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Association of intestinal helminthic infection, atopy and allergic disorder in the setting of mass deworming among selected government primary school children in Sululta Woreda, Oromia, Ethiopia

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This is to certify that the thesis prepared by Dessie Abera entitled "**Association of helminthic infection, atopy and allergic disorder in the setting of mass deworming among selected government primary school children in Sululta woreda, Oromia, Ethiopia"** submitted in partial fulfillment of the requirements for the Degree of Masters of Sciences in Clinical Laboratory Sciences (Diagnostic and Public Health Microbiology) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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List of Abbreviations

Epg	Eggs per gram
HDMS	House dust mites
IgE	Immunoglobulin E (Immunoglobulin with Epsilon chain
IL	Interleukin
ISAAC	International study of asthma and allergies in children
KG	Kindergarten
MDG	Millennium development goal
SAC	School age children
SAF	Sodium acetate acetic acid formalin
SOP	Standard operating procedure
SPSS	Statistical package for social science
SPT	Skin prick test
SSA	Sub-Saharan Africa
STH	Soil transmitted helminthes
TH	T helper cells
Treg	Regulatory T cells
WHO	World health organization

Operational definition

Allergic symptom Questionnaire based self-reported symptoms of allergy in school children (like wheeze,rhinitis ,eczema).

Atopy Skin test reactivity with a wheal size of 3mm and above for house dust mite and German cockroach allergens

School children Refers to children in the school whose ages between 5-14years

Primary school Refers to schools from kindergarten up to grade 8

Helminthic infected Helminthes detected either by direct wet mount, concentration technique or kato-katz

Abstract

Background: Intestinal helminthic infections have been suggested to play protective role from allergic sensitization and atopic diseases. There is a concern that deworming could increase the prevalence of atopic disease in endemic populations. There is inconsistent and little information about the relationship between helminthic infection and allergic disease in Ethiopia.

Objective: The aim of this study was to assess the association of intestinal helminthes and atopy /allergic disorder in the setting of mass deworming among school children in Sululta, Oromia, Ethiopia.

Methods: A cross sectional study was conducted among 526 school children aged 5 to 14 years from three selected government primary schools in Sululta woreda. Information on socio-demographic characteristic, associated risk factors and allergic symptoms were obtained using questionnaire. From all children, fresh stool samples were collected and processed by direct wet mount, formol-ether concentration and Kato-Katz technique and atopy was assessed by skin prick test (SPT). In addition, venous blood was collected for eosinophil count. Data was entered and analyzed using SPSS version21 statistical software. The odds ratio and 95% confidence interval was calculated to assess the strength of the association. P-value less than 0.05 were considered as statistically significant.

Results: Of the total 526 school children 58.2% (306) were females. Overall 24% (n=126/526) had questionnaire based allergic symptoms while 5.1 % (n=27/526) had skin prick test reactivity, 16.9% of them (n=89/526) had intestinal helminthic infection. All helminthic infections were with low intensity level. There was no association between helminthic infection and allergic symptoms (OR=1.30, 95% CI=0.778-2.171, P=0.317). *Ascaris lumbricoides* infection was positively associated with skin test reactivity (AOR=4.307, 95%CI=1.143-16.222, P=0.031). Atopy was significantly associated with increased allergy symptoms (AOR=2.787, 95%CI=1.253-6.197, P=0.012). Short term deworming had no effect on atopy but has a protective effect on allergic symptom.

Conclusion: Low intensity and low prevalence of helminthic infection in our findings may have contributed to the non-significant association of allergy and helminthes infection; however, *Ascaris lumbricoides* was positively associated with atopy. Further longitudinal study is recommended to examine the mechanism of *Ascaris lumbricoides* infection for the development of atopy.

Keywords: **Atopy, Allergy, Intestinal helminthes, School children**

1. Introduction

1.1. Background

The most common cause of intestinal helminthic infections that are linked to allergic disorder are *Ascaris lumbricoides,* hookworm *and Trichuris trichuria* in both tropical and subtropical countries (1).

Intestinal helminthiases are transmitted by eggs excreted in human faeces which contaminate the soil and water sources in areas that lack adequate sanitation. People are infected either through ingestion of infective eggs, larvae contaminating food, water and hands or penetration of the skin by infective larvae contaminating the soil (2).

Intestinal helminthic infections and allergy have similar immune response which is associated with an increase production of cytokines, particularly IL-4-, IL-5 and IL-13-mediated IgE and mast cell production as well as eosinophilia (3). Helminthic infection in endemic areas in early childhood stimulate strong regulatory immunological effects and allergens exposure among children not exposed to helminthes infections in early life will induce allergic phenotypes. As a result, these children may develop allergic diseases mediated by an increase in T-helper type2 (TH2) cells and T-helper type2 cytokines; a decrease in T-helper type1 (TH1) cells and an excessive decrease in T-regs which are IL-10 producing cells. However, the development of allergic response in some children may not relate to helminthes infection (4).

Allergic diseases out comes like, asthma, eczema and rhinitis are inflammatory diseases associated with allergic sensitization to environmental allergens. Allergic diseases are important causes of chronic diseases in childhood. Now a days, allergic diseases are the main cause of morbidity in urbanizing populations in developing countries(5). The reactions against helminthes which also resembling allergy are reactions induced by human defense mechanisms directly against the antigens of helminthes. The migration of helminthes from one organ to another induce a body reaction with clinical symptoms similar to allergic diseases such as Loffler's syndrome, which occurs during the movement of *Ascaris lumbricoides* larvae through the lungs; this causes symptoms similar to asthma (4).

Atopy is an immune disorder which is characterized by raised IgE levels, which leads to clinical disorders such as asthma, eczema and rhinitis. IL-4 and IL-13 derived from T-helper cell type 2 (Th2) subsets, are central in mediating IgE production and the development of immediate hypersensitivity. There has been an increase in severity, and probably in prevalence, of atopic disorders in developed countries. One factor associated with this rise is the decline of many infectious diseases, such as helminthic infections, as a result of improved living standards, immunization and anthelminthic drugs (6).

A child with atopy produces specific IgE antibodies after exposure to common environmental allergens and is said to be sensitized to that allergen and the presence of specific IgE antibodies is measured by means of a skin prick test(7). House dust mites and German cockroaches are the most common indoor allergens which are associated with the development of early child hood allergic disease. House dust mites are found in dust and products with woven material or stuffing such as mattresses, pillows, stuffed animals, and bedding. The most common species are *Dermatophagoides pteronyssinus* and *Dermatophagoides farina* (8). The German cockroach (*Blatella germanica*) and the American cockroach (*Periplaneta americana*) are also the most common species to cause allergies. Proteins derived from cockroach feces, saliva, eggs, and shed cuticles have been associated as leading causes of allergic diseases (9).

The association between helminthic infection and allergic disease can be influenced by time, intensity of infection and host genetics. Early and chronic infections are important in down modulating allergic disease, whereas later infections or transient infections may enhance allergic clinical symptoms. High intensity of infection may induce immune suppression, whereas mild infections may enhance allergic disease. Individuals who are genetically susceptible to atopic disease may be more likely to develop allergic responses to helminthes and allergens and may be genetically more resistant to infection (10).

The World Health Organization (WHO) has been supporting of deworming the population at risk particularly school age children (SAC) to meet the millennium development goals (MDG). Currently, only 10 percent of SAC and 20 percent of pre-school children who are at risk of acquiring intestinal helminthes infections are dewormed. This coverage is against the target set by WHO to regularly treat at least 75 % of SAC at risk of morbidity (11).

1.2. Statement of the problem

Helminthic infections and allergic diseases are important public health problems in developing countries (5). Allergic disease is one of the most common causes of chronic morbidity in childhood in developed countries and has increased rapidly in prevalence in low-and middle-income countries (12). Allergic diseases are very common and helminthic infection relatively uncommon in developed and urbanized populations while the reverse is true in developing countries and rural areas (3).

Different epidemiological researches have provided an evidence for the inverse association between soil-transmitted helminthic infections and allergen skin test reactivity in areas where these infections are highly endemic. It has been suggested that helminthes may suppress allergy in those populations. This has raised the concern that the mass treatment of helminthic infections through anthelminthic treatment program may increase the prevalence of allergic disease in populations where these infections are endemic (13).

Ethiopia launched national mass drug administration at school age children in 2015 (14). Though, some studies have been conducted on the association between helminthic infection, atopy and allergy in non-deworming conditions (28). However, there is little information about the association of helminthic infection, atopy and allergic disorder in the setting of mass deworming in our country. Hence, this study assessed the association between helminthic infection, atopy and allergic disease in mass deworming setting among selected government primary school children in Sululta woreda, Oromia, Ethiopia.

1.3. Significance of the study

- This study provides information to local concerned bodies and researchers on the association of helminthic infection, atopy and allergic disease in the deworming setting.
- Additionally, this study provides information on the prevalence of helminthes among school children in deworming setting; hence early intervention can be taken.
- This study gives information to policy makers to consider and integrate the management of allergic diseases along with intestinal helminthic infections.
- The finding of this study can also be used as base line data for further study.

2. Literature review

The relationship between helminthic infection, atopy and allergic disorder was inconsistent and showed positive, negative or no association (3). Therefore, this literature review section provides over view of different effects of helminthes on allergic disease.

2.1. **Helminthes are positively associated with atopy or allergic symptoms**

A cross-sectional study conducted in rural china among 2,164 children age between 8-18 years old to investigate the relationship between *Ascaris lumbricoides* infection, Asthma and Atopy. Positive history or stool examination of *Ascaris lumbricoides* infection in 15.9% and 12.2% of subjects, respectively. The number of positive skin tests ranged from 0 to 9%; 40% of subjects were sensitized to at least one aeroallergen. They found and conclude that *Ascaris lumbricoides* infection was associated with increased risk of child hood asthma and atopy(15).

Another cross sectional study was conducted in Uganda among 2316 individuals to assess the association between helminthic infection and allergy. They found that prevalence of reported wheeze was 2% in under-fives and 5% in participants ≥5 years; 19% had a positive SPT. They conclude that individuals with certain helminthic infection were more prone to Atopy (16).

Hawlader *et al,* in 2014 aimed to examine the effect of helminthic infection on asthma and atopy among Bangladesh children. A total of 912 children were included in a cross-sectional study nested into a randomized control trial. According to their finding, ever-asthma in 18.0%, current wheezes in 19.7% and ever-wheezing in 45.2%. Anti-*Ascaris* IgE was positive for 69.7%, skin prick test with mite antigen 15.7% *Ascaris lumbricoides* 17.4% *Trichuris trichiura* 17.5%. Their finding suggested that repeated *Ascaris lumbricoides* infection was a risk factor for asthma and atopy in rural Bangladeshi children (17).

A randomized, double-blind, placebo-controlled trial conducted in Vietnam among 1566 school children aged 6–17. Participants were randomly allocated to receive either anti-helminthic therapy or a placebo at 0, 3, 6, and 9 months, to test the hypothesis that infection with helminthes protects against allergic disease and allergen skin sensitization. They found a significant reduction in worm burden over a 12-month period in helminthic-infected children increases the risk of allergen skin sensitization but not of clinical allergic disease (18).

A cross-sectional study conducted in Bolivia among school children to assess the association between environmental factors and current asthma, rhinoconjunctivitis and eczema symptoms. They found that wood or coal as cooking fuel showed a positive association with asthma symptoms and two or more precarious household conditions and dog contact showed a positive association with rhinoconjunctivitis symptoms and finally, they concluded that lower hygiene conditions did not have protective effect against asthma and rhinoconjunctivitis and eczema symptoms(19).

2.2. **Helminthes are negatively associated with atopy or allergic symptoms**

A cross-sectional study was conducted among 1320 Cuban children aged 4-14 years to assess the relationship of past and current intestinal helminthic infection with asthma, allergic rhinoconjuctivitis, atopy dermatitis and atopy. The prevalence of self-reported allergic symptoms were 21%, 14 % , 8%, 20% for asthma, rhinoconjuctivitis, atopic dermatitis and skin prick positive test respectively. According to their finding they conclude that Current *Ascaris lumbricoides* infection protects against atopic dermatitis, while past infection with *E. vermicularis* and hookworm are risk factors for allergic rhinoconjunctivitis and atopic dermatitis (20).

Another cross-sectional study conducted among 4,433 school children from 71 schools to examine the relationship between geohelminth infections, atopy and symptoms of allergic disease. They found that recent wheeze 2.1%, rhinitis (4.1%), eczema (3.7%) and skin test reactivity was 18.2%. They concluded from their finding that presence of geohelminth infection was protective against allergen skin test reactivity and symptoms of exercise-induced wheeze but not against other wheeze symptoms or symptoms of allergic rhinitis or atopic eczema (21).

Similar cross-sectional study was conducted in the same area, Ecuador among 2865 children aged 5 to 19 years from 55 schools to assess whether geohelmithic infections protect against atopy and whether this protection is dependent on infection chronicity. From their finding they conclude, active infections with geohelminth parasites and the presence of serologic markers of chronic infections are independent protective factors against allergen skin test reactivity among school-age children (22).

A prospective cohort study was conducted in Israel in 2016 among a total of 126 newly arriving Ethiopian immigrants. Those who were highly infected with parasites were studied prospectively for the presence of allergy and response to allergens on arrival and after a year of follow up in Israel. The investigators found a significant inverse association between the presence of parasitic infection and allergy on arrival while after a year of living in Israel accompanied by a lowered parasitic infection load, they observed general increase of allergy in all immigrant groups and not only in those that had parasitic infections on arrival (23). The study concluded that the findings were supporting the suppressive effect of intestinal parasites on allergy but suggest that additional factors most probably environmental also play a role in the generation of allergy (23).

Lubis *et al* in 2014 from Indonesia recruited sixty eight children in their cross sectional study to determine the association between soil-transmitted helminthic (STH) infection and skin prick test reactivity. Data from 34 children with soil-transmitted infection was compared to those generated from34 children without soil-transmitted infection. There was a significant association between STH infection and skin prick test negativity. They concluded that children with STH infection tend to have negative skin prick test(24).

Among the limited available published data from Africa a randomized controlled trial was conducted in Gabonese school children in 2004 to assess the effect of repeated anthelminthic treatment on allergic sensitivity to house dust mites (HDMS) in chronically infected children. A total 317 school children were included in the trial study with a high prevalence of intestinal helminthes. Intervention consisted of treatment every 3months with praziquantel and mebendazole and with placebo in the control group. They found that a significant increase in the rate of developing skin sensitivity to HDMS, which was mediated in part by reduction in *Ascarislumbricoids* and /or *Trichuris tricuria* infection, levels of IgE were reduced but this did not mediate the effect of treatment on skin test reactivity. They generalized that anthelminthic treatment of chronically infected children results increased atopic reactivity which indicated that helminthes directly suppress allergic reactions (25).

2.3. Helminthes do not have effect on atopy or allergic symptoms

Data from a longitudinal study in Cuban school children by Werff *et al* in 2013 supports the argument that deworming is not a risk factor for the development of atopic diseases. The study involved 108 soil-transmitted helminthes positive school children aged 5-13years and followed them for 24 months in six-monthly intervals. One single dose of 500mg mebendazole was given to those who were positive for helminthic infection. Their result, showed that after deworming the frequency of asthma significantly decreased while the frequency of allergic rhinoconjuctivitis and atopic dermatitis was not affected. They concluded that atopic disease does not increase after anthelminthic treatment, whereas allergic sensitization on the other hand increases after deworming, as this increase appears only temporarily. Deworming of school children does not seem to be a risk factor for the development of allergic sensitization nor for atopic disease (26).

Another cluster- randomized controlled trial study was performed in 2006 by Cooper P *et al* in school children from 68 rural schools of Ecuador. Though, Albendazole treatment caused large reductions in geohelminthes prevalence over the study period. But there was no evidence that treatment was associated with an increase in atopy prevalence or clinical allergy in the albendazole compared with the no treatment group. They concluded that there is no increase in the prevalence of atopic or clinical allergy associated with albendazole treatment. Therefore, deworming program for school children are unlikely to be accompanied by an increase in allergy (27).

A study was conducted in Western Province of Sri Lanka from 17schools among children attending grade 5 to investigate the relationship between allergic diseases and helminthic infection. A total of 640 children were recruited to the study, of them 33.7% had evidence of allergic disease and 15.5 % had helminthic infections. Low intensity of infection was recorded in this study (68.9%), allergic disease and helminthic infections were not significantly associated; but there was a trend toward protective role of helminthic infections against allergic diseases was noted. They conclude that though not significant, a reduced risk of allergy in helminthic-infected children was observed (28).

A cohort study was conducted among 50 children aged 5to12 years to investigate the association of geohelminthes with allergic sensitization in the areas of Batangas, Philippines. Mixed infection of *Ascaris lumbricoides* and *Trichuris trichiura* were found to be the most common (40%) followed by *Trichuriasis* (34%) and *Ascariasis* (26%). They found that the occurrence of geohelminthic infection and allergy may affect any child regardless of age. More over serum total IgE level does not considerably vary with age, geohelminthiasis and presence of allergy. They conclude that there was no association between helminthic infection and allergic sensitization (29).

