



Occurrence of Intestinal Parasite Infections among Presumptive Pulmonary Tuberculosis Patients Attending COOUTH Amaku, Awka, Anambra State, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The aim of this study was to determine the occurrence of intestinal parasites in presumptive pulmonary tuberculosis (PTB) patients attending clinics at Chukwuemeka Odumegwu Ojukwu University Teaching Hospital (COOUTH) in Awka, Anambra State, Nigeria.

Study Design: The study is a cross-sectional, hospital-based descriptive study.

Place and Duration of Study: The study was conducted at Chukwuemeka Odumegwu Ojukwu University Teaching Hospital in Awka, Anambra State, Nigeria. Between April and June 2023, the

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laboratory investigations and analyses were carried out at the Department of Medical Microbiology, Medical Laboratory Service, Chukwuemeka Odumegwu Ojukwu University Teaching Hospital, Awka, Anambra State, Nigeria.

Methodology: A total of 323 participants aged five to ninety years old who were attending clinics at Chukwuemeka Odumegwu Ojukwu University Teaching Hospital in Awka, Anambra State, were systematically chosen for the study. Structured questionnaires were used to collect demographic information, and stool samples were tested for intestinal parasites using direct wet mounting, Formol-Ether Sedimentation Techniques, and the Modified Ziehl-Neelsen (ZN) method for Oocysts. Sputum samples were also tested for PTB using the Xpert MTB Rif assay method. SPSS version 25.0 was used for statistical analysis, and the chi-squared test was used to determine significant associations.

Results: Out of 323 presumptive PTB patients screened, 22% had intestinal parasite infections and 13.6% had confirmed PTB. Co-infection with intestinal parasites and PTB was found in 5.6% of the participants. *Ascaris lumbricoides* was the most common intestinal parasite (8.7%), and *Giardia lamblia* was the least common (2.2%). The 27-36-year-old age group had the highest infection rates (5.5%; $P = 0.05$), according to age-related analysis. Males had a higher (22.7%) infection prevalence than females ($P = 0.12$). Importantly, patients with tuberculosis were more susceptible to intestinal parasites.

Conclusion: This study underscores the significance of screening presumptive PTB patients for parasitic infections and treating co-infected individuals in order to slow the progression of both diseases and reduce morbidity among TB patients.

Keywords: Prevalence; tuberculosis; pulmonary; intestinal parasites; Awka.

1. INTRODUCTION

An intestinal parasite infection occurs when a parasite infects the gastrointestinal tract of humans or other animals. These parasites can live anywhere in the body, but they prefer to live on the intestinal wall. Ingestion of under-cooked meat, drinking contaminated water, faecal-oral transmission, and skin absorption are all possible routes of exposure and infection. Because they live in the intestines, some helminths and protozoa are classified as intestinal parasites that cause infection. These infections have the potential to harm or sicken the host (humans or other animals).

“Intestinal parasites cause a wide range of symptoms in those who are infected, the majority of which manifest as gastrointestinal complications and general weakness” [1]. “Gastrointestinal conditions include inflammation of the small and/or large intestine, diarrhea/dysentery, abdominal pains, and nausea/vomiting, all of which have a negative impact on nutritional status, including decreased absorption of micronutrients, loss of appetite, weight loss, and intestinal blood loss that causes anemia. It may also cause physical and mental disabilities, delayed growth in children, and skin irritation around the anus and vulva”, [2].

“Intestinal parasite infections (IPs) caused by protozoa and helminths are among the most

