# RESEARCH

Bacilli load in PTB- intestinal helminths coinfected and PTB non -infected patients at selected public health facilities in Jimma zone, Oromia, Ethiopia: comparative crosssectional study

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# Abstract

**Background** Tuberculosis (TB) and intestinal helminths are diseases that pose a dual burden on public health in lowincome countries. Previous studies have shown that helminths can affect the shedding of bacteria or the bacterial load in the sputum of active TB patients. However, there is limited information on bacterial load in TB patients with helminth infections.

**Objective** This study aimed to compare bacterial load in helminths-infected and non-infected pulmonary tuberculosis patients at selected public health facilities in Jimma zone, Oromia, Ethiopia.

**Methods** The study was conducted in Jimma Zone, Oromia, Ethiopia. A facility-based comparative cross-sectional study was employed from August 01, 2020, to January 2021. A total of 124 (55 intestinal helminths-infected and 69 non-infected) newly diagnosed smear-positive pulmonary tuberculosis (PTB) patients were included in the study. A convenience sampling technique was employed to recruit study participants, and a semi-structured questionnaire was used to collect data regarding socio-demographic characteristics and possible risk factors for intestinal helminths co-infection. Stool examination was performed using both wet mount and Kato Katz technique. Additionally, weight and height measurements, sputum, and blood samples were taken to determine body mass index, bacilli load, and diabetic mellitus, respectively. Data were entered into Epi-Data software version 3.1 and analyzed using Statistical Packages for Social Sciences (SPSS) Version 25. A statistically significant difference was defined as a *P*-value of less than 0.05.

**Results** Intestinal helminths reduced bacilli load 3 times more than intestinal helminths non-infected PTB (AOR = 3.44; 95% CI; 1.52, 7.79; *P* = 0.003) However, diabetes mellitus, HIV, drinking alcohol and cigarette smoking were not associated with bacilli load. The rate of co-infection TB with intestinal helminths was 44%. The three most prevalent parasites detected were *Trichuris trichiura* 29 (66%), hookworm 19 (43%), and *Ascaris lumbricoides* 11(25%)).

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Among co-infected patients about 36 (81.8%) had a single parasite infection, and 19 (43.2%) had multiple infections. A body mass index < 18.5 (AOR = 3.26; 95% CI; 1.25, 8.56;P = 0.016) and untrimmed fingernail status (AOR = 3.63; 95%CI;1.32,9.93;P = 0.012) were significantly associated with PTB- intestinal helminth –co-infection.

**Conclusion** Helminth infection was associated with a lower bacilli load compared to helmenths non-infected PTB. The rate of co-infection TB with intestinal helminths was 44%. *Trichuris trichiura* was the most prevalent helminth. Untrimmed fingernail and a body mass index were associated with PTB-intestinal helminth co-infection.

Keywords Bacilli load, PTB, Co-infection, Helminths, Ethiopia

# Background

Tuberculosis (TB) remains a significant global health issue and is the top infectious disease-related cause of mortality globally. With over 80% of cases occurring in 30 countries with significant TB burdens, low- and middleincome nations bear the brunt of the disease [1].

The TB epidemic in many African nations is worsened by co-infection with helminths. These two health problems often coexist in the same geographical areas and are among the most widespread infectious diseases, especially in middle and low-income nations [2].

There is a geographical variation in the prevalence of intestinal helminths-TB co-infection, with a global population prevalence of 29.69% [3]. In Ethiopia, the prevalence of co-infection with TB and intestinal helminths ranges from 20.2 to 32% [4–6].

Intestinal helminth-TB co-infection is associated with a significantly lower sputum smear positivity, particularly in Africa where the TB burden is high and diagnostic facilities are lacking [7, 8]. Helminth-induced immune regulation involves mechanisms like alternatively activated macrophages, regulatory T cells, and suppression of inflammatory cytokines [9–11]. These changes in the host immune microenvironment can alter the physiology and virulence of coinfecting bacterial pathogens [12].

Consequently, the pathogenesis of TB is different in helminth-coinfected individuals, resulting in the lack of complete cavitation and reduced TB disease [13]. These helminth-driven reductions in sputum bacilli reduce the sensitivity of TB diagnosis and subsequently delay treatment.

In contrast, various studies have found a significant correlation between intestinal helminth infections and TB. For instance, a prior study revealed that coexistent helminth infection was associated with higher sputum smear grade and TB disease severity [14], as well as high levels of Treg cells in active PTB patients facilitating mycobacterial replication and tissue damage [15]. Furthermore, the co-infection of TB and helminths was associated with increased transmission and poor treatment outcomes (including failure and relapse) [16–18].

Thus, there are contradictory findings that needs further investigation in resolving the complex challenges posed by intestinal helminth-TB co-infection regarding diagnosis strategies in tuberculosis.

Co-infection of helminths with mycobacterium tuberculosis could pose diagnostic challenges also. Because sputum smear microscopy is still the most common way of diagnosing infectious PTB, knowledge of its sensitivity, particularly in the case of helminth infection, is crucial in controlling TB.

Therefore, this study was aimed to determine the bacilli load among smear-positive PTB patients co-infected with intestinal helminths and PTB non-infected with helmenths patients attending selected hospitals and health centers in Jimma Zone, Oromia, Ethiopia.