A cross sectional survey study conducted in 2005 among 7649 people aged 5 years in Butajira, Ethiopia to assess wheeze, allergic sensitization and geohelminthes infection. They found no significant protective effects of any geohelminth infection against wheeze or asthma. They concluded that there were weak associations between allergic sensitization and wheeze but no evidence of a protective effect of geohelminthes against wheeze or asthma (30).

On the other hand a nested case control study was conducted in 2003 to investigate the relation between parasite infections, wheeze and allergen skin sensitization from a survey of 7,155 children aged1to4 years living in urban and rural areas of Jimma, Ethiopia. They found *Trichuristricuria* (54%),*Ascarislumbricoides* (38%) and hook worm (10%) and skin sensitization was more prevalent in rural than urban children ,and was unrelated to wheeze. From their research they concluded that *Ascaris lumbricoides* and hookworm infection protects against wheeze in young Ethiopian children and that this effect is not mediated by inhibition of allergen sensitization (31).

Similar cross-sectional survey and nested case-control study was conducted in the same area of Jimma, Ethiopia among children age 1 to 5 years to assess early life risk factors of atopic dermatitis. They collected information on lifestyle and other potential risk factors by parental questionnaire, and stool samples were analyzed for parasites. They found that the risk of atopic dermatitis was unrelated to family size, crowding in the home, or breast-feeding. They concluded that neither intestinal parasite infection nor other proposed risk factors for atopic dermatitis appear to be related to the presence of the condition in young children in Ethiopia(32).

3. Conceptual frame work

This conceptual frame work shows associated risk factors for allergic disease and helminthic infection. It is hypothesized that helminthic infection has an influence on immune status and allergic disease. Poor environmental hygiene, lack of latrine, socio-demographic characteristics and lack of clean water have an impact on allergic disease and helminthic infection (33).

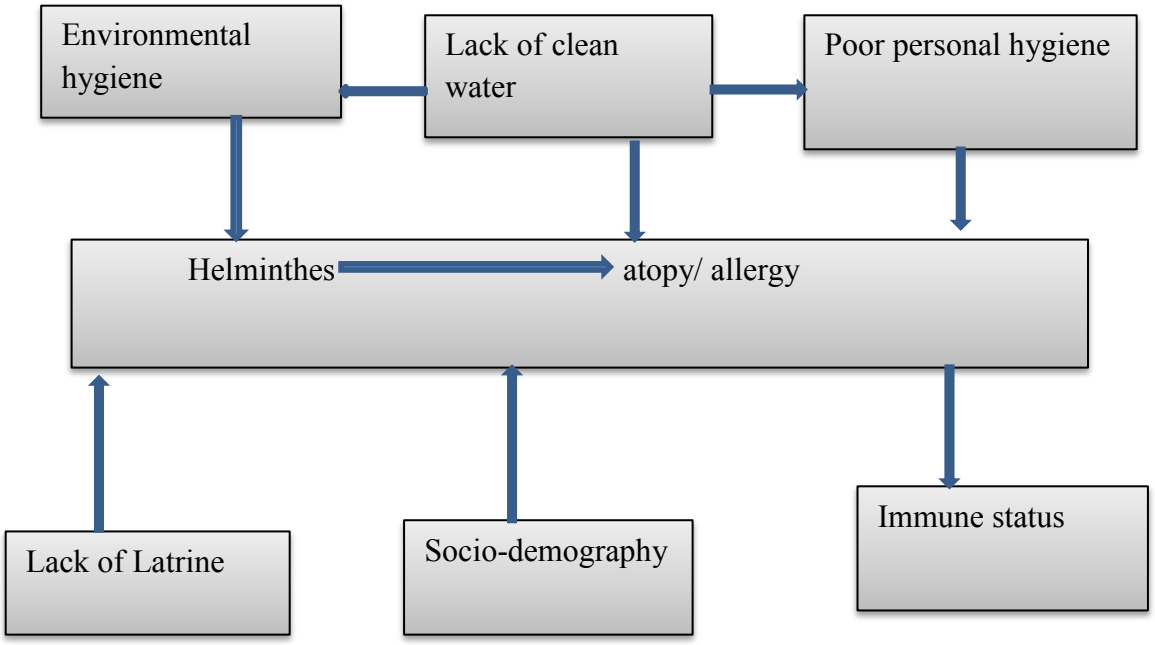

Figure 1.Conceptual framework for associated risk factors of helminthic infection and allergy

4. Objectives

4.1. General objective

To assess the association of intestinal helminthic infection, atopy and allergic disorder in the setting of mass deworming among selected government primary school children in Sululta Woreda, Oromia, Ethiopia

4.2. Specific objectives

- To determine the relationship between intestinal helminthes and allergic out comes
- To determine the association between intestinal helminthes and atopy
- To assess the association between allergy symptoms and atopy
- To compare atopy among children with deworming and non-deworming group.
- To assess associated risk factors with helminthes, allergic symptoms/atopy

5. Hypothesis

There is no difference in the burden of Atopy and allergic disease between deworming and non-deworming school children as compared to previous studies.

6. Materials and Methods

6.1. Study area

The study was conducted in Sululta district which is located in Finfine zuria special zone of Oromia regional state. It is 21km far from Addis Ababa located at 9^0 3' to $9^0$31N latitude and 38^0 29' to 38^0 58'E longitude. It has a total area of 158km^2 and the total population is 129,000 according to 2007 Ethiopian census (34).

There are nine governmental elementary schools in Sululta district having a total of 6922 students and of them, three schools (namely Abdiboru, Lagadimma, and Waserbe elementary schools) which have a total of 3193 students. Abdiboru primary school has a total of 1069 students (1033 are grade 1-4 and 36 were KG students). Laga dima primary school has a total of 1183 students of them 70 were kindergarten and the rest are from grade1-8.Waserbe primary school has a total of 941 students of them 38 were kindergarten students.

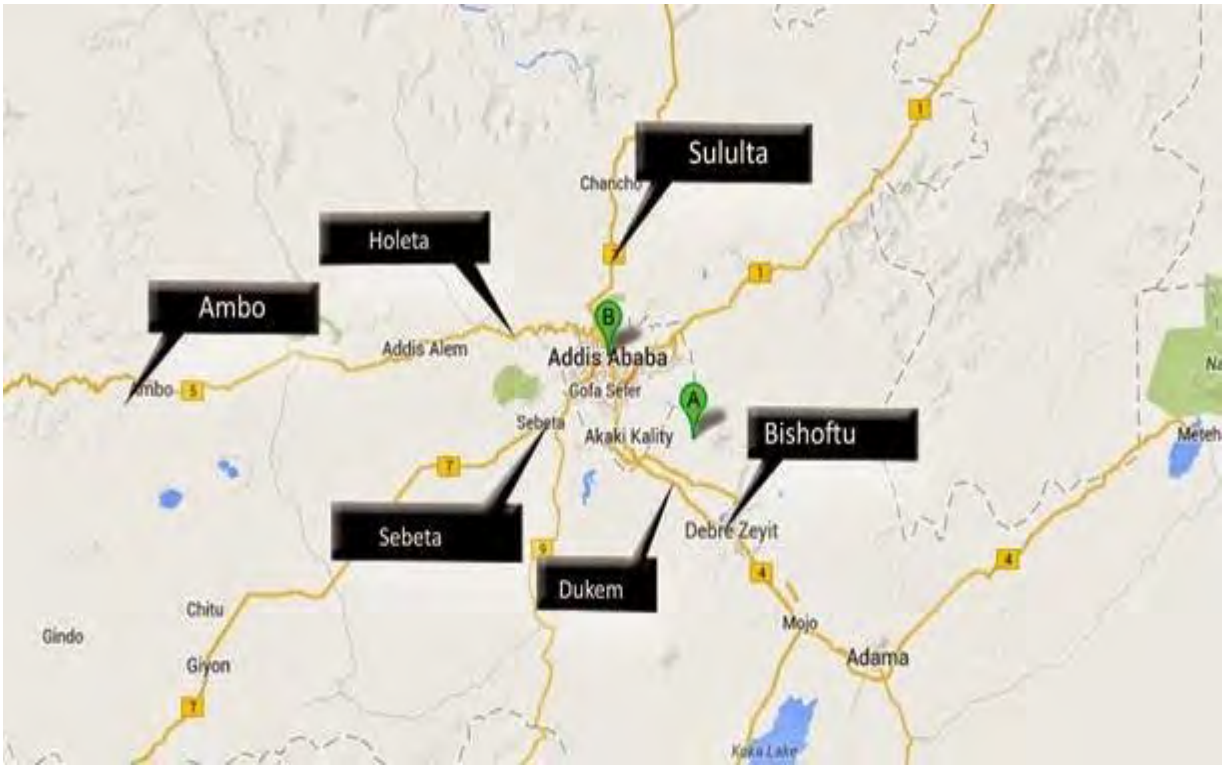

Figure 2. Sululta town geographical map (source: Sululta town administration)

6.1.1. School information

Information regarding to deworming was taken from school principal. Accordingly the deworming program was the first cycle in Sululta woreda and almost 81% of the students received anthelminthic treatment 3 months before this data collection. Regarding to paternal and maternal information, we asked school principals to write a letter by stating the purpose of the research and children were took the letter to their families. Volunteer families were came and discussed with us. We further explained the research purpose and their right to with draw.

6.2. Study design and period

A cross sectional study was conducted from April 2017 to June 2017.

6.3. Population

6.3.1. Source of population

All governmental primary school children in Sululta town were source of populations.

6.3. 2.Study population

Three selected governmental primary school children with the age of 5-14 years and who fulfilled the inclusion criteria in the study site.

6.4. Eligibility criteria

6.4.1. Inclusion criteria

School children age 5 to 14years old and who were volunteer to participate in the study

6.4.2. Exclusion criteria

Children who received antihistamine drugs, which may interfere (suppress skin test reactivity) with skin testing for the last five days were excluded (Bromphemiramine, Cetirizine, Chlorphemiramine), this was further confirmed by explaining symptoms of allergic disease and whether they had taken drugs or not for those conditions. Children whose parents didn't give consent or who were not willing to participate were excluded.

6.5. Study Variables

6.5.1. Dependent variables

- Atopic and allergic symptoms
- Intestinal helminthes

6.5.2. Independent variables

- Socio-demographic characteristics
- Parents' history of allergic disease
- Residence (rural/urban)
- Deworming status
- Latrine availability, hand washing habit after toilet, before meal.
- Grade level of children
- Fuel use etc.

6.6. Measurement and Data collection

6.6.1. Sample size determination

The minimum sample size (n) was estimated using the single population proportion formula.

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

Where: n= minimum sample size

d = margin of error (4%) at 95% confidence interval

Z = standard score corresponds to 1.96

P = prevalence of intestinal helminthes from previous study (27.2 %) at Babile town, Ethiopia (39).

$n = (1.96)^2 \times 0.272\,(0.728)/0.0016 = 475$

To minimize errors arising from the likelihood of non-compliance, ten percent of the sample size was added which gave a final sample size of 523.

6.6.2. Sampling method

From the total of nine government primary schools three primary schools were selected randomly using lottery methods. Allocation of students was made proportional to the number of students for each school. To select the study participants, the students were first stratified according to their educational level (kg to grade 8) and they were taken from each class category by systematic random sampling using class roster as a sampling frame.

The number of students in Abdiboru, Lagadima and waserbe were 172, 194 and 157 respectively based on systematic random sampling technique.

6.6.3. Data collection procedure

Informed consent and assent were signed by parents /guardians and children. Parents/ guardians of the enrolled children were asked to complete questionnaire regarding to socio-demographics, their daily habits, household information and allergy symptoms. Children were instructed to avoid contamination of the stool with water and urine and then each child was provided a clean, dry, disinfectant free, wide mouthed plastic container to collect about 20 g of stool specimen into the container for intestinal parasites detection.

After receiving stool specimens, venous blood was collected from each child. Thin blood smear, direct wet mount and kato-katz were performed on the same day of collection at the school setup (Separate sections in the same room were prepared for interviewing, sample collection, processing and analysis). In addition, electric source was available to examine direct wet mount and kato-katz. The remaining portions of stools were preserved using sodium acetate- acetic acid- formalin (SAF) and transported to Addis Ababa University, department of medical laboratory science and processed for formol-ether concentration technique.

6.6.4. Laboratory analysis

Each stool sample was processed and examined using direct wet mount, formol-ether concentration technique and kato-katz method.

A. Direct wet mount

Direct wet mount was examined using approximately 1gram of stool specimen from each children at the study site to detect intestinal helminthes eggs, larvae and protozoan trophozoites, and cysts. Direct wet mount preparations of fresh unpreserved stool were performed and examined as soon as possible under low power objective (10x) and high power objective (40x) (35).

B. **Formol-ether concentration technique**

Formol-ether concentration technique was performed after stool samples were preserved by SAF and transported to AAU, Department of medical laboratory sciences. Stool specimens (approximately, 1gram) were added to 15ml conical test tube and 4ml of formol- water (10%) were added after mixing another 4ml of formol-water was added and the mixed suspension were filtered using gauze to avoid large particles. after that 3-4ml ether was added and mixed suspension was centrifuged at 3000rpm for one minute. The supernatant was discarded and the sediment was examined under microscope for the detection of ova, larvae and cyst of parasites (35).

C. **Kato-Katze technique**

Small portion of fresh stool samples were examined using Kato- Katz technique at the study site. Stool was added on square paper and pressed through mesh screen to remove large particles. Then sieved sample was transferred to the hole of template on a slide and the template was removed after filled and then covered by cellophane which was socked over night with malachite green. The preparation was examined after 30minutes, under 10 × low powers filled and 40× high power filled objectives. Eggs per gram (epg) of faeces were counted and multiplied by 24 to determine the intensity of infection based on WHO grading system (1).

D. **Skin prick test (SPT)**

Two common allergens; house dust mite (*Dermatophagoides pteronyssinus*) and German cockroach (*Blattella germanica*) were used to measure Atopy. One drop of each allergen (house dust mite, German cockroach, positive and negative control) was added after cleaning the lower forearm of children with 70% alcohol. Skin prick was performed using lancets, after 15 minutes of pricking we measured the wheal diameter using graduated ruler. A positive test was defined as an average of two perpendicular wheal diameters. Allergic sensitization was taken as positive when at least one of the two allergens and positive control measured were greater than or equal to 3mm and negative control was negative. Histamine positive control and Saline negative controls were used to minimize false reactions (36).

Skin prick test provides information about the presence of specific IgE to protein and peptide antigens. Small amounts of allergens 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 (36).

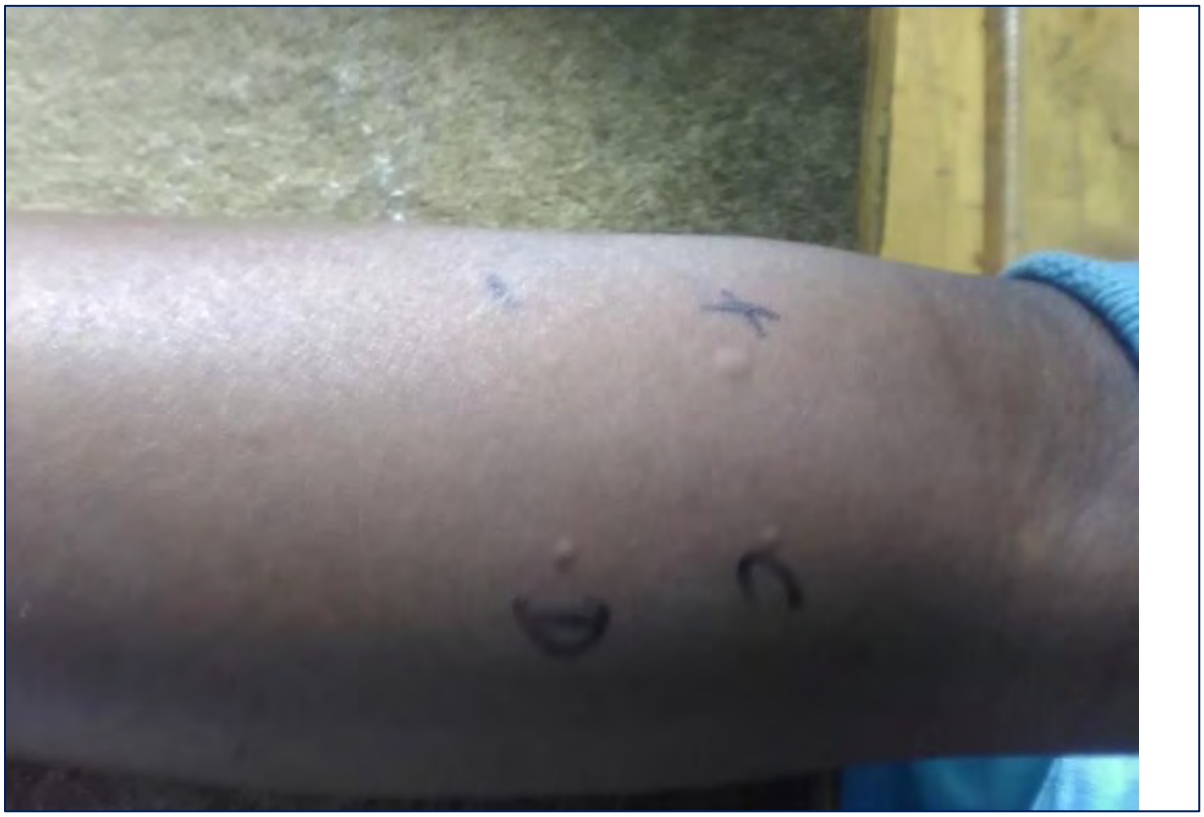

Figure 3. Skin prick test from school child in Sululta town

E. **Eosinophil count**

Venous blood was drown from each child and thin smears were prepared and fixed with methanol at study site and packed after dried, transported to Addis Ababa University, Department of medical laboratory science then stained using giemsa, each eosinophil was counted until 100 cells counted and percentage of eosinophil was multiplied by total white blood

cell to get absolute eosinophil count. Total white blood cell was analyzed using sysmex kx-21 automated machine.