common human infectious diseases worldwide, affecting approximately 3.5 billion people, with 450 million becoming ill as a result of the infections” [1]. “The bulk of these parasite diseases are produced by developing countries, particularly sub-Saharan Africa, due to low socio-economic status, inadequate water supply, poor environmental sanitation, and quick population expansion” [2]. “Globally, it is estimated that more than 10.5 million new cases are recorded annually, and *Ascaris lumbricoides*, hookworms, *Trichuris trichiura*, *Giardia lamblia*, *Entamoeba histolytica*, and *Schistosoma* species are determined to be the most prevalent intestinal parasites (IPs) detected” [2]. “More than 173 million people in underdeveloped regions, primarily Africa, are infected with *A. lumbricoides*, whereas hookworms and *T. trichiura* infect 198 million and 162 million people, respectively” [3]. “In Nigeria, intestinal worms have remained prevalent. As a developing country, helminthiasis is a public health concern and is classed as a neglected tropical disease that requires close attention due to the fact that children and immunocompromised adults are particularly vulnerable. Surprisingly, predisposing factors for intestinal worms are both directly and indirectly related to issues of continuous sanitation and sustainable hygiene. According to a World Health Organisation report, Nigeria is obviously above 50% infected” [4]. This is a major public health concern, especially given that many developed

countries have long effectively eliminated the scourge.

“Similarly, tuberculosis (TB) is an infectious disease caused mostly by *Mycobacterium tuberculosis* (MTB) that normally affects the lungs (pulmonary TB) but can sometimes affect other sections of the body (extra-pulmonary TB). TB is one of the top ten major causes of death, impacting over one-fourth of the world's population” [4]. “According to a recent World Health Organisation report, there would be around 10 million new TB infections in 2020, with 1.5 million deaths from all forms of TB. About a quarter of the reported TB cases were from African countries. Currently, there is some progress in Nigeria as an example of a country that managed to significantly increase national TB case finding by 50% by 2021 using innovative approaches such as expanding daily observed treatment protocols, using digital technologies, Community Active Case Finding, and enlisting Public Private Mix initiatives. However, Nigeria continues to have the highest TB burden in Africa and ranks sixth globally” [4].

“IPIs and tuberculosis are both chronic infectious illnesses that cause substantial harm to humans [4]. The geographic distributions of helminths and tuberculosis overlap significantly, increasing the possibility of co-infection with both infections” [5]. “Asymptomatic helminth infection is associated with higher regulatory T-cell and Th2-type response during active TB; helminth infection is also significantly associated with a lower rate of sputum smear positivity” [5]. “Co-infection with tuberculosis and intestinal parasites accelerates illness development and increases morbidity in PTB patients” [4].

This study delves into the intriguing intersection of these two health concerns, concentrating specifically on the prevalence and impact of intestinal parasite infections among PTB patients. PTB is still a major public health concern, and its control and management are crucial to lowering global TB incidence. Co-infections, such as intestinal parasites, can, however, complicate disease management, alter clinical outcomes, and need alternative approaches to care.

A hospital-based cross-sectional study was done at Chukwuemeka Odumegwu Ojukwu University Teaching Hospital (COOUTH) in Awka to address this issue. The study sought to give information on the prevalence and patterns of

intestinal parasite infections among individuals who have presumptive PTB, a population that is frequently disregarded in the setting of co-infections. Understanding the co-occurrence of these diseases, as well as their potential synergistic effects on health outcomes, is critical for developing effective and comprehensive healthcare solutions.

2. MATERIALS AND METHODS

2.1 Study Area

The study was conducted at Chukwuemeka Ojumegegwu University Teaching Hospital (COOUTH) in Awka, Awka South LGA. Awka is located in Nigeria's South Eastern Region. It is specifically located in Anambra State's Awka South Local Government Area. It is located at 60°12'E and 6°025'N, as well as 7°004'N and 7°004'E. Awka is located around 600 kilometres east of Lagos in the heavily populated Igbo heartland of south-eastern Nigeria [6]. The West-East Federal Highway connects Lagos, Benin City, Asaba, Onitsha, and Enugu to Awka, and other secondary roads connect it to smaller towns including Agulu, Nibo, Amawbia, Enugwu-ukwu, and Abagana.

Awka is made up of three parts: Awka urban, Ifite-Awka, and Umuokpu Awka. Awka is made up of thirty-four (34) villages in total, while Ifite-Awka and Umuokpu Awka are geographically isolated from Awka urban but are traditionally regarded villages inside Awka. Awka has attracted individuals from other states in Nigeria and abroad throughout the years, and it now has a substantial number of immigrants from Northern Nigeria, the Delta region, Cameroon, and Ghana. Awka has a population of 301,657 people, according to the 2006 Nigerian Census [7]. Since the founding of the State and the designation of Awka as its capital, as well as the building of a Federal and State University and other Federal and financial institutions, the population of Awka has grown at a rate estimated to be greater than 30% [7].