# Methods and materials

# Study area

The study was conducted in Jimma Zone, which is located in the Oromia region of Ethiopia. Jimma Zone is 356 km away from Addis Ababa, the capital city of Ethiopia. The town itself is the largest in Southwestern Ethiopia and has an average altitude of 1780 m above sea level. It is characterized by a tropical climate, with heavy rainfall and warm temperatures. The mean annual temperature in the area is 24.9 °C, while the average rainfall ranges from 800 to 2500 mm.

The projected total population of the Jimma Zone for 2019/2020 was 3,436,79, and there were a total of 126 health facilities, including 6 hospitals and 120 health centers [19].

# Study design and period

A facility-based comparative cross-sectional study was conducted from August 01, 2020, to January 2021.

#### Source population and study population

All newly diagnosed TB patients visited TB diagnostic centers from August 01, 2020, to January 2021 at selected hospitals and health centers in Jimma Zone were source population. All newly diagnosed smear-positive PTB patients with and without intestinal helminths infection during the study period (from August 01, 2020, to Jan 2021) at selected hospitals and health centers in Jimma Zone were study population.

#### Inclusion and exclusion criteria

Patients diagnosed with pulmonary tuberculosis (PTB), aged 18 years or older, and willing to participate were included in the study. This study excluded PTB patients admitted to hospitals, pregnant women, and patients with a history of anti-helminthic treatment within the past three months.

#### Identification and study procedures

In the Jimma zone, there are many health facilities. For this study, we purposefully selected 8 health facilities based on the total number of smear-positive PTB patients and patient flow data obtained from the Jimma Zone health bureau. Furthermore; the selection of facilities is due to they are most relevant to the study's objectives and ensuring that the data collected is meaningful and relevant to the research question.

The selected facilities include Jimma University Medical Center (JUMC), Jimma Town Health Center, Higher 2 Health Center, and Mendera Kochi Health Center from Jimma Town. Furthermore, Yebu Health Center, Seka Hospital, and Dedo Hospital were selected from outside Jimma town. These facilities were chosen for the study because they were easily accessible and transportation costs were feasible for data collection. Remote and difficult areas were excluded from study due to unreliable and often impassable roads, which can hinder transportation and data collection, and finding suitable accommodation can be challenging.

Participants were identified at selected health facilities when they came to the TB clinic for TB diagnosis. Smearpositive PTB patients were identified based on laboratory results. In addition, the following examinations were conducted: anthropometric measurements to determine the body mass index, stool examination to identify intestinal helminths, and blood glucose measurement to diagnose diabetes mellitus.

Sputum and stool samples obtained from patients were immediately transported to Jimma University's medical microbiology teaching laboratory. The sputum samples, after staining with ZNs techniques, were examined with light microscopy. The results were then graded as a low bacilli load (if smear grade $\leq$ +1) and a high bacilli load (if smear grade $\geq$ +2). Additionally, the stool specimen was first examined by wet mount, then with the Kato-Katz technique for egg load determination. Finally, patients were grouped intestinal helminths (IH) infected and IHnon-infected based on the detection of eggs of the intestinal helminths in their stool specimen or not.

Sputum smear grade or bacilli load was determined between the group infected with intestinal helminths and the non-infected group. Additionally, smear grades were assessed among the group infected with intestinal helminths based on the total egg load.

#### Sample size determination and sampling technique

The sample size was calculated using OpenEpi, version 3 (sample size calculation for cross-sectional study by Fleiss with continuity correction) [20]. The proportion of PTB with intestinal helminth infection was taken as 33%, and the proportion of PTB without intestinal helminth infection was taken as 66%. The parameters used were power 90%, 95%CI, and a ratio of unexposed to exposed 1:1, taken from the study done in Gondar town [21]. Adding a 5% non-response rate, the final total sample size is 110 (55 for each group).

Consecutive newly diagnosed smear-positive pulmonary TB patients were included in this study until the required sample size was obtained in both groups from the selected health facilities.

#### Data collection process

After patients were provided with sufficient information by trained laboratory staff in each health facility about the purposes of the study, socio-demographic data and associated risk factors for intestinal helminth infection were gathered by using a semi-structured questionnaire. Patients were interviewed in their language (Afan Oromo or Amharic).

# Stool sample collection and examination

For the proper collection of samples and the detection and identification of intestinal parasites, appropriate specimen containers, and applicator sticks were provided to patients by trained laboratory staff in each health facility to bring a sufficient amount of stool specimens from each patient included in the study. They were instructed to fill half of the container and discard the scoop after use. Instructions were given to take precautions before collection of feces, such as not mixing urine with a stool sample. All stool specimens were immediately brought to Jimma University's microbiology teaching laboratory, following standards of transportation protocols, within 30 min of collection, and examined for intestinal parasitic infections All containers, along with the specimens, were properly labeled.

A macroscopic examination of the fecal sample was conducted to assess its consistency and the presence or absence of blood and adult parasites. Microscopic examinations were performed using both the direct saline wet mount method and the Kato-Katz concentration methods [22]. The saline wet mount method was utilized to detect motile trophozoites of intestinal protozoa and helmint larvae, while the Kato-Katz method was used to count the eggs. Specimens were examined for parasitic infections using a binocular microscope under 10X magnification and confirmed by observation under 40X magnification. The reporting of gross and microscopic features of the stool was completed, and the presence of any parasitic stage, if diagnosed, was considered after being confirmed by a second observer.