F. **Questionnaire**

Interviewer administered questionnaire was used to interview parents/guardians. The questionnaire was modified and adopted from international study of asthma and allergy questionnaire of children (ISAAC) to assess allergic diseases like asthma, rhinitis, eczema and wheeze (37).

6.7. Data quality assurance

6.7.1. Pre analytical quality assurance

The questionnaire was assessed using Amharic and Afan Oromo (local language) and most of the children and their parents were able to communicate with Amharic and teachers were helping us to translate those who were not communicate with Amharic. Data were checked for completeness at the end of each data collection. Clean and dry containers were used and contamination of stool with water and urine was avoided. The principal investigator was also check during submission. The quality of reagents such as normal saline (turbidity of saline might contain moveable artifacts that might confused us when examined under microscope) and turbidity of formalin and ether were checked daily. Standard operating procedures were strictly followed for each laboratory activities.

6.7.2. Analytical quality assurance

The objectives and eye pieces of the microscope were cleaned before and after examination. The stool preparations were examined systematically, using 10x objective and 40 objectives. The examination at low magnification (10 x objectives) were carried out with the light-source voltage regulator at a minimum and examined at high magnification (40 x objectives), increasing the intensity of the light source. The wet mount preparations were examined immediately in order to assess the motility of the trophozoites, this also avoids evaporation of the fecal suspension and the formation of air bubbles. Egg counts using kato-katz was also performed on the site within 30minutes.

6.7.3. Post analytical quality assurance

Data entry quality was maintained by entering in to epidata and exports to SPSS version 21software and verified its quality against the collected hard copied data during entry. Finally all the necessary data was kept in a locked place. All results were recorded and kept its confidentiality in the appropriate prepared log sheet.

6.8. Data analysis and interpretation

The completed data collection tool was checked for completeness, consistency and was coded by the principal investigator. Data cleanup was performed to check for accuracy and consistencies. Any error identified was corrected immediately. Statistical analysis was performed using SPSS version 21. Descriptive statistics was used to describe the socio-demographic data. Binary and multiple regression analysis (odds ratio/adjusted odds ratio) were employed to assess the significant association of atopy /allergy with each type of helminthes. P-value less than 0.05 was considered as statistically significant.

6.9. Ethical consideration

Before starting the research work, ethical clearance was obtained from Department of Laboratory Sciences Departmental Research and Ethics Review Committee (DRERC), School of Allied Health Sciences, College of Health Sciences, Addis Ababa University. In addition a formal letter of cooperation was collected from Sululta woreda, education Bureau.

The purpose of the study, the study procedures, possible risks/benefits, the rights and responsibilities of participants including their right to withdraw from the study at any time were described. The study was carried out after obtaining informed consent and assent as needed. Confidentiality of data was kept throughout the study and children positive for intestinal helminthes were linked to the nearby health facility.

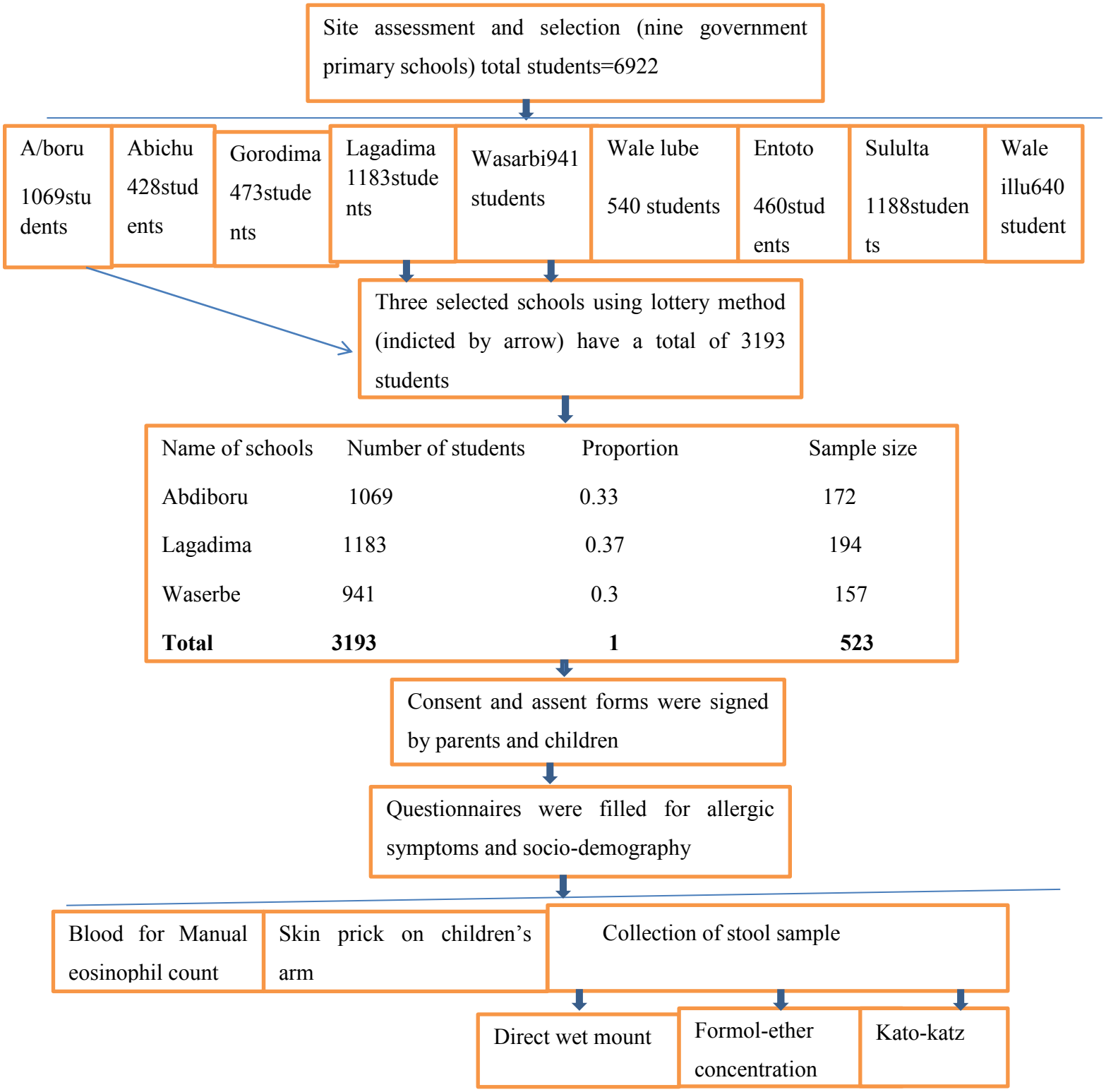

Figure 4: Work flow of data collection and analysis

7. Results

7.1. Socio-demographic characteristics of study participants

A total of 526 school children were participated from 3 primary schools, of which 58.2% (n=306/526) were females. Age ranges of students were 5 - 14years with mean (+/-SD) age of 10.80. Majority of the students were in the age of 10-14 years 70 %(n=368/526) and 57.1% of students were grade 1-4.

The age classification was based on Ethiopia Mini Demographic and Health Survey (38). Most of the students live in semi-urban areas 76% (n=400/526), based on schools' recorded document 81.2% (n=427/526) students received anthelminthic treatment in the last three months (before data collection). With regarding to maternal and paternal educational level, 52.1 % of students' mothers and 17.5 % of students' fathers were illiterate. Majority of mothers were house wife 58.6% (n=308/526) and 26.6% (n=138/526) of fathers were merchants as shown in Table 1.

Table1: Socio-demographic characteristics of children and their parents in selected primary schools in Sululta, (n=526) 2017.

Variables		Total number	Percent
Sex	Male	220	**41.8**
	Female	306	**58.2**
Age category in years	5-9	158	**30.0**
	10-14	368	**70.0**
Mother's education	Illiterate	274	**52.1**
	Write &read	92	**17.5**
	Primary	110	**20.9**
	high school	30	**5.7**
	higher education	20	**3.8**
Father's education	Illiterate	92	**17.5**
	Write &read	176	**33.5**
	Primary	155	**29.5**
	high school	74	**14.1**
	higher education	29	**5.5**
Residence	Urban	400	**76**
	Rural	126	**24**
Grade level	Kg	59	**11.2**
	1-4	302	**57.4**
	5-8	165	**31.4**
Deworming status	Yes	427	**81.2**
	No	99	**18.8**
Maternal occupation	Civil servant	35	**6.7**
	House wife	308	**58.6**
	Private	35	**6.7**
	Farmer	12	**2.3**
	Daily laborer	71	**13.5**
	Merchant	65	**12.4**
Paternal occupation	Civil servant	86	**16.3**
	Private	98	**18.6**
	Merchant	138	**26.2**
	Daily laborer	129	**24.5**
	Farmer	51	**9.7**
	No work	24	**4.6**

7.2. Prevalence of intestinal helminthes

Out of 526 school children 138 school children were positive at least for one intestinal parasite, which gave an overall prevalence of 26.2 % (n=138/526). The prevalence of intestinal helminthes alone was 16.9% (n=89/526). The most predominant intestinal helminthes identified were *Hymenolepis nana* 6.3% (33/526) followed by *Ascaris lumbricoides* 3.4% (n=18/526) (Figure 5).

Out of 89 intestinal helminthic infected children 3.4% (n=3/89) had double infection where *Ascaris lumbricoides and Tricuris tricuria* 1.1%(n=1/89), *Ascaris lumbricoides and Hymenolepis nana* 1.1% (n=1/89), Hook worm and *Hymenolepis nana* 1.1% (n=1/89).

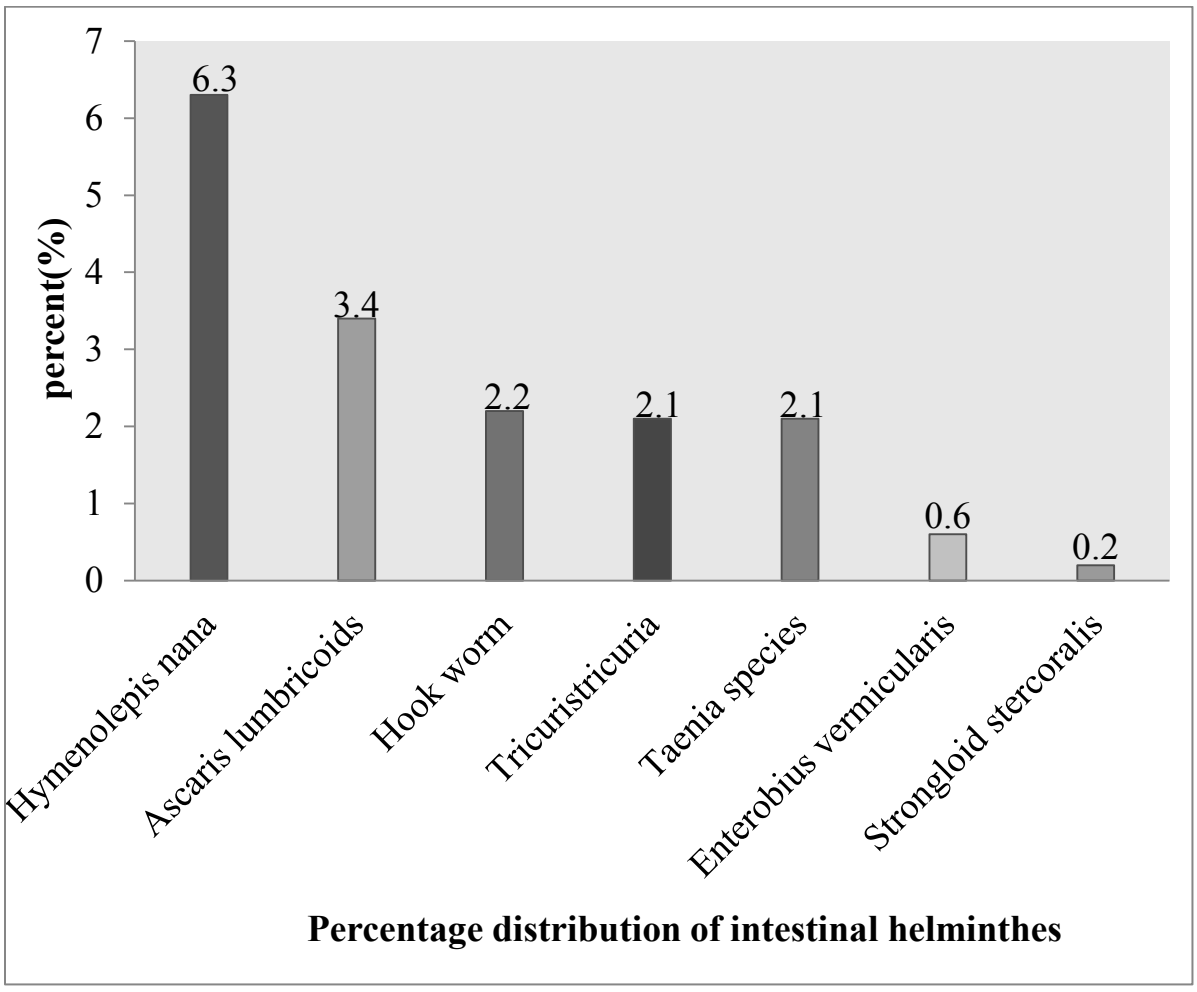

Figure 5: Distribution of intestinal helminthes in selected primary school children in Sululta, 2017 (n=526).

7.3. Prevalence of self-reported allergic symptoms and atopy

Based on self-reported allergy symptoms in the study group the overall prevalence of any allergy symptom was 24% (n=126/526). skin itch and rhinitis were the most reported allergy symptoms that accounted 18.8 %(n=99/526) and 8.9 %(n=47/526) respectively (Figure 6).

The prevalence of skin test reactivity (atopy) to cockroach or house dust mite allergen was 5.1% (n=27/526). The commonest allergen that showed skin test reactivity was cockroach accounted 51.9% (n=14/27) and both reactivity was 33.3% (n=9/27).

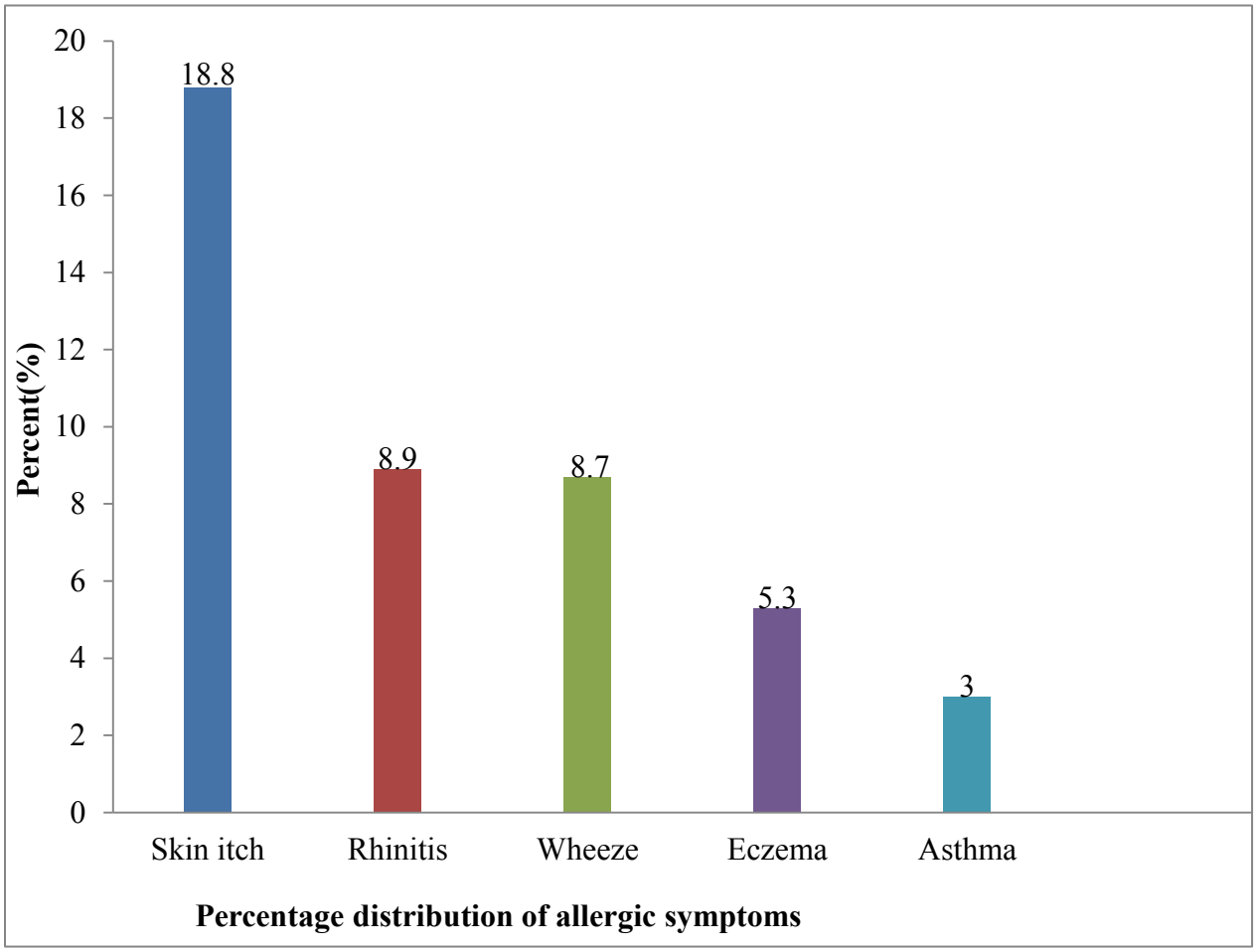

Figure 6: Distribution of allergic symptoms in selected primary school children in Sululta, 2017(n=526).