Awka is located in Nigeria's tropical zone and has two distinct seasons caused by the area's major winds: the south-west monsoon winds from the Atlantic Ocean and the northeastern dry winds from across the Sahara Desert. From April to October, there are seven months of strong tropical rainfall, followed by five months of drought from November to March. During this time, the harmattan, a very dry and dusty

season, frequently occurs. The temperature is often hot and humid, ranging between 27-28°C from July to December, but reaching to 35°C between February and April. Awka is located in a valley, though most of the natural jungle has been cleared for farming and human settlement. The rainforest has been preserved in locations such as the Imo-Oka Shrine. To the north and east of the city, wooded savannah grassland predominates.

Before the twentieth century, the inhabitants of Awka, Anambra State, were well recognised for their metal craftsmanship and blacksmiths. Agriculture, craftsmanship, construction activities, educational activities, state and federal establishments, hotels of all classes, petroleum and allied companies, pharmaceuticals and other manufacturing industries, as well as major financial institutions such as banks with branches in and around Awka, have recently begun. Awka additionally offers big rudimentary open-air marketplaces where everything from basic food to clothes, cosmetics, electronics, and home products is sold.

2.2 Study Design

From April to June 2023, a hospital-based cross-sectional study was done at Chukwuemeka Odumegwu Ojukwu University Teaching Hospital (COOUTH), Awka, Anambra State, Nigeria. COOUTH is the sole government-owned tertiary hospital in Awka metropolis that provides public healthcare services. The study included a laboratory evaluation of stool and sputum samples, as well as the distribution of questionnaires.

2.3 Ethics Approval and Consent to Participate

The study was carried out with ethical approval from the COOUTH Awka research ethics review board (Ref:COOUTH/CMAC/ETH.C/Vol.1/FN:04/264). Permission was also acquired from each study participant. Any information gathered over the course of the study was kept confidential.

2.4 Study Population

All patients attending the outpatient department (OPD), in-patient department (IPD), ANC (Antenatal clinics), ART (Anti-retroviral), and CHER (Children emergency) clinics were included in the study population. The study

population/participants were those who were chosen using a systematic random sampling technique.

2.4.1 Inclusion criteria

Presumptive tuberculosis (TB) cases from the OPD, IPD, ART, ANC, CHER, AND REFERRALS who provided written informed consent demonstrated a willingness to participate in the study aged five and above and of both sexes.

2.4.2 Exclusion criteria

Individuals who had already begun anti-TB treatment, participants who had used anti-parasitic drugs two weeks prior to specimen collection, and patients who refused to participate were all excluded from the study.

2.5 Sample Size

The minimum sample size needed was estimated using the single population proportion formula [8] $(Z/2)^2(p)(1-p)/d^2$, with the following assumptions: a prevalence (p) of 26.3% from a prior study [9], a 95% confidence level, and a 5% margin of error. As a result, the minimal sample size (n) was determined to be 323. Participants in the study were chosen based on the number of people on the presumptive TB registry. In the previous six months, the monthly average number of clients was 1987. The number of patients enrolled in the trial was then spread evenly across each week. As a result, the number of TB presumptives attending/referred to the Chest Clinic per week and per day throughout the study period was 83 and 16, respectively. Based on this analysis, the projected number of patients expected to visit the Chest Clinic during the following six months was determined. Finally, participants were chosen using systematic random sampling approaches ($k = 1987/480 = 4$).

2.6 Collection of Sociodemographic Data

A semi-structured questionnaire was used to obtain sociodemographic information such as age, gender, and residency from Directly Observed Treatment Short Course (DOTS) nurses in the COOUTH Awka Chest Clinics.

2.7 Sample collection

Stool samples weighing about 5 g were collected in adequately labelled, wide-mouthed, leak-proof universal containers.

