# Addis Ababa University

# College of Health Sciences

# School of Allied Health Sciences

# Department of Medical Laboratory Sciences


**Intestinal Parasites Co-infection and Associated factors Among Active Pulmonary Tuberculosis Patients in Selected Health Centers, Addis Ababa, Ethiopia: A Case control study**

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Department of Medical Laboratory Sciences, School of Allied Health Sciences, College of Health Sciences, Addis Ababa University, Approved by the Examining Board

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# List of Abbreviations

| | |
|---|---|
| AFB | Acid Fast Bacilli |
| AIDS | Acquired Immunodeficiency Syndrome |
| BCG | Bacilli Calmete Gurine |
| BMI | Body Mass Index |
| CMI | Cell Mediated Immunity |
| CBC | Complete Blood Count |
| CTL | Cytolytic T Lymphocytes |
| DOTs | Direct Observed Treatment short course |
| FM | Fluorescence Microscopy |
| Hgb | Hemoglobin |
| HCT | Hematocrit |
| HBCs | High Burdon Countries |
| HIV | Human Immunodeficiency Virus |
| IgE | Immunoglobulin E |
| IP | Intestinal Parasites |
| LJ | Lowenstein Jenson |
| MCH | Mean Cell Hemoglobin |
| MCHC | Mean Cell Hemoglobin Concentration |
| MCV | Mean Cell Volume |
| MGIT | Mycobacterium Growth Indicator Tube |

| | |
|---|---|
| MDR-TB | Multidrug Resistant Tuberculosis |
| NALC | N-Acetyl L-Cysteine |
| PBS | Phosphate Buffer Solution |
| PLT | Platelet |
| PTB | Pulmonary Tuberculosis |
| RBC | Red Blood Cell |
| SPSS | Statistical Package for Social Sciences |
| NaOH | Sodium Hydroxide |
| Th1 | T helper type 1 |
| Th2 | T helper type 2 |
| TB | Tuberculosis |
| WBC | White Blood Cell |
| WHO | World Health Organization |
| ZN | Ziehl Neelsen |

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**Abstract**

**Background:** In co-endemic areas, co-infection rate of intestinal parasites(IP) and tuberculosis (TB) thought to be high. There are limited studies on the epidemiology of IP /TB co-infection in Ethiopia.The present study was done to generate evidence on the co-infection rate in urban setting.

**Objective:** To determine intestinal parasite co-infection rate and associated factors among active pulmonary TB patients in selected health centers of *Kolfe Keraniyo* Sub-city, Addis Ababa, Ethiopia.

**Methods:** Unmatched case-control study was conducted during the period between Jan 2017 and Jan 2018. Ninety one TB patients were enrolled in the case group, and 89 TB free individuals in control group. Socio-demographic characteristics and associated factors were collected using structured questionnaire. Sputum, stool and blood specimens were collected, processed and examined for TB, IP and hematological profiles, respectively. Data was entered and analyzed by SPSS Ver. 20. Descriptive statistics, Fisher's exact test, binary logistic regression, odds ratio, 95% confidence interval and independent-samples T test were used. A *P-value* of <0.05 was considered as statistically significant.

**Results:** The infection rate of intestinal parasites in TB patients and controls was 22% and 9%, respectively.There was statisically significant difference among cases and controls (COR=2.85, 95% CI=1.183-6.87). The most prevalent intestinal parasite was *Gardia lamblia* (8.8%), and followed equivalently by *Ascaris lumbricoides*, *Haymenolopsis nana* and *Entamoeba histolytica/dispar* (4.4%) in TB patients. Co-infection in TB patients was associated with residence (Fisher's exact test=**7.260;** *p=0.046)*, BMI (AOR=6.715; 95% CI=1.655-27.251) and presence of dirty material in the finger (AOR=8.997; 95% CI=2.469-32.788). Anemia, leukocytosis and thrombocytosis were observed in 29.2%, 29.2% and 72% of TB patients respectively. Low value of hemoglobin was associated with intestinal parasitic infection (COR=8.333, 95% CI=2.454-28.297-6.8). Relatively higher mean eosinophil count was observed in helmenthis infected TB patients ($\bar{x}$=334 cells/mm$^3$) compared with non infected ones ($\bar{x}$=262 cells/mm$^3$) but the difference was not statistically significant (t=-1.011, *p=0.327*).

**Conclusion:** There was a statistical significant difference in the infection rate of intestinal parasites in TB patients compared to healthy household contacts. Variety of hematological changes was observed in TB patients; which might help for the diagnosis and prognosis of TB. Anemia was modified by intestinal parasitic infection. Routine stool examination before anti-TB treatment would be important.

**Keywords:** Pulmonary tuberculosis, intestinal parasites, co-infection

# 1. Introduction

## 1.1. Background Information

Tuberculosis (TB) is one of infectious diseases caused by *Mycobacterium tuberculosis* complex bacilli, which is mainly the disease of the lung (pulmonary TB) but also affects other parts of the body (extra pulmonary TB) (1, 2).The ability to control *Mycobacterium tuberculosis* infection is strongly correlated with intact immune functions of the infected human host (3). It is believed that the host innate immunity provides the initial resistance to infections with intracellular pathogens, such as Mycobacteria, before the adaptive type 1 cell-mediated immunity fully develops (4). Cellular immunity and, in particular, T-cell–mediated responses are central in the regulation of specific host– *Mycobacterium tuberculosis* interactions (5). The complex mechanisms that play during the development of acute and chronic TB involve host immunity and mycobacterial manipulation of both innate and adaptive immunity (3).

Although one third of the world population is estimated to be infected with *Mycobacterium tuberculosis*, relatively a small proportion of individuals develop TB disease (1, 6, 7). The host's immune system is a key for the progression of *Mycobacterium tuberculosis* infection and may result in: immediate elimination of the mycobacterium, latency or immune system failure, leading to the development of active disease (8). It is generally accepted that protection against TB is strongly associated with enhanced Th1 CMI responses while susceptibility to the disease is associated with reduced Th1 type responses (7).The high rate of chronic malnutrition, widespread poverty, overcrowding, and high sero-prevalence of human immunodeficiency virus (HIV) infection has created an environment which made TB a formidable threat in Sub Saharan countries like Ethiopia (2, 9).

Intestinal parasitic infections which are caused either by protozoa or helminthes or both are among the most widespread of human infections (10).The interaction between helminthes and the host's immune system evokes particular immune modulatory and regulatory mechanisms that ensure their survival in the host for years (11). Helmenthic infections are characterized by immune activation together with biased Th2 response and down regulated Th1 and CTL activity (11). These changes in the immunological milieu of the host might impair the immunological response to pathogens which mostly need Th1 responses to limit the severity and progression of infection (11).

In sub-Saharan Africa, where the prevalence of parasitic infections is very high, a dominant Th2 polarized immune response has been reported and suggested to increase susceptibility to both *Mycobacterium tuberculosis* and HIV (12). Helminth infections are typically associated with hyper eosinophilia and considerable IgE production (13).In addition parasitic infections can affect the nutritional status of the individual leading to immunological alterations that promote a decrease in the efficacy of the immune response, favoring the occurrence of other bacterial infections (9).

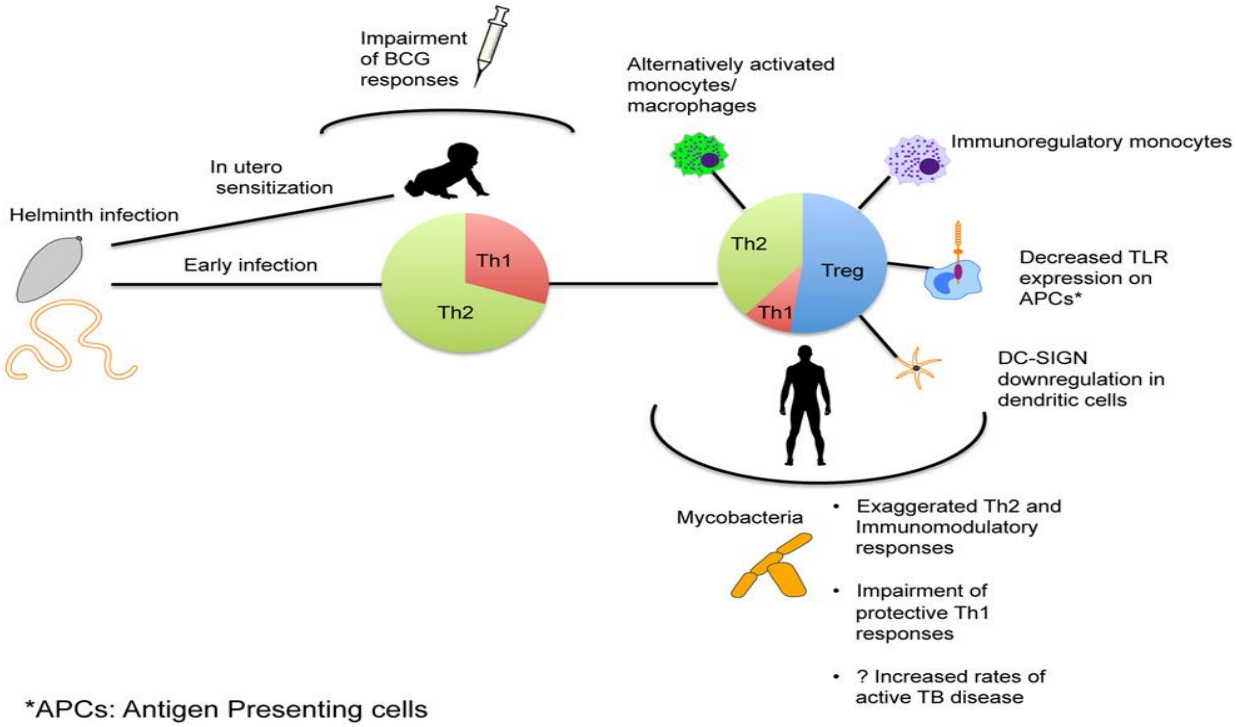


Figure 1: Mechanism of immune modulation caused by helminth infections affecting immune responses and susceptibility to TB. Adapted from a journal titled Helminth-Induced Immune Regulation: Implications for Immune Responses to Tuberculosis (14).

Hematological abnormalities are commonly associated with pulmonary tuberculosis *(15*). TB cause profound bone marrow and peripheral blood abnormalities by modulating normal hematopoiesis (15, 16). The most common hematological manifestation of PTB patients are anemia, iron deficiency and leukocytosis (17). These hematological changes are useful indicators for the diagnosis, prognosis and response to therapy(15, 18). During treatment improvements in the hematological values such as, rise in hemoglobin (Hgb) and hematocrit (HCT) values, were used as indicators reflecting good response to the treatment (19).

## 1.2. Statement of the problem

TB is still one of the infectious diseases known by its significant cause of morbidity and mortality affecting millions of people worldwide (20).TB is the ninth leading cause of death worldwide and the leading cause from a single infectious agent, ranking above HIV/AIDS (1).According to WHO 2017 report in 2016, there werean estimated 10.4 million incident cases of TB globally and 1,674,00 million TB deaths.The African Region had 25% of the world's cases(1). The 30 high TB, MDR-TB and TB/HIV burden Countries (HBCs)accounted for 90 % of all estimated incident cases worldwide (1).Although it is a global epidemic, TB predominantly affects developing countries, where 98% of worldwide TB death occurs (20). Ethiopia is among high TB, MDRTB and TB/HIV burden countries. According to the WHO 2017 Global TB Report, Ethiopia ranks 11$^{th}$ among the world's HBCs. The estimated incidence rate was 182 per 100,000 population (1)..

Similarly most of the world's population is infected with intestinal parasites. It is estimated that nearly 3.5 billion people are affected, and 450 million are ill due to parasite infections (8, 10).

Among the parasites intestinal helminthes are the most important problems in developing countries(9). The common soil transmitted helminthes which cause chronic gut infection in humans are *A.lumbricoides, T. trichiura* and *Hookworm* (21). There is a high prevalence of intestinal parasites in Ethiopia (22). The most prevalent and important helminthes in Ethiopia are those of the soil transmitted nematodes(21). TB and intestinal parasites affect primarily low social and economic level populations, living clustered in precarious settings (8). The distribution of TB and intestinal helminthes infections are geographically overlap substantially(2,7, 23). Co-infection with TB and intestinal parasites in humans is an important publich health problems in co-endemic areas, especially in developing countries (2, 24), which is extensive in sub-Saharan Africa (25). Inevitably, co-infection would increase the complexity of control and prevention on TB and parasitic diseases (24, 26).

The immune response was modified in the co-infection of intestinal parsites and TB (24, 26). In sub-Saharan Africa, where the prevalence of parasitic infections is very high, a dominant Th2 polarized immune response has been reported and suggested to increase susceptibility to *Mycobacterium tuberculosis*(12). Helmenthic infection is one of the factors that down regulates Th1 type immune response (23). Co-infection of intestinal parsites and TB also hastens progression of their respective diseases(12).The frequency of infections by at least a nematode is

significantly higher in patients with PTB(27). The immune modulation induced by worms may facilitate the infection with *Mycobacterium tuberculosis* and disease progression (27). It is observed that there was eosinophilia and high levels of IgE in patients with PTB that could be possibly caused by intestinal helminth infections (27). There is a decreased TB specific cellular responses in humans due to helmenthic infections (7). BCG has a limited effect against TB epidemic in developing countries where the prevalence of intestinal helmenthiasis is high (4). Infection with parasites can alter the protective immune response to BCG vaccination against *Mycobacterium tuberculosis* (26). The immunogenicity of BCG vaccination has been shown to be impaired in helminth-infected individuals (28, 29).

In addition, intestinal parasitic infections cause poor nutritional status (22), which leads to micronutrient deficiency that promotes a decrease in the efficacy of the immune response, favoring the occurrence of other bacterial infections (8, 12, 30). A statistically significant association between intestinal helmenthic infections and different bacterial infections, such as PTB and multi-bacillary leprosy was reported (31). On the one hand, it is reported that anemia would be caused by intestinal parasites (32) and this might be worsen if an individual is co-infected by intestinal parasites and TB. However, there is paucity of information on intestinal parasites and TB co-infection specifically in the present area and this research would fill this gap.

## 1.3. Significance of the Study

IP and TB are the major public health problems and highly estimated burden at national level and regional level in Ethiopia (2, 9, 11). The likely hood of co-infection is thought to be high, but there are limited studies on the epidemiology of co-infection. Most of the studies done in Ethiopia are limited to similar geographical location. Understanding co-infection rate has an input to design effective mechanism to reduce mortality due to dual effect and for designing effective prevention and control mechanism. In Addis Ababa or similar urban settings no published study was conducted to assess intestinal parasites co-infection rate and associated factors among active PTB patients. Thus, this study aimed at filling the knowledge gap that would inform local health authorities and program coordinators at different levels. The finding of this study would contribute for collaborative activities of TB Control pragram and Neglected Tropical Diseases Elimination program to achieve the sustainable development goals. In this study the PTB was ruled out by using sensitive TB diagnostic methods like Xpert MTB/RIF assay and liquid culture (MGIT 960).

## 2. Literature Review

Helmenthic infections compromise the host's ability to control *Mycobacterium tuberculosis* infection. Mice infected with the intestinal helminth *Nippostrongylus brasiliensis* exhibit a transitory impairment of resistance to airborne *Mycobacterium tuberculosis* infection (33).

A study was conducted in Brazil to assess the immune modulation induced by nematodes is a factor that enhances TB infection/ progression and eosinophilia seen in TB. Infection by at least one nematode was stastically significantly higher in patients with PTB (57.8%) than among the control group (20.9%). In TB patients eosinophilia was also statistically significantly higher among those with IPs (69.8%) compared to those without this condition (45.6%) (27).

However; in a study conducted in China rural community, there was no statistical significant difference on the prevalence of intestinal parasites between persons with PTB and healthy controls after adjusting for potential confounding factors. The overall prevalence of intestinal parasites in persons with PTB was 14.9%, including intestinal protozoa (7.9%) and helminthes (7.6%). The infection spectrum of intestinal parasites was *Entamoeba spp.* (1.4%), *Blastocystis hominis* (6.2%), *Trichomonas hominis* (0.3%), *Clonorchis sinensis* (0.3%), *A.lumbricoides* (0.5%), *T.trichiura* (2.2%), and *hookworm (*4.6%) (24).