7.4. Association of allergic symptoms with atopy, helminthic infection and risk factors

Male students were reported to have slightly higher allergic symptoms 25 %(n=55/220) than female students 23% (n= 71/306); however, the difference was not statistically significant (OR=1.103, 95%CI=0.736-1.653, P=0.634). Children who reported allergic symptoms of 24.6 % (n=85/346) had contact with animals, but there was no significant association between allergic symptoms and animal contact (OR = 1.104, 95% CI = 0.721-1.690, P = 0.648).Among 89 helminthic infected children 28.1 % (n=25/89) children had any allergic symptoms. There was no significant association between helminthic infection and allergic symptoms (OR=1.30, 95%CI=0.778-2.171, P=0.317) (Table 2).

Ascaris lumbricoides and *Trichuris trichuria* had no significant association with allergic symptoms (OR = 0.626, 95% CI=0.178-2.198, P = 0.465 and OR =0.312, 95% CI =0.0400-2.461, P = 0.269) respectively. But hookworm infection was significantly associated with allergic symptoms (AOR=4.309, 95%CI=1.321-14.056, P=0.015). Almost all children with allergic symptoms were from families who used charcoal as a source of fuel (n=125/521) but no statistical significant association was observed (OR = 1.263, 95% CI =0.140-11.401, P = 0.835). Out of 27 skin prick positive children 44.4% (n=12/27) had any allergic symptoms and was statistically significant (AOR = 2.787, 95% CI = 1.253-6.197, P =0.012) (Table 2).

Children who did not take anthelminthic treatment with in the last 3 months were 99, of them 32.3% (32/99) children were reported any allergic symptoms, whereas from the deworming group 22% (94/427) had allergic symptoms. Statistical significant association was observed between non-deworming and allergic symptoms (AOR=1.690, 95% CI=1.039-2.749, P =0.034). Related to history of allergic symptoms, 56.9% (n=25/58) of children with allergic symptoms were from families who had allergic symptoms and this association was statistically significant (AOR =2.647, 95% CI=1.494-4.691, P=0.001) (Table2).

Table 2: Association of any allergic symptoms with atopy, helminthic infection and risk factors in selected primary schools in Sululta, 2017.

Variables		Total	Any allergy		COR	95% CI	P-value	AOR	95%CI	P-value
			Yes (%)	No (%)						
Sex	Male	220	55 (25.0)	165 (75.0)	1.103	0.736-1.653	0.634			
	Female	306	71 (23.2)	235(76.8)	1					
Age category	5-9	158	33 (20.9)	125 (79.1)	1					
	10-14	368	93 (25.3)	275 (74.7)	1.281	0.817-2.009	0.281			
Residence	Urban	400	97(24.3)	303(75.7)	1.071	0.667-1.720	0.777			
	Rural	126	29 (23)	97(77)	1					
Animals	Yes	346	85(24.6)	261(75.4)	1.104	0.721-1.690	0.648			
	No	180	41(22.8)	139(77.2)	1					
Charcoal	Yes	521	125(24.0)	396 (76.0)	1.263	0.140-11.401	0.835			
	No	5	1 (20.0)	4 (60.0)	1					
Wood	Yes	490	115(23.5)	375 (76.5)	0.697	0.333-1.460	0.339			
	No	36	11 (30.6)	25 (69.4)	1					
Electric	Yes	174	48 (27.6)	126 (72.4)	1.338	0.882-2.030	0.171			
	No	352	78 (22.2)	274 (77.8)	1					
Helminthes	Yes	89	25 (28.1)	64 (71.9)	1.30	0.778-2.171	0.317			
	No	437	101(23.1)	336 (76.9)	1					
Ascaris lumbricoids	Yes	18	3 (16.7)	15 (83.3)	0.626	0.178-2.198	0.465			
	No	508	123(24.2)	385(75.8)	1					
Tricuris tricuria	Yes	11	1 (9.1)	10(90.9)	0.312	0.040-2.461	0.269			
	No	515	125(24.3)	390(75.7)	1					
Hook worm	Yes	12	7 (58.3)	5(41.7)	4.677	1.448-14.910	0.010	4.39	1.321-14.056	0.015
	No	514	119(23.2)	395 (76.8)	1			1		
Deworming	Yes	427	94 (22.0)	333 (80.0)	1			1		
	No	99	32 (32.3)	67 (67.8)	1.692	1.048-2.733	0.032	1.690	1.039-2.749	0.034
Any allergen	Yes	27	12(44.4)	15(55.6)	2.702	1.229-5.937	0.013	2.787	1.253-6.197	0.012
	No	499	114(22.8)	385(77.2)	1			1		
Family allergy	Yes	58	25(56.9)	33(43.1)	2.753	1.565-4.841	0.000	2.647	1.494-4.691	0.001
	No	468	101(21.6)	367(78.4)	1					

7.5. The relationship of atopy with allergic symptoms and helminthic infection

Male students were showed 6.8% (n=15/220) skin prick test reactivity which was higher than female students 3.9% (n=12/306) skin prick test reactivity. However, there was no statistically significant association between gender and atopy (OR=0.588, 95%CI=0.256-1.217,P=0.142) Among 89 helminthes positive children 6.7% (n=6/89) of them were positive for skin prick test whereas from 437 helminthes negative children 4.8% (n=21/437) were positive for skin prick test. There was no significant association between helminthic infection and atopy (OR=1.432, 95% CI=0.561-3.656, P=0.453). However, when specific helminthes considered, there was statistically significant association between *Ascaris lumbricoides* infection and skin prick test reactivity (AOR=4.307, 95%CI=1.143-16.222, P=0.031) but not with hookworm and *Trichuris trichuria* infection (OR=1.706, 95%CI=0.212-13.723, P=0.615 and OR=1.881, 95%CI=0.232-15.254, P =0.554) respectively. Children who reported one or more allergic symptoms were more likely to be positive for skin prick test (AOR=2.843,95% CI=1.281-6.307,P=0.010) (Table 3).

Children who had history of wheeze, and itch skin showed significant association to skin prick test reactivity or atopy in unadjusted odd ratio (COR=3.279,95%CI=1.251-8.589,P=0.016 and COR=2.710,95%CI=1.201-6.116,P=0.016) respectively but adjusted odd ratio in multivariate analysis showed non-significant association. From 99 non dewormed school children only 5.1 %(n= 5/99) children showed skin test reactivity. Out of 427 dewormed children 5.2% (n=22/427) children were positive for skin prick test. There was no difference in the prevalence of atopy between dewormed and non-dewormed children (OR=1.021, 95%CI=0.377-2.767, P=0.967) (Table 3).

Table 3: Association of atopy with socio-demography, intestinal helminthic infection, allergy symptoms and selected risk factors in primary school children in Sululta, (n=526) 2017.

Variables		Total	Atopic	Non atopic	COR	CI (95%)	P-value	AOR	CI (95%)	p-value
Sex	Male	220	15(6.8)	205(93.2)	1					
	Female	306	12 (3.9)	294 (96.1)	0.588	0.256-1.217	0.142			
Age category	5-9	158	11(7.0)	147 (93.0)	1					
	10-14	368	16(4.3)	352(95.7)	0.607	0.275-1.340	0.217			
Residence	Urban	400	22(5.5)	378(94.5)	1.408	0.522-3.800	0.499			
	Rural	126	5(4.0)	121(96.0)	1					
Helminthes	Yes	89	6(6.7)	83(93.3)	1.432	0.561-3.656	0.453			
	No	437	21(4.8)	416(95.2)	1					
Ascaris lumbricoides	Yes	18	3 (16.7)	15(83.3)	4.003	1.093-14.883	**0.036**	4.307	1.143-16.222	0.031
	No	508	24(4.7)	484(95.3)	1					
Hookworm	Yes	12	1(8.3)	11(91.7)	1.706	0.212-13.723	0.615			
	No	514	26(5.1)	488(94.9)	1					
Trichuris trichuria	Yes	11	1(9.1)	10(90.9)	1.881	0.232-15.254	0.554			
	No	515	26(5.0)	489(95.0)	1					
Deworming	Yes	427	22(5.2)	405(94.8)	1.021	0.377-2.767	0.967			
	No	99	5 (5.1)	94(94.9)	1					
Any allergy	Yes	126	12(9.5)	114(90.5)	2.702	1.229-5.937	0.013	2.843	1.281-6.307	0.010
	No	400	15(3.8)	385(96.2)	1					
Wheeze	Yes	46	6 (13.0)	40(87.0)	3.279	1.251-8.589	**0.016**	2.430	0.874-6.757	**0.089**
	No	480	21(4.4)	459(95.6)	1					
Rhinitis	Yes	47	4 (8.5)	43(91.5)	1.844	0.610-5.579	0.278			
	No	479	23(4.8)	456(95.2)	1					
Eczema	Yes	28	2 (7.1)	26(92.9)	1.455	0.327-6.480	0.622			
	No	498	25(5.0)	473(95.0)	1					
Itch skin	Yes	99	10(10.1)	89(89.9)	2.710	1.201-6.116	**0.016**	2.292	0.964-5.453	**0.061**
	No	427	17(4.0)	410(96.0)	1					

7.6. Infection intensity of intestinal helminthes

The intensity of helminthic infection was ranged between 24 and 1800 eggs per gram (Mean=332) for *Ascaris lumbricoides*, 24 and 624 epg (Mean=168) for hookworm, 24 and 144(Mean=69.78) for *Trichuris trichuria* 24 and 720 (Mean=106.0) for *Hymenolepis nana*, 48 and 48 (Mean=48) for *Enterobius vermicularis*, 48 and 4800 (Mean=645.82) for *Taenia spp*, 48 and 48(Mean=48.0) for *Strongloid stercoralis*.

From 18 *Ascaris lumbricoides* positive children the egg count was performed for 15children and from 11*Trichuris trichuria* positive children the egg count was performed on 9 children because the remaining children were positive in the direct microscopy or in formol-ether concentration method but not in kato-katz method. Based on WHO grading system (1), all helminthes eggs counted using kato-katz were light infection as shown in (Table4). There was no significant association between infection intensity and allergen sensitization.

Table 4: Association between helminthic infection intensity and Atopy in selected primary schools in Sululta , 2017.

Helminthes	Intensity of infection (epg)	Atopy		COR	95% CI	P-value
		Yes (%)	No (%)			
Ascaris lumbricoides	Light(1-4999)	2 (13.3)	13(86.7)	2.991	0.640-13.979	0.164
	Negative	25(4.9)	486(95.1)	1		
	Mild (5000-49999)	0	0			
	Heavy(>50000)	0	0			
Hookworm	Light(1-1999)	1(9.1)	10(90.9)	1.881	0.232-15.254	0.554
	Negative	26(5.0)	489(95.0)	1		
	Mild(2000-3999)	0				
	Heavy (>4000)		0			
Trichuris trichuria	Light(1-999)	0	9(100)			
	Negative	27(5.2)	490(94.8)			
	Mild (1000-9999)	0	0			
	Heavy (>10000)	0	0			

7.7. Eosinophil count, in helminthic infection and atopy status

From 85 helminthic positive children 51.8 % (44/85) had increased eosinophil count and 6.6 % (28/424) of helminthic negative children had high eosinophil count. Majority of (60%) atopy positive children had a normal eosinophil count as shown in (Figure7).

Relatively, high eosinophil count was observed in *Trichuris trichuria* infection. *Ascaris lumbricoids* and *Trichuris trichuria* infections were significantly associated with an increased eosinophil count (AOR=6.003, 95%CI=2.169-16.611,P=0.001 and AOR=18.273,95% CI=4.646-71.869,P=0.000) respectively.

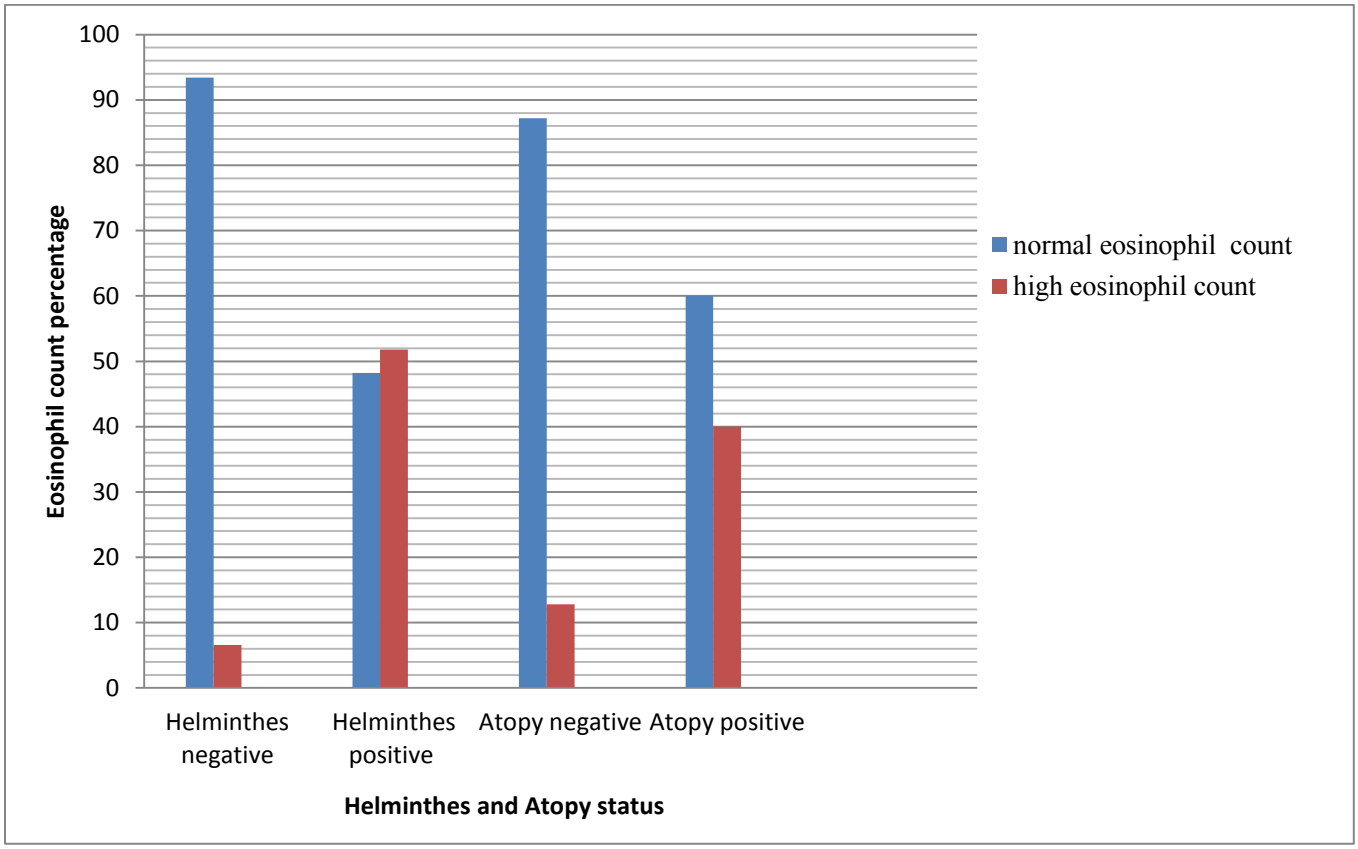

Figure 7: Eosinophil count against helminthic infection and atopy status

7.8. Association of intestinal helminthic infection and potential risk factors

From 220 males 18.2 % (n=40/220) and from 306 females 16.0% (n=49/306) were positive for at least one or more helminthes. There was no significant association between helminthic infection and gender of the study participants (OR=1.166, 95%CI=0.736-1.845, P=0.513).The main source of drinking water in the study participants was public tap water (common pipe water source), which accounted 51.1 %(n=269/526) and followed by pipe water (inside their house) 31.9% (n=168/526). High helminthic infection 25% (n=8/32) was observed among children whose families used open well water followed by those who used covered well 17.5% (n=7/40).There was no significant association between source of drinking water and helminthic infection (Table 5).