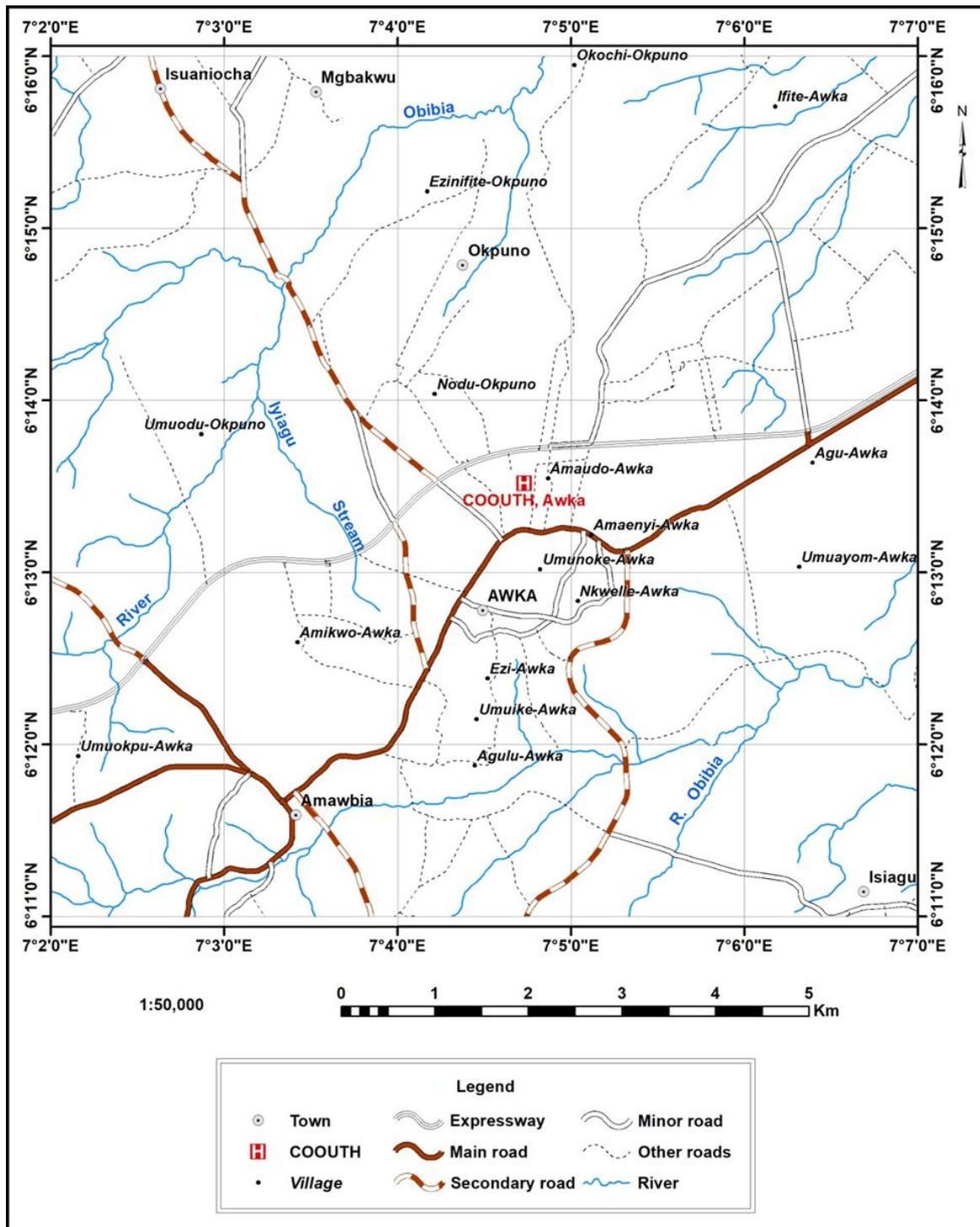


Fig. 1. Map of Awka, with emphasis on COOUTH Awka Hospital

2.8 Laboratory Procedures

2.8.1 Direct wet mounting procedure

Glass slides were labelled, and a drop of saline was placed on one end of the slide and an iodine drop was placed on the other. A small amount

(pea size) of stool specimen was mixed with an applicator stick, and 2 mg of the specimen was picked and emulsified on each drop of saline and iodine, respectively, to form a smooth thin preparation. A cover slip was placed over each preparation. The preparation was examined under the microscope with 10X and 40X

objectives for the detection and identification of parasite eggs, cysts, and oocysts [10].