#### Sputum sample collection and examination

Patients were provided with sputum plastic caps labeled with the patients' code, types of tests, and dates to bring spot-spot sputum samples to the laboratory for the diagnosis of tuberculosis using the Ziehl Neelsen Acid-fast bacilli (ZNs AFB) technique.

Sputum smear examinations were done on spot-spot sputum samples using the AFB technique and the ZNs method after the samples were collected and transported to the Jimma University teaching microbiology laboratory.

A sputum smear was prepared by first labeling the slide, then preparing the smear, allowing it to air dry, fixing it, and finally staining it with 1% carbolfuchsin for 5 min, acid alcohol decolorizer for 3 min, and 0.1% methylene blue as a counterstain for 1 min. The dried smear was then examined by a binocular microscope with 100x objective and 10x eyepiece magnification. Patients were considered smear-positive if a single spot sputum sample was positive for AFB.

The Acid-fast bacilli (AFB) load was measured and described using the World Health Organization protocol as negative (0 AFB/100 oil fields), scanty (1–9 AFB/100 oil fields), + (10–99 AFB/100 oil fields), ++ (1–10 AFB/oil field), and +++ (>10 AFB/oil field) [23].

#### Anthropometric measurement

Body Mass Index (BMI)measurement was performed using a measurements of height (cm) and weight (kg) by trained nurses. According to the WHO definition, individuals with a BMI<18.5 are malnourished, those with a BMI<16 are severely malnourished, and those with a BMI $\geq$ 18.5 and <25.0 are normal [24, 25].

#### **Blood glucose measurement**

Is measured mainly in the diagnosis and management of diabetes mellitus. The screening was based on determining whether patients are already known to have diabetus mellitus (DM) and in those with no known DM measuring glucose in capillary blood using a reagent test strip and Gmate glucometer machine which is calibrated plasma equivalent to roll out the diabetic status. A raised blood glucose level is called hyperglycemia. When definite it is diagnostic of diabetes mellitus. If blood glucose results exceeded the cutoff value patients were referred to the diabetus clinic from the TB clinic.

All patients with previously diagnosed DM and newly diagnosed DM were enrolled in diabetus care where the diabetes doctors decide on the type of therapy. Care for the dual-affected patient (TB and DM) was thus given at the TB clinic and also in the DM clinic.

Provider initiative counseling and testing (PIHCT) for HIV is being practiced for all TB suspects hence, HIV results were collected from health facility records with patient card numbers (TB clinic) [26].

#### Data processing and analysis

After collecting the data, it was checked for completeness, coded, and entered into EpiData software. It was then analyzed using SPSS Version 25. Descriptive statistics, such as frequencies, proportions, and means, were used to explain socio-demographic factors, associated factors, and intestinal helminths co-infection. The relationship between the outcome variables and selected determinant factors was analyzed using binary logistic regression analysis. For variables with a *P*-value of  $\leq 0.25$ in binary logistic analysis, multivariate analyses were conducted sequentially by removing the variable with the largest P-value until statistically significant associations were found. The strength of an association was measured using odds ratio and 95% confidence interval. A *P*-value<0.05 was considered indicative of a statistically significant difference.

#### **Quality assurance**

Training was provided to data collectors, and questionnaires were translated into the local languages (Afan Oromo, Amharic) and back-translated into English for consistency. Standard operating procedures (SOPs) were implemented for the collection and transportation of specimens to ensure the quality of reagents and equipment.

The questionnaire was tested on 5% of participants at Shenen Gibe primary hospital in Jimma Zone, and both the questionnaire and slides were retained for rechecking. A well-experienced individual blindly rechecked both negative and positive results. Stains and reagents were controlled, and slides, cover glasses, and other glassware and plastic ware were ensured to be clean. Equipment, particularly the microscope, was used correctly. A procedure manual, which contains a detailed description of the necessary procedures for processing specimens and identifying various parasites and their diagnostic stages, was utilized.

The results were interpreted properly and reported using a standardized laboratory reporting form, which is necessary to optimally manage the patient.

# Results

# Socio-demographic characteristics of study participants

This study involved the enrollment of 124 smear-positive PTB patients, of which 55 had intestinal helminth (IH) infection and 69 did not. Apart from the co-infection

Variables	Category	IH infected	IH-non infected
		n (%)	n(%)
Age	18–64	53(96.4)	51(92.7)
	≥65	2(3.6)	4(7.3)
Sex	Male	34(61.8)	29(52.7)
	Female	21(38.2)	26(47.3)
Occupational status	Student	13(23.6)	9(16.4)
	Farmer	12(21.8)	11 [20]
	Unemployed	16(29.1)	12(21.8)
	Merchant	10(18.2)	14(25.5)
	Employed	4(7.3)	9(16.4)
Educational status	Primary and above	40(72.7)	41(74.5)
	No formal education	15(27.3)	14(25.5)
Marital status	Single	24(43.6)	19(34.5)
	Married	31(56.4)	30(54.5)
	Divorced/widowed	0	6(10.9)
Source of income	Has income	27(49.1)	37(67.3)
	No income	28(50.9)	18(32.7)
Residence	Urban	32(58.2)	29(52.7)
	Rural	23(41.8)	26(47.3)

 Table 1
 Socio-demographic characteristics of smear-positive PTB patients attending selected public health facilities in Jimma zone,

 Oromia, Ethiopia, from August 2020 to January 2021

 Table 2
 Distribution of egg load per gram of stool among intestinal helminths infected PTB patients

Bacilli load	Total egg load/gm of stool	correlation
Lower bacilli load	46,536 eggs/gm of stool	r = -0.142, p = 0.301
Higher bacilli load	10,224 eggs/gm of stool	

analysis, fourteen [14] individuals from the IH-noninfected group were not included. As a result, additional analyses included 55 patients who were infected with IH and 55 who were not.