Regarding to toilet facilities, majority of the house hold used traditional type of toilet which was 75.5% (n=397/526) followed by open field defecation 13.3% (n=70/526). Children who were defecate in the open field had 18.6% (n=13/70) intestinal helminthic infection which was higher than those who used pit latrine 17.8% (n=8/45) and traditional type of toilet 16.7% (n=65/389) There was no significant association between helminthic infection, toilet facilities; hand wash practice and shoe wearing. From 427 children who received anthelminthic treatment 16.9% (n=72/427) were positive for helminthes whereas children who didn't take anthelminthic treatment were17.2% (n=17/99), however the difference was not statistically significant (OR=1.022, 95%CI=0.572-1.827, P=0.941). Children who didn't clean their finger nail were more likely to be infected with helminthes (AOR=1.875, 95%CI=1.166-3.014, P=0.009) (Table 5).

Table 5: Association of intestinal helminthic infection and potential risk factors in selected primary school children in Sululta, 2017.

Variables		Total	Helminthes Yes (%)	No (%)	COR	95% CI	P-value	AOR	95% CI	P-value
Sex	Male	220	40 (18.2)	180(81.8)	1.166	0.736-1.845	0.513			
	Female	306	49(16.0)	257 (84.0)	1					
Age category	5-9	158	20(12.7)	138(87.3)	1					
	10-14	268	69(18.8)	299(81.2)	1.592	0.931-2.724	0.090			
Grade level	Kg	59	7(11.9)	52(88.1)	1					
	1-4	302	58(19.2)	244(80.8)	1.766	0.763-4.088	0.184			
	5-8	165	24(14.5)	141(85.5)	1.264	0.514-3.110	0.609			
Deworming	Yes	427	72(16.9)	355(83.1)	1					
	No	99	17(17.2)	82(82.8)	1.022	0.572-1.827	0.941			
Water source	Pipe(inside	168	29(17.3)	139(82.7)	3.338	0.426-26.178	0.251			
	Public tap	269	44(16.4)	225(83.6)	3.129	0.404-24.207	0.275			
	Open well	32	8(25.0)	24(75.0)	3.394	0.384-29.985	0.272			
	Covered well	40	7(17.5)	33(82.5)	5.333	0.607-46.850	0.131			
	River	17	1(5.9)	16(94.1)	1					
Toilet facility	Flush	22	3(13.6)	19(86.4)	1					
	Pit latrine	45	8(17.8)	37(82.2)	1.369	0.325-5.766	0.668			
	Traditional	389	65(16.7)	324(83.3)	1.271	0.365-4.419	0.707			
	Bush /field	70	13(18.6)	57(81.4)	1.444	0.371-5.619	0.596			
Hand wash after toilet	Always	358	54(15.1)	304(84.9)	1				1	
	Sometimes	146	33(22.6)	113(77.4)	1.644	1.013-2.667	0.044	1.541	0.934-2.541	0.091
	Never	22	2(9.1)	20(90.9)	0.563	0.128-2.478	0.447			
Shoe wear	Closed	250	42(16.8)	208(83.2)	1					
	Open	276	47(17.0)	229(83.0)	0.984	0.623-1.553	0.944			
Finger nail	Not clean	159	37(23.3)	122(76.7)	1.837	1.148-2.941	0.011	1.875	1.166-3.014	0.009
	Clean	367	52(14.2)	315(85.8)	1			1		

8. Discussions

In our study the overall prevalence of intestinal parasites, questionnaire based self-reported allergic symptom and atopy using skin prick test (SPT) were 26.2%, 24% and 5.1% respectively. All helminthic infections were light infection and related to risk factors children who were not cleaned their figure were more likely to be infected intestinal helminthic infection. History of parental allergy was a risk factor for the development of child hood allergy.

In this study, the overall prevalence of intestinal helminthes was 16.9%, this was low compared to a study conducted in Babile town, Ethiopia 27.2% and Tilili town, north west Ethiopia 44.2% (39, 40). This difference might be due to mass deworming effect which was conducted in the last 3months before data collection in our case, good hygiene practice. This finding was higher than a study conducted by SahRB *et al* in Nepal and Babile town eastern Ethiopia by Tefera E *et al* which showed an overall prevalence of 13.0% and 13.8% (41, 42). This difference might be due to geographical location, socio-economic and hygiene conditions as well as deworming effect in the case of Babile.

In our finding, the prevalence of atopy, rhinitis, wheeze, eczema and asthma were 5.1%, 8.9%, 8.7%, 5.3%, 3% respectively. This finding was relatively low when compared to a study conducted by Wordemann MD *et al* in Cuban children which were 21% for asthma, 14% for rhinitis, 8% for eczema and 20% had atopy (20). This difference might be due to large sample size used in their study and geographical location. Except atopy, it was higher than a study conducted by Cooper P *et al* in Ecuador which reported that wheeze 2.1%, rhinitis 4.1%, eczema 3.7% and atopy 18.2% (21).

In this study, there was no statisticall significant association between helminthic infection and allergic symptoms ($P>0.05$). Possible explanation for lack of association between helminthic infection and allergic disease may be related to low prevalence and low intensity of helminthic infection in our finding. This finding was comparable to a study conducted in SriLanka which revealed reduced risk of allergy in helminthes infected children though their finding was not statistically significant(28). It was also in line with a study conducted by Cooper P *et al* in Ecuador and Magbojos CR *et al* in Philippines which showed that there was no association between geohelminthes and allergy symptoms (27,29).

The current finding disagreed with observations from prospective cohort and randomized trial study conducted in Ethiopian immigrants in Israel and in Gabonese school children which revealed significant inverse association of helminthic infection and allergy (23,25). The difference might be due to study design and geographical location.

To the contrary, cross sectional study results were reported in Ugandan fishing community and in rural Bangladeshi children that revealed certain helminthic infections were positively associated with allergy-related outcomes and positive association between helminthic infection and allergic disorder respectively (16,17). This difference might be due to large sample size, geographical location and low intensity of infection in our case.

There was no significant association between helminthic infection and skin prick test reactivity among this study group (P>0.05). This finding disagreed with a cross sectional study conducted in Indonesia, reported significant inverse association between helminthic infection and skin test negativity (24).The difference might be due to geographical location and study design.

In our study, we found individuals with *Ascaris lumbricoides* infection had significant positive association with skin prick test reactivity (P=0.031). Though not significant, hookworm and *Trichuris trichuia* infections were positively associated with skin prick test reactivity (OR= 1.706 and 1.881). This finding was in line with a cross sectional study conducted in rural China and Bangladesh, which suggested that *Ascaris lumbricoides* infection had positive association with atopy (15,17).

It was also comparable to a randomized controlled trial placebo study conducted in Vietnam which revealed, significant reduction in worm burden over a 12-month period in helminthic-infected children increases the risk of allergen skin sensitization but not of clinical allergic disease (18).

It was incomparable to a study conducted in Cuban children and two similar Ecuador studies, revealed that significant negative association of *Ascaris lumbricoides* infection with skin test reactivity and presence of geohelminth infection was protective against allergen skin test reactivity (20,21,22). Possible explanation for this difference might be due to large sample size and geographical location, as well as high prevalence and high intensity of helminthic infection in their study.

Skin test reactivity (atopy) had a positive significant association with any allergy symptoms (p=0.012). This result was consistent with a study conducted in Uganda in fishing communities and Ecuador which revealed that skin prick test reactivity was associated with an increased risk of all allergic symptoms (16, 27). It was disagreed a study conducted by Davey G *et al* in Ethiopia that reported weak association between allergen sensitization and wheeze (30).

The proportion of atopy among children who received anthelminthic treatment was 22(5.2%) which was similar to those who didn't received anthelminthic treatment 5(5.1%). In this study, there was no significant association between atopy and anthelminthic treatment (P>0.05). This result was consistent with a study conducted in Cuban school children and Ecuador which revealed, deworming of school children was not a risk factor for the development of allergic sensitization (26, 27).

On the contrary, evidence from randomized control trial in Vietnam children showed Significant reduction in worm burden over a 12-month period in helminth-infected children increases the risk of allergen skin sensitization but not of clinical allergic disease and in Gabonese children which showed that long term anthelminthic treatment had an effect on the increased skin test reactivity(18, 25).

Surprisingly, in our finding allergic symptoms were inversely associated with anthelminthic treatment (showing that deworming has a protective effect on the development of allergic disease). This was in disagreement with a study conducted in Cuban and Ecuador children which revealed, deworming of school children does not seem to be a risk factor for the development of allergic sensitization nor for atopic disease and deworming program for school children are unlikely to be accompanied by an increase in allergy respectively (26,27). Taken together, our data and others revealed that the effect of helmithes or their treatment on allergic symptoms and atopy varries from study to study as well as depends on the specific helminthes type and duration of treatmen

Intestinal helminthic infection in male students was 40(18.2%) which was slightly higher than female students 49(16.0%). However, there was no statistical significant difference (p>0.05) in prevalence of intestinal helminthes and gender. This slight difference could be due to the fact that male's exposure to different risk factors of intestinal helminthes infection during outdoor activities. This finding was in agreement with a study conducted in Jimma, south western Ethiopia, male students 43.1% and female students 40.5% (43).

To the contrary, this finding was disagreed with previous study conducted in Nepal which showed male 9.7% and female 16.5% (41). This difference might be due to geographical location.

Helminthic infection in the age group of 10-14 years old was 69 (18.8%) which was higher than the age group of 5-9 years old 20(12.7%),but there was no statistically significant association between age and helminthic infection ($p>0.05$). This difference could be due to the fact that as the child grows older the exposure to different risk factors for helminthic infection increase. This finding was in line with a study conducted in chencha town Ethiopia by AbbosieA *et al* which reporeted high prevalence of helminthic infection in high age groups (44).

In contrast, other study showed that the prevalence was found to be significantly high in children with lower age (45). This might be due to older age children are comparatively more knowledgeable and aware than the lower age children to be infected with intestinal parasite. Related to deworming, prevalence of helminthes among children who didn't receive anthelminthic treatment was 17(17.2%) which was slightly higher than those who were treated 72(16.9%). However, the difference was not statistically significant. This might indicate that deworming should be supported with health education to prevent reinfection. This finding was supported by Zerdo Z *et al* a study conducted in chencha town, Ethiopia, which showed that the prevalence of intestinal helminthes within three months of mass chemotherapy among school children was 36.8% which is (39.4%) before treatment, which was high rate of reinfection (46).

Childern who didn't cleaned their finger nail 37(23.3%) were positive for one or more helminthes which was higher than those who cleaned their finger nail 52 (14.2%),the difference was statistically significant ($p=0.009$). This difference might be due to lack of awareness and poor hygeine practice.This result was also supported by previous findings (40).

Residence, gender, age and animal ownership did not have significant association with any allergic symptoms of children ($P>0.05$). This finding agreed with a study conducted in Ethiopia which revealed, neither intestinal parasite infection nor other proposed risk factors for atopic dermatitis appear to be related to the presence of the condition in young children in Ethiopia (32).

Parental history of allergic symptoms were a risk factor for the development of child hood allergy ($P=0.001$), this was in agreement with a study conducted in Ethiopia by Amberbir A *et al* (47). In our finding, environmental allergens (house dust mite and cockroach) were significant risk factors for the

development of allergic symptoms (P=0.012). This was similar to previous study and a study conducted in rural area of Ecuador which revealed that any allergen or specific allergen was a significant risk factor for allergic outcomes (7, 21).

The use of charcoal and electric as a source of fuel had an increased risk of allergic disease; however, the association was not statistically significant. This finding was in agreement with a study conducted in Bolivia that cooking fuels wood or coal was associated with increased risk of allergic disease but not statistically significant (19). *Ascaris lumbricoides* and *Trichuris trichuria* infections were not associated for the risk of allergic symptoms but children who had hookworm infection were more likely to develop allergic symptoms (P=0.015). This finding was disagreed a study conducted in Jimma, Ethiopia which showed that *Ascaris lumbricoides* possibly hookworm infection were protective against wheeze in young Ethiopian children (31). This difference might be due to large sample size in their study and study design and in addition to intensity of infection differences.

9. Strength and limitation of the study

9.1. Strength of the study

- Atopy was measured using skin prick test
- Prevalence of intestinal helminthes was determined by three parasitological methods (Direct wet mount, formol ether concentration and kato-katz).

9.2. Limitation of the study

- The number of allergens used to determine atopy was only two; however, we used the most common ones to overcome this limitation.
- There was no base line data about helminthes and atopy/allergy before anthelminthic treatment to investigate the effect of anthelminthic treatment
- Total and specific serum IgE was not performed.

9. Conclusion and recommendation

9.1. Conclusion

The most predominant intestinal helminthes identified was *Hymenolopis nana* and the least identified helminth was *Strongloid stercoralis.* Intensity of infection was low in all intestinal helminthic infections and it was not associated with atopy but children with individual helminthic infections like *Ascaris lumbricoides* were more likely to be reactive for skin prick test. There was no significant relationship between helminthic infection and allergic symptoms. Significant association was observed between atopy and allergic symptoms. Anthelminthic treatment was not related to the increase or decrease of atopy but showed significant invers association with allergic symptoms.

9. 2.Recommendation

- Further large scale and longitudinal study is appropriate to investigate the relationship of helminthes and allergic disorder.
- It is better to include more allergens
- It is advisable to integrate Mass deworming with health education to prevent reinfection
- Other alternative drugs should be considered for *Hymenolepis nana*

10. References

1. World Health Organization. Eliminating soil-transmitted Helminthiases as a public health problem in children: progress report 2001–2010 and strategic plan 2011–2020. 2012.

2. World Health Organization. Helminth control in school-age children: a guide for managers of control programmes. 2nd ed: Geneva: World Health Organization; 2011.

3. Flohr C, Quinnell R, Britton J. Do helminth parasites protect against atopy and allergic disease? *Clin Exp Allergy*. 2009;39(1):20-32.

4. Sitcharungsi R, Sirivichayakul C. Allergic diseases and helminth infections. *pathog globhealth*. 2013;107(3):110-5.

5. Cooper P, Barreto M, Rodrigues L. Human allergy and geohelminth infections: a review of the literature and a proposed conceptual model to guide the investigation of possible causal associations. *Br Med Bull*. 2006;79(1):203-18.

6. Mao X, Sun D, Miyoshi A, Feng Z, Handzel Z, Hopkin J, *et al*. The link between helminthic infection and atopy. *Parasitology Today*. 2000;16(5):186-8.

7. Gold MS, Kemp AS. Atopic disease in childhood. *MJA*. 2005;182(6):298-304.

8. Baxi SN, Phipatanakul W. The role of allergen exposure and avoidance in asthma. *Adolesc Med State Art Rev*. 2010;21(1):57.

9. Sohn MH, Kim K-E. The cockroach and allergic diseases. *Allergy Asthma Immunol Res*. 2012;4(5):264-9.

10. Smits HH, Everts B, Hartgers FC, Yazdanbakhsh M. Chronic helminth infections protect against allergic diseases by active regulatory processes. *Curr Allergy Asthma Rep*. 2010;10(1):3-12.

11. Montresor A, Crompton D, Gyorkos T, Savioli L. Helminth control in school-age children. Geneva: World Health Organization. 2002:19-20.

12. Pawankar R, Canonica GW, Holgate ST, Lockey RF. Allergic diseases and asthma: a major global health concern. *Curr Opin Allergy Clin Immunol*. 2012;12(1):39-41.

13. Endara P, Vaca M, Chico M, Erazo S, Oviedo G, Quinzo I, *et al*. Long-term periodic anthelmintic treatments are associated with increased allergen skin reactivity. *Clin Exp Allergy*. 2010;40(11):1669-77.

14. Mengitsu B, Shafi O, Kebede B, Kebede F, Worku DT, Herero M, *et al*. Ethiopia and its steps to mobilize resources to achieve 2020 elimination and control goals for neglected tropical diseases: . *International health.* 2016;8(suppl_1):34-52.

15. Palmer LJ, Celedón JC, Weiss ST, Wang B, Fang Z, Xu X. Ascaris lumbricoides infection is associated with increased risk of childhood asthma and atopy in rural China. *Am J Respir Crit Care Med* 2002;165(11):1489-93.

16. Webb E, Nampijja M, Kaweesa J, Kizindo R, Namutebi M, Nakazibwe E, *et al*. Helminths are positively associated with atopy and wheeze in Ugandan fishing communities: results from a cross-sectional survey. *Allergy*. 2016;71(8):1156-69.

17. Hawlader MD, Ma E, Noguchi E, Itoh M, Arifeen SE, Persson LÅ, *et al*. Ascaris lumbricoids infection as a risk factor for asthma and atopy in rural Bangladeshi children. Tropical medicine and health. 2014;42(2):77-85.

