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Prevalence of Intestinal Parasites among Diarrhogenic Patients at Qilinto Prison Inmates of Addis Ababa,Ethiopia

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This is to certify that the thesis prepared by Senya Asfir, entitled: ***Prevalence of Intestinal parasites among diarrhogenic patients at Qilinto prison inmates of Addis Ababa*** and submitted in partial fulfillment of the requirements for Master of science degree **in Clinical Laboratory Sciences (diagnostic and public health microbiology specialty track**) complies with the regulations of the university and meets the accepted standards with respect to originality and quality.

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Abbreviations

CFM ----------Conventional Fluorescence Microscope

EPHI ----------Ethiopian Public health Institute

HCL ----------Hydro Chloric Acid

IPIs ----------Intestinal Parasitic Infections

NaCl---------- Sodium Chloride

PI----------Principal Investigators

LED---------- Light Emitting Diode

QC---------- Quality Control

SOP ----------Standard Operating Procedure

TB----------Tuberculosis

WHO ---------- World Health Organization

ZN ----------Zeihl-nelson

Abstract

Background: Intestinal parasites are one of the most neglected but the most wide spread and major public health problems in resource poor countries like Ethiopia. Studies have shown that intestinal parasites are prevalent among prison inmates. The lack of prompt and definitive laboratory method to diagnose intestinal parasitic infection is also one of the major problems in the control of the disease.

Objective: The aim of this study was to determine the prevalence of intestinal parasitic species using different diagnostic approaches among diarrhogenic patients in prison inmates.

Methods: A cross-sectional study was conducted from February to June 2017 in Addis Ababa Qilinto prison. A total of 335patients who were willing to participate, had diarrhea and not taken treatment had been included in the present study. Stool samples were collected from each study participant in clean stool collection cup and intestinal parasites were detected using wet mount, modified Zeihl_Nelsen staining and Auramine O stain. The data were double entered into excel file, cleaned, and analyzed using SPSS version 20.0. The prevalence of intestinal parasites was presented by using proportions. The difference between parasitological tests was compared using Pearson chi-square test.

Results: From the total 335 study participants 185 (55.2%) came from urban area. About 90(26.9 %) of the prisoners had no formal education. The outcome of direct microscopic examination of stool sample showed that infections with various intestinal parasitic species were common in the study subjects. About 134(40%) prisoners were found positive for one or more of the intestinal parasites. *E. histolytic*, *Gardia lamblia*, *Taenia* species were the most common parasites detected in the prison, 56(16.7%), 15(4.5%), and 18(5.4%) respectively.

Conclusion: The prevalence of Intestinal parasitic infection is the major public health problem in Qilinto prison, Addis Ababa, Ethiopia. Regular screening of prison inmates for intestinal parasites and potentially deworming strategy is a potential intervention that has to be considered by the concerned bodies.

Keywords: Prison inmates, Intestinal Parsites, Zeihl Nelson stain, Auramine O Stain

1. Introduction

1.1 Background

Parasites are organisms that are harbored on or within another organism, the host, in order to find the environment and nutrients obligatory for their own growth and production (1). The term intestinal parasites denote that the parasitic worms found in intestine of humans or animals (2). Intestinal parasites are among the most usual and broadly spread animal parasites of humans. Among the risk factors for parasitic infections are: immunosuppresion, overcrowding, close contact with soil and poor conditions of hygiene that promote the transmission of intestinal parasites (3).

Intestinal parasites are inducing a number of morbidity and mortality, and so they are among the most known public health problems worldwide (6). Globally, it is estimated that 3.5 billion people are at risk and 450 million are infected of these parasites (7). Similarly, a large number of people living in the Sub-Saharan Africa are at risk and infected with at least one parasitic species together with persistent poverty and poor socio-economic progress (8).

Ethiopia is one of the sub-Saharan African countries with high rate of intestinal parasitic infections (9). Researchers have reported the occurrence of high prevalence of intestinal helminthes in different parts of Ethiopia (10). Ethiopia is a country with the poor quality drinking water supply and latrine coverage in the world (11).

