Prevalence and Impact of Intestinal Parasites Infections among Presumptive Pulmonary Tuberculosis Patients: A Cross-Sectional Study

Aims: The aim of the study was to determine the prevalence and impact of intestinalparasites among Presumptive Pulmonary Tuberculosis Patients (PTB) attending clinics at Chukwuemeka Odumegwu Ojukwu University Teaching Hospital, Awka, Anambra State, Nigeria.
Study design: This study is a cross-sectional, hospital-based, descriptive study.
Place and duration of Study: The study was carried out in Chukwuemeka Odumegwu Ojukwu University Teaching Hospital, Awka, Anambra State, Nigeria. The laboratory investigations and analysis were done in the Department of Medical Microbiology, Medical Laboratory Service, Chukwuemeka Odumegwu Ojukwu University Teaching Hospital, Awka, Anambra State, Nigeria.Awka, between April and June 2023.
Methodology: A total of three hundred and twenty-three (323) participants attending clinics at Chukwuemeka Odumegwu Ojukwu University Teaching Hospital, Awka, Anambra State,were randomly sampled for the study with an age range from five to ninety years. Demographic data were collected using structured questionnaires, and stool samples were examined for intestinal parasites using various techniques. Additionally, sputum samples were tested for PTB using the Xpert MTB Rif assay method. Statistical analysis was performed using SPSS version 20, and the chi-squared test was employed to assess associations.
Results: Out of 323 presumptive PTB patients screened, 22% had intestinal parasite infections, and 13.6% had confirmed PTB. Co-infection of intestinal parasites and PTB was detected in 5.6% of participants.The most prevalent intestinal parasites were *Ascaris lumbricoides, Entamoeba histolytica*, and Hookworm. Age-related analysis revealed the highest infection rates among the 27-36-year-old age group. Males had a higher prevalence of infection compared to females, although this difference was not statistically significant. Importantly, tuberculosis patients 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 to reduce the progression of both diseases and mitigate morbidity among TB patients.

Keywords: Prevalence, Tuberculosis, pulmonary, intestinal parasites

1. INTRODUCTION

An intestinal parasite infection is a condition in which a parasite infects gastro-intestinal tract of humans and other animals. Such parasites can live anywhere in the body, but most prefer the intestinal wall. Routes of exposure and infection include ingestion of undercooked meat, drinking infected water, faecal-oral transmission and skin absorption. Some type of helminths and protozoa are classified as intestinal parasites that cause infection because they reside in the intestines. These infections can damage or sicken the host (humans or other animals). If the intestinal parasite infection is caused by helminths, the infection is called helminthiasis.

Intestinal parasites produce a variety of symptoms in those affected, most of which manifest themselves in gastrointestinal complications and general weakness, (WHO, 2017) Gastrointestinal conditions include inflammation of the small and/or large intestine, diarrhea/dysentery, abdominal pains, and nausea/vomiting. These symptoms negatively impact nutritional status, including decreased absorption of micronutrients, loss of appetite, weight loss, and intestinal blood loss that can often result in anemia. It may also cause physical and mental disabilities, delayed growth in children, and skin irritation around the anus and vulva, (Ashtiani et al., 2011).

Intestinal parasite infections (IPIs) caused by protozoa and helminths are among the most widespread human infectious diseases throughout the world affecting around 3.5 billion people; of whom 450 million are ill as a result of the infections, (WHO, 2017). Majority of this parasite infections accounted by developing countries, mainly sub-Saharan Africa due to low socio- economic status, inadequate water supply, poor environmental sanitation and fast population growth (Ashtiani et al., 2011). Globally, it is estimated that more than 10.5 million new cases are reported annually and Ascaris lumbricoides, hookworms, Trichuris trichiura, Giardia lamblia, *Entamoebahistolytica* and *Schistosoma* species are found to be the most common intestinal parasites (IPs) detected (Ashtiani et al., 2011). In developing regions, particularly in Africa, more than 173 million people are infected with *A. lumbricoides* while 198 million and 162 million people are infected with hookworms and *T. trichiura*, respectively (Cappello, 2004). In Nigeria, intestinal worm has remained high and endemic. As a developing nation, helminthiasis is a public health concern and is classified as a neglected tropical disease which requires close attention due to the fact that the worst hit are children and immunocompromised adults inclusive. Interestingly, pre-disposing factors to intestinal worms are directly and indirectly related to matters of sustainable hygiene and consistent environmental sanitation. World Health Organization report grouped Nigeria as highly infected and clearly above 50% (WHO, 2012). This is of serious public health concern knowing that many developed countries have long effectively eliminated the scourge.