Similarly in study conducted in Brazil, there was no statistical significant association between helmenthic infection and a favorable tuberculosis outcome, and between parasitism and tuberculin skin test response. The prevalence of enteroparasites observed was 19.6%. Helminthes and protozoa prevalence in investigated patients were 10.1% and 9.8%, respectively. The parasites found were: *S. stercoralis* (7.3%), *E. histolytica* (3.0%), and *G.lamblia* (2.7%). But the association between the eosinophilia and the presence of helminthes was statistically significant both for HIV negative and positive TB cases (8).

A pre-existing infection by intestinal helminthes may facilitate the establishment of *M. leprae* infection or its progression to more severe forms of leprosy, a study conducted in Brazil showed that the frequency of individuals harboring at least one intestinal helminth was statistically significantly higher among leprosy patients when compared to household contacts. Infection with at least one intestinal helminth species was diagnosed in 22% of 105 leprosy patients. *A. lumbricoides* infection was reported in 15 patients, *S.stercoralis* in five and *H.worms* in four patients. By contrast,only 10 (6·8%) of 146 household contacts were parasitized, three with *A. lumbricoides*, three with *S. stercoralis*, three with *T.trichiura* and one with *Enterobius*

*vermicularis*. Intestinal helmenthic infection among multibacillary patients was 34·1% when compared to either pauci bacillary patients (15·6%) or household contacts (6·8%) (31).

Co-infection with IP has been suggested to worsen the outcome of infection by polarizing the immune response towards Th2. A study was conducted in Ethiopia to investigate the serum IgE level on 241 TB patients with or without intestinal helmenthic infection and/or HIV infection. The IgE level was statistically significantly higher in patients co infected with intestinal helminthes and HIV compared to those infected with helminthes or without co infection. Intestinal helmenthic parasites were detected in 42 (33·9%) of the 124 HIV sero positive and 48 (41%) of the 117 HIV sero negative TB patients (34).

Intestinal helminthes infection may be one of the risk factors for the development of active PTB in addition to HIV infection. A study was conducted in Ethiopia, Gondar to determine the prevalence of intestinal helminthes infections in active TB patients and their healthy household contacts and to assess its association with active TB on 230 smear-positive TB patients and 510 healthy household contacts. There was statistical significant association between TB and intestinal helminthes infection. Of the 230 smear positive TB patients included in the study, 70.9% were positive for one or more intestinal helminthes but 36.3% of the controls did. The proportions of TB patients infected with 1, 2, 3 or more species of worms were 44.8% 20.4% and 5.7%, respectively; in controls these proportions were 25.7%, 9.4% and 1.2%. Helminth species prevalent in the study population were: *A.lumbricoides, H.worm, S.sstercoralis, T.trichiura, S. mansoni* and *E.vermicularis* (7).

A cross sectional study was conducted in Ethiopia, Gondar to determine plasma IgE level and blood eosinophil count in smear positive TB patients with and without helmenthic infection. The peripheral eosinophil count in smear positive TB patients with intestinal helmenthic infection ($352/mm^3$) was statistically significantly higher than those without helmenthic infection ($112/mm^3$). Intestinal helmenthic parasite was detected in 28.6% of the smear positive TB patients. *A.lumbricoides, Hookworm, S.stercoralis, T.trichiura*, and *S. mansoni* were more prevalent (35).

A study was conducted in Ethiopia, Gondar on 295 study participants comprises 112 TB patients, 112 community controls and 71 household contacts to assess the impact of asymptomatic helminthes infection on the immunological response. High burden of intestinal parasites was observed among TB patient and asymptomatic helminthes infection correlated to increased

eosinophil count. Eosinophilia were statistically significantly associated with asymptomatic helminthes infection which was not confounded by sex or HIV-serostatus. But there was no statistical difference in the prevalence of helminthes between TB patients and a combined or separate analysis of the community controls and household contacts groups. Intestinal helminthes were identified from 29% (32/112) of the TB patients, 21% (23/ 112) of the community controls and 21% (15/71) of the house hold contacts. *A.lumbricoides* was the most common intestinal parasite observed in all three groups followed by *H.worm* (36).

The prevalence of smear positive TB and IP co-infection predominantly *H.worm* was relatively higher among tuberculosis suspects. In a cross sectional study conducted in Ethiopia, Gondar; intestinal parasites were detected in 24 (33.3%) smear positive PTB patients and 96 (27.9%) pulmonary TB negative patients. *H.worm* and *S.stercoralis* infection were common in smear positive TB patients, with prevalence of 8 (11.1%) and 5(6.9%), respectively. Smear positive TB patients were frequently co-infected with parasitic infection. TB had significant association with shoe wearing and finger nail(9).

A facility based cross-sectional study conducted in Arba Minch showed that 26.3% of PTB patients were co-infected with intestinal parasites. The infection rate of intestinal helminthes and intestinal protozoas were 24.4% and 6.1% respectively. *A.lumbricoides* accounted the highest frequency (11.3%). Living in rural residence, eating vegetables/ fruits without washing or peeling off and having BMI <18.5 were associated with intestinal helminth infection (37).

Variety of hematological abnormalities has been demonstrated in patients with PTB. A study done at India on 100 PTB patients showed that anemia, leukocytosis and thrombocytosis was observed in 74% , 26% and 24% of patients respectively (38).

Hematological abnormalities might be an index for diagnosis of PTB. In a study done at Pakistan Hgb was recorded lower than normal value in 55% and 53% of male and female population respectively, while neutrophilia was recorded as 60% and 64% in male and female patients respectively (15).

In a study done at Sudan statistically significantly lower values in Hgb, Red Cell Count and HCT and normal Means MCV, Mean MCH and MCHC in TB patients compared with controls group was observed. Total leukocyte count, absolute Neutrophil count, absolute Monocyte count and platelet count was found statistically significantly increased in TB patients compared to controls. And the most type of anemia was normocytic normochromic anemia (16).

# 3. Objectives

## 3.1. General Objective:

The general objective of this study is to assess intestinal parasite co-infection rate and associated factors for co-infection among active pulmonary tuberculosis patients.

## 3.2. Specific objectives:

- To determine the prevalence of intestinal parasites co-infection among active pulmonary tuberculosis patients and controls/household contacts.
- To identify different factors associated with intestinal parasites co-infection among active pulmonary tuberculosis patients.
- To determine the level of hematological parameters among active pulmonary tuberculosis patients
- To compare the level of hemoglobin(anemia) and eosinophil count in intestinal parasites co-infected and intestinal parasite not infected active pulmonary tuberculosis patients.

## 3.3. Hypothesis

There is no difference in the prevalence of intestinal parasites infection between active pulmonary tuberculosis patients and healthy household contacts.

# 4. Materials and Methods

## 4.1. Study Area

This study was conducted in selected health centers of *Kolfe Keraniyo* Sub-city, Addis Ababa. Addis Ababa is the Capital City of Ethiopia, which is located at the heart of the country, with an altitude ranging from 2,100 meters to 3,000meters above sea level. Addis Ababa lies 9°1′48″N latitude and 38°44′24″E longitude and the area covers 540 Sq.Km(39).The city had an estimated population of 3,156,077 in June 2013(40).

Among the ten Sub-cities in Addis Ababa, *Kolfe Keraniyo* Sub-city was selected using purposive sampling technique due to easier access to Health Centers and patient flow. The land area of the *Kolfe Keraniyo* Sub-city is 6348.09 hectar (39). The sub-city is the largest populous sub-city with a population of 524,759 (40). In the sub city there are 11 health centers. Among these, 3 health centers were randomly selected and namely; *Kolfe, Wereda 11* and *Lomi meda* health centers. These health centers provide outpatient services, including care and treatment for TB and HIV/AIDS patients and there is TB DOTs program.

## 4.2. Study Design and Period

An unmatched case-control study was conducted during the period between 0 Jan 2017 to Jan 2018.

## 4.3. Source Population

Patients visiting *Kolfe, Wereda 11* and *Lomi meda* health centers for medical care.

## 4.4. Study Population

All patients visiting TB clinics in *Kolfe, Wereda 11* and *Lomi meda* health centers.

## 4.5. Study Participants

Cases: Patients with bacteriologically confirmed active PTB and fulfill the inclusion criteria.

Controls: Healthy household contacts to active PTB patients with no clinically and bacteriologically diagnosed TB.

## 4.6. Participant Inclusion and Exclusion Criteria

### 4.6.1. Inclusion Criteria

The criteria for incclusion of individulas for cases were:

- Individuals with bacteriological confirmed active PTB infection.

- Patients who were volunteered to take part and gave written consent to take part in the study.
- All age groups who expectorate sputum sample

The criteria for incclusion of individulas for controls were:

- Healthy house hold contacts of all age groups who were not had signs and symptoms of TB (not had clinically diagnosed TB), volunteered to take part, gave written consent and were confirmed negative by bacteriological TB diagnostic methods.

### 4.6.2. Exclusion Criteria

The criteria for exclusion of individulas for cases were:

- Individuals already started anti-TB treatment, with severe illness & were unable to provide specimens were excluded.
- Individuals positive for HIV antibody test.

## 4.7. Study Variables

### 4.7.1. Dependent variables

The dependent variable is intestinal parasites and TB co-infection

### 4.7.2. Independent variables

The independent variables are socio-demographic characteristics including age, gender, residence, marital status, educational status, occupation, monthly income. The associated factors are BCG vaccination, BMI, latrine availability, swimming habit, shoe wearing habit, hand washing habit, bathing , drinking water source, eating raw meat, eating unwashed vegetables, raised poultry/livestock, dirty material in the finger.

## 4.8. Sample Size Determination

The required sample size was calculated by using the formula for unmatched case-control study which is a difference in proportion between cases and controls

$$n= \frac{r+1}{r}\frac{(P*)(1-P*)\left(Z\beta+z\frac{\alpha}{2}\right)2}{(p1-p2)2}$$

Where n = sample size

r = Ratio of control to cases

P= Average proportion of exposed=Proportion of exposed cases + Proportion of control/2

$Z_\beta$= Standard normal value for power

$Z\frac{\alpha}{2}$ = Standard normal value for level of significance.

$P_1$-$P_2$=Effect size or different in proportion expected based on a study conducted at Gondar. By taking average proportion from studies done at Gondar (7, 36).

$P_1$= Proportion in cases

$P_2$= Proportion in controls

Sample Size for Unmatched Case-Control Study

For:

| | |
|---|---|
| Two-sided confidence level(1-alpha) | 95 |
| Power(% chance of detecting) | 80 |
| Ratio of Controls to Cases | 1 |
| Hypothetical proportion of controls with exposure | 28.5 |
| Hypothetical proportion of cases with exposure: | 50 |
| Least extreme Odds Ratio to be detected: | 2.51 |
| | **Fleiss with CC** |
| Sample Size – Cases | 89 |
| Sample Size – Controls | 89 |
| Total sample size: | 178 |

*(Fleiss, Statistical Methods for Rates and Proportions, formulas 3.18 &3.19)*

CC = continuity correction

Results are rounded up to the nearest integer

Therefore by using Fleiss with CC sample size for cases was 89 and for controls it was 89.

## 4.9. Sampling Procedure

Nonrandom convenience sampling technique was used. Consecutive active PTB patients were enrolled in the case group of the study. Household contacts of TB patients had no signs and symptoms of TB and bacteriologically confirmed negative for TB were included in the control.

## 4.10. Data collection method

### 4.10.1.Enrollment Procedure

The objective of the study was explained whenever eligible individuals visited the TB clinics. Written informed consent was administered for volunteers willing to participate in the study after full explanation of the information sheet.

### 4.10.2.Data collection procedure

Socio-demographic characteristics and associated factors were collected using a structured questionnaire (*Annex II*) via face to face interview by trained nurse. The questionnaire was pretested and revised before administering to the real data collection process. Variables like sex, age, occupation, education, marital status, monthly income and other associated factors were collected during the patient visit. Also, BCG vaccination status was collected by nurses based on the presence and absence of BCG scar on subjects' arms. Laboratory examinations like sputum smear microscopy, Xpert MTB/RIF assay, sputum culture, stool examination, CBC and HIV antibody test was conducted by an experienced and senior laboratory technologists.

### 4.10.3.Sample collection, processing and laboratory testing methods

Spot, spot sputum specimens were collected for sputum smear microscopy and one morning sputum specimen was collected for Xpert MTB/RIF assay and sputum culture respectively based on the Ethiopian tuberculosis and leprosy control program manual(41). Smear microscopy was done at *Kolfe* Health Center,*Wereda 11* Health Center and *Lomi meda* Health Centers. Sputum sample for culture and Xpert MTB/RIF assay was transported to Ethiopian Public Health Institute National Tuberculosis Reference Laboratory. The samples were stored in a refrigerator at 2-8$^{o}$c until transportation.

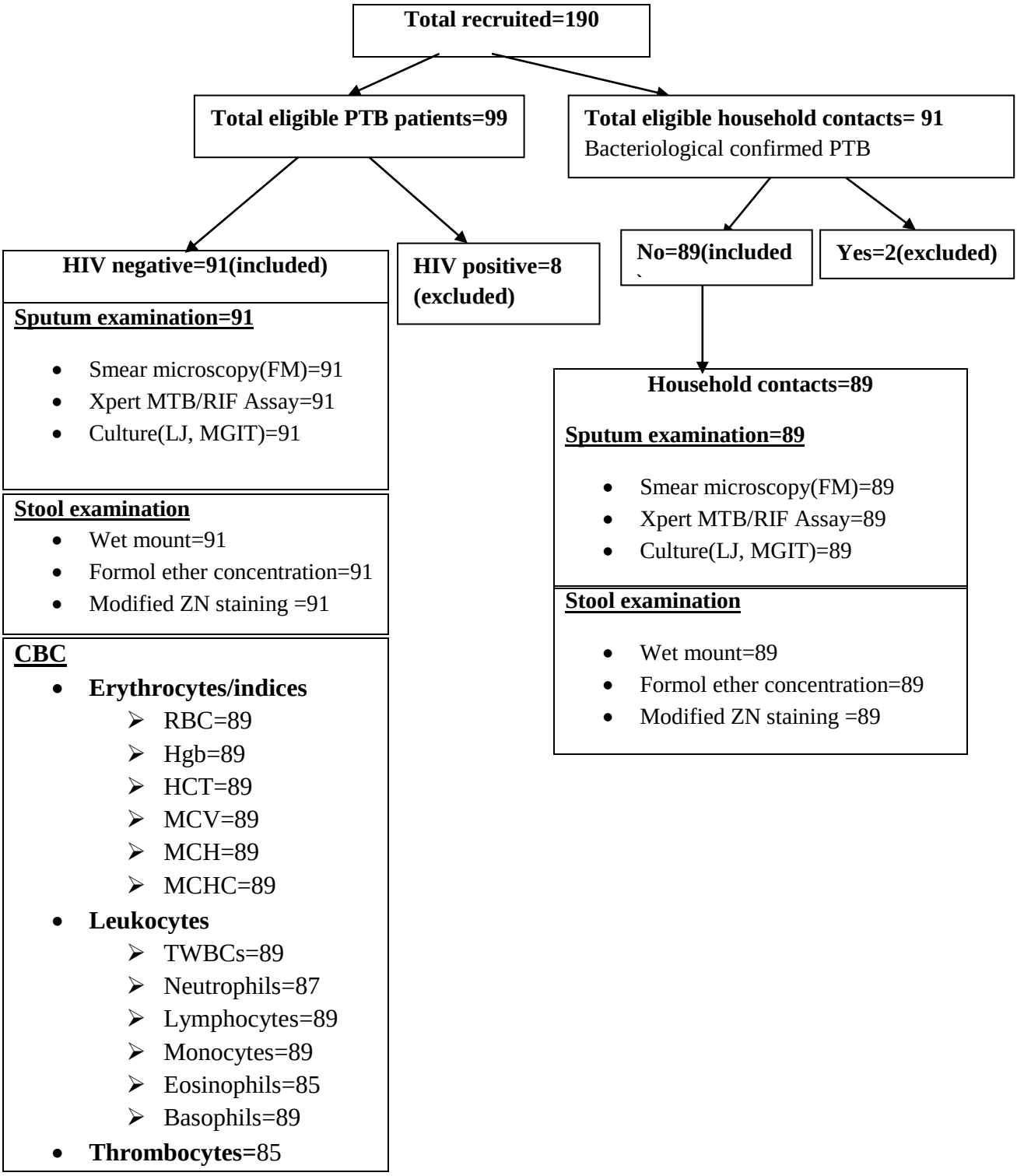


Figure 2: Flow chart for enrollment and data collection procedure for pulmonary tuberculosis patients and household contacts in selected health centers of *Kolfe Keraniyo Sub-city*, Addis Ababa, Ethiopia, Jan 2017 to Jan 2018.