Such conditions could be hazardous and insufficient facility of water along with unhealthy living conditions and contaminated waste management permit intestinal parasites and other communicable diseases to flourish in several localities. Because of this, intestinal parasites are common in many parts of Ethiopia including in prison inmates (12).

The transmission of some helminthes, protozoa and microsporidia to humans is via the fecal-oral route and can occur through direct contact with infected persons (anthroponotic transmission) or animals (zoonotic transmission), or by ingestion of contaminated food (food borne transmission) or water (waterborne transmission) (4). They are also easily contracted through drinking un boiled faecally contaminated water and through eating raw or undercooked foods (5).

Intestinal parasitic infections are usual in the communities of poor and socio-economically disadvantaged people of the tropics and sub tropics because of scarcity of sufficient private and public latrines and poor environmental sanitation. Children as young as six months can be infected with intestinal parasites which affect learning capability with bigger absenteeism from school (6).

Some of the most commonly detected pathogens associated with diarrhea in humans are the zoonotic protozoans *Cryptosporidium parvum* and *Giardia lamblia*(16).

Infections are often missed especially for coccidian parasitic infections in a direct smear examination. Previous studies have evaluated and compared the utility of certain staining techniques such as modified Zeihl-Neelsen (ZN) staining, wet mount, hot safaranin method and Auramine O for detections of coccidian parasites (13).

Modified ZN staining, wet mount and hot safaranin are simple to perform by using conventional light microscopy. Auramine O staining however requires source of conventional fluorescence microscope (CFM) for the demonstration of these parasites. CFM is not possible in most health services due to its high cost, short life of the specialized mercury lamp (200 h), higher lamp warm-up time, maintenance and alignment and the need for dark examination rooms(14).

To overcome this disadvantage, WHO recently introduced Light Emitting Diode (LED) microscope for the diagnosis of tuberculosis (TB) in TB burden settings. LED using auramine O staining has the combined advantages of light and fluorescent microscopy. However, the utility of auramine O staining using LED microscope has not yet been evaluated for the detection of some acid fast coccidian parasites which causes diarrheal infection (15).

Thus, this study aims to determine the prevalence of intestinal parasites among diarrogenic patients and assess the risk factors which may facilitate the spread and occurrence of diarrheal infection. In line with this, it was aimed to evaluate Modified ZN and Auramine O for the detection of parasitic infections among prison inmates.

1.2 Statement of the problem

Intestinal parasitic infections are exceedingly communicable in a congested environment and in a closed-contact society such as in day-care centers and prisons (17). Prison inmates have higher chance of obtaining intestinal parasitic infections due to the background from which they come from and by the prison in which the way they live (18).

Studies indicated that the common problems associated with prison inmates are lack of nutrition, scanty health care facilities and knowledge which may worsen the spread of intestinal parasitic infections among prisoners (19).

In spite of the fact that intestinal parasitic infections can infect all members of the community, it is obvious that there are particular groups who are at higher risk of illness than others, and who are more susceptible to the dangerous effects of chronic infections. Among this group are inmates who carry a much burden of disease than other people (5).

Prisoners in developing countries live in highly poor conditions with shortage of accommodations. The prisoners are also affected by malnutrition, lack of clean water, dirty environment and very poor personal and environmental hygiene, these factors aggravates the intestinal parasitic infections in prison (2).

Infectious diseases in general and intestinal parasitic infections specifically are targets of prison health because inmates are vulnerable to disease through poor healthcare, overcrowding, demographics, high-risk behaviors, low-level immunity due to stress and shortage or poor nutritional quality, and overall low-living standards compared to the general population (20).

Various types of laboratory methods, such as parasitological, molecular, serologic and culture approaches have been developed for the diagnosis of intestinal parasites (21). In most cases the preferential diagnostic methods depend on the types of parasitic species causing the infection. In line with this, the routine direct microscopy method does not support the detection of some coccidian parasites such as *Cryptosporidium, Isospora* and *Cyclospora* species from diarrhogenic patients, which may lead to false negative result. This is the main gap observed in most health institution diagnostic laboratories in Ethiopia. Therefore, the present study needs to investigate if the introduction of multiple laboratory methods would enable to improve the detection capability of the laboratory by introducing the appropriate methods for the detection of coccidian parasites.