Similarly, tuberculosis (TB) is another infectious disease mainly caused by *Mycobacterium tuberculosis* (MTB) that typically affects the lungs (pulmonary TB), but it can also affect other body parts (extra-pulmonary TB). TB still continuous as one of the top ten leading causes of mortality that affecting around one-fourth of the world's population (WHO, 2012). Based on recent World Health Organization report, in 2020, globally there were around 10 million new TB cases and the death toll was 1.5 million from all forms of TB. About a quarter of this reported TB cases were from African regions. Currently, although there is some progress in Nigeria as an example of a country that managed to significantly increases national TB case finding by 50 percent in 2021 using innovative approaches such as the expansion of the daily observed treatment protocols, use of digital technologies, Community Active Case Finding, and enlisting Public Private Mix initiatives. However, Nigeria still remains at the Top in the TB burden in Africa and sixth in the world (WHO, 2012).

Both IPIs and TB are chronic infectious diseases, causing serious harm to humans (WHO, 2012). The geographic distribution of helminths and TB overlap substantially, increasing the likelihood of co-infection with both the pathogens (Stephenson et al., 2000). During active TB, asymptomatic helminth infection is associated with increased regulatory T-cell and Th2-type response; helminth infection is also significantly correlated to a reduced rate of sputum smear positivity (Stephenson et al., 2000). Co-infection of TB and intestinal parasites hastens progression of the disease and increases morbidity in PTB patients (WHO 2012).

This study delves into the intriguing intersection of these two health concerns, specifically focusing on the prevalence and impact of intestinal parasite infections among presumptive pulmonary tuberculosis (PTB) patients. PTB remains a critical public health concern, and its control and management are pivotal in reducing global TB incidence. However, the presence of co-infections, such as intestinal parasites, can complicate disease management, alter clinical outcomes, and necessitate novel approaches to care.

To address this issue, a hospital-based cross-sectional study was conducted at Chukwuemeka Odumegwu Ojukwu University Teaching Hospital, Awka.The study aimed to shed light on the prevalence and patterns of intestinal parasite infections among individuals presenting with presumptive PTB, a population that is often overlooked in the context of co-infections. Understanding the co-occurrence of these diseases and their potential synergistic effects on health outcomes is crucial for designing effective and comprehensive healthcare interventions.