Among male PTB+patients, 34 (61.8%) of the IHinfected group and 29 (52.7%) of the IH-non-infected group were the majority in each group. The IH-infected group's mean age was  $32.25\pm14.63$  years, which was less than the IH non-infected PTB+patients' mean age of  $35.11\pm14.67$  years. In both groups, almost 90% of the PTB+patients under study were between the ages of 18 and 65. Urban locations accounted for more than half of the examined PTB+patients; 29 (52.7%) in the IHinfected group and 32 (58.2%) in the non-infected group (Table 1).

# Intestinal helminths infection associated with reduced bacilli load

The total egg load of intestinal helminthes (*A*. lumbricoides, Hook worm, *T. trichiura*, Taenia species, *H. Dimunita*, *S. mansonia* and *E. vermicularis*) was significantly higher (46,536 eggs/gm of stool) in patients with a lower bacilli load (a sputum smear grade $\leq$ +1) compared to those with a higher bacilli load (a sputum smear grade $\geq$ +2), which had a total egg load of 10,224 per gm of stool. Although not statistically significant, there was a

negative correlation between the egg load and bacilli load (r = -0.142, p = 0.301) (Table 2).

Bivariate analysis showed that intestinal helminth infection, cigarette smoking, and drinking alcohol were associated with a lower bacilli load. However, in multivariate analysis, only intestinal helminth infection was associated with a lower bacilli load or reduced risk of a bacilli load (AOR=3.44; 95% CI, 1.52-7.79; P=0.003). This means that the odds of having a lower bacilli load are three times greater for individuals with intestinal helminth infections compared to those who are not infected (Table 3).

#### Factors associated with intestinal helminth co-infection

According to the bivariate analysis model, respondents who commonly cook their food at home had a habit of swimming, eating vegetables/fruits without washing, had no income, had no hand washing habit after defecation, washed their clothes in rivers/lakes/streams, had nutritional status, and had dirty material under their fingernails associated with intestinal helminth infection.

However, in the multivariate regression model, nutritional status (AOR=3.26, CI: 1.25, 8.56,p=0.016) had a strong association with intestinal helminthiasis The odds of infection were 3.3 times higher in participants with BMI<18.5 compared to those with BMI≥18.5(AOR=3.26, 95% CI: 1.25, 8.56;P=0.016). untrimmed fingernail status was strongly associated with intestinal helminthiasis. The odds of infection were 3.6 times higher in those who had dirty material under their fingernail (AOR=3.63, 95%CI: 1.32, 9.93,p=0.012) than in those who did not. The other considered

Table 3 Factors associated with bacilli load among smear-positive PTB patients attending selected public health facilities in Jimma zone, Oromia, Ethiopia, Aug 2020 to Jan 2021

Variables	Category	Bacilli load		COR 95% CI	AOR 95% CI	P-value
		Lower	Higher			
Age	18-64	53(48.2%)	51(46.4%)	1	1	
	≥65	3(2.7%)	3(2.7%)	0.96(0.18,4.99)	0.84(0.14,3.65)	0.963
Sex	Female	23(48.9%)	24(51.1%)	0.87(0.41,1.85)	0.49 (0.23,1.58)	0.721
	Male	33(52.4%)	30(47.6%)	1	1	
BMI	< 18.5	34(30.9%)	28(45.2%)	1.44(0.67,3.06)	1.35(0.37, 2.85)	0.349
	≥8.5	22(20%)	26(54.2%)	1	1	
DM status	Yes	3 (2.7%)	3 (2.7%)	0.96(0.14,4.99)	0.69(0.16, 3.19)	0.963
	No	53(48.2%)	51(46.4%)	1	1	
HIV status	Yes	1(1.8)	2(3.6)	0.48((0.35,3.29	0.27(0.34,3.01)	0.272
	No	54(48.2)	53(96.4)	1		
Smoking cigarette	Yes	12(10.9%)	5(4.5%)	2.67(0.87,8.19)	2.36(0.67,8.24)	0.177
	No	44(40.0%)	49(44.5%)	1	1	
Drink alcohol	Yes	11(68.8%)	5(31.3%)	2.39(0.77,7.43)	2.43(0.674,8.79)	0.175
	No	45(47.9%)	49(52.1%)	1	1	
Intestinal helminths infection	Yes	35(31.8%)	20(18.2%)	2.83(1.31,6.14)	3.44(1.52,7.79)*	0.003
	No	21(19.1%)	34(30.9%)	1	1	

socio-demographic and behavioral characteristics did not show a significant level of association with intestinal helminth infection (Table 4).

#### **Distribution of IH among PTB patients**

Among 124 smear-positive PTB patients, 55 were infected with intestinal helminths with a co-infection rate of 44%, 2 had additional protozoa infection. Multiple parasitic infections were found in 19(43%) PTB+patients. Among all intestinal parasites identified in PTB patients; *T. trichiura* was frequently found 29(66%), followed by Hookworm 19(43%) and *A. lumbricoides*11 (25%). *Entamoeba histolytica (E. histolytica*) were the only intestinal protozoans detected from 2(4.5%)participants (Table 5).