18. Flohr C, Tuyen L, Quinnell R, Lewis S, Minh T, Campbell J, *et al*. Reduced helminth burden increases allergen skin sensitization but not clinical allergy: a randomized, double-blind, placebo-controlled trial in Vietnam. *Clin Exp Allergy*. 2010;40(1):131-42.

19. Solis-Soto MT, Patiño A, Nowak D, Radon K. Association between environmental factors and current asthma, rhinoconjunctivitis and eczema symptoms in school-aged children from Oropeza Province–Bolivia: a cross-sectional study. *Environmental Health*. 2013;12(1):95.

20. Wördemann M, Diaz RJ, Heredia LM, Madurga AMC, Espinosa AR, Prado RC, *et al*. Association of atopy, asthma, allergic rhinoconjunctivitis, atopic dermatitis and intestinal helminth infections in Cuban children. *Trop Med Int Health*. 2008;13(2):180-6.

21. Cooper PJ, Chico ME, Bland M, Griffin GE, Nutman TB. Allergic symptoms, atopy, and geohelminth infections in a rural area of Ecuador. *Am J Respir Crit Care Med*. 2003;168(3):313-7.

22. Cooper PJ, Chico ME, Rodrigues LC, Ordonez M, Strachan D, Griffin GE, *et al*. Reduced risk of atopy among school-age children infected with geohelminth parasites in a rural area of the tropics. *J Allergy Clin Immunol* 2003;111(5):995-1000.

23. Stein M, Greenberg Z, Boaz M, Handzel ZT, Meshesha MK, Bentwich Z. The role of helminth infection and environment in the development of allergy: A prospective study of newly-arrived Ethiopian immigrants in Israel. *PLoS Negl Trop Dis*. 2016;10(1):e0004208.

24. Lubis SR, Irsa L, Evalina R. Soil... transmitted helminth infection and skin prick test reactivity in children. *Paediatr Indones*. 2014;54(1):52-6.

25. van den Biggelaar AH, Rodrigues LC, van Ree R, van der Zee JS, Hoeksma-Kruize YC, Souverijn JH, *et al*. Long-term treatment of intestinal helminths increases mite skin-test reactivity in Gabonese schoolchildren. *J Infect Dis*. 2004;189(5):892-900.

26. Werff S, Twisk J, Wördemann M, Ponce MC, Díaz RJ, Núñez F, *et al*. Deworming is not a risk factor for the development of atopic diseases: a longitudinal study in Cuban school children. *Clin Exp Allergy*. 2013;43(6):665-71.

27. Cooper PJ, Chico ME, Vaca MG, Moncayo A-L, Bland JM, Mafla E, *et al*. Effect of albendazole treatments on the prevalence of atopy in children living in communities endemic for geohelminth parasites: a cluster-randomised trial. The *Lancet*. 2006;367(9522):1598-603.

28. Amarasekera M, Gunawardena NK, de Silva NR, Douglass JA, O'Hehir RE, Weerasinghe A. Impact of helminth infection on childhood allergic diseases in an area in transition from high to low infection burden. *Asia Pac Allergy* 2012;2(2):122-8.

29. Magbojos CR, Chua SO, Alegria CR, Macalalad FC, Malayba ACR, Mandigma AAD, *et al*. Determination of geohelminthiasis and its association with allergic sensitization among selected children in Batangas, Philippines. *Glob J Health Sci*. 2016;9(2):33.

30. Davey G, Venn A, Belete H, Berhane Y, Britton J. Wheeze, allergic sensitization and geohelminth infection in Butajira, Ethiopia. *Clin Exp Allergy*. 2005;35(3):301-7.

31. Dagoye D, Bekele Z, Woldemichael K, Nida H, Yimam M, Hall A, *et al*. Wheezing, allergy, and parasite infection in children in urban and rural Ethiopia. *Am J Respir Crit Care Med.* 2003;167(10):1369-73.

32. Haileamlak A, Dagoye D, Williams H, Venn AJ, Hubbard R, Britton J, *et al*. Early life risk factors for atopic dermatitis in Ethiopian children. *J Allergy Clin Immunol*. 2005;115(2):370-6.

33. Hamid F, Wiria AE, Wammes LJ, Kaisar MM, Lell B, Ariawan I, *et al*. A longitudinal study of allergy and intestinal helminth infections in semi urban and rural areas of Flores, Indonesia (ImmunoSPIN Study). *BMC infectious diseases*. 2011;11(1):83.

34. central statistics agency. Population and Housing Census of Ethiopia. 2007.

35. Cheesbrough M. District Laboratory Practice in Tropical Countries 2nd ed. New York: Cambridge University Press; 2006.

36. Heinzerling L, Mari A, Bergmann K-C, Bresciani M, Burbach G, Darsow U, *et al*. The skin prick test–European standards. *J Allergy Clin Immunol.* 2013;3(1):3.

37. Asher M, Keil U, Anderson H, Beasley R, Crane J, Martinez F, *et al*. International Study of Asthma and Allergies in Childhood (ISAAC): rationale and methods. *Eur Respir J*. 1995;8(3):483-91.
38. Central Statistical Agency .Ethiopia mini demography health survey .Addis Ababa, Ethiopia 2014.
39. Tadesse G. The prevalence of intestinal helminthic infections and associated risk factors among school children in Babile town, eastern Ethiopia. *EthiopJHealth Dev*. 2005;19(2):140-7.
40. Abera A, Nibret E. Prevalence of gastrointestinal helminthic infections and associated risk factors among schoolchildren in Tilili town, northwest Ethiopia. *Asian Pac J Trop Med*. 2014;7(7):525-30.
41. Sah RB, Bhattarai S, Yadav S, Baral R, Jha N, Pokharel PK. A study of prevalence of intestinal parasites and associated risk factors among the school children of Itahari, Eastern Region of Nepal. *Trop Parasitol*. 2013;3(2):140.
42. Tefera E, Mohammed J, Mitiku H. Intestinal helminthic infections among elementary students of Babile town, eastern Ethiopia. *Pan Afr Med J*. 2015;20(1).
43. Emana D, Jemal K, Bajiro M, Mekonen Z. Prevalence and Intensity of Soil-Transmitted Helminths Among School-Aged Children in Sigmo Primary School, Jimma Zone, South-Western Ethiopia. *Clinic Med Res.* 2015;4(4):98-103.
44. Abossie A, Seid M. Assessment of the prevalence of intestinal parasitosis and associated risk factors among primary school children in Chencha town, Southern Ethiopia. *BMC Public Health*. 2014;14(1):166.
45. Khanal L, Choudhury D, Rai S, Sapkota J, Barakoti A, Amatya R, *et al*. Prevalence of intestinal worm infestations among school children in Kathmandu, Nepal. *Nepal Med Coll J*. 2011;13(4):272-4.
46. Zerdo Z, Yohanes T, Tariku B. Soil-transmitted helminth reinfection and associated risk factors among school-age children in Chencha District, southern Ethiopia: a cross-sectional study. *J Parasitol Res*. 2016;2016.
47. Amberbir A, Medhin G, Erku W, Alem A, Simms R, Robinson K, *et al*. Effects of Helicobacter pylori, geohelminth infection and selected commensal bacteria on the risk of allergic disease and sensitization in 3-year-old Ethiopian children. *Clin Exp Allergy*. 2011;41(10):1422-30.

Annex

Annex-I. Information sheet for study participants (English version)

Principal Investigator: Dessie Abera,: Addis Ababa University College of Health Sciences

Purpose: the purpose of this study is to investigate the association between helminthic infection, atopy and allergic disorder in a setting of mass deworming among selected governmental primary school children in Sululta, Oromia Reginal State.

Procedures to be carried on: after obtaining consent from you and/ your parent / guardian, An interviewer-administrated questionnaire will be used to elicit and gather relevant information and you will also be requested to give stool and blood samples as well as skin prick.

Risks associated with the study: There is no risk and serious invasive procedure at the beginning as well as at the end of the study except minor pain associated with skin pricking. It will take about 30minutes to participate in this study.

Benefits of the study: There will be no financial benefit to you. But the result of the study will be used for your clinical care as well as plays a role in the infectious and inflammatory disease managing and control program. It also helps to consider allergic disorders. There will be no compensation for participating in this study; however, you will be linked to the nearby health facility by providing your laboratory results for free.

Confidentiality of your information: The results of the laboratory findings will be kept confidential and could only be accessed by the researcher and the responsible person

Termination of the study: your decision will be respected if you later on change your mind.

Based on the above information I agree to participate in the research

Signature:_____Date:____ Name of Data collector _____Signature _____

If you have any question you can ask the principal investigator

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Annex- II. Information sheet for study participants (Amharic version)

የተሳታፊዎች መረጃ ቅጽ

ጥናቱን የሚያጠናው ፤ ደሴ አበራ

በአዲስ አበባ ዩኒቨርሲቲ ጤና ሳይንስ ኮሌጅ የህክምና ላቦራቶሪ ሳይንስ ዲፓርትመንት

የጥናቱአላማ

የጥናቱ አላማ ከተማሪዎች የሰገራ ናሙና በመውሰድየአንጀት ጥገኛ ትላትል ምርመራ ማካሄድ እንዲሁም የትላትሉን መጠንና አይነት መለየት ከ አለርጂክ በሽታ ጋር ያለውን ግኑኙነት ለማወቅ እና ያላቸውን ግንኙነት ማነፃፀር አሰፈላጊ ሆኖ በመገኘቱ ተጨማሪ ሀሣብ ለመስጠት ነው፡: ጥናቱም ኦሮሚያ ክልላዊ መንግስት በሱሉለታ ወረዳ መጀመሪያ ደረጃ ት/ቤት ይካሄዳል.

በጥናቱ ወቅት ከእርስዎ የሚጠበቀው በጥናቱ ለመሳተፍ ፈቃደኛ ከሆኑ የሰገራ ናሙና መስጠትነው፡፡

ለጥናቱ ተሳታፊዎችያለው ልዩ ጥቅም

በጥናቱ ለሚሳተፉ ፍቃደኛ ተሳታፊዎች ምንም አይነት የገንዘብ ክፍያ የለውም ነገር ግን ከጥናቱ የሚገኘው ውጤት ለርስዎ ህክምና ተጨማሪ መረጃ ለማግኘትና ተላላፊ የሆኑ በሽታዎች ለመቆጣጠር ይጠቅማል እንዲሁም ውጤቱ ያለ ክፍያ ይሰጠወት እና በአካባቢው ባለ ጤና ጣቢያ ህክምና እንዲያገኙ ይደረጋል::

በጥናቱ ተሳታፊዎች ላይያ ለውጉዳት

ቆዳ ላይ በሚደረግ መርመራ ከሚሰማ ትንሺ ህመም በስተቀር በጥናቱ መጀመሪያም ይሁን መጨረሻ በዚህ ጥናት ላይ በመሳተፍዎ ሊደርስብዎ የሚችል አንድም ጉዳት አይኖርም፡፡ በጥናቱ ለመሳተፍ 30 ደቂቃ ሊወስድ ይችላል ፡፡

የመረጃ ሚስጥራዊ አጠባበቅ

የሚሰጡት መረጃ በጥናቱ ወቅትም ሆነ ከዚያ በኋላ ባሉት ጊዜያት ሙሉ በሙሉ ሚስጥራዊነቱ የሚጠበቅና መረጃውም የሚያዘዉ በስም ሳይሆን በመለያ ቁጥር ይሆናል፡፡ በጥናቱ ላይ ያለ መሳተፍ መብት አለዎት፡፡

ይህ መረጃ በጥንቃቄ የሚያዝ ይሆናል፡፡ በመጨረሻም የጥናቱ ውጤት ለሚመለከተዉ አካል ለጥናቱ አላማና ለህክምና ባለሙያዎች ብቻ የሚገለፅ ይሆናል፡፡ ያስታውሱ፤ ስለዚህ ጥናት ማንኛውም ጥያቄ ካለዎት በማንኛውም ጊዜ ከዚህ በታች በተጠቀሱት አድራሻዎች መጠየቅ ይችላሉ፡፡ እኔም የጥናቱ ተሳታፊ ይህንን በመገንዘብ ጥናቱ ላይ ለመሳተፍ ተስማምቼ ያለሁ፡፡

ፊርማ ----------

መረጃውን የሰበሰበው ግለሰብ ስም----------

ፊርማ ----------

የዋናተመራማሪው አድራሻ፤ ደሴ አበራ ኢ-ሜይል፣dessabera@gmail.com ስልክ ፣ +251091199353

የሕክምና ላቦራቶሪ ቴክኖሎጂ ዲፓርትመንት፣ የጤናሳይንስ ኮሌጅ፣ አዲስ አበባ ዩኒቨርሲቲ- አዲስአበባ፣ ኢትዮጵያ

Annex- III. Information sheet for study participants (Afaan Oromo version)

Lakk. Addaa maatii_____

Mucaan koo qorannoo haala faca'insa dhukkuba raamo fi alarjii ijoolle umrii wagga 5-14 magalaa Sulultaa mana barumsa motummaa filataman taasifamu irrattii akka hirmaatu fedhii koo gafatameen jira.Qorannoon kun miidha fayyaa daa'ima koo irratti geesissu akka hin jirree fi bu'aan qorannoo fayyaa ijoolle foyyessuu keessattii ga'e gudda akka qabuu naaf ibsameera.Akkasumas bu'aan qorannoo icittiin isaa akka egamuu naaf ibsameera .Haala kan hubachuun ,daa'ima koo qorannoo kana irratti akka hirmaatu fedhii koo akka ta'ee fi aanis odeeffanno barbachisuu kennuf qophii akkan ta'ee fi kanaafis mallatoo kiyyaan mirkaneessa.

Mallattoo Maatii _____ Guyya_____

Mallattoo ragaa _____ Guyya_____

Qorannoo kan ilaalchisee yoo gaafi ykn yaada qabaatan

Maqaa qorataa: Dasee Abaraa

E-mail:- dessabera@gmail.com.

Lakkofsa bilbilaa: +251091199353

Annex –IV. Assent form for participants (Amharic Version)

የተሳታፊዎች ስምምነት ማረጋገጫ ቅጽ

የሚስጥር ቁጥር ----------

የተሳታፊዉ ስም ----------

እኔ ስሜ ከላይ የተጠቀሰው ተሳታፊ የደም ናሙና እና የሰገራ ናሙና በመውሰድ የአንጀት ጥገኛ ትላትል ምርመራ መማካሄድ እንዲሁም የትላትሉን መጠንና አይነት መለየት ከ አለርጂክ በሽታ ጋር ያለውን ግኑኙነት ለማወቅ እና ያላቸውን ግንኙነት ማነፃፀር በልጅነት የዕድሜ ክልል በሚገኙ ህጻናት ስለሚደረገው ጥናት በቂ ገለጻ ተደርጎልኛል፡፡ ለጥናቱም ከእኔ ክንድ ላይ የሜደረግ የአለርጂ ምርመራ ፣የደም እና 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 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 ----------

የምስክር ሙሉ ስም ፊርማ

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

(የስምምነት ቅጹን ማንበብ ለማይችሉ ተሳታፊዎች)

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

ጥናቱን የሚያካሂደዉ ሰዉ ማረጋገጫ

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

ደሴ አበራ ኢ-ሜይል፣dessabera@gmail.com ስልክ ፣ +251091199353

የሕክምና ላቦራቶሪ ቴክኖሎጂ ዲፓርትመንት፣ የጤናሳይንስ ኮሌጅ፣ አዲስ አበባ ዩኒቨርሲቲ- አዲስአበባ፣ ኢትዮጵያ

Annex-V. Assent form for participants (Afaan Oromo version)

Guca walii galtee hirmaattota qorannoo

Maqaa hirmataa qorannoo _____

Lakkoofsa iccitii_____

Ani maqaan koo kan armaan olitti heerame dhukkuboota raammo fi alarjii ijoolle umri wagga kudha shanii gadii mana barnoota keenyaa irratti geessisu keessatii taasifamu irratii akkan hirmaadhu gaafatamen jira. Qorannoon kun bobba'a fi dhiiga irratti kan taasifamu akka ta'e naaf ibsameera. Dhukkubbin xiqqoo yeroo dhiiga fudhatamu kana hafe miidha ana irra gahuu akka hin jirre hubadheera .Kanafuu, qorannoo kana irratii kanan hirmaadhu fedhii kotin fi barbaachisuma qoranno sirritti ergan beeke booda akka ta'e hubadhera. Dhuma qorannoo kana irrattis bu'aan isaa akka naaf ibsamuu fi iccitiin isaan akka egamuu akkasumas dhukkubnii yoo narratti argamee yaala barbaachisu akka argadhu haalli akka mija'us naaf ibsameera. kanaafu qorannoo kana irratti hiramachuuf fedhii koo akka ta'e ibsaa, kanaafis mallattoo kiyyan mirkaneessa.

Mallattoo hiramaata _____.Guyya_____

Mallatoo ragaa _____.Guyya_____

Qorannoo kan ilaalchisee yoo gaafii ykn yaada qabaatan

Maqaa qoorata: Dasee Abaraa

E-mail:- dessabera@gmail.com.