2. MATERIAL AND METHODS

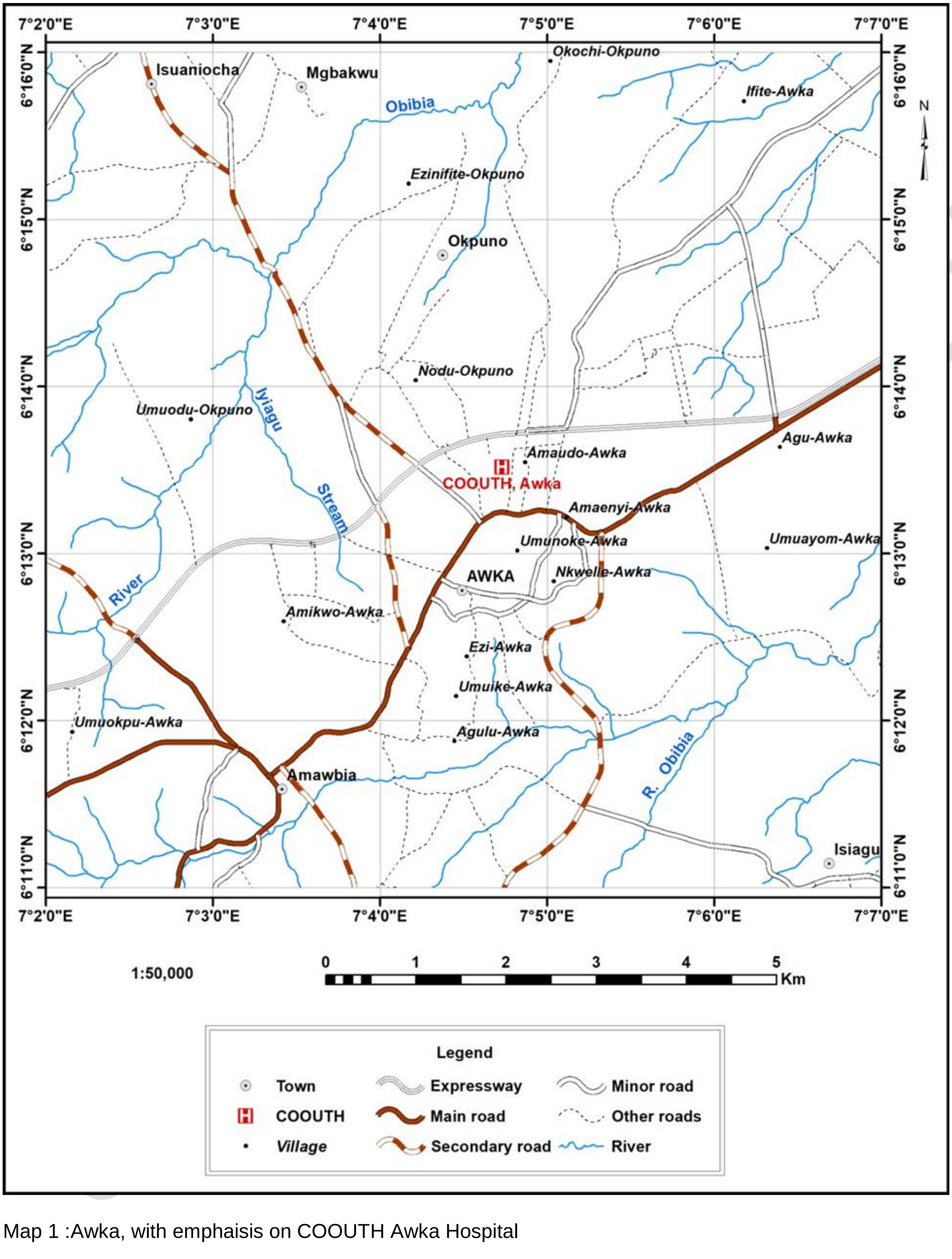
2.1 Study Area

The study was carried out in Chukwuemeka Ojumegwu University Teaching Hospital, Awka, in Awka South LGA. Awka is situated in the South Eastern Region of Nigeria. It is specifically located in Awka South Local Government Area of Anambra State. It is located at Latitude 60o12`E and 60o25`N and longitude 70o04`N and 70o04`E. Awka is at about 600km east of Lagos in the centre of the densely populated Igbo heartland in south-eastern Nigeria (Muoghalu, 2006). The West-East Federal Highway links Lagos, Benin City, Asaba, Onitsha and Enugu to Awka, and several location roads link it to smaller towns such as Agulu, Nibo, Amawbia, Enugwu-ukwu and Abagana.

Awka comprises of Awka urban, Ifite-Awka and UmuokpuAwka. All together, Awka comprises of thirty-four (34) villages, although Ifite-Awka and UmuokpuAwka are territorially separated from Awka urban but are traditionally considered as villages within Awka. Over the years, Awka has attracted people from other states of Nigeria, and beyond, even and has a significant number of immigrants from Northern Nigeria, Delta region, Cameroon and Ghana. According to the 2006 Nigerian Census, Awka has an estimated population of 301,657 (National Population Commission, 2006). Since the creation of the State, and making of Awka its capital and establishment of a Federal and State University and other Federal and financial institutions, the population of Awka has continued to grow at a rate estimated at over 30% (National Population Commission, 2006).

Awka is in the tropical zone of Nigeria and experiences two distinct seasons brought about by the predominant winds that rule the area: the south-west monsoon winds from the Atlantic Ocean and the northeastern dry winds from across the Sahara Desert. Seven months of heavy tropical rains starting from April to October are followed by five months of dryness from November to March. The harmattan, a particularly dry and dusty period usually occurs during this period. Thetemperature is generally hot and humid in the range of 27- 28oc during July through December, but rising to 35oc between February and April. Awka is sited in a valley, although most of the original rainforest has been lost due to clearing for farming and human settlement. A few examples of the rainforest remains at places like the Imo-Oka Shrine. Wooded savannah grassland predominates primarily to the north and east of the city.

The people of Awka in Anambra State are widely known for metal work and its blacksmith before the twentieth century in earlier times. The economic activities embarked upon in Awka recently includes: 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 institution such as Banks with branches within and around Awka. AlsoAwka is equally endowed with large rudimentary open-air markets where everything from basic food produce to clothes, cosmetic, electronics, household items are sold.



