህን ጥያቄ ለማኝኛውም አይነት በሽ☐ ☐ታዘዘለትን/ላትን መድሃኒት ያካትታል ነገር ግን** **ፓራሴንታሞል/ፓናዶልን ወይም አስፕሪንን አይጨምርም፡፡)**	አዎን	1	→G25A	ANTIB6A
		☐ሉም	0	→G26	
G25A	መልስዎ አዎ **ከሆነ የሚወስዱትን መድሃኒት አይነቱንና ስሙን በማየት ይሞላ፡፡**	1.----------			BANTA6A
		2.----------			BANTB6A
		3.----------			BANTC6A
G26	ህፃኑ/ኗ ባለፉት ስድስት ወራት ለሆድ ትላትል መከላከያ መድሃኒት ወስዶአል? **(የሆድ ትላትል መከላከያ ሲባል በዋነኛነት በጤና ባለሙጻ በአመት ሁለት ጊዜ ቤት ለቤት በነፃ ☐ሚታደል ማለት ነው፡፡)**	አዎን	1		DEWOR6A
		☐ሉም	0		
G27	በቤት ውስጥ ምንያህል ሰዎች ይኖራሉ?	[ ][ ]			PEOP6A
G28	ህፃኑን/ኗ ስንት ☐ላቅ ወንድምና ☐ህቶች በሕይወት አሉት/አሏት?	[ ][ ]			SIBS6A
G29	ህፃኑ/ኗ በሚኖርበት/በምትኖርበት ቤት ውስጥ ሲጋራ/ትምባሆ የሚያጨስ ሰው አለ?	አዎን	1	→G29A	HCIGR6A
		☐ሉም	0	→G30	
G29A	መልሱ አዎ ከሆነ የሚያጨስ ሰው **ብዛት ጠይቀሽ መዝግቢ**	[ ][ ]			HCIGRN6A

G30	ህፃኑ/ኗ በምን ላይ ነዉ የሚተኛ ዉ(የምትተኛ□)?	አልጋ	1	CHSLP6A
		መ□ብ	2	
		ወለል	3	
		ፀ ባ	4	
		ስፉጽ	5	
		ሌላ (ጁፅለ□)	9	
G31	ህፃኑ/ኗን ለመኝ□ የሚጠቀሙዉ/የምትጠቀመዉ አልጋ ከሆነ የተሠራው ከምንድ ነው?	ከሸቦ	1	CHBED6A
		ከእንጨት	2	
		ከቦንዳ	3	
		ከገመድ	4	
		ከቁርበት	5	
		አልጋ የለኝም	6	
		ሌላ (ጁፅለ□)	9	
G32	ህፃኑ/ኗን ለመኝ□ የሚጠቀሙዉ/የምትጠቀመዉ ፍራሽ ከሆነ የተሠራው ከምንድ ነው?	ከጥጥ	1	CHMAT6A
		ከስፖንጅ	2	
		ከሳር	3	
		ከአበባ የሚገኝ ጥጥ መሰል ነገር	4	
		ፍራሽ የለኝም	5	
		ሌላ (ጁፅለ□	9	
G33	ህፃኑ/ኗን ለመኝ□ የሚጠቀሙዉ/የምትጠቀመዉ ትራስ ከሆነ የተሠራው ከምንድ ነው?	ከጥጥ	1	CHPIL6A
		ከስፖንጅ	2	
		ከሳር	3	
		ከአበባ የሚገኝ ጥጥ መሰል ነገር	4	
		ፊ ርቅ ወጁም ልብስ	5	