**AFB staining**: A smear was prepared, dried, heat-fixed and stained with 0.1% auramine stain for 20 minutes. Decolorized with 0.5% v/v acid alcohol for 3 minutes and after rinsing covered with 0.5% potassium permanganate stain for 1 minute. Examined with a fluorescent microscope (FM), using 20x and 40x objective to cover 30 fields and 40 fields respectively. AFB appeared as yellowish fluorescent bacilli against a dark background (42). The smear result was interpreted based on the Ethiopian TB and Leprosy control program guideline (41). The result was noted on laboratory data collection format and the patient result was reported accordingly.

**Sputum processing for culture and Identification:**

Sputum samples were processed by using N-Acetyl-L-Cysteine–Sodium Hydroxide (NALC-NaOH) decontamination method. An approximately equal volume of 3% NALC-NaOH solution was added to specimen in the centrifugation tube and vortexed for not more than 20 seconds. Kept for 15 minutes and mixed after 8 minutes. Filled within up to 45 ml with phosphate buffer (pH 6.8) and mixed by inverting the tube. Centrifuged at 3000*g* for 15 minutes. Carefully poured off the supernatant. Smear were prepared from the deposit and resuspended with 2 ml of phosphate buffer. Using a transfer pasture pipette, 2-4 drops and 0.5ml of sediment was inoculated to LJ slant and MGIT tube respectively. Prior to innoculation 800µl from a mix of 15ml MGIT growth supplement and PANTA was added to MGIT tubes. The inoculums in the LJ slants were distributed over the entire surface of the slant (43) and MGIT tube was inverted 2-4 times. The smear was stained with Ziehil Nelseen staining method (44) and examined using oil immersion objective. The result was noted on laboratory data collection format.

Inoculated LJ slants were incubated at 37°C. MGIT tubes were loaded to MGIT 960 instrument. LJ slants were inspected at 3$^{rd}$ day and once per week for the remaining 8 weeks. The tubes from the MGIT 960 machine were unloaded based on the day of positivity or negativity.The *Mycobacterium tuberculosis complex* isolates were confirmed by colony morphology and antigen identification(45). After unloading, positive MGIT tubes were inspected for typical flakes. Smear was prepared and inoculated at Brain Heart Infusion media to look for contamination. The smears from LJ slants and MGIT tubes were stained by using Ziehl–Neelsen staining method (45).

LJ slants not had colonies growing at eight weeks and no growth unit on MGIT tubes at 42 days were defined as negative (45). "SD BIOLINE TB Ag MPT 64®" test was done for the confirmation of *Mycobacterium tuberculosis complex* (46). When observed by microscopy, TB bacilli were

frequently arranged in serpentine cords of varying length or show linear clumping. Positive cultures were reported immediately (47)..

**Xpert MTB/RIF assay:** For Xpert MTB/RIF assay 0.5 ml of sediment from sputum specimen processed by using NALC-NaOH decontamination method was taken and vortexed with 1.5 ml of sample reagent buffer (supplied in kit) in a 3:1 (SR buffer: resuspended sputum sediment.) volume ratio. After 15 minutes an approximate of >2ml of the specimen was dispensed it into the open port of the Xpert MTB/RIF cartridge and loaded to GeneXpert instrument. And the result was viewed after 2 hrs (48).

**Stool Examination:** The approximate of 3 gram of stool sample was collected from all study participants for parasitic examination. Specimen was examined by using direct saline microscopic method, formal-ether concentration technique and modified ZN method**.**

**Wet mount:** For direct saline method 50 mg of feces mixed with one or two drops of normal saline placed on a clean slide. A uniform thin suspension was made and covered with cover slip. The entire film was screened systematically for the presence of helminth ova and larvae or protozoan cysts and trophozoites (49).

**Formol-ether concentration:** In addition, using an applicator stick about 1 g of faeces was placed in a clean 15 ml conical centrifuge tube containing 7 ml formalin saline for formal-ether concentration technique. The resulting suspension was filtered through a sieve into another conical centrifuge tube. The debris trapped on the sieve was discarded. After adding 3 ml of diethyl ether to the formalin solution, the contents were centrifuged at 3, 200 rpm for 3 minutes. The supernatant was poured away and the tube was replaced in its rack. Smear of the sediment was made on clean glass slide and covered by cover slip. Then, the entire area under the cover slip was systematically examined by using 10x and 40x objective lenses (9).

**Modified ZN method:** Prepared faecal smears was made from the concentration deposit and allowed to air dry. Then fixed with methanol for 3 minutes. The fixed smear was stained with 1% carbol fuchsin for 15-20 minutes. Then washed off the stain with clean water and drain, covered the smear with 1% v/v acid alcohol (HCl in methanol)for 15-20 seconds, rinsed with clean water and drained, covered with 0.25% malachite green for 30-60 seconds and washed off the stain with clean water and drained. Finally wiped the back of the slide clean, air dried and an experienced laboratory technologist/microbiologist using 100x objective (50).

**CBC:** About 5ml of whole blood was collected and total white blood cell count was done for each study subjects using automated hematology analyzer (XT-1800*i*)with the anti-coagulated blood as per the manufacturer's protocol (51).

**HIV antibody test** on TB patients was done by using rapid kits (Wanti HIV1+2, Uni-Gold$^{TM}$ HIV and VIKIA HIV 1/2) based on implementation guideline for TB/HIV collaborative activities in Ethiopia (52).

### 4.10.4.Data Quality Control

A structured face to face interview questionnaire was prepared in English and translated in to Amharic and then back-translated into English to check for any inconsistencies or distortions in the meaning of words and concepts. Questionnaire was pre-tested on patients who did not included in the study.

Health professionals working in TB clinic and laboratory technologists of each health center were selected and trained on the instrument of data collection for basic interview techniques, the objective of the study, how to collect and analyze specimensand concerning their duties and responsibilities. The overall activities of data collection was monitored by the principal investigatorto keep the validity of the data during data collection.The collected data was checked for completeness, accuracy, clarity and consistency by the supervisors, on daily basis by the principal investigator.

To ensure the quality of instrument performance, controls were run and preventive maintenance were performed. For quality assurance instruments and reagents were checked for reliability and reproducibility of the test before any test was started. Standard operating procedures was used for specimen collection and processing for maintaining a good quality study. Patients was instructed on how to produce appropriate sputum and stool specimen. After collection of specimen, it was processed and tested according to standard operating procedures. Diagnosis of smear positive PTB was based on the national TB diagnosis guidelines. For all smear positive TB patients Xpert MTB/RIF and sputum culture were performed and was used to confirm presence of PTB infection. Stool examination was conducted by individuals who were blinded to the source of the specimen whether it was from TB patients or from household contacts to ensure quality control. And control slides were used. The result of laboratory examination was

recorded on well prepared format carefully and finally it was attached with the questionnaire. Prepared LJ media was checked visually for the color, texture and homogeneity and performance check and sterility test was performed before usage and was used only if it was appropriate. Activities like reagent and media preparation were carried out as standard operating procedure describe by Kent and Kubica (53). For Liquid culture everything was done based on MGIT instrument manual (54).

### 4.11. Data entry, storage and management

Data that were obtained through questionnaire and laboratory test results were coded, checked, entered and stored in to Statistical Packages for Social Sciences(SPSS) Ver. 20.

### 4.12. Data analyses

Analysis was performed by SPSS version 20 statistical software package. Q-Q plot was used to check the normal distribution of quantitative variables. Descriptive statistics was used to explain socio demographic, associated factors, co-infection rate of intestinal parasites among active PTB patients and household contacts. Fisher's exact test was used to compare the study variables for the presence or absence of association.The association between IP co-infection with TB was evaluated by using binary logistic regression. Odds ratio and 95% confidence interval was used to measure the strength of an association. Independent students-T test was used to analyze mean differences.A P-value of $<0.05$ was considered indicative of a statistically significant difference. Finally, the results of the study were presented on words, graphs and tables**.**

### 4.13. Ethical consideration

The study optained ethical approval from Department Research and Ethical Review Committee of the Department of Medical Laboratory Science, College of Allied Health Sciences, Addis Ababa University**;** after being approved written was obtained from the department before the actual work is started. Permission was obtained from Ethiopian Public Health Institute. In addition permission was also obtained from the administrators of *Kolfe, Wereda 11* and *Lomi meda* health centers of *Kolfe Keranyo* Sub-city. Subjects were recruited after they became informed about the objectives and use of the study and after they gave informed consent. For participants less than 18 years of age, their parents were asked to sign a written parental permission form. Full explanation about the purpose of the study was clearly explained for administers and concerned bodies of *Kolfe,Wereda 11* and *Lomi meda* health centers of *Kolfe Keranyo* Sub-city prior to the actual data collection time. The respondents were informed of their

right to refuse or agree to be part of the study, or discontinue their participation whenever they feel the need. Confidentiality of the data was maintained during data collection. Blood sample collection was carried out under aseptic conditions by well experienced laboratory technologist.All participants who werepositive for PTB by using any of bacteriological methods were linked with DOTs at respective health centerwith free of charge and subjects positive for intestinal parasites were treated accordingly.

## 4.14. Dissemination of results

The result of this study will be submitted to the department of Medical Laboratory Science, College of Allied Health Sciences, Addis Ababa University**;** as a partial fulfillment for the Degree of Masters in Clinical Laboratory Science (Diagnostic and Public health Microbiology specialty track). Further, it will be communicated to Ministry of Health and other concerned bodies through report or publication on reputable scientific journals.The principal investigator will submit the study abstract to local associations (like EMA, EPHA and EMLA) and other international associations to present the results of the project during continuous medical educational events or conferences organized by these associations. The findings of this study will be submitted to the international or national peer reviewed journal for publication.

## 4.15. Operational Definitions

**Active PTB:** A patient with PTB who was usually infectious and may spread the bacteria to other people.

**Bacteriological confirmed TB Case:** A patient from whom at least one biological specimen is positive for *Mycobacterium tuberculosis* by either smear microscopy, Xpert MTB/RIF or culture.

**Cases:** Bacteriological confirmed Active PTB patients who were negative for HIV antibody test

**Controls:** House hold contacts to PTB patients who were not had signs and symptoms of TB, and were confirmed negative by bacteriological TB diagnostic methods.

**Co-infection:** Presence of any of intestinal parasites among active PTB patients

**Multiple parasitic infection:** harboring 2 or more intestinal parasites

**New case *:***A patient who never had treatment for TB, or has been on previous anti-TB treatment for less than four weeks.

# 5. Results

## 5.1. Socio-demographic characteristics of study participants

A total of 190 study subjects were enrolled in the study; among them 99 were cases with bacteriological confirmed active PTB and 91were controls who were healthy household contacts to PTB patients. Among the 99 cases, eight were excluded due to HIV co-infection. Similarly, two household contacts were bacteriologically confirmed TB patients and excluded from the control group. Therefore, 91 cases and 89 controls were included in the analysis. Majority of the cases (61.5%) and controls (62.9%) were males and females, respectively. The mean age of PTB patients and household contacts was 26.7(±7.7) and 26.69 (±9.19) years, respectively. Among all of PTB patients, 93.4% were younger than 40 years and this was true for 94.4% of the controls. The residence for 97.8% of both PTB patients and controls were from urban. Regarding the marital status, 56(61.5%) and 35(38.5%) of PTB patients were single and married respectively. From the controls, 43(48.3%) and 46(51.7%) were single and married respectively (Table 1).

From PTB patients (cases) 65.9% were unemployed similar to 66.3% of the controls. Half of the PTB patients (50.5%) had a monthly income <500 Ethiopian birr(ETB). However, 59.6% of the controls had a monthly income <1500 ETB. Both TB patients and household contacts had been utilizing tap water as a source of drinking water equally (97.8%). Eighty five (93.4%) patients in case group and 100% of the controls reported that they had latrine or use latrine (Table 1).

All TB patients and majority of the controls (98.9%) a shoe wearing habit. Similarly All from both groups have been washing their hands before taking meal at breakfast, lunch and dinner time. Majority of TB patients, 85(93.4%) and controls, 88(98.9%) were washing their cloths in their home on one hand; in 21(23.1%) of TB patients and 13(14.6%) of the controls there was a dirty material in their finger (Table 2). The mean BMI of TB patients was 18.26 (±2.14). Among TB patients 17.6% were BCG vaccinated (Table 1).

Table 1: Socio-demographic characteristics of tuberculosis patients (n=91) and controls(n=89) in selected health centers of *Kolfe Keraniyo* Sub-city, Addis Ababa, Ethiopia, Jan 2017 to Jan 2018.

| Variables | Cases n (%) | Controls n (%) |
|---|---|---|
| Age | | |
| Mean | 26.7 | 26.96 |
| SD | 7.69 | 9.19 |
| Age group | | |
| ≤24 | 44(48.4) | 38(42.7) |
| 25-34 | 31(34.1) | 34(38.2) |
| ≥35 | 16(17.6) | 17(19.1) |
| Total | 91(100.0) | 89(100.0) |
| Gender | | |
| Male | 56(61.5) | 33(37.1) |
| Female | 35(38.5) | 56(62.9) |
| Total | 91(100.0) | 89(100.0) |
| Residence | | |
| Urban | 89(97.8) | 87(97.8) |
| Rural | 2(2.2) | 2(2.2) |
| Total | 91(100.0) | 89(100.0) |
| Marital status | | |
| Single | 56(61.5) | 43(48.3) |
| Married | 35(38.5) | 46(51.7) |
| Total | 91(100.00) | 89(100.0) |
| Educational status | | |
| No formal education | 26(28.6) | 27(30.3) |
| Primary completed | 42(46.2) | 25(28.1) |
| High school completed | 19(20.9) | 20(22.5) |
| College &above | 4(4.4) | 17(19.1) |
| Total | 91(100.0) | 89(100.0) |
| Monthly income | | |
| Low | 46(50.5) | 28(31.5) |
| Medium | 22(24.2) | 25(28.1) |
| Satisfactory | 23(25.3) | 36(40.5) |
| Total | 91(100.0) | 89(100.0) |
| Latrine availability | | |
| Yes | 85(93.4) | 89(100.0) |
| No | 6(6.6) | 0(0.0) |
| Total | 91(100.0) | 89(100.0) |
| Raised poultry | | |
| Yes | 8(8.8) | 22(24.7) |
| No | 83(91.2) | 67(75.3) |
| Total | 91(100.0) | 89(100.0) |
| Occupation | | |
| Employed | 31(34.1) | 30(33.7) |
| Unemployed | 60(65.9) | 59(66.3) |
| Total | 91(100.0) | 89(100.0) |

Table 2: Behavioral characteristics of tuberculosis patients and controls visiting selected health centers of *Kolfe Keraniyo* Sub city,Addis Ababa, Ethiopia, Jan 2017 to Jan 2018.