Map 1 :Awka, with emphaisis on COOUTH Awka Hospital

2.2 Study Design

Institution based cross-sectional study was conducted at Chukwuemeka Odumegwu Ojukwu University Teaching Hospital (COOUTH), Awka, Anambra State, Nigeria from November, 2022 to March, 2023. COOUTH is the only government owned tertiary hospital in Awka metropolis providing healthcare services to the public. The research involved laboratory examination of stool and sputum samples as well as questionnaire administration.

2.3 Ethics Approval and Consent to Participate.

This study was carried out following ethical approval obtained from the research ethics review board of the COOUTH Awka (Ref:COOUTH/CMAC/ETH.C/Vol.1/FN:04/264). The permission was also obtained from each study participants. Any information obtained at each course of the study was kept confidential.

2.4 Study Population

The study population included; all patients attending the outpatient department (OPD), in-patient department (IPD), ANC (Antenatal clinics), ART (Anti- retroviral), CHER (Children emergency) clinics. Those individuals, who were selected using systematic random sampling technique, were the study population/participants.

2.4.1 Inclusion Criteria

Presumptive TB cases from OPD, IPD, ART, ANC, CHER AND REFFERRALS from who gave written informed consent showed willingness to participate in the study aged five year and above and of both sexes.

2.4.2 Exclusion Criteria

Individuals who had already started anti-TB treatment, participants who had taken anti-parasitic drugs two weeks prior to the specimen collection and patients who declined participating were excluded from the sudy.

2.5 Sample Size

The minimum sample size was calculated using single population proportion formular (Daniel, 1999), (Zα/2)2(p)(1-p)/d2, with the following assumptions; prevalence (p) of 26.3% from a previous study (Alemu et al 2017), 95% confidence level and 5% margin error. Accordingly, the minimum sample size (n) was found to be 323. Study participants were selected based on the information we had on the number of presumptive TB register. The monthly average number of clients in the previous six months was 1987. Then the number of patients included in the study was distributed uniformly to each week (there was a total of 24 weeks and 120 working days in COOUTH Awka). Accordingly the number of TB presumptives attending/referred to the Chest Clinic during the study period per week and per day was 83 and 16, respectively. Based on this analysis, approximately numbers of patients who were expected to visit Chest clinic during the next six months were determined. Finally, systematic random sampling techniques (k = 1987/480 = 4) was used to select participants.

2.6 Sociodemographic Data Collection.

Sociodemographics like: age, sex and residence were collected by using a semi structured questionnaire from Directly Observe Treatment Short course (DOTS) nurses in the Chest clinics, COOUTH Awka.

2.7 Sample collection

Five grams of Stool samples was collected in a properly labeled, wide mouthed, leak proof universal containers.

2.8 Laboratory Procedures

2.8.1 Procedure for Direct Wet Mount

Glass slides were labeled and a drop of saline was placed on one end of the slide and a drop of iodine was placed on the other end of the slide. An applicator stick was used to mix small amount (pea size) of stool specimen and 2 mg of the specimen was picked and emulsified on each drop of the saline and iodine, respectively, to make a smooth thin preparation. Each preparation was covered with a cover slip. The preparation was mounted on the microscope and examined using 10X and40X objectives for the detection and identification of eggs, cysts and oocyst of parasites (Cheesbrough, 2005)

2.8.2 Procedure for Formol-Ether Sedimentation Technique

Centrifuge tubes were labeled and 4mls of 10% formol water was dispensed in each tube. An applicator stick was used to pick 1g (pea size) of the stool specimen and emulsified in each tube. Three milliliters of 10% formol water was added into each tube, mixed well and shaked. The emulsified faeceswas sieved into another centrifuge tubes using fine mesh gauze. Four milliliters of diethyl-ether was added into each tube. The tubes were covered with stopper and mixed for 1 min, the stopper was removed using tissue paper. The tube containing the stool suspension was centrifuged at 3000 rpm. An applicator stick was used to loosen the layers of faecal debris from the side of the tubes and the tubes were inverted, and the ether, faecal debris and formol water were discarded. The sediments remained and the tubes were returned to their upward position to allow the liquid from the side to drain to the bottom. The bottom of each tube was tapped to re-suspend and mix the sediments. The sediments of each tube was transferred to a clean glass slide and covered with a cover slip, mounted on a microscope and examined using the 10X and 40X objectives for the detection and identification of eggs and cyst of parasites (Cheesbrough, 2005)