		ከጨርቅ የተሰራ ትራስ	6	
		ትራስ የለውም/ላትም	7	
		ሌላ (ጁፅለ□	9	

1.2 የህፃኑ/ኗ እናትን/አባትን የተመለከተ

G34	ባለፉት 12 ወራት በደራትዎ ዉስጥ ሲጥ ሲጥ የሚል ወይም የፉጨት ድምፅ ነበረብዎት?	አዎ	1	→G35	MWHZ6A
		□ሕም	0	→G36	
G35	ባለፉት 12 ወራት በደራትዎ ዉስጥ ሲጥ ሲጥ የሚል ወይም የፉጨት ድምፅ ተሰምቶዎት የነበረዉ ስንት ጊዜ ነበር?	0	0		MWHFR6A
		1-3	1		
		4-12	2		
		ከ13 በላይ	3		
G36	ባለፉት 12 ወራት አስም ነበረብዎት?	አዎ	1	→G37	MOAS6A
		□ሕም	0	→G38	
G37	□ርስዎ አስም እንዳለብዎት በሐኪም ተረፉ□ል?	አዎ	1		MASDR6A
		□ሕም	0		
G38	ባለፉት 12 ወራት □ልዓ (ልፀ ታ) አባት በ□ረታቸው ዉስጥ ሲጥ ሲጥ የሚል ወይም የፉጨት ድምፅ ነበረባቸው?	አዎ	1		FWHZ6A
		□ሕም	0		
		አይመለከትም	9		
G39	ባለፉት 12 ወራት የልጁ(ልጅታ) አባት አስም ነበረባቸዉ?	አዎ	1	→G40	FAAS6A
		□ሕም	0	→G41	
		አይመለከትም	9		
G40	□ልዓ (ልፀ ታ) አባት አስም □ንዳለባቸው በሐኪም ተረፉ□ል?	አዎ	1		FASDR6A
		□ሕም	0		
G41	ባለፉት 12 ወራት ዉስጥ ንፍጥ የበዛበት ጉንፋን፤ የማያቋርጥ ማስነጠስ፤ አፍንጫ ወይም ዓይን ማቃጠል ነበረብዎት?	አዎ	1		MOHAY6A
		□ሕም	0		
G42	ባለፉት 12 ወራት ዉስጥ የልጁ(ልጅታ) አባት፤ ንፍጥ የበዛበት ጉንፋን፤ የማያቋርጥ ማስነጠስ፤ አፍንጫ ወይም ዓይን ማቃጠል ነበረባቸዉ?	አዎ	1		FAHAY6A
		□ሕም	0		
		አይመለከትም	9		
G43	ባለፉት 12 ወራት የሚያሳክክና ፤በተለጁም የአጥንት መታጠፊያ አካባቢዎች ያሉትን የሰውነት ክፍሎችን/ለምሳሌ የክንድ፤ ከጉልበት በሰተኋላ ታጣፊ ቆዳዎችን/ የሚያጠቃ የቆዳ ሽአ□ ነበረብዎት?	አዎ	1		MOEZCA6A
		□ሕም	0		
		አዎ	1		

G44	ባለፉት 12 ወራት የልጁ(ልጅቷ) አባት ፤በተለይም የአጥንት መታጠፊያ አካባቢዎች ያሉትን የሰውነት ክፍሎችን/ለምሳሌ የክንድ፤ ከጉልበት በሰተኋላ ታጣፊ ቆዳዎችን/ የሚያጠቃ □ቆ□ ሽአ□ ነበረባቸዉ?	□ለም	0	FAEZC6A
		አይመለከትም	9	
G45	በላፈው ዓመት ፓራሴታሞል/ፓናዶል ወስደው ጸዉቃሉ?	አዎ	1	MOPAR6A
		□ለም	0	
G46	በላፈው ወር ሰንት የፓራሴ□ሞል ወጁም ፓናዶል ኪኒኖች ዉጠዋል?	[ ] [ ] ወስዳስች፡፡		MOPAFR6A
G47	ማንኛውም አይነት መድኃኒት ለየትኛዉም አይነት በሽታ በሕክምና በቅርብ ጊዜ ታዞልዎት ጸዉቃል? **(ይህን ጥያቄ ለማኝኛውም አይነት በሽታ የታዘዘልዎትን መድሃኒት ያካትታል ነገር ግን ፓራሴንታሞል/ፓናዶልን ወይም አስፕሪንን አይጨምርም፡፡)**	አዎን	1	MANTIB6A
		□ለም	0	
G48	መልስዎ አዎ **ከሆነ የሚወስዱትን መድሃኒት አይነቱንና ስሙን በማየት ጁሞላ፡፡**	1.----------		MANTA6A
		2.----------		MANTB6A
		3.----------		MANTC6A