# Discussion

Helminth infections are highly prevalent in populations where Mtb infection is endemic, and it has been widely conjectured that the strong mucosal Th2 and Treg cell responses can cause down-modulation of protective immune responses against Mtb [27]. However, there is a limited study that has addressed the coincidence and assessed whether helminth infections affect bacilli load in smear-positive PTB patients or not?.

In the present study, the presence of intestinal helminths is associated with a lower bacilli load at the time of diagnosis. The total egg load of intestinal helminths was significantly higher (46,536 eggs/gm of stool) in patients with a lower bacilli load (sputum smeargrade  $\leq$ +1) compared to those with a higher bacilli load (sputum smear grade $\geq$ +2), with a total egg count of 10,224 per gm of stool. This finding is consistent with a study conducted by Abate et al. in Ethiopia, where helminth infection was associated with a lower sputum smear grade [7].

The mean egg load was significantly lower in patients with a smear grade of +2 compared to patients with a smear grade of +1 or sputum smear-negative patients. This is due to the antinematode response mounted by individuals with low fecal egg count (FECs) being stronger than the response mounted by individuals with high FECs. Thus, when individuals are exposed to helminths, the TB bacilli load decreases in individuals with high FECs. This is in line with a study conducted in Tanzania in 2017 [28]. This may be due to the higher levels of Tregs and increased levels of the regulatory cytokine, IL-10, which could suppress the inflammatory and Th1-associated immune responses, leading to reduced cavity formation and, consequently, a reduced sputum smear load [29].

The co-infection of intestinal helminths among PTB+patients in this study was 44% and higher compared to the cross-sectional study results conducted in Gondar, Northwest Ethiopia by Alemayehu et al. (33.3%) [5] and Afework et al. (40.5%) [30]. Hence, the difference in the study population may be one factor for the differences in intestinal parasite co-infection.

Also the present findings show a higher co-infection rate than in China (7.3%) [31]. Variations in socio-demographic characteristics and understanding of intestinal parasite transmission and prevention may be a significant determinant factors. In this study, the total intestinal helminth co-infection rate at least with one helminth was about [36(81.8%)]. This is higher than the findings from Gondar, Ethiopia by Eba et al. (36.8%) [7], Brazil (27.5%) [32], Northwest Ethiopia (29%) [6], China (7%) [31], and Table 4 Factors associated with intestinal helminths infections among smear-positive PTB patients attending selected public health facilities in Jimma zone, Oromia, Ethiopia, Aug 2020 to Jan 2021

facilities in Jimma zone, Oromia, Ethiopia, A Variables	Variable category	Intestinal helminthes		COR 95% CI	AOR 95% CI	P <sup>a</sup>
		Yes No		-		
Sex	Female	21(38.2)	26(47.3)	0.68(0.32,1.47)	0.36(0.30,1.17)	0.336
	Male	34(61.8)	29(52.7)	1	1	
Age	18–64	53(96.4)	51(92.7)	2.07(0.36,11.84)	1.75(0.29,8.76)	0.410
	≥65	2(3.6)	4(7.3)	1	1	
Educational level	Primary and above	40(72.7)	41(74.5)	1	1	
	No formal education	15(27.3)	14(25.5)	1.09(0.47,2.56)	0.98(0.32,1.96)	0.298
Source of Income	Has income	27(49.1)	37(67.3)	1	1	
	Has no income	28(50.9)	18(32.7)	2.13(0.98,4.62)	1.58(0.89,3.36)	0.155
Resident	Rural	23(41.8)	26(47.3)	0.80(0.37,1.70)	0.61(0.28,1.68)	0.565
	Urban	32(58.2)	29(52.7)	1	1	
Shoe wearing habit	Yes	43(78.2)	44(80)	1	1	
-	No	12(21.8)	11 [ <mark>20</mark> ]	1.12(0.45,2.80)	0.92(0.38,2.19)	0.815
Hand washing habit before a meal	Always or often	49(89.1)	51(92.7)	1	1	
2	Occasionally or never	6(10.9)	4(7.3)	1.56(0.42-5.87)	1.38(0.24,4.98)	0.510
Hand washing habit after defecation	Always or often	36(65.5)	48(87.3)	1	1	
2	Occasionally or never	19(34.5)	7(12.7)	1.56(0.42-5.87)	1.38(0.24,4.98)	0.190
Swimming habit	Yes	19(34.5)	7(12.7)	3.62(1.37,9.53)	2.63(0.89,7.23)	0.090
5	No	36(65.5)	48(87.3)	1	1	
Common food source	Cooked at home	49(89.1)	44(80)	2.04(0.69,5.98)	1.52(0.35,3.98)	0.193
	Hotel	6(10.9)	11(20)	1	1	
A water source for bathing	Pipe	35(63.6)	36(65.5)	1	1	
5	River/lake/stream	16(29.1)	12(21.8)	1.37(0.56,3.31)	1.03(0.45,2.97)	0.482
	Pipe and River/lake/stream	4(7.3)	7(12.7)	0.58(0.16,2.18)	0.41(0.12,1.49)	0.428
Untrimmed fingernail status	Yes	28(50.9)	10(18.2)	4.66(1.96,11.08)	3.63(1.32,9.93)*	0.012
5	No	27(49.1)	45(81.8)	1	1	
Washing raw vegetables or fruits before eating	Always or often	28(50.9)	43(78.2)	1	1	
5 5 5	Occasionally or never	27(49.1)	12(21.8)	3.45(1.51,7.92)	2.76(0.96,6.86)	0.230
A habit of eating raw meat	Always or often	18(32.7)	13(23.6)	1.57(0.67,3.64)	1.48(0.72,2.91)	0.293
	Occasionally or never	37(67.3)	42(76.4)	1	1	
Washcloths	Pipe	27(49.1)	35(63.6)	1	1	
	River/lake/stream	20(36.4)	13(23.6)	1.99(0.84,4.71)	1.54(0.56,3.14)	0.116
	Pipe and River/lake/stream	8(14.5)	7(12.7)	1.48(0.47,4.59)	1.17(0.21, 4.12)	0.496
Raise poultry/livestock	Yes	26(47.3)	22(40)	1.35(0.63,2.86)	1.12(0.23,1.85)	0.442
	No	29(52.7)	33(60)	1	1	
BCG vaccination	Yes	33(60)	32(58.2)	0.93(0.43,1.98)	0.56(0.34,1.52)	0.846
	No	22(40)	23(41.8)	1	1	
Use night soil	Yes	6(10.9)	5(9.1)	1.22(0.35,4.27)	0.94(0.26, 2.89)	0.751
	No	49(89.1)	50(90.9)	1	1	0.751
BMI	< 18.5	39(63	23(37.1)	' 3.391(1.538,7.480	3.26(1.25,8.56)*	0.001
	≥ 18.5	16(33.3)	32(66.7)	1	1	0.001