1.3 Significance of the study

The present study is very important to increase consciousness among health care personnel, the prison inmates and public about Intestinal parasitic infections. The study will also indicate direction on better managements of parasitic infections among Qilinto prision inmates and will minimizing the adverse effects. Based on the finding from the current study, the Qilinto prison management may consider improving preventive and control strategies for intestinal parasitic infections within the prison. Strategic interventions such as deworming could be taken by appropriate bodies to reduce the burden of intestinal parasitic infection.

2. Literature review

There are about twenty major helminthes infections of human affecting more than one third of the world population (22).The major intestinal parasitic infections, namely Amoebiasis, giardiasis, roundworm infection or A*scariasis by Ascaris* (*A.lumbricoides*)), whipworm infection or *trichuriasis* by *Trichuris* (T. *trichuira*), and hookworm *by Necator (N.americanus), and Ancylostoma (A. duodenal),* and infection caused by coccidian parasites account for most of the global disease burden (23).

Study conducted by Okolie, N. (2008), among 260 inmates of Owerri prision using direct wet preparation and formol ether concentration techniques showed 77% over all prevalence of intestinal parasite. According to this study the prevalence of *Strongyloides stercoralis* (0.4%), *Taenia* spp (0.8%), *Schistosoma mansoni* (0.8%), *Trichuris trichiura* (6.5%), *Ascaris lumbricoides* (10.8%), *Entamoeba coli* (11.9%), *Hookworm* (13.1%), *Entamoeba histolytica* (15.0%), and *Giadia lambia*(17.7%) varies. The combination of *Hookworms* / *Entamoeba histolytica* (3.8%) and *Ascaris lumbricoides* / *Entamoeba histolytica* (2.3%) dominated the cases of multiple infections. The resason for this types of prevalance has been suggested for Owerri prision is that there was inadequate water supply, unsanitary defecation methods and poor personal hygiene among the inmates (17).

Astudy done by Length,F. (2014) in inmates of Jos prison(Plateau State ,Nigeria), the prevalence of intestinal parasitic infections was 27(9%). among this *A.lumbricoides(2%), A.duodenalis(2.3%), S.mansoni(4.3%)* and *S.stercoralis(0.3%* (3).

The prevalence of intestinal parasitic infection(*Blastocystis* sp., *Strongyloides stercoralis*, *Entamoeba* spp., *Cryptosporidium* spp., *Giardia* spp., and *Trichuris trichiura)* among Malaysia prision inmates was 26.5%, this was done by Angel,.et al (2015), in inmates from kajang prison, Selangor, from this study the prevalence of intestinal parasitic infections is slightly higher among HIV positive inmates (27.5%) than HIV negative inmates (25.8%) (20).

from food hundler in the city of Nairobi Kenya (2012), the over all prevalence of different intestinal parasitic infections were 15.7%. among this *E. histolytica* was (12.5%) of the subjects while *G.lamblia* was found in (1.3%) which was done by Kamau, P. *et al.* (24).

Duedu, K. et al at Ghanaian psychiatry hospital (2015), from atotal of 111 study participants the prevalence of intestinal parasites was 13.5 % with gender specific prevalence is high in male 18.8 and females 4.8 % (25).

Astudy by Mamo H, in Shewa Robit prison and tobacco leaf development farm North-Central part of Ethiopia (2014),showed the overall prevalence of 61.8%, which were infected with one or more intestinal parasites belonging to eight different species, *A.lumbricoides*, *E. histolytica/dispar/, Hookworm species, Hymenolepis nana, Schistosoma mansoni*, *Strongyloides stercoralis*, *Taenia species, T. trichiura.* From these 55.4% of the cases had mixed infections among which 48.8% were peoples at prison and 65.5% were peoples in tobacco farm, while the rest had single infection (17).

A study done by Terefe,B et al at Bedele prision,(2015),the prevalence of intestinal helmenthes were identified; *A.lumbricoides* (42.6%), *hookworms* (32.4 % *),T. trichiura* (21.6%), *E. vermicularis* (4), *H. nana*(1)inamates and 29.7 % had multiple helminthes infections (26).