1.3 ቤትዎን የተመለከተ

G49	የቤትዎ ጣራ የተሰራው ከምንድን ነው?	ሣር	1	GROOF6A
		ቆርቆሮ	2	
		ሌላ (ጁ.ዘለ□)	9	
G50	ግድግዳው ከምን የተሠራ ነው?	እንጨትና ጭቃ	1	GWALL6A
		እንጨት፣ ጭራሮና ሳር	2	
		ድንጋይና ሲሚንቶ	3	
		ብሎኬት	4	
		ጂ.ብ	5	
		ቆርቆሮ	6	
		ሌላ (ጁ.ዘለ□)	9	

G51	**የህፃኑ/ኗ መኖሪያ ቤት ወለል የተሰራው ከምንድን ነው?**			GFLOOR6A
		ከሲሚንቶ	***1***	
		ከጣውላ ወይም □ንጨት	***2***	
		ከሸክላ	***3***	
		ከአፈር	***4***	
		ሌላ(ጁፅለ□)	***9***	
G52	**የህፃኑ/ኗ መኖሪያ ቤት ወለል በምንጣፍ ወይም በሌላ ነገር ተሸፍኗል?**	**አዎን**	***1***	GCOVER6A
		□ለም	***0***	

G53	**ቤተሰቡ ምግብ በብዛት የሚያበስሉት የት ነው?** **(አንዱ ላይ ብቻ ምልክት አድርጊ)**	**በዋናው ቤት ውስጥ**	***1***	GCOOK6A
		□ቤት ውስጥ ሆኖ ከዋናው ቤት ሌላ	***2***	
		ከቤት ውጭ ማዕድ ቤት	***3***	
		ከቤት ውጭ ክፍት ቦ□	***4***	

G54 **የ የህፃኑ/ኗን ቤተሰበ ከሚከተሉት የማገዶ ዓይነቶች ምግብ ለማብሰል በየስንት**

ቂ□ው እንድሚጠቀሙ ቢገቂፁል?

ማገዶ/ነዳጅ	**አልፎቀም ም**	**አንዳንድ ቂ□**	**ምየቀነ ፡**	
1. ከሠል	***1***	***2***	***3***	GFUEL6AA
2. እንጨት	***1***	***2***	***3***	GFUEL6AB
3. ቅቷል	***1***	***2***	***3***	GFUEL6AC
4. ኩበት	***1***	***2***	***3***	GFUEL6AD
5. ናፍጣ/ላንባ	***1***	***2***	***3***	GFUEL6AE
6. ቡ□ፉ□	***1***	***2***	***3***	GFUEL6AF
7. መብራት/ኮርነቲ	***1***	***2***	***3***	GFUEL6AG GFUEL6AH

		9. ሌላ (ጽፅለ□)	1	2	3	
G55	የ የህፃኑ/ኗን ቤተሰበ ከሚከተሉት የማገዶ ዓይነቶች ምግብ ከማብሰል ውጭ ለሌላ ጉዳይ ይጠቀማል (ለምሳሌ ለመሞቀትና ለመብራት)?					
		ማገዶ/ነዳጅ	አልጠቀምም	አንዳንድ ቲ□	በየቀኑ	GFUEL6AAA
		1. ከሠል	1	2	3	GFUEL6AAB
		2. እንጨት	1	2	3	GFUEL6AAC
		3. ቅቷል	1	2	3	GFUEL6AAD
		4. ኩበት	1	2	3	GFUEL6AAE
		5. ናፍጣ/ላንባ	1	2	3	GFUEL6AAF
		6. በ□ፉ□	1	2	3	GFUEL6AAG
		7. ባትሪ ድንጋይ	1	2	3	GFUEL6AAH
		8. መብራት/ኮርነቲ	1	2	3	GFUEL6AAI
		9. ሌላ (ጽፅለ□)	1	2	3	
G56	የህፃኑ/ኗን ብተሰብ ከሚከተሉት እንሳሳትና ከብቶች ዓይነቶች የትኞቹ አሏችው?					
		□ንስሳ	በቤት ውስጥ ጁቷበቃሉ/ጸት ራሉ	ከቤት ውጪ ጁቷበቃሉ		GANIM6AA
		1. ድመት	1	2		GANIM6AB
		2. ውሻ	1	2		GANIM6AC
		3. □ሮ	1	2		
		4. ላም/በሬ	1	2		
		5. በፅ	1	2		