2.8.3 Modified Ziehl-Neelsen (ZN) method for Oocysts

The smear obtained from formol-ether sedimentation, modified ZN staining method was used stained to identify coccidian parasites. After fixing the smear with methanol; 1% carbol fuchsin, 1% v/v acid alcohol and 0.25% malachite green were used as a primary stain, decolorizer and counter stain respectively. The smear was examined using 100X objective (Casemore, 1991)

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

Two (2) mls of sputum samples were collected from Presumptives PTB patients after counseling and education on how proper sample can be gotten. The samples were taken to the sputum processing

Bench. Unscrewed the lid of the sputums and double volume of genexpert buffer reagents were added per sputum ie 1:2 ratio of sputum sample to buffer reagents. The lids were closed and the mixture agitated vigorously up to 20 times and were incubated at room temperature for 10 minutes. At the elapsed of time, the mixture was agitated for the second time for up to 20 times and incubated further at room temperature for 5 minutes. The mixtures have liquefied and there were no visible clumps. Calibrated 3mls pipettes were used to collect 2mls of the liquefied solution and dispensed into genexpert cartridges and were taken to the genxpert machine where they were logged in and the Genexpert machine started processing the specimen for the detection of MTB/Rif in the sputum samples (Boehme et al., 2011).

2.9 Data Quality Control

The trustworthiness of the study results were assured by applying and following quality control measures during the total process of the laboratory procedures (pre analytical, analytical and post analytical quality control steps were followed). All materials, equipment and procedures were adequately controlled. Each Xpert MTB/RIF cartridge includes a Sample processing control (SPC) and Probe Check control (PCC). Print out of the test result indicates the validation of controls.

2.10 Data Management and Analysis

Data were collected and then entered and analyzed using Statistical Package for Social Science (SPSS version 20). Simple descriptive statistics was used to explain socio-demographic, prevalence rate of intestinal parasite infection, MTB infection, and their co infection. Chi-square test was used to compare the study variables for the presence or absence of association in all cases. P values of <0.05 were considered indicative of a statistically significant difference.

3. RESULTS

A total of 323 Pulmonary Tuberculosis (PTB) presumptive participants were included in this study with response rate of 88.3%. Out of 323 participants, 163 (50.5%) of the respondents 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. From all the study participants, 200 (61.9%) live in rural area, whereas 123 (38.1%) live in urban.

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 the prevalence rate of 8.7% and 5.9% respectively; whereas *Giardia lamblia* was the least, 2.2%. Out of the 323 participants 13.6% had MTB infections (Table 1).

Table 1: Prevalence of Intestinal parasites infections among presumptive PTB patients attending Clinics at COOUTH Awka.

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

Out the 323 study participants screened for intestinal parasites, age range of 27-36, had the highest prevalent of 5.5% infection rate while age range above 77 – 86, and had the lowest prevalence (0.6%). There is a statistical significance association prevalence in the Intestinal parasites with age (Table 2).

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

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

	INTESTINAL PARASITES					
AGE	**No Infected (%)**	***Ascaris***	**Hookworm**	***E. histolytica***	***G. lamblia***	**Total**
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

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

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

	INTESTINALPARASITES				
GENDER	***Ascaris***	**Hookworm**	***E. histolytica***	**G. *lamblia***	**Total**
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

Out the 323 study participants screened, 200 came from (Rural) outside Awka with 13.6% of them being infected while 123 study participants were from Awka with 8.4% infection rate (Table 4).

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

INTESTINAL PARASITES

Settlement	No Infected (%)	*Ascaris*	Hookworm	*E. histolytica*	*G. lamblia*	Total
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

The prevalence of co-infection of intestinal parasites and MTB was detected 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 5: Prevalence of co-infection of IPs and MTB among Presumptive PTB patients at COOUTH Awka.