Among patients who attend at Teda health center north west Ethiopia, Abate A. et al (2013), from 48.3% male and 51.7% female study participants prevalence of intestinal parasitic infection was 62.2%..*Ascaris lumbricoides* was the most predominant parasite (23.2%) followed by *Giardia intestinalis* (12.4%), *Entamoeba histolytica/dispar* (4.6%), *Schistosoma mansoni* (8.9%), hookworm (6.6%), *Hymenolepis nana* (1.5%), *Enterobius vermicular is* (0.4%), and *Strongyloides stercoralis* (0.2%). Absence of toilet and hand washing after toilet was shown to be associated with intestinal parasitic infection ($P< 0.05$ for both) (27).

A study done by Assefa, S. et al at Hawassa screened 55.0% (208/378) of intestinal parasites for at least one intestinal parasite from a total of 378 individuals. The most frequently detected parasites were *E. histolytica/dispar* (26.5%) and *A. lumbricodes* (12.4%) (28).

A total of 342 study participants were present among highland and lowland dwellers in Gamo area (Wegayehu, T. et al) South Ethiopia, 39.9% were found positive for intestinal parasite. The prevalence of *Entamoeba histolytica*/*dispar* was the highest 98 (11.4%), followed by *Giardia lamblia* 91 (10.6%), *Ascaris lumbricoides* 67(7.8%), *Strongyloides stercoralis* 51(5.9%), Hookworm 42(4.9%), *Trichuris trichiura* 24 (2.8%), *Taenia* species 18(2.1%), *Hymenolepis nana*7 (0.6%) and *Schistosoma mansoni* 1(0.12%). No statistically significant difference was observed in the prevalence of intestinal parasitic infections among lowland (37.9%) and highland (42.3%)(19).

Kiros, H. et al. Bahir Dar at Felegehiwot Referral Hospital, (2015), an overall prevalence IPI was 30.6%. The highest prevalence of enteric protozoan infection was due to *Entamoeba histolytica*/*E. dispar* (19.3%), followed by *Cryptosporidium* spp (5.8%), *Giardia lamblia* (4.3%), and *Isospora belli* (1.3%) (6).

3. Objectives

3.1General Objective

- To investigate the overall burden of intestinal parasites among diarrhogenic patients in Qilinto prison inmates.

3.2 Specific Objectives

- To determine the prevalence of intestinal parasites among dirrhogenic patients
- To compare the yield of intestinal parasites using MZ and Auramine O staining techniques
- To assess the risk factors associated with intestinal parasitic infections among prison inmates

4. Hypothesis

- The prevalence of intestinal parasites among prision inmates is not different from studies in Ethiopia.

5. Materials and Methods

5.1 Study design

A cross-sectional study was conducted to determine the prevalence of parasitic infection and associated risk factors among prison inmates at Qilinto, Addis Ababa, Ethiopia.

5.2 Study Area and Period

The study was conducted from February 2017- June 2017 in Addis Ababa, Qilinto prison. It is one of the federal prisons of Ethiopia. It serves as the main prison of the country. It is 11 km south of central Addis Ababa, in Akaki Kality, the southernmost sub city of the nation's capital. In the prison there is clinic which gives patient care activities daily. The clinic has emergency psychiatry, pharmacy, laboratory services. The setup of laboratory is only one room and performs routine laboratory tests. Macroscopic and Direct microscopic examination was used for diagnosis of intestinal parasitic infections.

5.3 Source of population

The prisoners who were living in prison inmates at Addis Ababa Qilinto prison were the source of study populations.

5.4 Inclusion and Exclusion criteria

5.4.1Inclusion criteria

- Those Prisoners who had diarrhea and had not taken treatment
- Willing to participate,
- Greater or equal to 18years old was included in the study.

5.4.2 Exclusion criteria

- Visitors of clinic who were not prison inmates
- Participants who could not provide samples due to unconsciousness

5.5 Study variables

5.5.1. Dependent variable

- Burden of Intestinal parasites using different methods.