		6. □ረስ	1	2	GANIM6AD
		7. አ□ል	1	2	GANIM6AE
		8. በቅሎ/አህያ	1	2	GANIM6AF
		9. ሌላ	1	2	GANIM6AG
					GANIM6AH
					GANIM6AI
G57	የህፃኑ/ኗን ቤተሰብ የመጠጥ ውሃ በዋንኝነት የሚያገኙት ከየት ነው?	በግቢው ውስጥ ከሚገኝ ቧንቧ	1		WAT6A
		ከግቢ ውጪ ከሚገኝ ቧንቧ	2		
		ከጉድጓድ ወይም ምንጭ	3		
		ከተጠበቀ ጉድጓድ ወይም ምንጭ	4		
		ከወንዝ፣ ከኩሬ፣ከጉድጓድ	5		
		ከዝናብ ውሃ	6		
G58	የህፃኑ/ኗን ቤተሰቡ የሚገለገልበት መፀዳጀ ቤት ምን አይነት ነው?	በውሃ የሚሰራ ሽንት ቤት	1		SANIT6A
		ሽታ አልባ መጸዳጃ	2		
		የተለመደ ዓይነት የሽንት ቤት ቱትጃት	3		
		ሜ□ ላጁ ወጁም □ ካ	4		
		በየቀኑ	2		
		ቢያንስ በሳምንት አንድ ጊዜ	3		
		ቢያንስ በ15 ቀን አንድ ጊዜ	4		
		በበዓል ቀን ወይንም ለየት ባለ ቀን	5		
		አላውቅም	6		
		መልስ መስጠት አልፈለኩም	7		
G59	የህፃኑ/ኗ ቤተሰብ ቆሻሻ ለመጣያነት የሚገለገሉበት ምንድን ነው?	በቱትጃት ዉስጣ	1		GSAND6A
		ሜ□ ላጁ	2		
		አንድ ላይ ሰብስቦ በማቃጠል	3		
		በቆሻሻ መ□ጸ □ቃ	4		

		ሌላ (ጽፏለን)	9	
G60	የህፃኑ/ኗ ቤተሰብ ከሚከተሉት የተባይ ማጓኝጸ መት□ኒቶች በቤቱ ውስጥ የትኛውን ይጠቀማሉ? (ከአንድ በላይ መልስ መስጠት ይቻላል) (መልሱ ይነበብ)	ዲዲት	1 0	GINSE6AA
		ማላታይን	1 0	GINSE6AB
		ፍሊት	1 0	GINSE6AC
		ሌላ (ጽፅለ□)	1 0	GINSE6AD
G61	□ንደ ፊሊት የመሳሰሉት ጸረተባይ መድሀኒቶች የት ነው የሚያስቀምጡት?(ስፍራዉን □ንዲያሳዩሽ ጠይቂያቸዉ) አሁን ቤት ውስጥ ጸረተባይ መድ□ኒቶች ከሌላቸው ቢኖራቸው ኖሮ የት □ንደሚያስቀምጡ ጠይቂያቸው	ልጆች ሊደርሱበት በሚችሉበት ቦ□	1	PROT6A
		ልጆች ሊደርሱበት በማይችሉበት ቦ□	0	

ANNEX VIII: LABORATORY PROTOCOLS

1. Formol ether concentration technique

1. Using a rod or stick, emulsify an estimated 1 g (pea-size) of faeces in about 4 ml of 10% formol water contained in a screw-cap bottle or tube.

   Note: Include in the sample, faeces from the surface and several places in the specimen.
2. Add a further 3–4 ml of 10% v/v formol water, cap the bottle, and mix well by shaking.
3. Sieve the emulsified faeces, collecting the sieved suspension in a beaker.
4. Transfer the suspension to a conical (centrifuge) tube made of strong glass, copolymer, or polypropylene. Add 3–4 ml of diethyl ether or ethyl acetate.

   Caution: Ether is highly flammable and ethyl acetate is flammable, therefore use well away from an open flame, e.g. flame from the burner of a gas refrigerator, Bunsen burner, or spirit lamp. Ether vapour is anaesthetic, therefore make sure the laboratory is well-ventilated.
5. Stopper* the tube and mix for 1 minute. If using a Vortex mixer, leave the tube unstoppered and mix for about 15 seconds (it is best to use a boiling tube).