	PARASITES

Mycobacterium tubaculosis	**No Infected (%)**	*Ascaris*	*Hookworm*	*E. histolytica*	*G. lamblia*	**Total**
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

4. DISCUSSION

This hospital-based, cross-sectional study was done with the aim of accessing the prevalence of intestinal parasitic infections amongst Presumptive Pulmonary Tuberculosis patients attending clinics at COOUTH Awka. Among 323 study participants, the overall prevalence of intestinal parasites infection among presumptive PTB cases was 22%. This finding is higher than a study conducted in Ethiopia (Yalewaker et al., 2017), China (14.9%) (Chen et al., 2014) and Nigeria (Akoko and Chessed, 2022). The difference in prevalence may be due to the difference in study period, method of stool examination, geographical area, sample size and differences in the selection criteria of the study participants: the study participants were cases identified as presumptive PTB patients and had not taken any anti-TB and or anti-parasitic medications. On the other hand, a study conducted in different areas of Ethiopia Arba Minch; 26.3% (Alemu et al., 2017), Northwest Ethiopia;29.0% (Abate et al., 2012), Gondar; 28.9% (Alemayehu et al., 2014) shows higher prevalence compared with the current study and even higher in Sub-saharan Africa; 40.3% (Alemu et al., 2017). The level of awareness about parasitic transmission, prevention and economical status of the population might be essential determining factor for the decreased prevalence of intestinal parasite infection in the current study.

Ascaris lumbriciodes was the predominant parasitic infection in this study 8.7%. This finding was higher than that of a similar study reported by Yalewayker et al. (2017) 5.9% and Akoko and Chessed, (2022) 4.4%; however lower than that of study reported by Alemayehu et al. (2014) and Yalekwargyter et al. (2014) with the prevalence of 7.7% and 5.9% respectively. What our study hadin common was *Ascaris lumbricoides* being the most predominant parasitic infection, while findings from other writers had Hookworm infection as their most predominant parasites.

The present study suggested that there exists a significant detection rate of *Ascaris lumbricoides* and hookworm among PTB presumptive patients. It is known that some developmental stages of these parasites involve heart to lung migration. Due to this fact, lung related symptoms (bronchospasm, fever, cough, dyspnoea, wheezing and Haemoptysis) reported by patients might be due to these parasites (Ransom et al., 1921; Yoshida 1920). Thus, due consideration of such infection as a differential diagnosis is important.

In the current study *Entamoeba histolytica* was the highest intestinal protozoan infection (5.9%) and *Giardia lamblia* (2.2%) was also detected. This finding is slightly higher than a study carried out by Yalewaker et al., (2017) which is 5.46%. This might be due to difference in feeding habit, environmental sanitation, economic variations, water supply and awareness on the ways of transmission, prevention and control measure of this parasitic infection.

The overall intestinal parasite co-infection rate among presumptive PTB patients in this study was 5.6%. This finding was lower than the studies done across Ethiopias; Addis Ababa 22% (Alemu, et al., 2019), Arbaminch (Alemu, 2017), Gondar (Elia et al., 2006, Kassu, 2004) and Brazil (Neto, 2009). Three consecutive stool samples examined in these studies might be a reason for the lower results in the present study as against spot stool sample and also the study participants were those confirmed to have active PTB patients as against presumptive PTB patients in the current study. However, the present study finding is higher than that of study done Gonder, Northwest Ethiopia 2% Yalewaker et al. (2018). This observed difference might be due to laboratory protocols followed for detection of PTB and sample size. In this previous study, AFB microscopy was implored for detection of PTB while the present study used Xpert MTB Rif assay test which has about 97% sensitivity and 99.9 % specificity to MTB, also the present study has more study participants.

The present study has also shown factors associated with intestinal parasitic infections among PTB presumptive patients. The prevalence of IPIs showed a significant association among different age groups. The age group of 27 – 36 years and 47 – 56 years were the most affected age group. This might be due to occupational related exposure of these age groups. There is no statistical significance association in the prevalence of IPIs with sex of the respondents, though prevalence of intestinal parasites was relatively higher in males than females. This is in agreement with studies done in Gondar North West (Yalewaker et al., 2018) and Arba Minch (Alemayehu et al., 2014). There is also a statistical significance association with the prevalence of IPIs with the MTB status of the respondents.

5. CONCLUSION

The present study has demonstrated that a significant number of intestinal parasitic cases were detected in varying degrees among PTB presumptive cases in the study area. *Ascaris lumbricoides* was the most common parasites isolated, followed by *Entamoeba histolytica* and Hookworm. Intestinal parasites and MTB co-infections were also identified and this may indicates that the patient's morbidity and mortality rates may be increased, therefore all Presumptive PTB patients should be checked for parasitic infection and be 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).

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