| Variables | Cases n (%) | Controls n (%) |
|---|---|---|
| Swimming habit | | |
| Yes | 6(6.6) | 3(3.4) |
| No | 85(93.4) | 86(96.6) |
| Total | 91(100.0) | 89(100.0) |
| Habit of shoe wearing | | |
| Yes | 91(100.0) | 88(98.9) |
| No | 0(0,0) | 1(1.1) |
| Total | 91(100.0) | 89(100.0) |
| Bathing | | |
| Home | 87(95.6) | 87(97.8) |
| River | 4(4.4) | 1(1.1) |
| Home and River | 0(0.0) | 1(1.1) |
| Total | 91(100.0) | 89(100.0) |
| Hand wash before meal | | |
| Yes | 91(100.0) | 89(100.0) |
| No | 0(0.0) | 0(0.0) |
| Total | 91(100.0) | 89(100.0) |
| Hand wash style after toilet | | |
| With water | 30(40) | 35(41.2) |
| With water and soap | 45(60) | 50(58.8) |
| Total | 75(100.0) | 85(100.0) |
| Water source for drink | | |
| Tap | 89(97.8) | 87(97.8) |
| River | 0(0.0) | 0(0.0) |
| Tap and River | 2(2.2) | 2(2.2) |
| Total | 91(100.0) | 89(100.0) |
| Washing cloth | | |
| Home | 85(93.4) | 88(98.9) |
| River | 2(2.2) | 0(0.0) |
| Home and River | 4(4.4) | 1(1.1) |
| Total | 91(100.0) | 89(100.0) |
| Dirty material in the finger | | |
| Yes | 21(23.1) | 13(14.6) |
| No | 70(76.9) | 76(85.4) |
| Total | 91(100.0) | 89(100.0) |
| Eating unwashed vegetables | | |
| Yes | 28(30.8) | 19(21.3) |
| No | 63(69.2) | 70(78.7) |
| Total | 91(100.0) | 89(100.0) |
| Eating raw meat | | |
| Yes | 52(57.1) | 38(42.7) |
| No | 39(42.9) | 51(57.3) |
| Total | 91(100.0) | 89(100.0) |

## 5.2. Burden of intestinal parasites

The overall co- infection rate of one or more intestinal parasites among TB patients was 22% (20/91 ) and it was 9% (8/89) among the controls. The difference was statistically significant (COR=2.85; 95% CI= 1.183-6.87). A total of 24 intestinal parasites (14 intestinal protozoans and 10 intestinal helminthes) from TB patients and eight intestinal parasites (six intestinal protozoans and two intestinal helminthes) from the controls were identified. There was a statistical significance difference for helmenthic infection among TB patients and controls (COR=5.37; 95% CI= 1.142, 25.253) but not for Protozoans(*p=0.157*). There was a multiple parasitic infection in four(4.4%) of TB patients; but not identified among the controls. The difference was statistically significant (*p=0.045*). *E.histolytca/dispar, G.lamblia* and *Cryptosporidium parvum* from intestinal protozoans and *A.lumbricoides,S.sterocolaris, Taenia species, H.nana* and *T.trichuria* from intestinal helminthes were identified in the study from both groups. Among all intestinal parasites identified; *G.lamblia* was frequently detected (8, 33.3%), followed by equal prevalence of *A. lumbricoides, H.nana and E.histolytica/dispar* (4, 16.7%) from TB patients. *E.histolytica/dispar* was frequently detected from controls (5, 62.5%). Among intestinal parasites identified from TB patients and controls *G.lamblia*, *A.lumbricoides* and *H.nana* were found with a statistical significance difference between groups (*p=0.046*, *p=0.045* and *p= 0.045* respectively) (Table 3).

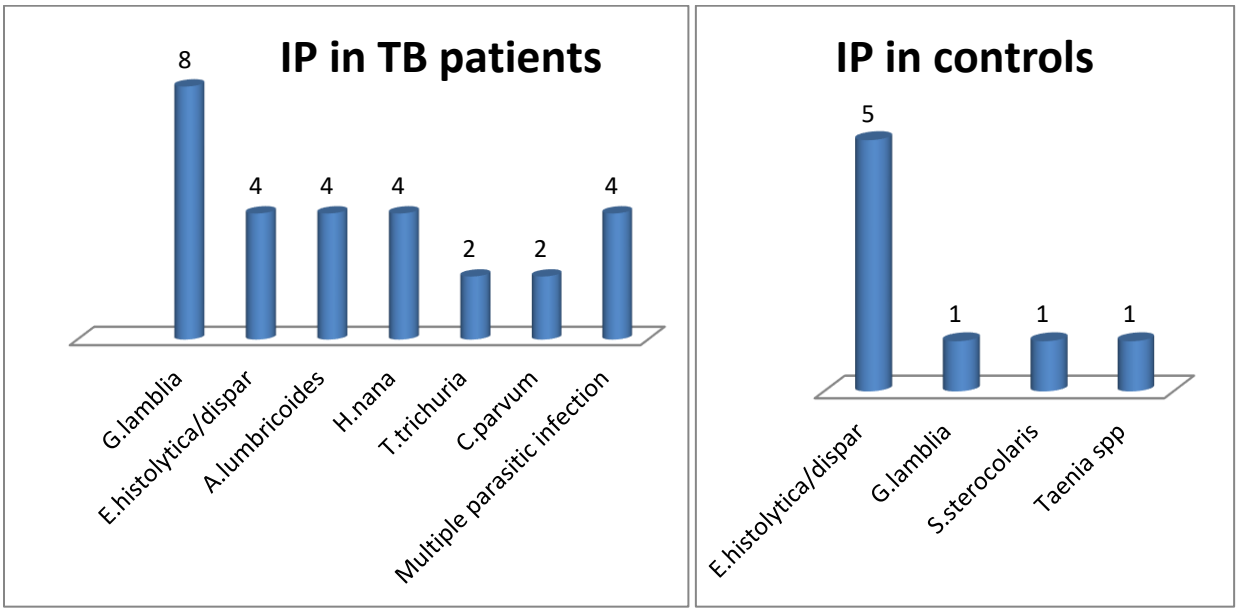


Fig 3: Distribution of intestinal parasites among TB patients and controls in selected health centers of *Kolfe Keraniyo* Sub-city, Addis Ababa, Ethiopia, Jan 2017 to Jan 2018.

Table 3: Infection rate of intestinal parasites among tuberculosis patients (n=91) and controls (n=89) in selected health centers of *Kolfe Keraniyo* Sub-city, Addis Ababa, Ethiopia, Jan 2017 to Jan 2018.

| Variables | Cases Number(%) | Controls Number(%) | COR(95%CI) | *p-value* |
|---|---|---|---|---|
| Parasites | | | | 0.020 |
| No | 71 (78.0) | 81(91.0) | 1.00 | |
| Yes | 20 (22.0) | 8(9.0) | 2.852(1.183-6.874) | |
| Total | 91(100.0) | 89(100.0) | | |
| Protozoa | | | 1.00 | 0.157 |
| No | 79(86.8) | 83(93.3) | 2.101(0.752-5.870) | |
| Yes | 12(13.2) | 6(6.7) | | |
| Total | 91(100.0) | 89(100.0) | | |
| Helmenths | | | | 0.033 |
| No | 81(89.0) | 87(97.75) | 1.00 | |
| Yes | 10(11.0) | 2(2.25) | 5.370 (1.142-25.253) | |
| Total | 91(100.0) | 89(100.0) | | |
| ≥2 parasites | | | - | 0.045 |
| No | 87(95.6) | 89(100.0) | | |
| Yes | 4(4.4) | 0(0.0) | | |
| Total | 91(100.0) | 89(100.0) | | |
| Each parasite | | | | |
| *E.histolytca/dispar* | 4(16.7) | 5(62.5) | | 0.707 |
| *G.lamblia* | 8(33.3) | 1(12.5) | 8.482 (1.038-69.291) | 0.046 |
| *C. parvum* | 2(8.3) | 0(0.0) | | 0.160 |
| *A.lumbricoids* | 4(16.7) | 0(0.0) | - | 0.045 |
| *S.sterocolaris* | 0(0.0) | 1(12.5) | | 0.311 |
| *Taenia spp* | 0(0.0) | 1(12.5) | | 0.311 |
| *H.nana* | 4(16.7) | 0(0.0) | - | 0.045 |
| *T.trichuria* | 2(8.3) | 0(0.0) | | 0.160 |
| Total | 24(100.0) | 8(100.0) | | |

COR=Crude Odds ratio, CI=Confidence Interval," -"=Not Done

## 5.3. Associated factors for intestinal parasites infection

Intestinal parasitic co-infection on PTB patients had statistically significant association with residence (*p=0.046)*, BMI (AOR=6.715;95%CI=1.655-27.251) and presence of dirty material in the participant's finger (AOR=8.997;95%CI=2.469-32.788) **(**Table 4, Table 6). However; intestinal parasitic infection in the control group had not statistically significant association with socio demographic or behavioral factors (Table 5, Table 6). Intestinal parasites are identified from 6(37.5%) of BCG vaccinated TB patients. From the identified parasites the majority (4, 66.7%) were intestinal helminthes.

Table 4: Associated factors for intestinal parasites co-infection among tuberculosis patients in selected health centers of *Kolfe Keraniyo* Sub-city,Addis Ababa, Ethiopia, Jan 2017 to Jan 2018.

| Variables | Cases | | | | |
|---|---|---|---|---|---|
| | Number (%) | COR(95%CI) | *p-value* | AOR(95%CI) | *p-value* |
| Gender | | | 0.873 | - | - |
| Male | 12(60.0) | 1.000 | | | |
| Female | 8(40.0) | 1.086(0.394-2.997) | | | |
| Age group | | | 0.700 | - | - |
| 10-24 | 8(40.0) | 0.667(0.170-2.164) | | | |
| 25-34 | 8(40.0) | 1.043(0.260-4.183) | | | |
| ≥35 | 4(20.0) | 1.000 | | | |
| BMI | | | 0.025 | | 0.008 |
| <18.49 | 16(80.0) | 3.889(1.183-12.787) | | 6.715(1.655-27.251) | |
| 18.5-24.99 | 4(20.0) | 1.000 | | 1.000 | |
| Marital status | | | 0.381 | | |
| Single | 14(70.0) | 1.000 | | | |
| Married | 6(30.0) | 0.621(0.214-1.804) | | | |
| Educational status | | | 0.329 | - | - |
| No formal education | 4(20.0) | 1.000 | | | |
| Primary completed | 8(40.0) | 1.294(0.348-4.818) | | | |
| Completed high school | 6(30.0) | 2.538(0.602-10.703) | | | |
| Collage & above | 2(10.0) | 5.500(0.591-51.190) | | | |
| Occupation | | | 0.141 | | 0.366 |
| Employed | 4(20.0) | 1.000 | | 1.000 | |
| Unemployed | 16(80.0) | 2.45(0.742-8.116) | | 1.867(0.482-7.226) | |
| Monthly income | | | 0.280 | | 0.186 |
| Low | 12(60.0) | 1.000(0.320-3.126) | | 1.465(0.349-6.149) | |
| Medium | 2(10.0) | 0.283(0.050-1.592) | | 0.237(0.032-1.731) | |
| Satisfactory | 6(30.0) | 1.000 | | | |
| Dirty material in the finger | | | 0.002 | | 0.001 |
| Yes | 10(50.0) | 5.455(1.839-16.174) | | 8.997(2.469-32.788) | |
| No | 10(50.0) | 1.000 | | 1.000 | |
| Eating unwashed vegetables | | | 0.933 | | |
| Yes | 14(70.0) | 0.955(0.324-2.812) | | | |
| No | 6(30.0) | 1.000 | | | |
| Eating raw meet | | | 0.466 | - | - |
| Yes | 10(50.0) | 0.690(0.255-1.870) | | | |
| No | 10(50.0) | 1.000 | | | |
| Raised poultry /livestock | | | 0.829 | - | - |
| Yes | 2(10.0) | 1.204(0.224-6.480) | | | |
| No | 18(90.0) | 1.000 | | | |

COR=Crude odds Ratio, AOR=Adjusted Odds Ratio, CI=Confidence Interval," -"=Not Done

Table 5: Associated factors for intestinal parasites co-infection among healthy household contacts in selected health centers of *Kolfe Keraniyo* Sub-city,Addis Ababa, Ethiopia, Jan 2017 to Jan 2018.

| Variables | Controls | | |
|---|---|---|---|
| | Number(%) | COR(95%CI) | *p-value* |
| Gender | | | 0.979 |
| Male | 3(37.5) | 1.020(0.227-4.575) | |
| Female | 5(62.5) | 1.000 | |
| Educational status | | | 0.387 |
| 1$^{o}$ completed or less | 4(50.0) | 0.389(0.078-1.947) | |
| Completed high school | 1(12.5) | 0.246(0.023-2.617) | |
| College & above | 3(37.5) | 1.000 | |
| Occupation | | | 0.315 |
| Employed | 4(50.0) | 1.000 | |
| Unemployed | 4(50.0) | 0.473(0.110-2.040) | |
| Dirty material in the finger | | | 0.392 |
| Yes | 2(25.0) | 2.121(0.379-11.869) | |
| No | 6(75.0) | 1.000 | |
| Eating unwashed vegetables | | | 0.254 |
| Yes | 3(37.5) | 2.437(0.527-11.283) | |
| No | 5(62.5) | 1.000 | |
| Eating raw meat | | | 0.247 |
| Yes | 5(62.5) | 2.424(0.542-10.847) | |
| No | 3(37.5) | 1.000 | |
| Raised poultry /livestock | | | 0.415 |
| Yes | 1(12.5) | 0.408(0.047-3.516) | |
| No | 7(87.5) | 1.000 | |

COR=Crudes Odds Ratio, CI=Confidence Interval

Table 6:Associated factors for intestinal parasites co-infection among tuberculosis patients(n=91) and controls(n=89) in selected health centers of *Kolfe Keraniyo* Sub-city,Addis Ababa, Ethiopia, Jan 2017 to Jan 2018.

| Variables | Cases | | | Controls | | |
|---|---|---|---|---|---|---|
| | Number (%) | Fisher's exact test | *p-value* | Number (%) | Fisher's exact test | *p-value* |
| Residence | | 7.260 | 0.046 | | 0.000 | 1.000 |
| Urban | 18(90.0) | | | 8(100.0) | | |
| Rural | 2(10.0) | | | 0(100.0) | | |
| Latrine availability | | 0.697 | 0.44 | | - | - |
| Yes | 20(100.0) | | | 8(100.0) | | |
| No | 0(0.0) | | | 0(0.0) | | |
| Swimming habit | | 0.697 | 0.404 | | 0.224 | 0.636 |
| Yes | 0(0.0) | | | 1(12.5) | | |
| No | 20(100.0) | | | 7(87.5) | | |
| Bathing | | 0.219 | 0.640 | | 6.338 | 0.173 |
| Home | 20(100.0) | | | 7(87.5) | | |
| River | 0(0.0) | | | 0(0.0) | | |
| Home and River | 0(0.0) | | | 1(12.5) | | |
| Hand wash style after toilet | | 1.136 | 0.286 | | 14.08 | 0.495 |
| With water | 10(50.0) | | | 2(25.0) | | |
| With water &soap | 10(50.0) | | | 6(75.0) | | |
| Water source for drink | | 0.000 | 1.000 | | 0.000 | 1.000 |
| Tap | 20(100.0) | | | 8(100.0) | | |
| River | 0(0.0) | | | 0(0.0) | | |
| Tap and River | 0(0.0) | | | 0(0.0) | | |
| Washing cloth | | 0.915 | 0.740 | | 0.000 | 1.000 |
| Home | 20(100.0) | | | 8(100.0) | | |
| River | 0(0.0) | | | 0(0.0) | | |
| Home &River | 0(0.0) | | | 0(0.0) | | |

## 5.4. Hematological profiles of tuberculosis patients

### 5.4.1. Red cell parameters

The overall mean of RBCs in TB patients was 4.98(±0.54) x$10^6$cells/$mm^3$. In males and females, the mean of RBCs was 5.05(±0.57)x$10^6$cells/$mm^3$ and 4.85±(0.47) x$10^6$cells/$mm^3$ respectively. The mean of Hgb in TB patients was 13.77(±1.75) g/dl. It was 14.18(±1.75) g/dl and 13.07(±1.53) g/dl for males and females respectively (Table 7).