2.8.2 Procedure for the formol-ether sedimentation technique

“Each centrifuge tube was labelled, and 4mls of 10% formol water was poured into it. Each tube received 1g (pea size) of faeces samples emulsified with an applicator stick. Each tube received three millilitres of 10% formol water, which was thoroughly mixed and shaken. The emulsified faeces were sieved through fine screen gauze into additional centrifuge tubes. Each tube received four millilitres of diethyl-ether. After mixing the tubes for 1 minute with a stopper, the stopper was removed with tissue paper. The faeces suspension was centrifuged in a tube at 3000 rpm. The ether, faecal material, and formol water were disposed after an applicator stick was used to loosen the layers of faecal debris off the wall of the tubes. The sediments remained, and the tubes were raised to allow the liquid from the side to drain to the bottom. To re-suspend and mix the sediments, the bottom of each tube was tapped. The sediments from each tube were transferred to a clean glass slide and covered with a cover slip before being viewed under a microscope with 10X and 40X magnifications for the detection and identification of parasitic eggs and cysts” [10].

2.8.3 Modified Ziehl-Neelsen (ZN) method for Oocysts

The smear obtained from formol-ether sedimentation was stained using a modified ZN staining approach to identify coccidian parasites. Following methanol fixation, 1% carbol fuchsin, 1% v/v acid alcohol, and 0.25% malachite green were employed as primary, decolorizer, and counter stains, respectively. The smear was examined with 100X magnification [11].

2.8.4 Collection of sputum sample, preparation and Xpert-MTB Rif assay methods

After counselling and teaching on how to obtain a suitable sample, two (2) ml of sputum were collected from Presumptive PTB patients. The sputum processing bench was where the samples were taken. Unscrewed the sputum lids and added a doubling volume of Genexpert buffer reagents per sputum, resulting in a 1:2 ratio of sputum sample to buffer reagents. The lids were closed, and the liquid was vigorously

stirred up to 20 times before being incubated at room temperature for 10 minutes. After 5 minutes, the mixture was stirred for the second time, up to 20 times, and incubated at room temperature for another 5 minutes. There were no obvious clumps in the mixtures since they had liquefied [12].

2.9 Data Quality Control

The reliability of the study results was ensured by implementing and adhering to quality control measures throughout the entire laboratory procedure process (pre analytical, analytical, and post analytical quality control processes were followed). All supplies, equipment, and procedures were adequately controlled. A Sample processing control (SPC) and a Probe Check control (PCC) are included with each Xpert MTB/RIF cartridge. The validation of controls is indicated by the printout of the test result.

2.10 Data Management and Analysis

Data were collected, entered, and analysed using the Statistical Package for Social Science (SPSS version 20). Simple descriptive statistics were employed to describe socio-demographic characteristics, the prevalence rate of intestinal parasite infection, MTB infection, and their co-infection. In all cases, the Chi-square test was applied to compare the research variables for the presence or absence of connection. P values of 0.05 were deemed statistically significant differences.

3. RESULTS AND DISCUSSION

A total of 323 Pulmonary TB presumptive participants were included in this study, with a response rate of 88.3%. Out of 323 participants, 163 (50.5%) were males and 160 (49.5%) were females. The study participants have an age range between 5 and 86 years, with a mean age of 35.5 years. Of all the study participants, 200 (61.9%) live in rural areas, whereas 123 (38.1%) live in urban areas.

Among 323 presumptive PTB patients, intestinal parasites were detected in 22% (71/323). *Ascaris lumbricoides* and *Entamoeba histolytica* were the most frequently isolated parasites, with a prevalence rate of 8.7% and 5.9%, respectively, whereas *Giardia lamblia* was the least, at 2.2%. Out of the 323 participants, 13.6% had MTB infections (Table 1).