   Do not use a rubber bung or a cap with a rubber liner because ether attacks rubber.
6. With a tissue or piece of cloth wrapped around the top of the tube, loosen the stopper (considerable pressure will have built up inside the tube).
7. Centrifuge immediately at 750–1 000 g (approx. 3000 rpm) for 1 minute. After centrifuging, the parasites will have sedimented to the bottom of the tube and the faecal debris will have collected in a layer between the ether and formol water
8. Using a stick or the stem of a plastic bulb pipette, loosen the layer of faecal debris from the side of the tube and invert the tube to discard the ether, faecal debris, and formol water. The sediment will remain.
9. Return the tube to its upright position and allow the fluid from the side of the tube to drain to the bottom. Tap the bottom of the tube to resuspend and mix the sediment. Transfer the sediment to a slide, and cover with a cover glass.
10. Examine the preparation microscopically using the 10x objective with the condenser iris closed sufficiently to give good contrast. Use the 40xobjective to examine small cysts and eggs. To assist in the identification of cysts, run a small drop of iodine under the cover glass. Although the motility of Strongyloides larvae will not be seen, the non-motile larvae can be easily recognized.

11. If required, count the number of each species of egg in the entire preparation. This will give the approximate number per gram of faeces [26].

2. Differential white cell count

A differential white cell count provides information on the different white cells present in the circulating blood, i.e. neutrophils, lymphocytes, monocytes, eosinophils, basophils (rarely seen). Providing the total WBC count is known, the absolute number of each white cell type, i.e. number of each cell per litre of blood, can be calculated and an assessment made of whether the number of a particular cell type is increased or decreased (compared with the accepted reference range).

Method

As previously discussed, it is only possible to report blood films reliably providing the thin blood film is well made and correctly stained. Allow the stained film to dry completely before examining it.
1. Place a drop of immersion oil on the lower third of the blood film and cover with a clean cover glass.
2. Examine the film microscopically. Focus the cells using the 10 objective with the condenser iris closed sufficiently to see the cells clearly. Check the staining and distribution of cells.
3. Move to a part of the film where the red cells are just beginning to overlap and bring the 40 objective into place. Open the iris diaphragm more.

4. Systematically examine the blood film and count the different white cells seen in each field, preferably using an automatic differential cell counter, or if this is not available, record the count in chart form

5. Calculate the absolute number of each white cell type by multiplying the number of each cell counted (expressed as a decimal fraction) by the total WBC count

Differential WBC reference range*

*These values are guideline figures only. They should be checked locally. Neutrophil counts are lower in Africans and Afro-Caribbean people.

Absolute number Percentage

ADULTS

Neutrophils. 1.5–7.5 109/l (40–75%)

Lymphocytes‡ . . . 1.2–4.0 " " (21–40%)

Monocytes . . . . . 0.2–1.0 " " (2–10%)

Eosinophils . . . . . 0.02–0.6 " " (1–6%)

Basophils. . . . . 0.01–0.1 " " (0–1%)

CHILDREN (2–6 y)

Neutrophils. 1.5–6.5 109/l (20–45%)

Lymphocytes‡ . . . 6.0–8.5 " " (45–70%)

Monocytes . . . . . 0.1–1.0 " " (2–10%)

Eosinophils . . . . . 0.3–1.0 " " (1–6%)

Basophils. . . . . 0.01–0.1 " " (0.1–1%)

‡In an adult, lymphocytes are mainly of the small type whereas in a child, large lymphocytes predominate.

3.ELISA (Total IgE)

Test Principle

The Diagnostic Automation, Inc. IgE Quantitative Test is a solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle. The test specimen (serum) is added to the IgE monoclonal antibodies immobilized on polystyrene microtiter wells (solid phase) and incubated with the Zero Buffer. If human IgE is present in the specimen, it will combine with the antibodies on the well. The well is then washed to remove any residual test specimen, and goat anti-IgE in the antibody-enzyme (horseradish peroxidase) conjugate reagent is added. The conjugate reagent will bind immunologically to the IgE on the well, resulting in the IgE molecules being sandwiched between the solid phase and the enzyme-linked antibodies.

After an incubation at room temperature, the solid phase is washed with water to remove unbound labeled antibody. A solution of 3,3',5,5'- Tetramethylbenzidine (TMB) is added and incubated for 20 minutes, resulting in the development of a blue color. The color development is stopped and the resulting yellow color is measured spectrophotometrically at 450 nm. The concentration of IgE is directly proportional to the color intensity of the test sample.