Table 7: Red blood cell parameters of tuberculosis patients(n=89) in selected health centers of *Kolfe Keraniyo* Sub-city, Addis Ababa, Ethiopia, Jan 2017 to Jan 2018.

| RBC parameters | Mean ± SD | Median ($95^{th}$ percentile Commonly used U.S based ref. |
|---|---|---|
| RBC x $10^6/mm^3$ | | |
| Male | 5.05±0.57 | 4.5-5.9 |
| Female | 4.85±0.47 | 4.0-5.2 |
| Combined | 4.98± 0.54 | 4.2-5.9 |
| Hgb g/dl | | |
| Male | 14.18± 1.75 | 13.5-17.5 |
| Female | 13.07±1.53 | 12.0-16.0 |
| Combined | 13.77±1.75 | |
| HCT % | | |
| Male | 42.25±4.36 | 41.00-53.00 |
| Female | 39.95±4.36 | 36.00-46.00 |
| MCV fl | 83.39±5.51 | 80.00-100.00 |
| MCH pg | 27.77±2.19 | 26.00-34.00 |
| MCHC g/dl | 33.15±1.14 | 31.00-37.00 |

Moderate anemia (Males; Hgb=10-12.99 g/dl, Females; 10-12.49g/dl) was detected in16 (28.6%) of males and 10(30.3%) of females respectively. Based on morphological classification from all TB patients who had anemia; normocytic normochromic anemia and microcytic hypochromic anemia were common on 14(53.85%) and 12(46.15%) TB patients respectively (Table 8).

Table 8: Hgb value(n=89) and type of anemia(n=26) among tuberculosis patients in selected health centers of *Kolfe Keraniyo* Sub city,Addis Ababa, Ethiopia, Jan 2017 to Jan 2018.

| Hgb value(male) | Number (percent) | Hgb value (female) | Number (percent) | Anemia type (morphological) | Number (percent) |
|---|---|---|---|---|---|
| 10-12.99 g/dl | 16(28.6%) | 10-12.49g/dl | 10(30.3%) | Normocytic normochromic | 14(53.85%) |
| ≥13g/dl | 40(71.4%) | ≥12.5g/dl | 23(69.7%) | Microcytic hypochromic | 12(46.15%) |
| Total | 56(100%) | Total | 33(100%) | Total | 26(100%) |

### 5.4.2. Leukocytes

The overall mean of white blood cells count(WBCs) in TB patients was 8.89(±3.79)x$10^3$cells/$mm^3$. In males and females it was 8.84(±3.90)x$10^3$cells/$mm^3$, and 8.97(±3.66) x$10^3$cells/$mm^3$ respectively (Table 9). Leukocytosis (WBC count>11,000 cells/$mm^3$) was observed in 29.2% of TB patients (Figure 4).

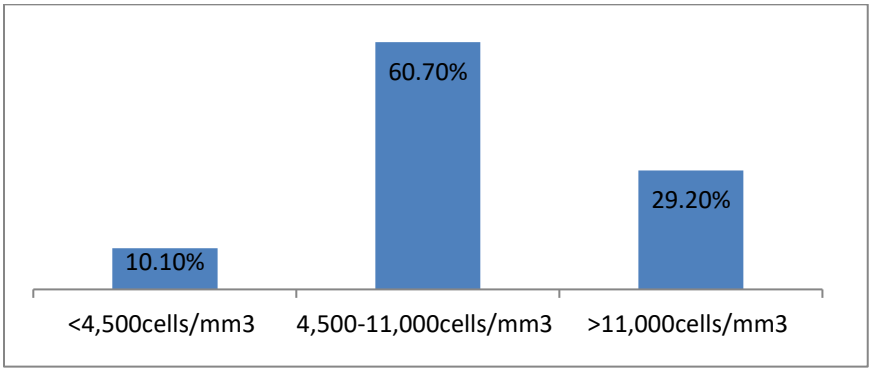


Fig 4: Values of Leukocytes in $mm^3$ among tuberculosis patients in selected health centers of *Kolfe Keraniyo* Sub-city,Addis Ababa, Ethiopia, Jan 2017 to Jan 2018.

Table 9: Leukocyte differential count of tuberculosis patients in selected health centers of *Kolfe Keraniyo* Sub-city, Addis Ababa, Ethiopia, Jan 2017 to Jan 2018.

| Leukocytes | Mean ± SD | Median ($95^{th}$ percentile Commonly used U.S based ref |
|---|---|---|
| TWBCs $10^3/mm^3$ | | |
| Male | 8.84± 3.90 | |
| Female | 8.97±3.66 | |
| Combined | 8.89±3.79 | 4.50-11.00 |
| Neutrophils | | |
| Percentage | 64.15±15.80 | 40.00-70.00 |
| Absolute($10^3/mm^3$) | 6.19±3.56 | 1.80-7.70 |
| Lymhocytes | | |
| Percentage | 22.31±11.98 | 22.00-44.00 |
| Absolute($10^3/mm^3$) | 1.66±0.71 | 1.00-4.80 |
| Monocytse | | |
| Percentage | 9.91±3.78 | 4.00-11.00 |
| Absolute($10^3/mm^3$) | 0.82±0.34 | 0.00-0.80 |
| Eosinophils | | |
| Percentage | 3.43±3.46 | 0.00-8.00 |
| Absolute($10^3/mm^3$) | 0.27± 0.29 | 0.00-0.45 |
| Basophils | | |
| Percentage | 0.43±0.30 | 0.00-3.00 |
| Absolute($10^3/mm^{3)}$ | 0.033±0.019 | 0.00-0.20 |

### 5.4.3. Thrombocytes

The mean of platletes in TB patients was 459.25(±150.39)x$10^3$cells/$mm^3$. Thrombocytosis (>350. 000 cells/$mm^{3)}$ was observed in 71.8% of TB patients (Figure 5).

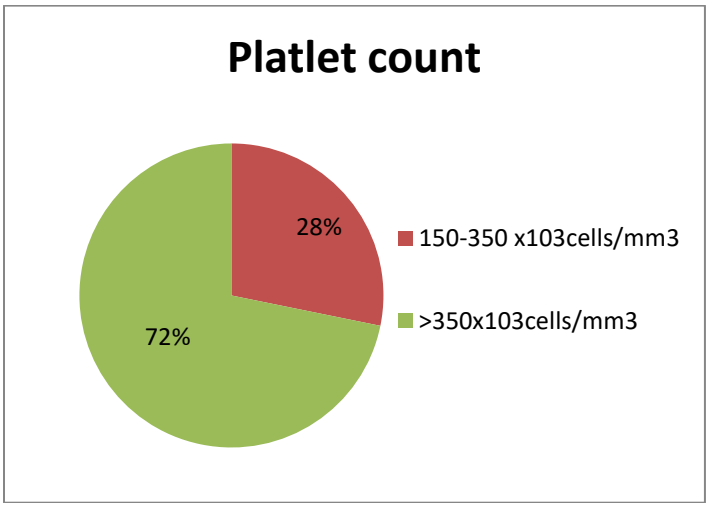


Fig 5: Value of Thrombocytes among tuberculosis patients in selected health centers of *Kolfe Keraniyo* Sub city,Addis Ababa, Ethiopia, Jan 2017 to Jan 2018.

## 5.5. Hemoglobin value and Eosinophil count

### 5.5.1. Hemoglobin

In this study, among 18 PTB patients co-infected with intestinal parasites, 14(77.8%) had low Hgb value(<12.9g/dl) compared to the 21(29.5%) of TB patients without intestinal parasites. The difference was statistically significant (COR=8.333; 95%CI=2.454-28.297). When Bivariate analysis was done a low value of Hgb was observed in intestinal protozoan infected TB patients compared to non-infected TB patients (COR=7.704; 95%CI=1.528-38.839). Similarly, there was also a statistical significant difference in intestinal helminthes co-infected TB patients (COR=7.704; 95%CI=1.528-38.839) (Table 10). Astatistically significant decrease in mean Hgb value was observed in *G. lamblia* (*p=0.002*), *C. parvum* (*p<0.001*), *A.lumbricoides* (*p<0.001*), and *T.trichuria(p<0.001)* co-infected PTB patients (Table 11).

Table 10: Hemoglobin value among intestinal parasites co-infected tuberculosis patients (n=89) in selected health centers of *Kolfe Keraniyo* Sub-city, Addis Ababa, Ethiopia, Jan 2017 to Jan 2018.

| | Hgb value | | | |
|---|---|---|---|---|
| | 10-12.9, N(%) | 13.00-18.00, N(%) | COR(95%CI) | *p-value* |
| Parasite | | | | 0.001 |
| No | 21(60.0) | 50(92.6) | 1.000 | |
| Yes | 14(40.0) | 4(7.4) | 8.333(2.454-28.297) | |
| Total | 35(100.0) | 54(100.0) | | |
| Protozoa | | | | 0.013 |
| No | 27(77.1) | 52(9.3) | 1.000 | |
| Yes | 8(22.9) | 2(3.7) | 7.704(1.528-38.839) | |
| Total | 35(100.0) | 54(100.0) | | |
| Helminthes | | | | 0.013 |
| No | 27(77.1) | 52(9.3) | 1.000 | |
| Yes | 8(22.9) | 2(3.7) | 7.704(1.528-38.839) | |
| Total | 35(100) | 54(100.0) | | |

COR-Crude Odds ratio, CI=Confidence Interval, N=Frequency

Table 11: Hemoglobin mean differences based on each parasitic infection among active PTB patients(n=89) in selected health centers of *Kolfe Keraniyo* Sub-city,Addis Ababa, Ethiopia, Jan 2017 to Jan 2018.

| Name of parasite | Number | Mean Hgb | Mean Difference | 95% CI of the Difference | *p-value* |
|---|---|---|---|---|---|
| *G.lamblia* | | | 1.86 | 0.92- 2.80 | 0.002 |
| No | 83 | 13.89 | | | |
| Yes | 6 | 12.03 | | | |
| Total | 89 | | | | |
| *A. lumbricoides* | | | 1.01 | 0.63-1.40 | <0.001 |
| No | 85 | 13.81 | | | |
| Yes | 4 | 12.80 | | | |
| Total | 89 | | | | |
| *T.trichuria* | | | 1.00 | 0.61-1.37 | <0.001 |
| No | 87 | 13.80 | | | |
| Yes | 2 | 12.80 | | | |
| Total | 89 | | | | |
| *C.parvum* | | | 2.93 | 2.57-3.30 | <0.001 |
| No | 87 | 13.83 | | | |
| Yes | 2 | 10.90 | | | |
| Total | 89 | | | | |

### 5.5.2. Eosinophils

The mean of eosinophil count in intestinal parasites co-infected PTB patients was 271 cells/$mm^3$,which is comparable with the count PTB patients without intestinal parasite co-infection (t=-0.012, *p=0.991*). Intestinal helminthes co-infected TB patients had relatively higher eosinophil count ($\bar{x}$ =334 cells/$mm^3$) than with the non-infected TB patients ($\bar{x}$=262 cells/$mm^3$ ), and it was statistically insignificant(t=-1.011, *p=0.327*). However, eosinophil count had statistically significant increase in PTB patients co-infected with intestinal helminthes ($\bar{x}$=365 cells/$mm^3$) compared to those co-infected with intestinal protozoans ($\bar{x}$= 167 cells/$mm^3$)(t=-2.639, *p=0.031*)(Table 12).

Table 12: Eosinophil level among intestinal parasites infected and non-infected tuberculosis patients in selected health centers of *Kolfe Keraniyo* Sub-city, Addis Ababa, Ethiopia, Jan 2017 to Jan 2018.

| Variable | Number(%) | Mean | t | *p-value* |
|---|---|---|---|---|
| Parasites | | | -0.012 | 0.991 |
| No | 69(81.2) | 0.270 | | |
| Yes | 16(18.8) | 0.271 | | |
| Total | 85(100.0) | | | |
| Helminthes | | | -1.011 | 0.327 |
| No | 75(88.2) | 0.262 | | |
| Yes | 10(11.8) | 0.334 | | |
| Total | 85(100.0) | | | |
| Parasites | | | -2.639 | 0.031 |
| Protozoan | 6(42.9) | 0.17 | | |
| Helminthes | 8(57.1) | 0.37 | | |
| Total | 14(100.0) | | | |

# 6. Discussion

Both TB and parasitic diseases overlap similar geographic distribution, especially in developing countries (2, 26,36). In this study, the overall any intestinal parasite co-infection rate among active PTB patients was 22%. Similar findings were reported in studies done at Arbaminch (37) and Brazil (8). However, the finding of the present study is lower than the studies in Gondar (7,34) and Brazil (27). This might be due to the time when these studies were conducted was before 15 years ago and currently though out the country there are health extension workers engaged in the primary health care activities. Three consecutive stool specimens were examined in these studies, which might be another reason for the lower results in the present study. The co-infection rate in the present study was also lower than studies conducted in Gondar by Alemayehu *et al.* (9) and Abate *et al.*(36) and in Tanzania by Mhimbira *et al.*(55). This might be due to the difference in the study area where the present study was conducted in urban set up. On the other hand the co-infection rate in the present study was higher than a study done at China(24), which might be due to the start of anti-TB chemotherapy before stool examination in the China's study and the difference in the study setting.

Intestinal parasites and TB, either way, might be a risk factor for co-infection (7). In the present study, there was a statistically significance difference in intestinal parasites and TB co-infection among TB patients (cases) with against the controls (TB free contacts)(COR=2.85; 95% CI=1.183-6.87). Similar findings were reported by different previous studies (7, 9, 27). However; a study from rural China showed there was no a significant difference in the prevalence of intestinal parasites between persons with PTB and healthy controls (24). This might be due to the low prevalence of intestinal parasites in the study area and might be also due to the start of anti-TB chemotherapy before stool specimen were examined TB patients. Moreover, highly sensitive diagnostic methods (Xpert MTB/RIF Assay and MGIT 960 liquid culture) were used to rule out TB in control group, but they used least sensitive smear microscopy technique to rule out TB.

In the present study, the prevalence of intestinal protozoans and helminthes in PTB patients was 12% and 11%, respectively. This finding is in line with a study done in China (24) and Brazil (8). However, Alemu *et al* (37) and Alemayehu *et al* (9) reported that higher prevalence of helminthes in Arbaminch and Gondar, respectively. This might be due to the difference in geographical prevalence of helminthes variation; a study conducted in Adds Ababa (56) showed that the prevalence of intestinal protozoan parasite is higher than helminthes. Majority of the

studies are focused only on the prevalence of helminthes among TB patients (7, 27,34,36, 55) so that I could not be able to compare the protozoan prevalence against the present finding.

Intestinal helminthes were found in TB patients with a significance difference compared to controls in the present study which is supported by a study done by Elias *et al*.(7) and by Tristão-Sá *et a.l* (27). However; in a study done in Gondar by Abate *et al* (36) and in Tanzania by Mhimbira *et al.*(55), there was no statistical significance difference. This might be due to the intestinal helminthes prevalence difference. In this study TB is ruled out by using sensitive methods (Xpert MTB/RIF Assay and MGIT 960 liquid culture) in controls which might be another possible reason.

In the present study the frequency of multiple parasitic infection in TB patients was 4.4%. In line with this finding, mixed or more than one species of intestinal parasites co-infection among TB patients were reported by Alemu *et al*.(37), by Alemayehu *et al.*(9) and by Mhimbira *et al*.(55). From intestinal parasites identified in the present study; *G.lamblia*, *A. lumbricoides* and *H.ana* were found with a significant difference in TB patients compared with controls. Likewise; a greater frequency of *G.lambia* infection in TB patients was reported by a study done at Gambo which is 250 kms south of Addis Ababa, Ethiopia(57). Similarly, *A.lumbricoides* was reported as the predominantly identified helminthes in previous studies done at Gondar (7, 34, 36) and at Arbaminch (37).

Even though *H. worm* was the predominantly identified helminthes in a cross sectional study done at Gondar (9) and in a study done at China (24), and was the second most frequently identified helminthes in many studies (7, 34, 36, 37), it was not identified in this study. This might be due to the difference in the study area where the present study was conducted in urban setting where 100% of TB patients participated in this study had a habit of wearing shoe. In studies from Brazil (8, 27) and Tanzania (55), *S.sterocolaris* was the predominantly identified helminthes. And it was also identified from TB patients in studies done at Ethiopia (7, 9,34, 37), however; it is not identified in the present study. The possible explanation might be the fact that HIV infected TB patients were not included in this study.