5.5.2 Independent variable

- Age, gender, marital status, occupation, residence place, life style**,** prison facility, length of staying, sharing food and drinking materials and food support from family, sharing of shelters, eating habit (like in Amharic culture gursha), abdominal pain, weight loss, hand washing before and after meals and after bathroom use , laboratory methods

5.6 Measurement and Data collection

5.6 .1 Sample size determination

The sample size was determined using single proportion formula based on previous prevalence study of 72.7% (17). By considering 95% confidence intervals and 5% sampling error.

$n = Z^2 P (1 - P)/ d^2$

Where:

n = sample size.

p =0.727

d = marginal error between the samples and population (0.05)

Z = critical value at 95% certainty (1.96)

n = (1.96)2 × 0.727 (1-0.727)/ (0.05)2

Thus, the sample size used in this study will be 305 when we add 10% contingency of 305 the total sample will be 335.

5.6.2 Sampling methods

Convenient sampling methods were used until the sample size is reached

5.7 Data Collection

5.7.1 socio demography data

Data on socio-demography and predisposing factors were collected using pre tested structured questionnaire. The questionnaire was prepared in English and translated to Amharic language.

The questionnaire addressed socio-demographic information and personal hygienic practices including hand washing before and after meal.

5.7.2 Sample collection

A pre-labeled stool cup was provided to each patient and about 5 grams of stool samples were collected. The collected stool samples were preserved in SAF and transported to parasitological laboratory of Ethiopian public health research institute on the same day of sample collection.

5.7.3 Laboratory methods

5.7.3.1 Macroscopic *examination*

The collected stool samples were physically characterized based on consistency (Watery, Mucoid and Bloody).Presence and absence of larval stages of parasites and other macroscopic features by principal researcher assisted by an experienced lab technician(17)

5.7.3.2. Microscopic examination

Diagnostic tests involving microscopy includes direct wet mount preparations, Modified Zeihl-Neelsen Method, and Auramine O staining method were used(5).

5.7.3.3 Wet mount method

Direct wet mount examination of fresh faecal specimens were done using physiological saline (saline wet mount) or iodine solution (iodine wet mount.In this method parasites were identified by motility of their larval forms and trophozoites(29).

5.7.3.4 Modified Zeihl-Neelsen Method

For detection of oocysts of opportunistic coccidial intestinal parasite-*Cryptosporidium* species, *Isospora belli* and *Cyclospora cayetanensis*, modified ZN staining was run accordingly. Fresh and non-contaminated faecal samples were collected, and then appropriate thin smears were prepared, and air-dried. The air dried stool samples were fixed with methanol for 5 minutes and stained with strong carbol fuchsin for 15-20 minutes.The slides were decolorized in 1% HCl acid-alcohol for 1 minute and counter stained with 1% methylene blue and left for 1 minute. Lastly, the stained smears were dried and examined microscopically using higher magnification (100X) (9).

5.7.3.5 Phenol- auramine stain method

This stain could be used as an alternative to the modified Ziehl-Neelsen stain for staining oocysts of *Cryptosporidium parvum.* First faecal smears were prepared and fixed in methanol then stained with phenol-auramine (Lemperts) for 10-15 minutes, after the phenol-auramine rinsed thoroughly by tap water acid alcohol was added to decolorize the phenol-auramine. Then the acid alcohol was rinsed thoroughly by tap water, and 0.1% potassium permanganate (counter stain) was added and left for 30 sec, finally the counter stain rinsed thoroughly by tap water, allowed to air dry. Then faecal smears were observed with blue light under an incident light fluorescent microscope and low power followed by oil immersion x100 objective if oocysts were suspected(30).

5.8 Data Quality assurance

For each steps Standard operational procedure (SOP) was followed. To ensure quality control, all the laboratory procedures including collection and handling of specimens were carried out in accordance with standard protocols. All the reagents were checked for contamination each time. To ensure general safety, disposable gloves were worn. Microscopic reading was examined by senior laboratory technologists. Data quality was assured by prior training of data collectors about the objective of the study and data collection procedure. In addition quality control performed with daily checking, known positive and negative control permanent stained slides were used.