Northwest Ethiopia by Elias et al. (71%) [33]. The differences in laboratory protocol followed, geographical variation, and sample size may justify these differences.

In this study, *T. trichiura* causes the highest co-infection rate of 29 (66%) among PTB+patients, followed by hookworms 19 (43%) and *A. lumbricoides* 11 (25%). The prevalence of hookworms infection in this study were 19 (43). Hookworms were the most frequent helminths in China and Northwest Ethiopia, with rates of 4.3% and

11.1% respectively [5, 31]. A stool survey from adult outpatient attendants in Kenya also revealed the highest prevalence of hookworms (13.1%) among intestinal helminths [34]. Such disparities could be due to variations in the relative distribution of helminth species based on environmental conditions. Maybe because most respondents are urban residents, the habit of wearing shoes was not considered an associated factor in the present study.

**Table 5**Distribution of IH among PTB patients attendingselected hospitals and health centers of Jimma zone, Oromia,Ethiopia, Aug 2020 to Jan 2021

Parasites		Number (%)
Helminths	A. lumbricoides	11 (25)
	Hook worm	19 (43)
	T. trichiura	29 (66)
	Taenia species	4 (9.1)
	H. Dimunita	1 (2.3)
	S. Mansonia	9 (20.45)
	E. Vermicularis	3 (6.8)
Number of helminths	1	36 (81.8)
species per participant	≥2	19 (43.2)
Protozoa	E. Histolytica	2 (4.5)

In the present study, we examined the factors associated with intestinal helminthiasis among smear-positive PTB patients. The multivariate regression model in the present study revealed that participants who had dirty material on their fingers and a BMI < 18.5 were at a higher risk of helminth infection in smear-positive TB patients.

Patients with BMI<18.5 were 3.3 times at higher risk of acquiring helminth co-infection. Findings from China go in line with our results [31]. Those who had dirty material in their fingernails were about three times as likely to have intestinal helminth infection compared to those who did not have. This was supported by a study done by Alemu et al., in Addis Ababa, and Abera et al., in Tilili, Ethiopia [35, 36] where 47.9% of students in Tilili, 25% in Addis Ababa. This might be due to variations in water supply, income, feeding habits, environmental sanitation, the sample size of the study population, and awareness of the means of transmission prevention and control measures of parasitic infection.

Studies from from China and Kenya reported that females were more likely to acquire intestinal parasite infections than males [31, 34]. Age, sex, educational level, residency, and shoe-wearing habits were not significantly associated with intestinal helminth infection in this study. This finding is in line with studies conducted in China and Ethiopia [5, 31]. This consistency might be due to the fact that occupational exposure status in different social settings might bring differences in exposure rate by age, sex, resident, and shoe-wearing habit.

# Limitations of the study

Due to the small sample size, there was a lack of statistical significance which limited our power to detect differences between the comparable groups. Convenient sampling technique which can not indicated the normal representatives was used to select sample units.

### Conclusion

IHs were associated with lower bacilli load. The bacilli load decreases with increasing total egg load. The overall co infection rate of intestinal helminths among PTB patients were 44%. Ihis were also prevalent in varying magnitude among smear-positive PTB patients. *T. trichuria* was the most prevalent helminth. As a result, more sensitive methods may be required in this study area and healthcare providers should screen TB patients for intestinal helminthiasis and treat them accordingly. The impact of helminth-TB co-infection should be considered for the TB controls program and also future studies should be taken into consideration to include immunological profile and radiological evidence of TB patients with and without helminth infection.