Table 1. Prevalence of intestinal parasite infections among presumptive PTB patients attending clinics at COOUTH Awka

Parasites	No examined	No positive	Prevalence (%)
<i>Ascaris lumbricoides</i>	323	28	8.7
Hookworms	323	17	5.3
<i>E. histolytica</i>	323	19	5.9
<i>Giardia lamblia</i>	323	7	2.2
Total	323	71	22.0

$P = 0.01, df = 4, \chi^2 = 15.081$

Out of the 323 study participants examined for intestinal parasites, age range of 27-36 had the highest prevalence of infection (5.5%), while age range above 77-86 had the lowest prevalence (0.6%). There is a statistically significant association between the prevalence of intestinal parasites and age (Table 2).

Among the 323 study participants screened for intestinal parasites, 160 were females and 163 were males. Females had 10.4% intestinal parasites, while 11.3% were males (Table 3).

Out of the 323 study participants screened, 200 were from (rural) areas outside Awka, with 13.6% infected, and 123 were from Awka, with an infection rate of 8.4% (Table 4).

The prevalence of co-infection of intestinal parasites and MTB was found in 5.6% (18) of the study participants. Out of 18 MTB and IPis co-infected patients, 56% (10) were of *Ascaris lumbricoides* while 22% (4) were with *E. histolytica* (Table 5).

Table 2. Prevalence of intestinal parasite infections among presumptive PTB patients attending COOUTH Awka by Age

Age	Intestinal Parasites					Total
	No Infected (%)	<i>Ascaris</i>	Hookworm	<i>E. histolytica</i>	<i>G. lamblia</i>	
5 – 16	11(3.4%)	9	1	1	0	52
17 – 26	8(2.5%)	1	1	4	2	38
27 – 36	18(5.5%)	4	9	4	1	53
37 – 46	10(3.1%)	4	2	3	1	68
47 – 56	9(2.7%)	3	1	2	3	39
57 – 66	8(2.5%)	4	1	3	0	39
67 – 76	5(1.5%)	3	1	1	0	22
77 – 86	2(0.6)	0	1	1	0	12
Total	71	28(8.7%)	17(5.3%)	19(5.9%)	7(2.2%)	323

$P = 0.05, df = 28, \chi^2 = 41.310$

Table 3. Prevalence of intestinal parasite infections among presumptive PTB patients attending COOUTH Awka by Gender

Gender	Intestinal Parasites				Total
	<i>Ascaris</i>	Hookworm	<i>E. histolytica</i>	<i>G. lamblia</i>	
FEMALE	13(4%)	12(3.7%)	5(1.5%)	4(1.2%)	160
MALE	15(4.6%)	5(1.5%)	14(4.3%)	3(0.9)	163
Total	28 (8.7%)	17(5.3%)	19 (5.9%)	7 (2.2%)	323

$P = 0.116, df = 4, \chi^2 = 7.404$

Table 4. Prevalence of intestinal parasite infections among presumptive PTB patients attending COOUTH Awka by place of settlement

Settlement	Intestinal Parasites					Total
	No Infected (%)	<i>Ascaris</i>	Hookworm	<i>E. histolytica</i>	<i>G. lamblia</i>	
RURAL	44(13.6)	17	12	12	3	200
URBAN	27(8.36)	11	5	7	4	123
Total	71	28	17	19	7	323

$P = 0.80, df = 4, \chi^2 = 1.650$

Table 5. Prevalence of co-infection of IPs and MTB among Presumptive PTB patients at COOUTH Awka

<i>Mycobacterium tuberculosis</i>	No Infected (%)	Parasites				Total
		<i>Ascaris</i>	<i>Hookworm</i>	<i>E. histolytica</i>	<i>G. lamblia</i>	
NEGATIVE	53(16.4%)	18	14	15	6	279
POSITIVE	18(5.6%)	10	3	4	1	44
Total	71	28	17	19	7	323