Even though *H.nana* was significantly associated with TB in the present study, supportive study findings are not found. This might be due to the absence of previous studies in the present area or similar setting. However, all the intestinal parasites identified in the present study except *C.parvum* was reported by different studies done in different settings and population (7, 8, 9,24,

34, 36, 37, 55 ). The detection of *C.parvum* in TB patients in the present study might be due to the use of Modified Ziehl Neelsen staining method which were not included in the previous studies.

In the present study, intestinal parasitic infection on TB patients had statistically significant association with BMI (AOR=6.715; 95% CI=1.655-27.251), which is supported by previous studies (24, 37, 55). In this study intestinal parasitic infection on TB patients had statistically significant association with residence (*p=0.046*). Similarly studies from different parts of Ethiopia showed people who were living in rural areas were at risk of harboring intestinal parasites compared to urban dwellers (9, 37, 58). In the present study those who had dirty material in their finger were nine times as likely to have intestinal parasites infection compared to those who did not have (AOR=8.997; 95% CI=2.469-32.788). This was supported by a study done by Abera *et al.*(59); where 47.9% of students who had dirty material in their finger nails were infected with helminth infection. In the control group there was no variable significantly associated with intestinal parasitic infection. However; in study done at China (24), annual labor time in farmlands > 2 months was the only the risk factor associated with overall infections in healthy controls**.**

In patients with TB, a variety of hematological changes have been described including anemia, and leukocytosis (60). In the present study, the overall mean of Hgb in TB patients was 13.77(±1.75) g/dl and Anemia was detected in 28.6% of males (Hgb<13.0 g/dl) and in 30.3% of females(Hgb<12.5gdl). The Hgb level was higher and the prevalence of anemia is lower in this study in comparing with studies done in India (18, 19, 38 ), in Iraq (17, 61) and in Khartoum (16). This might be due to the population difference, where individuals living in the highlands of Ethiopia were reported having higher level of Hgb due to the eating habit of Injera which has a high iron content (62). And this is observed in a study done on TB patients at Gondar (63). In the present study from all TB patients who had anemia; 53.85% and 46.15% had normocytic normochromic and microcytic hypochromic anemia respectively. This is comparable with previous studies (16, 17, 18). Macrocytic anemia which is not observed in the present study was reported by Banerjee et al (18). Among TB patients included in the present study; 28.1% and 32.6% had lower MCV and MCH values respectively. This is supported by a study done by Shareef *et al*.(17) and by Kassa *et al*.(63). Anemia in TB patients in this study is associated with

female gender ($X^2$=7.321, *p=0.007*) and older age(>40yrs) were mostly affected. This is similar to Mandal *et al*'s (19) finding.

Leukocytosis was observed in 29.2% of (16 males and 10 females) TB patients in the present study, similar to the previous studies (16, 18, 19, 38). But no significant differences wasobserved in TWBC counts, neutrophils, monocytes and lymphocytes between all patients and healthy group in a study done by Shareef *et al*.(17) and no significant change of WBCs after completion of anti-TB treatment was reported from Gondar (63). In this study neutrophilia was observed in 28.1% of PTB patients, and an increase in monocyte was observed in 48.3% of PTB patients supported with a study done at Khartoum(16). In this study a decrease in Lymphocyte count was observed in 16.1% of PTB patients similar to studies done before (15,19). The mean of eosinophil count observed in the present study is comparable with a study done by Mohammed S (16) and by Neto *et al*.(8); but lower compared to a study done by Tristão-Sá *et al*.(27). This might be due to high nematodes prevalence in the study done by Tristão-Sá *et al*.(27). The level of basophils observed in this study is similar to a study done by Mohammed S at Khartoum (16). Thrombocytes have been suggested to play a role in the evolution of inflammatory response against mycobacterium(64). A high mean level of Thrombocytes ((459.25((±150.39)x 103/mm3) and thrombocytosis(72%) was observed in the present study. This is supported by previous studies (16,17, 18,61).

Intestinal parasites affect the nutritional value of individuals (12) and the likely hood of causing anemia is high (58,65). In the present study, low level of Hgb is observed in TB patients coinfected with intestinal parasites compared with TB patients not infected with intestinal parasites (COR=8.333; 95%CI=2.454-28.297). Similar findings are reported by different studies (58,65, 66), even though these studies were done to look on hematological profiles on intestinal parasite infected individuals whom were not TB patients. In the present study a statistically significant decrease in Hgb level in TB patients is observed due to G.*lamblia, A.lumbricoides, T.trichuria* and *C. parvum* infection compared to non infected TB patients in concordance with previous studies (32, 65, 66). As it is well described by different scholars that peripheral eosinophilia is widely recognized as a useful indicator of parasitic diseases mainly of helminthes(35, 67), in the present study the level of eosinophils was comparably higher in helmenthic infected TB patients compared to non infected TB patients which is also reported by studies done before at different settings such as in Gondar (35, 36) and in Brazil (8, 27).

# 7. Strength and Limitation

The strengthes of this study were; no similar published studies in the study area, controls were household contacts, sensitive methods like Xpert MTB/RIF assay and MGIT liquid culture were used to rule out TB for cases and controls and the tests were done in an accredited laboratory(EPHI).

The limitations of this study were; hematological parameters were done only for TB patients, Katho katz technique was not used to assess the parasitic load, nutritional assessments except BMI were not done and HIV antibody testing was not done for controls

# 8. Conclusion

The infection rate of intestinal parasites in TB patients and healthy household contacts had statistically significant difference. The most frequently identified intestinal parasites in TB patients with a statistical significant difference compared to controls were G.*lamblia, A. lumbricoides* and *H.nana*. Multiple parasitic infection was observed in TB patients but not in controls. Intestinal parasitic co-infection on TB patients had significant association with residence, BMI and presence of dirty material in the patient's finger. Variety of hematological changes were observed in TB patients. Moderate anemia was detected in one third of TB patients. Leukocytosis, monocytosis, decrease in lymphocytes and thrombocytosis were observed in PTB patients. Parasitic infections modifies the occurrence of anemia in TB patients. Relatively higher level of eosinophils was observed in intestinal helminthes infected TB patients.

# 9. Recommendations

Generally, based on the findings of the present study, routine stool examination before anti-TB treatment would be important. A large-scale study with diverse population and wide geographical coverage should done.

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# 11. Annex

## Annex I: Consent Forms and Information Sheets

A: Patient information sheet form (English version)

My name is Ayinalem Alemu and I am MSc student in Clinical Laboratory Science (Diagnostic and Public health Microbiology specialty track) at Addis Ababa University College of Health Science, School of Allied Health Sciences, Department of Medical Laboratory Science. I am doing a research on the Co-infection of intestinal parasites and associated factors among active Pulmonary Tuberculosis patients visiting *Kolfe, Wereda* 11 and *Lomi meda* health centers of *Kolfe keraniyo* sub city**.**

**Purpose:** To determine the co-infection rate of intestinal parasites among Active Pulmonary Tuberculosis patients. The outcome can have its own role in the national infection prevention program, which can be very important to design effective mechanism to reduce morbidity and mortality of tuberculosis patients and important to provide guidance on the control and prevention of co-infection of tuberculosis and parasitic diseases. Therefore at the end of the study based on the result found all the necessary recommendations will be forwarded to all responsible bodies.

**Participation:** I am asking you to participate voluntarily in this study; if you agree to participate you will be asked to sign a consent form and response to short questionnaire interview.

**Risks associated:** With this study there are no risks associated during sample collection procedures.

**Benefits:** If there is any positive finding in laboratory examination the result will be reported to your clinician for appropriate treatment and management.

**Confidentiality:** All information you give and data obtained from laboratory analysis will be kept confidential and will be communicated only to responsible figure. Formats containing data will be kept locked.

**Sharing the result:** Report will be written about the finding of the study, either through publication or any other means. The result will not bear any information relevant to your personality in anyway.

Contact Address

If you have any question or doubt you can contact: Ayinalem Alemu

Addis Ababa University College of Health Science, School of Allied Health Sciences, Department of Medical Laboratory Science.

Tel: -+251912366676, E-mail:- ayinalemal@gmail.com

B: የምርምርጥናትመብራሪያ /አማርኛ ቅጂ

አይናለምአለሙእባላለሁ።።

የአዲስአበባዩኒቨርስቲየጤናሳይንስኮሌጅየህክምናላቦራቶሪዲፓርትመንትየክሊኒካልላቦራቶሪሳይንስ (የምርመራእናየማህበረሰብጤናየሕክምናማይክሮባዮሎጂ)

የማስተርስዲግሪማለትምየ2ኛአመትየማስተርስዲግሪተማሪነኝ።።

የሳንባቲቢእናየሆድትላትሎችስርጭትለማወቅእናአንዱበአንዱላይየሚያመጣዉንተፅዕኖለማወቅየሚካሄድ ጥናትነዉ።።

<u>የጥናቱተሳታፊዎችየመረጃቅጽ</u>

ሀ.<u>የጥናቱዓላማ፡-</u>

የሳንባቲቢስርጭት&የሆድትላትሎችአብሮየመገኘትመጠንእናእርስበራሳቸዉየሚያሳድሩትንጫናለማወቅየ ሚደረግጥናትነው።።

ለ.<u>ፈቃደኝነት፡-</u>

እርስዎንበጥናቱበሙሉፍቃደኝነትእንዲሳተፉእየጠየቅንበጥናቱላይለመሳተፍፍቃደኛከሆኑለሚቀርብሉዎት ንመጠይቅምላሽከሰጡበኋላየአክታናሙና&የሰገራናሙናእናየደምነሙናእንዲሰጡይጠየቃሉ::

ሐ.<u>የሚያገኙትጥቅም</u>፡-

በሽታአምጪተህዋሳያንበላቦራቶሪመኖራቸውከተረጋገጠጥተገቢውንህክምናእንዲያገኙየላቦራቶሪወጤትወ ደሀኪምተልኮተገቢዉንክትትልያገኛሉ።።

መ.<u>የሚያሰከትለዉጉዳት</u>፡-

በዚህጥናትበመሳተፍዎበእርስዎላይየሚያስከትለዉችግርየለምበጣምይጠቀማሉ።።

ሠ. <u>ሚስጥራዊነት</u>፡-የእርስዎየግልመረጃበሙሉሚስጥራዊነቱየተጠበቀይሆናል።።

ረ.<u>ውጤቱንስለመጠቀም</u>፡-ከዚህጥናትበኋላየበሽታውንስርጭትበተመለከተሪፖርትይፃፋል።።

ሆኖምየእርስዎንማንነትየሚገልፅመረጃየማይካተትሲሆንችግሩንለማሳወቅብቻየሚውልነው።።

አድራሻ

ማንኛውምጥያቄወይምጥርጣሬካለዎትይህንንአድርሻይጠቀሙ፡

የዋናውተመራማሪአድራሻ፡ -አይናለምአለሙ

አዲስአበባዩኒቨርስቲየጤናሳይንስኮሌጅየህክምናላቦራቶሪዲፓርትመንት፡አዲስአበባዩኒቨርስቲ

ሞባይል- +251912366676 ኢሜል ayinalemal@gmail.com

C. Adult Consent form

Your signature below indicates that you have read or listened to and understand the information provided to you about the study. Before you sign, please confirm that you understand the following:

Purpose of the study

Study procedures, including

Interview

Sputum sample

Blood sample

Stool sample

Process for feedback of results, if needed

Risks and benefits of participating in the study

Right to refuse or stop participation at any time

Confidentiality and privacy concerns

Who to contact if you have questions

By signing, you are making a decision to participate in this study. If you decide that you wish to withdraw or discontinue your participation in the study, you may do so at any time. Do you agree to participate?

I have read and/or listened to the description of the study and I understand what the procedures are and what will happen to me in the study. I agree to participate in it. I know that I can quit the study at any time.

_____ _____ _____

Printed Name Signature Date

_____ _____

Signature of Investigator representative Date

D. የአዋቂወች የስምምነት ቅፅ በአማርኛ

ሰለ ጥናቱ የተሰጡት ኢንፎርሜሽን እንዳነበቡ ወይም ግንዛቤ እንዳገኙ የሚከተለዉ ፊርማዎ ያመለክታል።

ከመፈረምዎ በፊት የሚከተሉትን መገንዘበዎን ያረጋግጡ ፡ -

የጥናቱ ዓላማ

የጥናቱ የሚከተሉትን ቅደምተከተሎች

ቃለ መጠየቅ

የአክታ ምረመራ

የደም ምረመራ

የሰገራ ምረመራ

ስለዉጤት ግብረመልስ አስፈላጊ ከሆነ

በጥናቱ በመሳተፍዎ ያሉትን ጥቅሞች እና ጉዳቶች

በፈለጉት ጊዜ ጥናቱን ማቆም እንደሚችሉ

ምስጢራዊነቱን

ለጥያቄ ማንን ማግኘት እንደሚችሉ

በጥናቱ ለመሳተፍ መወሰንዎን በመፈረም ያረጋግጣሉ' ላለመሳተፍ ወይም ለማቋረጥ ከወሰኑ በማንኛዉም ጊዜ ይችላሉ' የጥናቱ አካል ለመሆን ወስነዋል?

በተሰጠኝ ገለፃ መሰረት በጥናቱ ሂደት በእኔ ላይ ሊደረጉ የሚችሉ ኹነቶችን ተረድቻለሁ' በጥናቱ ለመሳተፍ ወስኛለሁ' በፈለኩት ጊዜ ማቋረጥ እንደምችል ተገንዝቢያለሁ'

_____ _____ _____

ሙሉ ስም ፊርማ ቀን

_____ _____

የተወከለ መርማሪ ፊርማ ቀን

E. Parental Consent Form for the Participation of Children aged <18

Your signature below indicates that you have read or listened to and understand the information provided to you about the study. Before you sign, please confirm that you understand the following:

Purpose of the study

Study procedures, including

Interview

Sputum sample

Blood sample

Stool sample

Process for feedback of results, if needed

Risks and benefits of participating in the study

Right to refuse or stop participation at any time

Confidentiality and privacy concerns

Who to contact if you have questions

By signing, you are making a decision to allow your child (son/daughter/ child/infant/adolescent youth) to participate in this study. If you decide that you wish your child to withdraw or discontinue participation in the study, you may do so at any time. Do you agree for your child to participate?

I have read and/or listened to the description of the study and I understand what the procedures are and what will happen to my child in the study. I agree to allow him/her to participate in it. I know that my child can quit the study at any time.

_____

Printed Name of (son/daughter/child/infant/adolescent youth)

_____ _____

Signature of Parent(s) or Legal Guardian Date

_____ _____

Signature of Investigator representative Date

F. ከ 18አመት በታች ላሉ ልጆች የወላጆች የስምምነት ቅፅ በአማርኛ

ሰለ ጥናቱ የተሰጡት ኢንፎርሜሽን እንዳነበቡ ወይም ግንዛቤ እንዳገኙ የሚከተለዉ ፊርማዎ ያመለክታል።

ከመፈረምዎ በፊት የሚከተሉትን መገንዘበዎን ያረጋግጡ ፡ -

የጥናቱ ዓላማ

የጥናቱ የሚከተሉትን ቅደምተከተሎች

ቃለ መጠየቅ

የአክታ ምረመራ

የደም ምረመራ

የሰገራ ምረመራ

ስለዉጤት ግብረመልስ አስፈላጊ ከሆነ

በጥናቱ በመሳተፍዎ ያሉትን ጥቅሞች እና ጉዳቶች

በፈለጉት ጊዜ ጥናቱን ማቆም እንደሚችሉ

ምስጢራዊነቱን

ለጥያቄ ማንን ማግኘት እንደሚችሉ

በጥናቱ ለመሳተፍ መወሰንዎን በመፈረም ያረጋግጣሉ' ላለመሳተፍ ወይም ለማቋረጥ ከወሰኑ በማንኛዉም ጊዜ ይችላሉ' የጥናቱ አካል ለመሆን ወስነዋል?