Furthermore, a large-scale study recruiting more participants is required to drive a definitive conclusion.

#### Abbreviations

AFB	Acid-fast bacilli
AOR	Adjusted Odds ratio
BCG	Bacillus Calmette Guérin
BMI	Body Mass Index
COR	Crude Odds ratio
CI	Confidence interval
DM	Diabetus mellitus
IH	intestinal helminthes
Gene Xpert mtB/r	Gene Xpert Mycobacterium tuberculosis (MTB) DNA and resistance to rifampicin (RIF)
PTB	pulmonary tuberculosis
SOPs	Standard operating procedures
ТВ	tuberculosis
ZNs AFB	Ziehl Neelsen Acid-fast bacilli

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#### Author contributions

Melese Mekuria conceived the idea. Dr. Ahmed Zeynudin, Prof. Gemeda Abebe, and Melese Mekuria collected, analyzed, and interpreted the data. Melese Mekuria and Habtamu Hasen drafted the manuscript. All contributed significantly and gave the final approval for the paper to be published.

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#### Data availability

Data is provided within the manuscript or supplementary information files. The datasets used and/or analysed during the current study are available from the corresponding author based on reasonable request.

#### Declarations

#### Ethics approval and consent to participate

Ethical clearance was obtained from Jimma University institutional review board (IRB). Written informed consent was taken from each participant. Any information concerning the patients was kept confidential and the specimens collected from the patients were analyzed for the intended purpose only. Positive patient results were communicated with physicians who were working at a TB treatment center for treatments and better management. The study was followed the principles of declaration of Helsinki.

#### **Consent for publication**

Not applicable.

#### **Competing interests**

The authors declare no competing interests.

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#### References

- 1. WHO. 2022b. Global Tuberclosis report 2022: Tb Moritality [Online]. World Health organization. Available: https://www.who.int/teams/globaltuberculosis-programme/tb-reports/global-tuberculosis-report-2022/ tb-disease-burden/2-2-tb-mortality
- Kyu HH, Abate D, Abate KH, Abay SM, Abbafati C, Abbasi N, Abbastabar H, Abd-Allah F, Abdela J, Abdelalim A. Global, regional, and national disability-adjusted life-years (DALYs) for 359 diseases and injuries and healthy life expectancy (HALE) for 195 countries and territories, 1990–2017: a systematic analysis for the global burden of Disease Study 2017. Lancet. 2018;392(10159):1859–922.
- Zenebe Y, Habtamu M, Abebe M, Tulu B, Atnafu A, Mekonnen D, Lang R, Munshea A. Intestinal helminth co-infection and associated factors among pulmonary tuberculosis patients in Africa and Asia: a systematic review and meta-analysis. BMC Infect Dis. 2023;23(1):739.
- Tesfaye S, Zerfu B, Desta K. Magnitude and associated factors of intestinal parasitosis and tuberculosis among tuberculosis suspected patients attending Kuyu General Hospital, North Shewa, Oromia, Ethiopia. PLoS Negl Trop Dis. 2022;16(1):e0010120.
- Alemayehu M, Birhan W, Belyhun Y, Sahle M, Tessema B. Prevalence of smear-positive tuberculosis, intestinal parasites and their co-infection among tuberculosis suspects in Gondar University Hospital and Gondar Poly Clinic, North West Ethiopia. J Microb Biochem Technol. 2014;6(4):179–84.
- Abate E, Belayneh M, Gelaw A, Idh J, Getachew A, Alemu S, Diro E, Fikre N, Britton S, Elias D. The impact of asymptomatic helminth co-infection in patients with newly diagnosed tuberculosis in north-west Ethiopia. PLoS ONE. 2012;7(8):e42901.
- Abate E, Belayneh M, Idh J, Diro E, Elias D, Britton S, Aseffa A, Stendahl O, Schön T. Asymptomatic helminth infection in active tuberculosis is associated with increased regulatory and Th-2 responses and a lower sputum smear positivity. PLoS Negl Trop Dis. 2015;9(8):e0003994.
- Manuel Ramos J, Reyes F, Tesfamariam A. Intestinal parasites in adults admitted to a rural Ethiopian hospital: relationship to tuberculosis and malaria. Scand J Infect Dis. 2006;38(6–7):460–2.
- Anthony RM, Rutitzky LI, Urban JF Jr, Stadecker MJ, Gause WCJNRI. Protective immune mechanisms in helminth infection. 2007;7(12):975–87.
- Oyesola OO, Souza COS, Loke PJFI. The influence of genetic and environmental factors and their interactions on immune response to helminth infections. 2022;13:869163.
- 11. Babu S, Nutman TB. Helminth-Tuberculosis Co-infection: an immunologic perspective. HHS Public Access. 2017;37(1):597–607.