The Diagnostic Automation, Inc. IgE ELISA provides a rapid, sensitive, and reliable assay for total serum IgE. Two carefully selected IgE antibodies are used to determine a minimal concentration of IgE of 5.0 IU/mL.

Specimen Collection and Preparation

1. Serum should be prepared from a whole blood specimen obtained by acceptable medical techniques. This kit is for use with serum samples without additives only. Avoid grossly hemolytic (bright red), lipemic (milky), or turbid samples.

2. Specimens should be capped and may be stored for up to 48 hours at 2-8°C. Specimens held for a longer time should be frozen only once at -20°C prior to assay. Thawed samples should be inverted several times prior to testing.

Reagent Preparation

1. All reagents should be allowed to reach room temperature (18-25°C) before use.

2. All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.

3. Samples with expected values greater than 800 IU/mL should be diluted with Zero Standard prior to assaying. A 1:100 initial dilution is recommended.

Assay Procedure

1. Secure the desired number of coated wells in the holder.

2. Dispense 20µL of standards, samples, and controls into appropriate wells.

3. Dispense 100 µL of Zero Buffer into each well.

4. Thoroughly mix for 10 seconds. It is very important to have complete mixing in this setup.

5. Incubate at room temperature (18-25°C) for 30 minutes.

6. Remove the incubation mixture by flicking plate content into a waste container.

7. Rinse and flick the microtiter plate 5 times with distilled or deionized water. (Please do not use tap water.)

8. Strike the microtiter plate sharply onto absorbent paper or paper towels to remove all residual water droplets.

9. Dispense 150µL of Enzyme Conjugate Reagent into each well. Gently mix for 10 seconds.

10. Incubate at room temperature (18-25°C) for 30 minutes.

11. Remove the incubation mixture by flicking well contents into a suitable waste container.

12. Rinse the wells 5 times with running distilled or deionized water. (Please do not use tap water.)

13. Strike the wells sharply on absorbent paper to remove residual water droplets.

14. Dispense 100 µL TMB Substrate Reagent into each well. Gently mix for 5 seconds.

15. Incubate at room temperature, in the dark, for 20 minutes.

16. Stop the reaction by adding 100µL of Stop Solution (1N HCl) into each well.

17. Gently mix for 30 seconds. It is important to make sure that all the blue color changes to yellow color completely.

18. Read OD at 450nm with a microtiter well reader within 15 minutes.

Results

1. Calculate the mean absorbance value (OD450) for each set of reference standards, controls and samples.

2. Construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in IU/mL on graph paper, with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis.

3. Using the mean absorbance value for each sample, determine the corresponding concentration of IgE in IU/mL from the standard curve. Depending on experience and/or the availability of computer capability, other methods of data reduction may be employed.

4. Sample Dilution - If a sample contains more than 800 IU/mL of IgE, make a 1:100 dilution or further dilutions with the zero standard. After assaying the diluted sample, multiply the calculated value by the appropriate dilution factor.

5. Any diluted samples must be further converted by the appropriate dilution factor.

Expected Values

The total Immunoglobulin E level in normal, allergy-free adults is less than 150 IU/mL in the serum. Variation in total IgE concentrations may be expected in certain age groups and clinical conditions, as briefly described in the “Introduction” above. Each laboratory should establish its own normal ranges based on patient population in the geographical areas served. These values have clinical significance

only after a statistically significant number of assays have been performed over a suitable period of time.

Normal ranges:

Age (years)	IgE (IU/mL)
1 – 5	< 60
6 – 9	< 90
10 – 16	< 200
16 +	< 100

ANNEX IX: Declaration

This thesis is as a partial fulfillment of the requirements for the degree of master of science from Addis Ababa University, I hereby grant to Addis Ababa University and its agents the non-exclusive license to archive, make accessible, and display my thesis in whole or in part in all forms of media, now or hereafter known, including display on the World Wide Web. I understand that I may select some access restrictions as part of the online submission of this thesis or dissertation. I retain all ownership rights to the copyright of the thesis. I also retain the right to use in future works (such as articles or books) all or part of this thesis.

Sosina Walelign: _____ Date: _____

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