በተሰጠኝ ገለፃ መሰረት በጥናቱ ሂደት በእኔ ላይ ሊደረጉ የሚችሉ ኹነቶችን ተረድቻለሁ' በጥናቱ ለመሳተፍ ወስኛለሁ' በፈለኩት ጊዜ ማቋረጥ እንደምችል ተገንዝቢያለሁ'

_____ _____ _____

የልጅ ሙሉ ስም የወላጅ ወይም የህጋዊ አሳዳጊ ፊርማ ቀን

_____ _____

የተወከለ መርማሪ ፊርማ ቀን

G. Assent form for child between <18 years of age

I have read and/or listened to the description of the study and I understand what the procedures are and what will happen to me in the study. I have received permission from my parent(s)/guardian(s) to participate in the study, and I agree to participate in it. I know that I can quit the study at any time.

_____ _____

Signature of Child Date

_____ _____

Signature Investigator representative Date

H. ከ18 ዓመት በታች ላሉ ልጆች የስምምነት ቅፅ

በተሰጠኝ ገለፃ መሰረት በጥናቱ ሂደት በእኔ ላይ ሊደረጉ የሚችሉ ኹነቶችን ተረድቻለሁ' በጥናቱ ለመሳተፍ ከወላጆቸ/አሳዳጊዎቸ ፈቃድ አግኝቻለሁ ስለዚህ ለመሳተፍ ፈቃደኛ ነኝ' በጥናቱ ለመሳተፍ ወስኛለሁ' በፈለኩት ጊዜ ማቋረጥ እንደምችልም ተገንዝቢያለሁ'

_____ _____

ፊርማ/የልጅ ቀን

_____ _____

የተወከለ መርማሪ ፊርማ ቀን

## Annex II. Questionnaire

Questionnaire (English Version)

Addis Ababa University College of Health Science School of Allied Health Sciences Department of Medical Laboratory Sciences for the study of Intestinal parasites Co-infection among active Pulmonary Tuberculosis patients visiting selected health centers of *Kolfe Keraniyo* sub city (*Kolfe, Wereda* 11 and *Lomi meda*), Addis Ababa, Ethiopia, from 01 Jan 2017 to 30 Jan 2018. I request kindly to give appropriate response for each question. Your response will be kept confidential

Patient Code (to be assigned by the investigator); _____

Data collection date: ———————————

Data collector name and signature: _____

I. Socio demographic and economic data

1. Gender: A. Male B. Female

Age:_____

Residency: A. Urban B. Rural

Occupation: A. Government B. Merchant C. House wife D. Student E. Other

Monthly income: A. Lowest B. Second C. Middle D. Fourth E. Highest

Educational status: A. Illiterate B. Primary complete C. Secondary complete D. Diploma and above

Marital status: A. Single B. Married C. Divorced D. Widowed

Family size: A. <4 B.5-7 C. 8-10 D. >10

BMI: _____

BCG vaccination: A. Yes B. No

In haw many rooms do you live?_____

Behavior of the study participants

Do you have a contact history of TB patient: A. Yes B. No

Do you have a chronic cough for the last two weeks: A. Yes B. No

Do you have a past history of PTB: A. Yes B. No

Have you ever smoked cigarette in the past one year? A. Yes B. No

If yes to question 4, how much cigarette do you smoke per day?

A. (Specify) _____ B. I don't remember C. Unwilling to tell

Have you ever drunk alcoholic beverage in the last one year? A. Yes B. No

If yes to Question 6, what type of Alcohol and how much do you consume per day?

A. Type and amount: _____ B. I don't remember C. Unwilling to tell

Have you ever been imprisoned in the past one year? A. Yes B. No Unwilling to tell

If yes to question 8, for how long? _____

If yes to question 9, was there any TB patient/chronically coughing person in the cell where you were staying? A. Yes B. No

Dou you use a latrine: A. Yes B. No

Do you have a habit of swimming: A. Yes B. No

If the answer in question12 is yes, what was the frequency of swimming per week? A. 1-2 days B. ≥3 days

Do you have a habit of shoe wearing? A. Yes B. No

If the answer in question14 is yes, what was the frequency of wearing shoe?

A. Always B. Sometimes

What was the habit of bath? A. Home B. River C. Home and River

Do you have the habit of hand washing before food? A. Yes B. No

If the answer in question17 is yes, what was the frequency of hand washing?

A. Sometimes B. Always

Do you have the habit of hand washing after defecation? A. Yes B. No

If the answer in question 19 is yes, what was the habit of hand washing?

A. With water only B. With water and soap

What is the source of water for drinking? A. Tap B. River C. Tap and River

What is the habit of washing cloth? A. Home B. River C. Home and River

Is there a dirty material in the finger? A. Yes B. No

Is there a habit of eating unwashable vegetables? A. Yes B. No

Is there a habit of eating raw meet? A. Yes B. No

Is there a raised poultry or livestock? A. Yes B. No

Thank you!

Name of supervisor:_____ Signature and Date:_____

ቃለ መጠይቅ በአማርኛ

በአዲስ አበባ ዩኒቨርስቲ የጤና ሳይንስ ኮሌጅ በህክምና ላቦራቶሪ ትምህርት ክፍል በሳምባ ቲቢ ተጠቅተው በኮልፌ ቀራንዮ ክፍለ ከተማ ኮልፌ÷ ወረዳ 11 እና ሎሚ ሜዳ ጤና ጣቢያ ጣቢያዎችን ጥር 01 2009 ዓም እስከ ጥር 30 2010 ዓም ለሚጎበኙ ታካሚዎች የሳምባ ቲቢ እና የሆድ ትላትሎች አብሮ የመገኘት መጠን እና ተዛማጅ ችግሮችን ለማወቅ የሚደረግ ጥናት ሲሆን ተሳታፊ በመሆንዎ እያመሰገን ለእያንዳንዱ ጥያቄ ተገቢዉን መልስ እንዲሰጡ በትህትና እንጠይቃለን' ምስጢራዊነቱ የተጠበቀ ነዉ'

የታካሚ መለያ በመርማሪዉ የሚሰጥ፡- _____

መረጃ የተሰበሰበበት ቀን፡- _____

የመረጃ ሰብሳቢ ስም እና ፊርማ፡- _____

የማህበራዊ እና የዲማግራፊ መረጃ

ጾታ: ሀ. ወንድ ለ. ሴት

ዕድሜ_____

ነዋሪነት: ሀ. ከተማ ለ. ገጠር

የስራ ዘርፍ: ሀ. የመንግስት ለ. ነጋዴ ሐ. የቤት እመቤት መ. ተማሪ ሠ. ሌላ

ወርሃዊ የገቢ መጠን: ሀ. ዝቅተኛ ለ. መጠነኛ ሐ. መካከለኛ መ. ከፍተኛ ሠ. እጅግ ከፍተኛ

የትምህርት ደረጃ: ሀ. ያልተማረ ለ. አንደኛ ደረጃ ያጠናቀቀ ሐ. ሁለተኛ ደረጃ ያጠናቀቀ መ. ዲፕሎማ እና ከዚያ በላይ

የጋብቻ ሁኔታ: ሀ. ያላገባ/ች ለ. ያገባ/ች ሐ. የፈታ/ችመ. ባሏ/ሚስቱ/የሞተባት/የሞተችበት

የቤተሰብ መጠን: ሀ. <4 ለ. 5-7 ሐ. 8-10 መ. >10

የሰዉነት ክብደት መጠን/ BMI_____

የቢሲጅ ክትባት ተከትበዋል: ሀ. አዎ ለ. አልተከተቡም

በስንት ክፍል ዉስጥ ይኖራሉ?_____

ስለ ጥናቱ ተሳታፊዎች ባህሪ

ከሳንባ ቲቢ ታማሚዎች ጋር የቀረበ ግንኙነት ነበርዎት? ሀ. አዎ ለ. የለም

ለሁለት ሳምንት እና ከዚያ በላይ የሚሆን ሳል ነበርዎት? ሀ. አዎ ለ. የለም

ከዚህ በፊት የሳንባ ቲቢ ታመዉ ያቃሉ? ሀ. አዎ ለ. የለም

ባለፈዉ አንድ አመት ዉስጥ ሲጋራ አጨሰዉ ያቃሉ? ሀ. አዎ ለ. የለም

ለጥያቄ ቁጥር4መልስዎ አዎ ከሆነ& በቀን ዉስጥ ስንት ሲጋራዎችን ያጨሳሉ? _____

ሀ. ይግለፁ ለ. አላስታዉስም ሐ. መናገር አልፈልግም

ባለፈዉ አንድ አመት ዉስጥ አልኮል ጠጥተዉ ያቃሉ? ሀ. አዎ ለ. የለም

ለጥያቄ ቁጥር 6 መልስዎ አዎ ከሆነ& ምን አይነት የአልኮል መጠጥ እና በቀን ዉስጥ ምን ያህል ይጠቀሙ ነበር? ሀ. አይነት እና መጠን ይግለፁ ለ. አላስታዉስም ሐ .መናገር አልፈልግም

ባለፈዉ አንድ አመት ውስጥ እስር ቤት አሳልፈዉ ያቃሉ? ሀ. አዎ ለ. የለም

ለጥያቄ ቁጥር 8 መልስዎ አዎ ከሆነ ለስንት ጊዜ?_____

ለጥያቄ ቁጥር 9 መልስዎ አዎ ከሆነ እርስዎ በነበሩቤተ ክፍል ዉስጥ የቲቢ ህመምተኛ ነበር?

ሀ. አዎ ለ. የለም

ሽንት ቤት አለዎት? ሀ. አዎ ለ. የለም

የመዋኘት ልምድ አለዎት? ሀ. አዎ ለ. የለም

ለጥያቄ ቁጥር 12 መልስዎ አዎ ከሆነ በሳምንት ለስንት ጊዜ ይዋኛሉ?ሀ. 1-2 ቀን ለ. ≥3 ቀን

ጫማ የማድረግ ልምድ አለዎት? ሀ. አዎ ለ. የለም

ለጥያቄ ቁጥር 14 መልስዎ አዎ ከሆነ ልምድዎ ምን ይመስላል? ሀ. ሁልዚዜ ለ. አልፎአልፎ

ገላዎን የሚታጠቡት የት ነው? ሀ. ቤት ዉስጥ ለ. ወንዝ ሐ. ቤት እና ወንዝ

ከመመገብዎ በፊት እጅዎን ይታጠባሉ? ሀ. አዎ ለ. የለም

ለጥያቄ ቁጥር 17 መልስዎ አዎ ከሆነልምድዎ ምን ይመስላል?ሀ. ሁልዚዜ ለ. አልፎአልፎ

ከሽንት ቤት መልስ እጅዎን ይታጠባሉ? ሀ. አዎ ለ. የለም

ለጥያቄ ቁጥር 19 መልስዎ አዎ ከሆነ በምን ይታጠባሉ? ሀ. በውሃ ብቻ ለ. በውሃ እና በሳሙና

የሚጠጣ ውሃ ከየት ነዉ የሚጠቀሙት? ሀ. የቧንቧ ለ. የወንዝ ሐ.የቧንቧ እና የወንዝ

ልብስ የሚያጥቡት ከየት ነዉ? ሀ. ቤት ለ. ወንዝ ሐ. ቤት እና ወንዝ

ጥፍር ዉስጥ ቆሻሻ አለ? ሀ. አዎ ለ. የለም

ያልታጠበ አትክልት የመመገብ ልማድ አለዎት? ሀ. አዎ ለ. የለም

ጥሬ ስጋ ይመገባሉ? ሀ. አዎ ለ. የለም

እንስሳት ከቤት ዉስጥ ያረባሉ? ሀ. አዎ ለ. የለም

እናመሰግናለን!

የተቆጣጣሪ ስም፡-_____ፊርማእና ቀን፡-_____

# Annex III. Laboratory Data Recording Format

## Laboratory result AFB/ FM/ staining

Date specimen received: ____/___/___ (Ethiopian Calendar)

Laboratory Number: _____

Microscopic examination result

| IUATLD/WHO SCALE (1000x field =HPF) Result | MICROSCOPY SYSTEM USED | | |
|---|---|---|---|
| | **BRIGHTFIELD (1000x magnification; one length = 2cm = 100 HPF)** | **FLUORESCENCE (200-250x magnification; one length = 30 fields = 300 HPF)** | **FLUORESCENCE (400x magnification; one length = 40 fields = 200 HPF)** |
| **Negative** | Zero AFB / 1 length | Zero AFB / 1 length | Zero AFB / 1 length |
| **Scanty** | 1-9 AFB / 1 length or 100 HPF | 1-29 AFB / 1 length | 1-19 AFB / 1 length |
| **1+** | 10-99 AFB / 1 length or 100 HPF | 30-299 AFB / 1 length | 20-199 AFB / 1 length |
| **2+** | 1-10 AFB / 1 HPF on average | 10-100 AFB / 1 field on average | 5-50 AFB / 1 field on average |
| **3+** | >10 AFB / 1 HPF on average | >100 AFB / 1 field on average | >50 AFB / 1 field on average |

| Negative | Positive | | | |
|---|---|---|---|---|
| | 1 – 9 | 1+ | 2+ | 3+ |
| | | | | |

Comment: _____

_____

_____

Date reported:____/____/____ Name and Signature of expert _____

Reviewed by: _____

**Laboratory result for TB Culture result**

Date specimen received: ____/___/___ (Ethiopian Calendar)

Laboratory Number: _____

<table>
<tr><td rowspan="4">Contaminated</td><td rowspan="4">Negative</td><td colspan="4">Positive</td></tr>
<tr><td colspan="4">Mycobacterium tuberculosis</td></tr>
<tr><td>1-9 colonies</td><td>10-99 colonies</td><td>More than 100 colonies</td><td>Confluent growth</td></tr>
<tr><td>Actual Count</td><td>1+</td><td>2+</td><td>3+</td></tr>
</table>

Comment: _____

_____

_____

Date reported:____/____/____ Name and Signature of expert _____

Reviewed by: _____

**Laboratory result for stool examination**

Date specimen received: ____/___/___ (Ethiopian Calendar)

Laboratory Number: _____

Result:

_____

_____

Comment: _____

_____

Date reported:____/____/____ Name and Signature of expert _____

Reviewed by: _____

**Laboratory result for hematological parameters**

Date specimen received: ____/___/___ (Ethiopian Calendar)

Laboratory Number: _____

Result(***attach the print out):***

_____

_____

Comment: _____

_____

Date reported:____/____/____ Name and Signature _____

Reviewed by: _____

## Annex IV. Laboratory Procedures

**1. Procedure for Ziehl-Neelsen staining technique**

- Place the slides with smear upwards on the staining rack over a sink about 1 cm apart
- Add 1% carbol-fuchsin staining solutions over the smears
- Prepare the torch by dipping its cotton wool end in burning spirit and light it
- Heat all slides keeping the torch a little below them until steam arises
- Repeat it twice at intervals of 3-5 minutes
- Do not let staining solution dry on the slides
- Tilt each slide using forceps to drain off the staining solution
- Rinse the slides well with clean water from a beaker
- Pour 3% acid solution over the smears covering them completely
- Allow to act for 3 minutes
- Tilt each slide with forceps to drain off the acid
- Gently rinse each slide again with clean water
- If needed, repeat until all macroscopically visible stain has been washed away
- Flood smear with 0.1% methylene blue solution for 1 minute
- Tilt each slide with forceps draining off the methylene blue solution

- Wash with clean water
- Using forceps take the slides from the rack
- Let drain off the water and stand the slide on edge to dry at the air on the drying rack always keep smears out of direct sunlight

Reading

Use the objective 100x apply one drop of synthetic immersion oil to the left edge of the stained smear. Scan the stained smear systematically from left to right side, covering one length (100 to 150 microscopic high-power fields). This is the minimum to be scanned before reporting a negative result. Place the slide smear-down on a piece of absorbent paper after examination clean the objective lens at the end of each day using lens or soft tissue

Result Interpretation

Recording

| | |
|---|---|
| Negative- | No AFB found in at least 100 fields |
| Exact figure/100- | 1 to 9 AFB per 100 fields |
| 1+- | 10 to 99 AFB per 100 fields |
| 2+- | 1 to 10 AFB per field (count at least 50 fields) |
| 3+- | More than 10 AFB per field (count at least 20 fields) |

**2. Procedure for Auramine staining technique**

- Place the slides on the staining rack over a sink and keep distance between every slide at least 1cm.
- Cover the smears completely with auramine solution
- Do not heat
- Leave for 20 minutes
- Wash the slides well with distilled or running water
- Pour the acid solution over them
- Allow to act for 3 minutes

- Gently rinse each slide again with distilled or tap water
- Flood smear with potassium permanganate or ink blue solution for 1 minute.
- Wash off with distilled or running water
- Stand the slide on edge to drain, and air dry on the slide rack out of strong light

keep stained smears in the dark (box or folder) till reading, and read as soon as possible since fluorescence fades quickly

Result Interpretation

Reading

Use the objective 20-25x for scanning and 40x for confirmation..One length has to be scanned before reporting a negative, corresponding to 300-200 high-power fields and taking 1-2 minutes (20x – 40 x objectives).'Acid-fast bacilli appear bright yellow against the dark background material.