5.8.1 Pre analytical

Patient identification and labeling was made with great care and samples were properly collected and transported without delay and others that were mentioned in the sample rejection criteria list were also controlled in this stage of quality assurance.

5.8.2 Analytical

Three types of stool sample diagnostic approaches; Direct wet mount , Modified ZN and Auramine O were used .Test was performed by following standard operating procedure. Quality of reagents were checked by using Positive and negative control slides which are

available at EPHI parasitology and TB laboratory. The principal investigator performed all tests with close supervision from the advisors.

5.8.3 Post analytical

Results were recorded and documented using SPSS version 20 with great care.

5.9 Data management and statistical Analysis

The data were double entered into excel file, cleaned, and analyzed using SPSS version 20.0. The prevalence of intestinal parasites was presented by using proportions. The difference between parasitological tests was compared using Pearson chi-square test .*Pvalue* of <0.05 was considered as statistically significant.

5.10 Operational definitions

Diarrhogenic: looseness of the bowels is increased in frequency, fluidity or volume of bowel movements compared to usual due to infection in intestinal parasites.

Patients: A person who are infected by intestinal parasites.

Diagnostic-approaches: The practice or techniques used to diagnose intestinal parasite infection.

Prevalence: The number of intestinal parasite case identified on examination during the study period in the study population.

7. Ethical Issue

Prior to commencement, the study was ethically reviewed and approved by the Department of Medical Laboratory Sciences, and Qilinto prison administration office. The reason of data collection was explained to the study participant and written consent was obtained from every study participants and confidentiality was assured for all the information provided. The recorded data were kept confidentially and was not accessed by a third person except by the principal investigator, participants positive for intestinal parasites were treated accordingly.

8. Results

8.1 Socio-Demographic characteristics of Study Participants

Socio-demographic characteristics of the study participants are summarized and presented in Table 1. A total of 335 individual were included in this study. All of the participants examined for intestinal parasitic infections were male subjects and half of the prisoners 169 (50.4%) stayed in the prison for less than 1 year. The mean age of the study subjects were 32 years with a minimum and maximum age of 18 and 72 years, respectively.

From the total of 335 study participants 185 (55.2%) came from urban areas, 160 (47.8%) of them were single, about 90(26.9%) had no formal education and 64 (19.1%) of them completed their education with certificate level and above, 324 (96.7%) and 208 (62.1%) of the study participants washed their hands before meal and after toilet respectively. 269 (80.3%) of the participants used food which came from outside the prison, 286 (85.4%) of the study subjects used tap water for drinking purpose (Table1).

Table.1 Socio-demographic features of the study participants (n=335) in Qilinto prison inmates, December, 2017.

Characteristics	Category	Frequency (n=335)	Percent (%)
Address	Urban	185	55.2
	Rural	150	44.8
Sex	Male	335	100
	Female	0	0
Age	18-25	70	20.9
	26-30	81	24.2
	31-35	99	29.6
	36-40	46	13.7
	41-45	22	6.6
	more than 45	17	5.1
Educational status	Non formal education	90	26.9
	Primary education	79	23.6
	Secondary education	102	30.4
	Certificate and above	64	19.1
Marital status	Single	160	47.8
	Married	153	45.7
	Divorced	17	5.1
	Widowed	5	1.5
Length of staying	Less than 1year	169	50.4
	1-2 years	100	29.9
	More than 2 years	66	19.7
Source of water	Tap water	286	85.4
	Filtered water	9	2.7
	Tap and filtered water	40	11.9
Hand washing before meal	Yes	324	96.7
	No	11	3.3
Hand washing after Toilet	Yes	208	62.1
	No	127	37.9
Foods outside the prison	Yes	269	80.3
	No	66	19.7
Sharing of food	Yes	206	61.5
	No	129	38.5

8.2 Prevalence of intestinal parasitic infections in prison inmates

The outcome of direct microscopic stool sample examination showed that infections with various intestinal parasitic species were common in the study subjects. About 134(40%) prisoners were found positive for one or more of the intestinal parasites (Table 2). *E. histolytic*, *Gardia lamblia*, *Taenia* species were the most common parasites detected in the prison, with prevalence rate of 56(16.7%), 15(4.5%), and 18(5.4%) respectively (Table.2).