$$P = 0.01, df = 4, X^2 = 15.081$$

This hospital-based, cross-sectional study aimed to assess the prevalence of intestinal parasite infections among presumptive pulmonary tuberculosis patients attending COOUTH Awka clinics. The overall prevalence of intestinal parasite infection among presumptive PTB cases was 22% among 323 study participants. This result is higher than in Ethiopia [13], China (14.9%) [14], and Nigeria [15]. The difference in prevalence could be attributed to differences in study period, method of stool examination, geographical area, sample size, and study participant selection criteria. Study participants were cases identified as presumptive PTB patients who had not taken any anti-TB or anti-parasitic medications. A study conducted in several areas of Ethiopia—Arba Minch, 26.3% [9], Northwest Ethiopia, 29.0% [16], and Gondar, 28.9% [17]—shows a higher prevalence than the current study and much higher in Sub-Saharan Africa, 40.3% [9]. The population's level of awareness of parasitic transmission, prevention, and economic position may have played a role in the current study's lower prevalence of intestinal parasite infection.

In this investigation, *Ascaris lumbricoides* was the most predominant parasite infection (8.7%). This finding was higher than that of a similar study reported by [13] with 5.9% and [15] with 4.4%, but lower than that of studies reported by [17] and [13] with 7.7% and 5.9%, respectively. What our study had in common was that *Ascaris lumbricoides* was the most prevalent parasitic infection, although other authors' data indicated that hookworm infection was the most common parasite.

“The current investigation found a substantial detection rate of *Ascaris lumbricoides* and hookworm among PTB presumptive patients. Some of these parasites' developmental phases are known to entail heart-to-lung migration. As a result, patients' reported lung symptoms (bronchospasm, fever, cough, dyspnoea, wheezing, and haemoptysis) may be caused by

these parasites” [18,19]. Thus, proper assessment of such an infection as a differential diagnosis is critical.

In the current study, the most common intestinal protozoan infection was *Entamoeba histolytica* (5.9%), with *Giardia lamblia* (2.2%) being found. This finding is slightly greater than the 5.46% found in research by [13]. This could be related to differences in dietary habits, environmental sanitation, economic variances, water supply, and awareness of the ways in which this parasite infection is transmitted, prevented, and controlled.

In the present study, the overall rate of intestinal parasite co-infection among probable PTB patients was 5.6%. This finding was lower than in other Ethiopian studies: Addis Ababa 22% [20], Arbaminch [9], Gondar [21,22], and Brazil [23]. Three successive stool samples evaluated in these studies may be a reason for the lower results in the current study as opposed to spot stool samples, and the study participants in the current study were those proven to have active PTB patients as opposed to presumptive PTB patients. However, the present study finding is higher than that of a study conducted in Gondar, Northwest Ethiopia, which found 2% [13]. This observed disparity could be attributed to laboratory techniques used for PTB detection as well as sample size. In the previous study, AFB microscopy was used to detect PTB; however, the current study used the Xpert MTB Rif assay test, which has about 97% sensitivity and 99.9% specificity to MTB and also contains more study participants.

The present study has also shown factors associated with intestinal parasitic infections among PTB presumptive patients. The prevalence of IPs was shown to be significantly related to age group. The most affected age groups were 27-36 years and 47-56 years. This could be because of occupational exposure in these age groups. There is no statistically

significant relationship between the prevalence of IPIs and the gender of the respondents, despite the fact that the prevalence of intestinal parasites was higher in males than females. This is consistent with research from Gondar North West [13] and Arba Minch [17].

4. CONCLUSION

The present study found a substantial number of intestinal parasite infections in varied degrees among PTB presumptive cases in the study area. The most prevalent parasite isolated was *Ascaris lumbricoides*, followed by *Entamoeba histolytica* and Hookworm. Intestinal parasites and MTB co-infections were also found, which could increase the patient's morbidity and fatality rates; consequently, all Presumptive PTB patients should be tested for parasitic infection and treated accordingly.

CONSENT AND ETHICAL APPROVAL

Consent was sort from the participants after a clear explanation of the study had been given to them. They were equally informed that the data generated from the study will be kept confidential and used for academic purposes and their identity will not be disclosed for any reason. Chukwuemeka Odumegwu Ojukwu Teaching Hospital, Amaku, Awka gave the ethical clearance for this work (COOUTH/CMAC/ETH.C/Vol.1/FN:04/264).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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