Recording

| IUATLD/WHO SCALE (1000x field =HPF) Result | MICROSCOPY SYSTEM USED | | |
|---|---|---|---|
| | **BRIGHTFIELD (1000x magnification; one length = 2cm = 100 HPF)** | **FLUORESCENCE (200-250x magnification; one length = 30 fields = 300 HPF)** | **FLUORESCENCE (400x magnification; one length = 40 fields = 200 HPF)** |
| **Negative** | Zero AFB / 1 length | Zero AFB / 1 length | Zero AFB / 1 length |
| **Scanty** | 1-9 AFB / 1 length or 100 HPF | 1-29 AFB / 1 length | 1-19 AFB / 1 length |
| **1+** | 10-99 AFB / 1 length or 100 HPF | 30-299 AFB / 1 length | 20-199 AFB / 1 length |
| **2+** | 1-10 AFB / 1 HPF on average | 10-100 AFB / 1 field on average | 5-50 AFB / 1 field on average |
| **3+** | >10 AFB / 1 HPF on average | >100 AFB / 1 field on average | >50 AFB / 1 field on average |

## 3. Procedure of sputum culture

### I. Preparation of Sputum Processing Reagents for TB Culture

1. Preparation of Lowenstein Jensen (LJ) Media

Dissolve in the following order:

- Mon potassium phosphate (anhydrous)……2.4 g
- Magnesium sulfate- $7H_20$…….…….0.24 g
- Magnesium Citrate…….……0.6 g
- Asparagine……..3.6 g
- Glycerol (reagent grade)…….12.0 ml
- Distilled water…….600.0 ml
- Potato Flour……..….....30.0 g
- Autoclave at 121ºC for 30 minute
- Cool the autoclaved salt solution to room temperature
- Add malachite green (2%) aqueous prepared fresh…….20.0 ml
- Add homogenized filtered eggs….....1000 ml
- Mix well to minimize bubbles, let the liquid mixture stand for 30 minutes prior to the tubing of the medium
- Dispense approximately 6 to 8 ml of media into each 20 x 150 mm tube.
- Ensure all glassware and caps have been autoclaved to ensure sterility before dispensing
- Keep all media in a dark and humid place preferably in the refrigerator (2-8º C)
- If LJ is prepared from fresh ingredients and stored at 2-8º C. then expected expiration is usually 6 months.

**II. TB Specimen Processing and Inoculation of LJ culture medium**

| | Action | Remarks |
|---|---|---|
| 1 | Login Specimens | |
| 2 | TB processing checklist to collect supplies, reagents, and specimens. Prepare bio safety | Disinfect BSC and perform daily maintenance<br>Label tubes and media<br>Set up BSC for specimen processing with racks of reagents, waste |

| | | |
|---|---|---|
| | cabinet(BSC) for work | containers, transfer pipettes, and slides |
| 3 | Prepare reagents for use | Prepare 3% NaOH-NaCitrate-NALC – Add 0.25g of NALC to 50 ml aliquots of NaOH-Citrate. |
| 4 | Sort specimens into batches with sterile water blanks as the first and last specimens | Batch size is based on the centrifuge load, minus two for the negative processing controls |
| 5 | Place specimens and 50 mL conical tubes in BSC | Place empty 50 ml conical tubes in a rack |
| 6 | Label processing tubes with lab serial number Transfer specimen into labeled processing tube | Transfer up to 5 mL of specimen to the 50 mL conical or processing tube.<br>Discard specimen cup in waste container<br>Repeat for all specimens |
| 7 | Add equal volume of NaOH-Citrate-NALC solution to each processing tube | Remove and discard the cap from a 3% NaOH-Citrate-NALC aliquot tube. Pick up the first processing tube with the left hand, using the pinky method remove the cap. Look at volume of specimen in tube and pour an equal volume of 3% NaOH-NALC solution with the right hand into the processing tube. Hold the processing tube at a slant and pour solution without touching tubes. Replace cap on processing tube. Discard the 3% NaOH-NALC tube.<br>Vortex processing tube at a slow speed for about 5 seconds or until homogeneous, replace in rack. Start timer for 15 minutes Repeat for all specimens Invert all specimens again after 8 minutes have elapsed |
| 8 | Pour PBS into | Remove cap on PBS aliquot tube, discard hold the processing tube in the left |

| | | |
|---|---|---|
| | processing tube to 45ml mark | hand, remove cap on processing tube with the right pinky using the pinky method. Hold the processing tube at a slant and slowly pour PBS into the tube with the right hand to the 45ml mark without touching or splashing. Replace cap on processing tube, tighten, slowly invert tube about 4 times to mix and place tube in rack. Discard PBS aliquot tube. Repeat for other specimens |
| 9 | Centrifuge tubes at 3000 g, 4ºC for 15 minutes | Place centrifuge safety cups in BSC. Remove safety cap and place tubes in cup- balance tubes if the cup is not full<br><br>Replace safety cap on cup and tighten- DO NOT over tighten Wipe off outside of safety cups with disinfectant soaked cotton Place cups on trolley and take to centrifuge.<br><br>Place cups inside the centrifuge .Close the door Check settings on centrifuge Brake set at zero |
| 11 | Open centrifuge cups in BSC | Remove safety cups from centrifuge. Place on trolley and carefully move without agitation to BSC- bench liner with disinfectant. Remove safety cap inside BSC. Lift up insert and check tubes for leakage. No leakage - remove tubes and place in rack. |
| 12 | Pour off supernatant<br><br>Add 2 ml PBS | Hold tube in right hand, remove cap on processing tube with left hand and hold in pinky. Carefully pour the supernatant into the liquid discard container. Wipe rim of tube with a disinfectant soaked cotton. Be careful- do not put the disinfectant inside the tube. Place tube in rack. Make a 2x3 cm smear from the sediment by dropping 1drop on the slide and spreading.<br><br>Remove cap from PBS tube and discard. Remove ~2 ml of PBS with a transfer pipette. Carefully express PBS in transfer pipette into processing tube. Discard transfer pipette. Replace cap on processing tube. |

| | | |
|---|---|---|
| 13 | Inoculate media | Vortex capped processing tube 5 seconds.<br><br>Remove adequate LJ culture media from the refrigerator the day before in order to reduce the amount of liquid at the bottom of the slant. Loosen caps of the LJ tube; use 1 LJ tube for one specimen.<br><br>For MGIT before inoculation add 0.8ml of reconstituted PANTA to 7 ml MGIT tube and use one tube for one specimen<br><br>Remove transfer pipette from package. Pick up cap on processing tube with left hand .Aspirate 1 ml of sample with transfer pipette with right hand. Keep transfer pipette in an upright position and hold with a steady pressure on bulb.<br><br>Replace cap on processing tube- do not try to tighten at this time. Inoculate 0.5 ml to 7ml MGIT tube and pick up LJ with the left hand; use the pinky method to remove the cap with right pinky. Hold the LJ slant at an angle. Dispense 2-4 drops on surface. Replace the cap and place the tube back in rack. Pick up lid on blood plate and place one drop on surface. Replace lid. Place one drop of specimen on slide. Spread inoculums by gentle rolling over the LJ slant below the shoulder of the tube and mix inoculated MGIT tube 4 times by inverting. Store residual sediments at -20°C for further or repeat testing. |
| 14 | Clean the BSC | Wipe down culture media with disinfectant and remove from BSC. Fold bench liner and discard. Wipe down all surfaces in the BSC with disinfectant. |

3. Incubation of LJ and MGIT culture media
   - Slightly loosen caps of inoculated LJ tubes in BSC
   - Incubate LJ tubes in slanted position at 35-37˚C for 48 hours to ensure even distribution of inoculum
   - After 48 hours tighten the caps

- Incubate LJ slants in upright position at 35-37°C for up to 8 weeks and for positives do smear
- For liquid media load to MGIT 960 instrument and wait until the instrument flags (+) or (-). For negatives it takes 42 days.
- After being positive in the MGIT instrument unload the positive tubes and do the MGIT work up(inoculate on BAP/incubate for 48 hrs at 37°c and do smear). If there is no growth in BAP and smear positive/cording proceed to identification test.

4. Antigen detection TB rapid test **"**SD BIOLINE TB Ag MPT 64®"

Can be used for isolates from both solid culture and liquid culture. It has internal control. It is Immunochromatographic assay, detects MPB64 antigen, secreted specifically by members of the MTBC. Monoclonal antibody technology monoclonal antibody labeled by colloidal gold particles reacts with MPB64 antigen in sample to form antigen-antibody complex. Complex is then captured by a second monoclonal antibody fixed in the middle of the test zone .Results available within 15 minutes

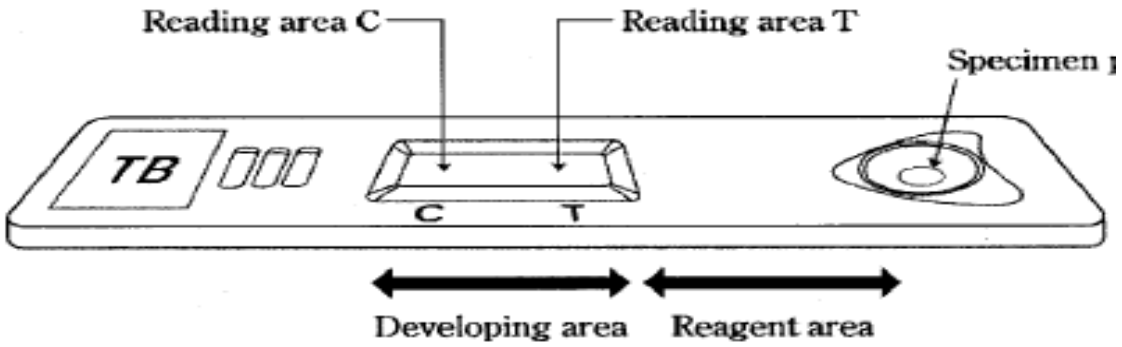
<u>For LJ</u>

- Label test cartridge
- Take 3 to 4 colonies by using sterile loop and resuspend in 200µl buffer and vortex
- Add 100µl to SD Bioline sample port
- Examine the reading area of the cartridge after 15 minutes

<u>For MGIT</u>

- Vortex MGIT tube for 1 minute and wait for 15 minutes
- Label test cartridge
- Take 100µl from the supernatant and to SD Bioline sample port
- Examine the reading area of the cartridge after 15 minutes

Note: Test results must be interpreted no later than 60 minutes after inoculation of the test cartridge due to the inoculums drying, which may alter results




Interpretation of Results

Positive: formation of a purple to red line on the reading areas labeled [T] and [C] of the cartridge

Negative: formation of a purple to red line on the reading area labeled [C] of the cartridge but not [T]

Invalid; if no line is observed on the reading area [C], technical errors or product damage has occurred. In this case the test should be considered invalid and repeated using a new cartridge

**4.Procedure for Complete Blood Cell Count (CBC) by using XT- 1800i**

Specimen

Collected in EDTA anticoagulant. Follow the manufacturer's guidelines regarding collection and stability.

Mixed well before processing.

Fresh whole blood specimens are recommended (process within eight hours after collection).WBC size distribution can shift if specimens are tested within the first 20 minutes following collection or more than eight hours after collection. A minimum of 50µL must be collected for micro-collection specimens. This ensures an adequate amount of blood for the 30 µL aspiration.

Cause for rejection

- Hemolysis
- Clotted specimen
- specimen diluted with IV fluid

- Tube not filled with minimum volume
- Improperly labeled specimen.

Entering and Running Patient Specimen

Note: prior to running patient specimens, perform daily start-up procedures

- When the READY message is displayed on the run screen, the instrument is ready to run specimens.
- Entering specimen ID
- Manual entry
- From RUN screen, press [SPECIMEN TYPE]
- In the SPECIMEN TYPE screen, press [PATIENT SPECIMEN]
- The cursor is placed in the <NEXT ID #> entry field. Use the alphanumeric keys on the PC keyboard to enter a specimen ID of up to 16 characters.
- Bar code scanner entry
- While in the RUN screen, hold the bar code scanner two to five inches away from the bar code labeled patient specimen tube, and aim directly at the bar code.
- Squeeze the trigger handle on the underside of the scanner to activate the red light beam. Aim the beam to scan horizontally across the entire bar code length. A successful scan will be indicated by an audible tone.
- Running patient specimen
- To run patient specimens, proceed as follows:
- With the cap tightly secured on the specimen tube, slowly invert the tube 10 to 15 times.
- Remove the cap from the pre-mixed specimen tube.
- Place the tube under the aspiration probe and raise tube so that the end of the probe is deeply immersed in the specimen.
- Press the touch plate to aspirate the run.
- When the sample has been aspirated from the tube, the probe will move up through the wash block. Remove the specimen tube and replace the cap.
- After the cycle is completed, run results are displayed on screen and the aspiration probe moves into position to accept a new specimen. The current run data is saved to the Data Log.

- If Automatic Graphics printout has been specified in the SETUP menu, a report is printed according to the parameters selected during the setup procedure.
- If Automatic Graphics printout has not been specified in the SETUP menu, press [PRINT REPORT] to obtain a copy of the results.

NOTE: if a system has been idle for 15 minutes or more, a normal background should be run immediately prior to running patient specimens

**5. Procedures for stool examination**

**A. Direct saline and iodine mount (wet preparation)**

- Place one drop of normal saline or iodine on a clean slide
- Take 50 mg of faeces with an applicator stick and mix.
- Make a uniform thin suspension and covered with cover slip.
- Look for the presence of helminth ova and larvae or protozoan cysts and trophozoites

**B. Formalin saline stool preservation**

- Add 7 ml formalin saline to a clean 15 ml conical centrifuge tube
- Place 1 g of faeces
- Dissolve and vortex
- Store until transport

D. **Formalin-ether concentration technique (FEC)**

- Add 7 ml formalin saline to a clean 15 ml conical centrifuge tube
- Place 1 g of faeces
- Dissolve and vortex
- Filter suspension through a sieve into a beaker and pour the filtrate back into the same tube
- Discarded the debris trapped on the sieve.
- Add 3 ml of diethyl ether to the formalized solution
- Centrifuge at 3,200rpm for 3 minutes.
- Decant the supernatant
- Made iodine stain preparation or use for modified Ziehl-nelseen staining method

- Examine systemically

**E. Modified Ziehl-NeelsenMethod**

- Make faecal smears either directly from the stool sample or from the concentration deposit.
- Allow to air dry.
- Fix in methanol for 3 minutes.
- Stain with strong carbol fuchsin for 15-20 minutes.
- Rinse thoroughly in tap water.
- Decolorize in acid alcohol (1% HCl in methanol) for 15-20 seconds.
- Rinse thoroughly in tap water.
- Counterstain with 0.25% malachite green (or methylene blue) for 30-60 seconds.
- Rinse thoroughly and air dry.
- Examine using x40 and x100 objectives.

Declaration:

I the undersigned, declare that this thesis is my original work and has never been presented for the degree in any other university and that all the source materials used for this proposal have duly acknowledged.

I certify that all the information given here above are true.

| Principal Investigator | Signature | Date |
|---|---|---|
| Ayinalem Alemu Shitie | _____ | _____ |

This research proposal has been submitted with our approval as academic advisors.

| Principal Advisors | Signature | Date |
|---|---|---|
| Kassu Desta (MSc, PhD Fellow) | _____ | _____ |
| Abebaw Kebede (MSc, PhD Fellow) | _____ | _____ |