Lakkofsa bilbilaa: +251091199353

Annex- VI. Questionnaire English version

I thank gratefully for your agreement to participate in this study. Now Im going to undertake interview with you and the interview is about general socio demographic characteristics and other health related questions. The information you give me is very essential for this study. Therefore, I politely ask you to give me the right response.

Section 1.SCHOOL, CHILD, FAMILY AND INTERVIEWER IDENTIFICATION

CODE:_____ **SCHOOL NAME**_____**GRADE**_____**SECTION:** _____

ADDRESS: **1)URBAN** **2) RURAL**

PHONE NUMBER_____**DATE:** _____

INTERVIEWER'S NAME & SIGNATURE_____

1	Sex	a) Female (1) b) Male
2	When is the child's birthday? (dd/mm/yy)	/ / Age

Section 2. Maternal and Paternal Characteristics

1.3 Maternal occupation 1) Civil servants 3) Private organization 5) Daily laborer
2) House wife 4) Farmer 6) Merchant

1.4 Maternal Educational status
0) Illiterate 1) read and write only 2) primary 3) high school 5) higher education

1.5 Paternal occupation 1) Civil servant 3) Merchant 5) Farmer
2) Private organization 4) Daily laborer 6) No work

1.6. Paternal Educational Status
0) Illiterate 1) read and write 2) primary 3) high school 5) higher education

Section 3.Allergy Characteristics of a child

G01	Has your child ever had wheezing or whistling in their chest in the last 1 year? if you have answered "no" please skip to question 3	Yes	1		WHZL6A
		No	2		
G02	How many times in the last year has your child had an attack of wheezing?		0		WHZFRQ6A
			1		
			2		
			3		
			4		
G03	Has your child ever had Asthma?	Yes	1		ASTL6A
		No	2		

G04	Has this been confirmed by a doctor?	Yes	1		ASTHDR6A
		No	2		
		No	2		
G05	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	→G8A	RASH6A
		No	2	→ G9	
G05A	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	

G06	Has your child ever had hay fever or persistent sneezing attacks?	Yes	1		HAYFL6A
		No	2		
G07	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		
G08	Does your child has eczema in the last 1 year	Yes	1		
		No	2		

G09	Has your child taken any deworming medication in the last 3 months?	Yes	1		DEWOR6A

G10	Is there anyone who smokes cigarettes in your home?	Yes	1	→ G13A	HCIGR6A
		No	2	→ G14	
G11A	If yes, please write the total number of people who smoke cigarettes in the home where the child is living?	_____			HCIGRN6A

Section 4. Allergy Maternal/Paternal Characteristics

G12	Have you had wheezing or whistling in your chest in the last 1 year?	Yes	1	→ G18	MOWHZ6A
		No	2	→ G19	
G13	How many times in the last year have you had an attack of wheezing?		0		MOWHFR6A
			1		
			2		
			3		
			4		
G14	Have you had asthma in the last 1 year?	Yes	1	→ G20	MOAS6A
		No	2	→ G21	
G15	Was this confirmed by a doctor?	Yes	1		MOASSDR6A
		No	2		
G16	Has the baby's father/mother had wheezing or whistling in the chest in the last 1year?	Yes	1		FAWHEZ6A
		No	2		
G17	Has the baby's father/mother had asthma in the last 1 year?	Yes	1	→ G23	FAAS6A
		No	2	→ G24	
G18	Was this confirmed by a doctor?	Yes	1		FAASDR6A
		No	2		
G19	In the last 1 year have you had hay fever?	Yes	1		MOHAY6A
		No	2		
G20	In the last 1 year has the child's father/mother had hay fever?	Yes	1		FAHAY6A
		No	2		
G21	Have you had eczema in the last 1 year?	Yes	1		MOEZC6A
		No	2		
G22	Has the baby's father/mother had eczema in the last 1 year?	Yes	1		FAEZC6A
		No	2		

G23 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. Nafta/Lamba	1	2	3
4. Gas (Butane)	1	2	3
5. Electricity	1	2	3

GFUEL6A

G24 Which of the following animals do you or your household keep? (Multiple answers possible)

Animal	Not available	Inside the house	Outside
1. Cat	1	2	3
2. Dog	1	2	3
3. Hen	1	2	3
4. Cow/ox	1	2	3
5. Sheep/goat	1	2	3
6.Horse, mule/donkey	1	2	3
7. Pig	1	2	3

GANIM6A

G25	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	
G26	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	
G27	Hand wash practice after toilet.	1) Always 2) Sometimes 3) Never		
G28	How do you wash your hands after going to toilet?	1) Water only 2) Soap and Water		
G28A	If with soap and wáter, frequency of soap use	1) Always 2) Sometimes 3) Never		
G39	Hand washing practice before meal	1) Always 2) Sometimes 3) Never		
G40A	Hand washing	1)Water only 2) with Soap		
G40B	If with soap, frequency	1) Always 2) Sometimes 3) Never		
G41	Do you eat raw meat?	1) Yes 2) No		

G42	Shoe wearing	1) Always 2) Sometimes 3) Never
G42A	Type of shoe (if answer to Q44 is 1 or 2)	1) Closed 2) Sandal
G43	Finger nail cleanness	1) Cleaned 2) Not cleaned
G44	Are you currently taking any anthelminthic drugs?	1) Yes 2) No
G45	Have you taken antihistamine drugs for the last five days (Bromphemiramine,Cetirizine,chlorphimiramine)	1) Yes 2) No

Parent's Signature:_____

Thank you very much for your participation in this study!

Annex-VII. Questionnaire Amharic version

አሁን ስልጅዎ ቀጣይ ስምናደርገው ምርመራ ደረዳን ዘንድ የሚከተሉትን ጥያቄወች ስስምንጠይቀወት ባክዎን ባስፉት ቲ□ያቶች ውስጥ ያጋጠሙወትን ከዚህ ጋር ተያያዝነት ያሳችውን ችግሮች በማስታወስ ትክክለኛ የሆነ ምሳሽ □ንዲሰጡን በትህትና ዕንጠይቀወተስን።

1. ስለልጁ እና ቤተሰቡ ጠቅላላ መረጃ

1.1. የት/ቤት ስም፡----------

1.2. አድራሻ፡ 1)ገጠር 2) ከተማ

1.3. ጾታ ፡ ወ □ ሴ □

1.4.የልጁ እድሜ ----------

1.5. የክፍል ደረጃ----------

2.የቤተሰብ ሁኔታ

2.1. የእናት ስራ ሁኔታ 1) የመንግሰት ሰራተኛ

2) የቤት እመቤት

3) የግል ተቀጣሪ

4) ገበሬ

5) የቀን ሰራተኛ

6) ነጋዴ

2.2. የእናት የት/ት ደረጃ

0) ያልተማረች 1) መጻፍና ማንበብ ብቻ 2) የመጀመሪያ ደረጃ ት/ት 3)ሁለተኛ ደረጃ ት/ት

5) ከፍተኛ ት/ት

2.3.የአባት የስራ ሁኔታ 1) የመንግሰት ሰራተኛ

2) ነጋዴ

3) የግል ተቀጣሪ

4) የቀን ሰራተኛ

5) ገበሬ

6) ስራ የለውም

2.4. የአባት የት/ት ደረጃ

0) ያልተማረ 1) መጻፍና ማንበብ ብቻ 2) የመጀመሪያ ደረጃ ት/ት 3)ሁለተኛ ደረጃ ት/ት

5) ከፍተኛ ት/ት

1.1 ህፃኑን/ኗን የተመለከተ

G01	ባለፉት 12 ወራት ዉስጥ በሕፃኑ/ኗ ደረት ውስጥ ሲጥ ሲጥ የሚል ወይም የፉጨት ድምፅ ተሰምቶ ጸዉቃል/□ዉቃለች?	አዎን	1	→ G04 → G05	WHZ6A
		□ለም	0		
G02	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		
		□ለም	0		
G03	ባለፉት 12 ወራት ዉስጥ ህፃኑ/ኗ አስም ኖሮት ጸዉቃል/□ዉቃለች?	አዎን	1	→G08	AST6A
		□ለም	0		
G04	ህፃኑ/ኗ አስም እንዳለበት/ባት በሐኪም ተረፃዕ□ል?	አዎን	1		ASTHDR6A
		□ለም	0		
G05	ባለፉት 12 ወራት ዉስጥ ልጁ(ልጅቷ) በአጥንት መ□ቷኝጸ ቦታዎቹ(□) (በክርን መታጠፍያ፤ከጉልበቱ ኋላ ባለ□ መታጠፍያ፤በቁርጭምጭሚት ፊት ለፊት፤ በአንገት □ሪጸ፤ እና በአይን አካባቢ) የሚያሳክክ ሽፍታ ወጥቶበት (ወጥቶባት) ነበር?	አዎን	1	→G11A	RASH36
		□ለም	0		
G05A	መልሱ አዎን ከሆነ፡ ሽአ ታዉ የነበረዉ በየትኛዎቹ ቦታዎች ላይ ነዉ?	በክርን መታ-ቷኝጸ	1		RASH6AA
			0		
		ከጉልበትዎ ኋላ	1		RASH6AB
			0		
		በቁርጭምጭሚት ፊትለፊት	1		RASH6AC
			0		
		ከመቀመጫ በታች	1		RASH6AD
			0		
		በአንገትዎ ዙሪያ	1		RASH6AE
			0		
		በአይንና በጆሮዎች ዙሪያ	1		RASH6AF
			0		
		□ለም	0		
G06	ባለፉት 12 ወራት ዉስጥ ልጅዎ ንፍጥ የሚያበዛ ጉንፋን፤የማያቋርጥ ማስነጠስ፤አፍንጫ ወይም ዐይንን የሚያቃጥል ሕመም ነበረበት(ባት)? (□ኛዚህ ምልክቶች የ□ዩት ህፃኑ በጉንፋን ሳይያዝ መሆን አለበት)።	አዎን	1		HAYF6A
		□ለም	0		
G07	ባለፉት 12 ወራት ውስጥ ልጅዎ ቆዳ ላይ ለምጥ ነበረው	አወን			
		የለም			

G08	ህፃኑ/ኗ ባለፉት ሶስት ወራት ለሆድ ትላትል መከላከያ መድሃኒት ወስዶአል?	አዎን	1		DEWOR6A
		☐አይም	0		
G08	ህፃኑ/ኗ በሚኖርበት/በምትኖርበት ቤት ውስጥ ሲጋራ/ትምባሆ የሚያጨስ ሰው አለ?	አዎን	1	→G29A	HCIGR6A
		☐አይም	0	→G30	
G08A	መልሱ አዎ ከሆነ የሚያጨስ ሰው ብዛት	[ ]			HCIGRN6A

1.2 የህፃኑ/ኗ ☐ክናትን/አባትን ☐ተመለከተ

G09	ባለፉት 12 ወራት በደራትዎ ዉስጥ ሲጥ ሲጥ የሚል ወይም የፉጨት ድምፅ ነበረብዎት?	አዎ	1	→G35	MWHZ6A
		☐አይም	0	→G36	
G10	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		
G11	ባለፉት 12 ወራት አስም ነበረብዎት?	አዎ	1	→G37	MOAS6A
		☐አይም	0	→G38	
G12	☐ርስዎ አስም ☐ንዳለብዎት በሐኪም ተረፉ☐ል?	አዎ	1		MASDR6A
		☐አይም	0		
G13	ባለፉት 12 ወራት ☐ልፃ (ልፀ ታ) አባት በ☐ረ☐ ቸው ዉስጥ ሲጥ ሲጥ የሚል ወይም የፉጨት ድምፅ ነበረባቸው?	አዎ	1		FWHZ6A
		☐አይም	0		
G14	ባለፉት 12 ወራት የልጁ(ልጅታ) አባት አስም ነበረባቸዉ?	አዎ	1	→G40	FAAS6A
		☐አይም	0	→G41	
G15	☐ልፃ (ልፀ ታ) አባት አስም ☐ንዳለባቸው በሐኪም ተረፉ☐ል?	አዎ	1		FASDR6A
		☐አይም	0		
G16	ባለፉት 12 ወራት ዉስጥ ንፍጥ የበዛበት ጉንፋን፤ የማያቋርጥ ማስነጠስ፤ አፍንጫ ወይም ዓይን ማቃጠል ነበረብዎት?	አዎ	1		MOHAY6A
		☐አይም	0		
G17	ባለፉት 12 ወራት ዉስጥ የልጁ(ልጅታ) አባት፤ ንፍጥ የበዛበት ጉንፋን፤ የማያቋርጥ ማስነጠስ፤ አፍንጫ ወይም ዓይን ማቃጠል ነበረባቸዉ?	አዎ	1		FAHAY6A
		☐አይም	0		
G18	ባለፉት 12 ወራት የሚያሳክክና ፤በተለጁም የአጥንት መተታኝጸ አካባቢዎች ጸሉትን የሰውነት ☐ፍሎችን/ለምሳሌ የክንድ፤ ከጉልበት ቆዳዎችን/ ☐ሚጸታቃ ☐ቆ☐ ችግር ነበር	አዎ	1		MOEZCA6A
		☐አይም	0		
G19	ባለፉት 12 ወራት የልጁ(ልጅታ) አባት ፤በተለጁም	አዎ	1		FAEZC6A

	የአጥንት መ□ ታኝጸ አካባቢዎች ጸሉትን □ስዉነት □አሎችን/ለምሳሌ የክንድ፤ ከጉልበት በሰተኋላ □□ኝ ቆዳዎችን/ □ሚጸታቃ □ቆ□ ሽአ□ ነበረባቸዉ?	□ስም	0	

G20	የህፃኑ/ኗን ቤተሰበ ከሚከተሉት የማገዶ ዓይነቶች ምግብ ለማብሰል በየስንት ቲ□ው፡ □ንድሚታቀሙ ቢቶቲናል?				
		ማገዶ/ነዳጅ	አልፎቀምም	አንዳንድ ቲ□	ምየቀኑ
		1. ከሠል	1	2	3
		2. □ንጨት	1	2	3
		3. ቅታል	1	2	3
		4. ኩበት	1	2	3
		5. ናፍጣ/ላንባ	1	2	3
		6. ቡተ□ፉ□	1	2	3
					GFUEL6AA GFUEL6AB GFUEL6AC GFUEL6ADGFUEL6AE GFUEL6AF
G21	የህፃኑ/ኗን ብተሰብ ከሚከተሉት □ንሳሳትና ከብቶች ዓይነቶች የትኞቹ አሏችው?				
		□ንስሳ	በቤት ውስጥ ጁታበቃሉ/ጸትራሉ	ከቤት ውጪ ጁታበቃሉ	
		1. ድመት	1	2	
		2. ዉሻ	1	2	
		3. □ሮ	1	2	GANIM6AA
		4. ላም/በሬ	1	2	GANIM6AB
		5. በፅ	1	2	GANIM6AC
		6. □ረስ	1	2	GANIM6AD
		7. አ□ል	1	2	GANIM6AE
		8. በቅሎ/አህያ	1	2	GANIM6AF
G22	የህፃኑ/ኗን ቤተሰብ የመጠጥ ውሃ	በግቢው ውስጥ ከሚገኝ		1	WAT6A

	በዋንኝነት የሚያገኙት ከየት ነው?	ቧንቧ		
		ከግቢ ውጪ ከሚገኝ ቧንቧ	2	
		ከጉድጓድ ወይም ምንጭ	3	
		ከተጠበቀ ጉድጓድ ወይም ምንጭ	4	
		ከወንዝ፣ ከኩሬ፣ከጉትጃት	5	
		ከዝናብ ውሃ	6	
G23	የህፃኑ/ኗን ቤተሰቡ የሚገለገልበት መፀዳጀ ቤት ምን አይነት ነው?	በውሃ የሚሰራ ሽንት ቤት	1	SANIT6A
		ውሀ አልባ መ□□□	2	
		የተለመደ ዓይነት የሽንት ቤት ቱትጃት	3	
		ሜ□ ላጁ ወጁም □ ካ	4	

G24	እጅን ከሽንት ቤት መልስ የመታጠብ ልምድ	1) ሁልጊዜ 2) አልፎአልፎ 3) አይታጠብም
G25	በውሀ ወየስ በሳሙና?	1) በውሀ ብቻ 2) በሳሙናና በውሀ
G25A	በሳሙናና በውሀ ከሆነ ድግግሞሹ	1) ሁልጊዜ 2) አልፎአልፎ 3) በጭራሽ
G26	ከምግብ በፊት እጅን የመታጠብ ልምድ	1) ሁልጊዜ 2) አልፎአልፎ 3) በጭራሽ
G26A	ከታጠበ በምንድነው የሚታጠበው?	1) በውሀ ብቻ 2) በሳሙናና በውሀ
G26B	በሳሙናና በውሀ ከሆነ ድግግሞሹ	1) ሁልጊዜ 2) አልፎአልፎ 3) በጭራሽ
G27	ጫማ ይለብሳል	1) ሁልጊዜ 2) አልፎአልፎ 3) በጭራሽ
G27A	ከለበሰ ጫማው ምን አይነት ነው	1) ሽፍን 2) ግልጥ
G28	የጥፍር ንጽህና	1) ንጹህ ነው 2) ንጹህ አይደለም

G29	ሰሞኑን የትላትል መዳህኒት ወስዶል?	1) አው 2) አልወሰደም
G30	ሰሞኑን የአለርጂ መዳህኒት ወስዶል	1) አው 2) አልወሰደም

የወላጅ ፊርማ ----------

ስለተሳትፉ አመሰግናለሁ

Annex-VIII. Questionnaire Afaan Oromo version

Qoranno kana irratti hiramaachuu keessaniif galatooma jechaa odeefanno sirri ta'e akka nuuf kennitan kabajan siin kafanna.