- 12. Frisan TJCM. Co-and polymicrobial infections in the gut mucosa: the hostmicrobiota-pathogen perspective. 2021;23(2):e13279.
- Elias D, Britton S, Kassu A, Akuffo H. Chronic helminth infections may negatively influence immunity against tuberculosis and other diseases of public health importance. Expert Rev anti-infective Therapy. 2007;5(3):475–84.
- Kumar NP, Kathamuthu GR, Moideen K, Banurekha VV, Nair D, Fay MP, Nutman TB, Babu S. Strongyloides Stercoralis coinfection is associated with greater disease severity, higher bacterial burden, and elevated plasma matrix metalloproteinases in pulmonary tuberculosis. J Infect Dis. 2020;222(6):1021–6.
- Marin ND, París SC, Vélez VM, Rojas CA, Rojas M, García LF. Regulatory T cell frequency and modulation of IFN-gamma and IL-17 in active and latent tuberculosis. Tuberculosis. 2010;90(4):252–61.
- Epstein MD, Schluger NW, Davidow AL, Bonk S, Rom WN, Hanna B. Time to detection of Mycobacterium tuberculosis in sputum culture correlates with outcome in patients receiving treatment for pulmonary tuberculosis. Chest. 1998;113(2):379–86.
- Visser ME, Stead MC, Walzl G, Warren R, Schomaker M, Grewal HM, Swart EC, Maartens G. Baseline predictors of sputum culture conversion in pulmonary tuberculosis: importance of cavities, smoking, time to detection and W-Beijing genotype. PLoS ONE. 2012;7(1):e29588.
- Monin L, Griffiths KL, Lam WY, Gopal R, Kang DD, Ahmed M, Rajamanickam A, Cruz-Lagunas A, Zúñiga J, Babu S. Helminth-induced arginase-1 exacerbates lung inflammation and disease severity in tuberculosis. J Clin Investig. 2015;125(12):4699–713.
- CSA (Central Statistical Agency). Population and Housing Census of Ethiopia. CSA, Addis Ababa, Ethiopia; 2015.
- 20. https://www.openepi.com/SampleSize/SSPropor.htm
- 21. Abate E. The impact of helminth infection in patients with active tuberculosis. Linköping University Electronic; 2013.
- Guerrant RL. Bench aids for the diagnosis of intestinal Parasites-World Health Organization, 1994, sw. Fr. 35.00 (sw. Fr. 24.50 in developing countries). Parasitol Today. 1995;6(11):238.
- 23. WHO: Blood safety and clinical technology. Standard Operating Procedures for Laboratory Diagnosis of Tuberculosis and M avium Complex Disease in HIV-Positive Patients 2006.
- 24. Weisell RC. Body mass index as an indicator of obesity. Asia Pac J Clin Nutr. 2002;11:S681-4.
- 25. WHO. Management of severe malnutrition: a manual for physicians and other senior health workers. World Health Organization; 1999.
- 26. Evans C, Ndirangu E. The nursing implications of routine provider-initiated HIV testing and counselling in sub-saharan Africa: a critical review of new policy guidance from WHO/UNAIDS. Int J Nurs Stud. 2009;46(5):723–31. Epub 2009 Jan 20. PMID: 19159879.
- 27. Chin KL, Fonte L, Lim BH, Sarmiento ME, Acosta AJFI. Immunomodulation resulting of helminth infection could be an opportunity for immunization against tuberculosis and mucosal pathogens. 2023;14:1091352.
- Mhimbira F, Hella J, Said K, Kamwela L, Sasamalo M, Maroa T, Chiryamkubi M, Mhalu G, Schindler C, Reither K. Prevalence and clinical relevance of helminth co-infections among tuberculosis patients in urban Tanzania. PLoS Negl Trop Dis. 2017;11(2):e0005342.
- Elkington PT, Ugarte-Gil CA. Friedland JSJErj. Matrix Metalloproteinases Tuberculosis. 2011;38(2):456–64.
- Kassu A, Mengistu G, Ayele B, Diro E, Mekonnen F, Ketema D, Moges F, Mesfin T, Getachew A, Ergicho B. HIV and intestinal parasites in adult TB patients in a teaching hospital in Northwest Ethiopia. Trop Doct. 2007;37(4):222–4.
- Li X-X, Chen J-X, Wang L-X, Tian L-G, Zhang Y-P, Dong S-P, Hu X-G, Liu J, Wang F-F, Wang Y. Prevalence and risk factors of intestinal protozoan and helminth infections among pulmonary tuberculosis patients without HIV infection in a rural county in PR China. Acta Trop. 2015;149:19–26.
- 32. Resende Co T, Hirsch CS, Toossi Z, Dietze R, Ribeiro-Rodrigues R. Intestinal helminth co-infection has a negative impact on both anti-Mycobacterium tuberculosis immunity and clinical response to tuberculosis therapy. Clin Experimental Immunol. 2007;147(1):45–52.
- Elias D, Mengistu G, Akuffo H, Britton S. Are intestinal helminths risk factors for developing active tuberculosis? Tropical Med Int Health. 2006;11(4):551–8.
- Andereck JW, Kipp AM, Ondiek M, Vermund SH. Helminth prevalence among adults in rural Kenya: a stool survey for soil-transmitted helminths and schistosomiasis in Nyanza Province. Trans R Soc Trop Med Hyg. 2014;108(12):804–9.
- 35. Alemu A, Kebede A, Dagne B, Amare M, Diriba G, Yenew B, Tesfaye E, Tadesse M, Sinshaw W, Challa D. Intestinal parasites co-infection and associated factors among active pulmonary tuberculosis patients in selected health

centers, Addis Ababa, Ethiopia: unmatched case-control study. BMC Infect Dis. 2019;19(1):1–10.

 Abera A, Nibret E. Prevalence of gastrointestinal helminthic infections and associated risk factors among schoolchildren in Tilili town, northwest Ethiopia. Asian Pac J Trop Med. 2014;7(7):525–30.

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