Table.2 Prevalence of intestinal parasitic infections among different age groups, December, 2017.

Parasite species	Age in years						
	18-25 n=70	26-30 n=81	31-35 n=99	36-40 n=46	41-45 n=22	> 45 n=17	Total n=335
Entameoba histolytica/dispar	8(14.3)	11(19.6)	21(37.5)	7(12.5)	5(8.9)	4(7.1)	56(16.7)
Gardia lamblia	2(13.3)	5(33.3)	3(20.0)	0(0.0)	5(33.3)	0(0.0)	15(4.5)
Taenia spps	2(11.1)	4(22.2)	6(33.3)	2(11.1)	1(5.6)	3(16.7)	18(5.4)
A.lumbricoides	1(9.1)	2(18.2)	3(27.3)	2(18.2)	2(18.2)	1(9.1)	11(3.3)
H. worm	3(60.0)	0(0.0)	1(20.0)	1(20.0)	0(0.0)	0(0.0)	5(1.5)
S. stercoralis	1(20.0)	0(0.0)	1(20.0)	3(60.0)	0(0.0)	0(0.0)	5(1.5)
H. nana	0(0.0)	4(80.0)	1(20.0)	0(0.0)	0(0.0)	0(0.0)	5(1.5)
Entameoba histolytica/dispar & T aenia spps	2(28.6)	0(0.0)	2(28.6)	3(42.9)	0(0.0)	0(0.0)	7(2.1)
Taenia spps. & T. trichuria	2(33.3)	0(0.0)	3(50.0)	1(16.7)	0(0.0)	0(0.0)	6(1.8)
Gardia lamblia and Entameoba histolytica/dispar	2(33.3)	0(0.0)	2(33.3)	1(16.7)	1(16.7)	0(0.0)	6(1.8)
Total	23(17.2)	26(19.4)	43(32.1)	20(14.9)	14(10.4)	8(6.0)	134(40)

Overall results	Negative	201(60%)
	Positive	134(40%)

8.3 Prevalence of intestinal parasites by using Modified ZN and Auramine O staining methods

All stool samples were examined by using Modified ZN and Auramine O staining methods. In MZ staining tequniques 18 (5.4%) and 2(0.6%) were positive for cryptosporidium and Isospora belli parasites respectively. From 335 stool samples examined by Auramine O staining method 15(4.5%) was positive for cryptosporidium. (Table.3).

Table. 3 Prevalence of intestinal parasites by using Modified ZN and Auramine O staining methods

parasitic species		Frequency(n=335)	Percent (%)
Modified ZN	Negative	315	94.0
	Cryptosporidium	18	5.4
	Isospora belli	2	0.6
Aura mine O	Negative	320	95.5
	Cryptosporidium	15	4.5
	Isospora belli	0	0

8.4 Risk Factors Associated With Intestinal parasitic infections

None of the risk factors including age, residence, educational level, source of drinking water, length of staying inside the prison, hand washing experience before meal, and after toilet, source of food, sharing of food, were significantly associated with intestinal parasitic infections (Table.4).

Table.4 Association between risk factors and the prevalence of intestinal parasitic infections

Risk factors		Total no(n=335)	No. Positive (%)	χ2	P-value
Age category	18-25	70	23 (17.2)	9.788	0.081
	26-30	81	26(19.4)		
	31-35	99	43(32.1)		
	36-40	46	20(14.9)		
	41-45	22	14(10.4)		
	moe than 45	17	8(6.0)		
Residence	Urban	185	74(55.2)	0	1
	Rural	150	60(44.8)		
Educational status	Non formal education	90	33(24.6)	1.312	0.726
	primary education	79	32(23.9)		
	secondary education	102	45(33.6)		
	Certificate or diploma	64	24(17.9)		
Source of water	Tap water	286	108(80.6)	4.108	0.128
	Bottled water	9	5(3.7)		
	Tap & bottled water	40	21(15.7)		
Length of staying	less than 1year	169	69(51.5)	0.238	0.888
	1-2 years	100	38(28.4)		
	more than 2 years	66	27(20.1)		
hand washing before meal	Yes	324	131(97.8)	0.768	0.293
	No	11	3(2.2)		
hand washing after toilet	Yes	208	85(63.4)	0.171	0.383
	No	127	49(36.6)		
Source of food	Yes	269	111(82.8)	0.909	0.209
	No	66	23(17.2)		
Sharing of food	Yes	206	88(65.7)	1.647	0.121
	No	129	46(34.3)		