1 .Odeefanno barataa/hirmataa

1.1.Maqaa mana barumsa _____
1.2.Iddoo jireenya:- 1) Magaala 2) Baadiya
1.3.Saala :- 1) Dhira 2) Dubarti
1.4.Umrii_____
1.5.Kuta_____

2. Odeefanno haala maati

2.1. Hojii haadha mana

1) Hojjata mootumma 3) Hojjata dhaabata dhuunfa keessa 5) Hojii guyyaa

2) Hojii mana keessa 4) Qote bula 6) Daldalaa

2.2. Haala barumsa haadha mana

0) Kana hin baranne 1) dubisu fi barressu qofa 2) sadarkaa 1ffaa xumure 3) sadarkaa 2ffaa xumuree 4) barumsa sadarkaa ol'ana xumure

2.3. Hojii Abba warraa

1) Hojjata mootumma 3) Daldalaa 5) Hojii guyya

2) Hojjata dhaabata dhuunfa keessa 4) Qote bula 6) Kana hojii hin qabanne

2.4. Haala barumsa Abba warraa

0) Kana hin baranne 1) dubisuu fi barressu qofa 2) sadarkaa 1ffaa xumure 3) sadarkaa 2ffaa xumure 4) barumsa sadarkaa ol'anaa xumure

3. **Odeefanno haala dhukkuba alarjjii daa'imanni**

3.1 Daa'iminni keessan mallattoo dhukkuba alarjji waggaa darbee keessattii irratti mulate beeka?

1) Eyyyen 2) hin mulanne

3.2 Daa'iminni keessan asimii dhukkubee beeka? 1) Eyyeen 2) dhukkubee hin beeku

3.3. Wagga darbee keessaa daa'iminni keessan gogaa isaa/ishee irraatti waan akka finisaa kan hooqsisu irrattti mulatee beeka? 1) Eyyeen 2) hin mulanne

3.4. Daa'iminni keessan qoricha raamo ji'oota sadan darbee keessa fudhatee beeka ? 1) Eyyeen 2) hin fudhanne

3.5. Mana keessan keessatti nama tambo xuuxu jira? 1) Eyyeen 2) hin jiru

3.6. Deebii keessan eyyen yoo ta'e lakkofsa namoota tamboo xuuxan meeqa? ____

4. **Haala dhukkuba alarjii Abbaa/Haadha**

4.1. Mallattoo dhukkuba alarjii wagga darbee keessattii siin irratti mulate beeka?

1) Eyyyen 2) hin mulanne

4.2 .Asimii siin dhukkubee beeka? 1) Eyyeen 2) hin beeku

4.3. Kanan duraa gogaa keessan irratti waan akka finisaa kan hoqsisu siin irrattti mulate beeka?
1)Eyyeen 2) hin mulanne

5. Madda annisa kannen armaan gaditti ibsaman si'a meeqa fayyadamtu

Madda Annisaa	Siruma	Darbee darbee	Guyya hunda
Kasalaa	1	2	3
Muka	1	2	3
Gaazii	1	2	3
Eelektirikii	1	2	3

6. Bineensota armaan gadii keessaa kamtuu mana keessanitti argamaa

Bineensota	Hin-jiruu	Mana keessattii	Mana alaa
Aduree	1	2	3
Saree	1	2	3
Hindaqoo	1	2	3
Sa'attii/sa'a	1	2	3
Hoola	1	2	3
Harree/farad	1	2	3

7. Madda bishaan dhugaati 1) bishaan bomba'a iddoo keessaa

2) bishaan bomba'a iddoon alatti

3) bishaan burqaa hin huwifamnee

4) bishaan burqaa huwifamee 5) bishaan laga 6) bishaan rooba

5. **Haala qulqullina ijoollee**

5.1. Si'a meeqa harka kee mana finacanii yeroo deebitu dhiqata? 1) yeroo hundaa 2) darbee darbee 3) siruma

5.2 Harka kee maaliin dhiqataa?

1) Bishaan qofa 2) bishaan fi saamuna

5.3. Yoo bishaani fi saamunan ta'e , si'a meeqa saamuna fayyadamata? 1) yeroo hunda 2) darbee darbee

5.4. Nyaata nyaachuun duraa si'a meeqa harka kee dhiqata? 1) Yeroo hunda 2) darbee darbee 3) sirumaa

5.5. Foon dheedhii ni nyaata? 1) Eyyeen 2) hin-nyaadhu

5.6. koophe kaawachu 1) yeroo hunda 2) darbee darbee 3) Sirumaa

5.7. Qulqullinaa qeensa 1) Qulqullu 2) Qulqullu miti

5.8. Yeroo ammaan kan qooricha raamoo fudhachaa jirata? 1) Eyyeen 2) hin-fudhaanne

5.9. Qooricha dhukkubaa alarjii (Bromphemiramine,Cetirizine,chlorphimiramine) guyyoota shanan darbee keessa fudhatee beekta? 1) Eyyeen 2) hin-fudhaanne

Mallattoo maati _____

Hirmaana keessanif guddaa galatooma

Annex-IX. Check list

Laboratory result form

Code ——————School name ——————Age———Grade——— Date————————

A.Skin prick test

Type of test	Wheal diameter	Remark	Signature
Positive control			
Negative control			
Test result			

B. Direct microscopy

Type of intestinal parasite	Ova	Larvae	Troph	Cyst	Remark	Signature
Ascaris						
Hook worm						
Tricuris						
H .nana						
S .stercoralis						
Taenia spp						
E .histolytica						
G .lambila						
Other parasites						

C.Kato Katz

Type of intestinal parasite	**Ova**	**Number**	**Remark**	**Signature**
Ascaris				
Hook worm				
Tricuris				
H .nana				
Other parasites				

D. Formol-ether concentration

Ascaris	**Ova**	**Cyst**	**Remark**	**Signature**
Hook worm				
Tricuris				
H .nana				
Other parasites				

Results:

a) Completed b) Incomplete c) Excluded

Action taken for the incomplete data

Rejected sample: Unlabeled ☐ Insufficient ☐ Contaminated ☐

Test Results

If you have any question you can ask the following individuals

Dessie Abera, Addis Ababa University College of Health Sciences, Cell phone: +251-911993530, e-mail:- dessabera@gmail.com Department of Medical Laboratory Sciences +251112755170.

Annex-X. SOPs for Different Laboratory Procedures

1. Direct or wet mount microscopy

Purpose :Faecal specimens is important to identify intestinal parasitic infections that re-quire treatment, i.e. those associated with serious ill health, persistent diarrhea, weight loss, intestinal malabsorption and the impairment of development and nutrition in children. To assist in the surveillance and control of local parasitic infections caused by geohelminths (soil transmitted nematodes). Helminthes eggs, larvae and protozoan trophozoites, and cysts identified using a saline wet mount identification technique. Direct wet mount was prepared using microscope slide and the stool specimen. The value of wet preparations lies in certain protozoa trophozoites retain their motility which may aid in their identification. Wet preparations on fresh unpreserved liquid stool performed and examined as soon as possible (within 30 minutes of passage) and on soft/formed stool within 60minute.

Principle

Small portion of the stool specimen (size of a match head) added using applicator stick on the microscope slide and mixed with the drop of saline. If the stool specimen is still somewhat solid, a drop or two drop of saline added to the specimen and mixed and covered with cover slide. The entire slide preparation examined systematically under low power objective (10x) and followed by high power (40 x) objective.

Specimen collection

Specimen collected into wide-mouthed leak proof plastic bags

Specimen transport and storage

Specimens stored and transported in sealed plastic bags .Laboratory processing, performed as soon as possible after specimen collection. Specimen refrigerated which was delayed in processing over two hours.

Materials and reagents required

Microscope, distill water Normal saline (0.85%), cover slip Applicator stick, 70% alcohol, bleach (10%).

Procedure

1. The sample was mixed well; a drop of saline placed on a clean microscope slide

2. Using a small wooden applicator pick up a small amount of faeces(approximately 1g of faeces)and transfer to the saline drop on the slide.

3. Placed a cover slip on top of the sample, making sure that the coverslip is flat. Scan the whole of the slide using the 10x objective and 40x objectives.

2. Formol-ether Concentration Method

Fecal concentration is part of the ova and parasite examination and allows the detection of small numbers of organisms that may be missed by using a direct wet smear. Sedimentation methods use centrifugation to concentrate the helminthic ova and Larva in the bottom of the tube. Ether is used as an extractor of debris and fat from the feces. Formol-ether concentration technique will be performed to separate the parasites from fecal debris. The technique not only increases the number of parasites in the sediment but also unmask them, making them more visible by removing organic and inorganic debris.

Principle

Formol-ether sedimentation technique use solutions of lower specific gravity than the parasitic organisms, hence concentrating the parasites in the sediment. Faeces emulsified in10%v/v formol water, the suspension strained to remove large faecal particles, ether or ethyl acetate added, and the mixed suspension was centrifuged. Cysts, oocysts, eggs, and larvae fixed and sedimented ,the faecal debris separated in a layer between the ether and the formol water, faecal fat dissolved in the ether. The supernatant removed and the sediment added to the slide, saline added and covered with coverslip and examined systematically the entire slide preparation under 10x objective and 40 x objectives.

Materials and reagents required

Normal saline (0.85%), Dietylether, Formalin (10%), Microscope slide.

Centrifuge, Microscope, Funnel filter, Applicator sticks.

Conical tubes, Pasteur pipette,Waste container , Cover slips.

Formol-ether concentration procedure

1. Approximately 1g of faece was emulsifying using applicator stick in about 4 ml of 10% formol water contained in a screw-cap bottle or tube.
2. Add further 3-4ml of 10%v/v formol-water, cap the bottle, and mixed well by shaking.
3 .The emulsified faeces sieved; the sieved suspension was collected into a beaker.
4. The suspension transferred to a conical tube made of strong glass. Add 3–4 ml of diethyl ether or ethyl acetate.
5. The tube was closed with stopper and mix for 1 minute.
6. Tissue or piece of cloth wrapped around the top of the tube, loosen the stopper
7. The tube was balanced with other tube oppositely and centrifuged at 750-1000g for 3000 revolution per minute.
8. The layer of faecal debris was loosening by applicator stick from the side of the tube and inverted the tube to discard the ether, faecal debris and formol water.
9. Return the tube to its upright position and allow the fluid from the side of the tube drain to the bottom. The bottom of the tube was taped to resuspend and mix the sediment. The sediment was transferred to a slide and covered with a cover glass.
11. The preparation was examined microscopically using the 10x objective and 40x objectives

3. Kato-Katz technique

Kato-Katz technique is used for qualitative and semi-quantitative diagnosis of intestinal helminthic infestations;caused by *Ascaris lumbricoides, Trichuris trichiura*, hookworm and *Schistosoma spp*.

Principle

In the kato-katz technique faeces were pressed through a mesh screen to remove large particles. A portion of sieved sample transferred to the hole of a template on a slide. After filling the hole, the template was removed and the remaining sample covered with a piece of cellophane soaked in glycerol. The glycerol clears the faecal material from around the eggs, the eggs were counted and the number calculated per gram of faece to determine the intensity of infection.

Materials required

Plastic bags, Absorbable paper, Wire net (stainless steel) Cellophane soaked in glycerin-malachite-green solution, Plastic spoon, Card board paper with 6 mm diameter hole.

Procedure

1. A small amount of fecal material placed on newspaper or scrap paper and a piece of nylon screen was pressed on top so that some of the feces sieved through the screen and accumulated on top.
2. Flat-sided spatula scraped across the upper surface of the screen to collect the sieved feces.
3. Template was placed on the slide and the sieved feces were added with the spatula so that the hole in the template completely filled.
4. The spatula passed over the filled template to remove excess feces from the edge of the hole. The template was removed carefully so that a cylinder of feces left on the slide.
5. The fecal material was covered with a pre-soaked cellophane strip.
6. The slide was inverted and the fecal sample will be pressed firmly against the hydrophilic cellophane strip to spread evenly.
7. The slide was placed on the bench with cellophane upwards to enable the evaporation of water while glycerol cleared the feces. For all helminthes, except hookworm eggs, the slide was kept for one or more hours at room temperature to clear the fecal material, prior to microscopic examination.

4. 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, eosinophil, 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 liter 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

Differential count procedures

1. Perform thin blood film and allow it to dry

2. Fix with methanol by dipping

3. Satin with geimsa solutions for 10minutes

4. Wash with tap water and allow it dry

5. Place a drop of immersion oil on the dried blood film

6. Examine the film microscopically. Focus the cells using the 10 objective with the condenser iris closed sufficiently to see the cells clearly.

7. Move to a part of the film where the red cells are evenly distributed and bring the 100 objective.

8. Systematically examine the blood film and count the different white cells seen in each field, using an automatic differential cell counter,

9. 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 (counted by sysmex kx-21)

5. Skin prick test

Purpose of skin prick test (SPT)

The purpose of SPT is to screen for a predisposition to develop atopic diseases, which can be done with a limited number of allergens, or to identify all sensitized subjects in a given population. SPT also can be used in epidemiologic studies to determine trends in sensitization rates or regional differences and to help standardize allergen extracts. SPT is used to test adults and children from birth onwards. Repeated testing may be necessary in order to detect new sensitizations, especially in children, when symptoms change, or if new environmental allergens are suspected.

Materials required

Allergens, Gauze, alcohol (70%), skin prick lancet

Principle of skin prick test

SPT interpretation utilizes the presence and degree of cutaneous reactivity as a surrogate marker for sensitization within target skin. When relevant allergens are introduced into the skin, specific IgE bound to the surface receptors on mast cells are cross-linked, mast cells degranulate, and histamine and other mediators are released. This produces a wheal and flare response which can be quantitated. Many different allergens can be tested simultaneously because the resultant reaction to a specific allergen is localized to the immediate area of the SPT.

Skin prick test (SPT) procedure

1. School children appropriately screened and discontinued on medications that interfere with test results (example, those who are taking antihistamine drugs).
2. The location of each allergen can be marked with a pen or by using a test grid on the forearm to properly identify test results
3. Tests were applied to the volar aspect of the forearm, at least 2 – 3 cm from the wrist and the antecubital fossae .The back also used for SPT, especially in infants. The skin on the back is more sensitive than the forearm which may result in larger wheals and thus possibly a greater number of positive test results .The distance between two skin prick tests (≥ 2 cm) was critical to avoid false-positive reactions due to direct contamination of a nearby test or secondary to an axon reflex .
4. A drop of each test solution was placed on the skin in identical order for each subject tested and immediately pricked.
5. A single-head metal lancet exhibits excellent reproducibility with few false-negative results and is thus the preferred testing instrument for SPT it is pressed through the drop of allergen extract and held against the skin for at least 1 second with equal pressure applied for each test.
6. The epithelial layer of the skin was penetrated without inducing bleeding, which can lead to false-positive results.

7. A new lancet was utilized for each allergen since wiping a previously used one between tests could result in cross contamination from the previous allergen tested.
8. Excess solution from drops on the skin can be blotted using a clean tissue. It is important to assure that there is no cross-contamination between drops of different allergen extracts, i.e., that the drops do not run together.
9. A timer, with an alarm, was utilized so that all tests, including the histamine and negative control test results will be measured using ruler about 15minutes following application
10. Positive test was defined as an average of two perpendicular wheal diameters greater than or equal to 3mm and negative when an average of two perpendicular wheal diameters are less than to 3mm.

Interpretation of SPT results

SPT results were appropriately interpreted based on skin prick test manuals. The probability of a given sensitization to be clinically relevant depends on the type of allergen and country where the patient lives.

Annex-XI. Declaration

I, the undersigned, declare that this MSc thesis is my original work, has not been presented for a degree in Addis Ababa University or any other universities. I also declare that all sources of materials used for the thesis work have been duly acknowledged.

Name of the candidate: Dessie Abera (BSc) Signature_____

Place: Addis Ababa University Department of Medical Laboratory Sciences, Ethiopia

Date of submission_____/_____/_____

Advisors

BinyamTaye (MPH, PhD) Signature _____

Place: Colgate University, New York, USA

Date of submission_____/_____/_____

Aster Tsegaye (MSc, PhD) Signature _____

Place: Addis Ababa University, Department of Medical Laboratory Sciences, Ethiopia

Date of submission_____/_____/_____

Mr. Kassu Desta (MSc, PhD fellow) Signature _____

Place: Addis Ababa University, Department of Medical Laboratory Sciences, Ethiopia

Date of submission_____/_____/_____

Mistire Wolde (MSc, PhD) Signature _____

Place: Addis Ababa University, Department of Medical Laboratory Sciences, Ethiopia

Date of submission_____/_____/_____