9. Discussion

A total of 335 Qilinto prisioners participated in the present study. The participants were from urban and rural areas.

The prevalence of intestinal parasitic infections in the present study was comparable with the study conducted by Terefe, B.et al (2015), 47.4 % among inmates of Bedele prision Ethiopia (26). And prevalence of IPIs was almost agrees to Wegayehu T.et al (2013), among highland and low land dwell in Gamo area, southern Ethiopia was 39.9% (19). This indicates the similarity in the hygienic and health service conditions between the two study locations of Ethiopia. Over all, the most frequently detected intestinal parasites were eight, namely, E.histolytic16.7% *Gardia lamblia 4.5% Taenia species , 5.4% A.lumbricoides 3.3% H.worm 1.5% S.stercoralis 1.5% H.nana 1.5% and T.trichria 2.1%. E.histolytica* is the most prevalent.

As shown in table.2, the total prevalence (40%) of intestinal parasites detected in the present study was lower than the study conducted by Mamo H. (2014), who was reported 61.8% among Shewa Robit, north central parts of Ethiopia,(17). and lower than study done by Abate et al, (2013), 62.2% on Teda health center north-west Ethiopia(27).and Assefa. et al (2009), 55.0% from the study conducted at Hawassa, southern Ethiopia(5).

The prevalence of intestinal parasite in the present study is higher than the study conducted by Length F. 2014, among inmates of Jos prison , Plateau State , Nigeria (9%) (3). and Angel,.et al 2015, in inmates from kajang prison , Selangor ,Malaysia (26.5%)(20),Kiros,.etal.at Felege hiwot Referral Hospital ,(2015), (30.6%)(6). The above variations on the prevalence of intestinal parasites could be owing to environmental hygiene differences, economic, educational status of the study subjects, climatic conditions, altitudinal difference, and level of general hygiene.

The prevalence of *E.histolytica* in this study is 16.7%. This result is lower than the study conducted by Kiros,H et al (19.3%) at Bahir Dar , Felegehiwot Referral Hospital (6). but higher than the study done by Angel,.et al (2.7%) in kajang prison (20). and Abate A. et al (4.6%) at Teda health center north-west Ethiopia(27).

Again the prevalence of *G.lamblia* in this study (4.5%), is lower than study at Owerri prison (17.7%), in São Paulo state, (9.68%).Gamo area (10.6%) and Teda health center north-west Ethiopia(12.4%) , but higher than study done in kajang prison ,Malaysia (0.3%)(20).

Furthermore, in the present study, the prevalence of Taenia sppss (5.4%) is more prevalent next to E.histolytica spps (16.7%). The magnitude of both parasites was higher than the study done by Wegayehu, T. et al(0.6%)(19).

In the present study the prevalence of cryptosporidium (5.4%), is comparable with the study done by Angel, et al (2.4%) (20). but similar with the study done by Kiros, et al.(6) (5.8%) at Felegehiwot Referral Hospital, BahirDar (6).

Moreover, the prevalence of I*sosporabelli* (0.6%) is comparable with the study conducted by Kiros, et al.(1.3%)(6).Besides the prevalence of E.histolytica in this study (16.7%) and G.lamblia (4.5%), is comparable with the study conducted by Okolie, N. at Owerri prison (15.0%)(31). And by Kiros, et al. (4.3%) (6). And the prevalence of *H.nana* in this study (1.5%) is similar with the study done by Abate, et al (1.5%) (27). This indicates the similarity in the hygienic and health service conditions between the two different study areas.

The prevalence of intestinal parasitic infection was different among different age groups 18-25,26-30,31-35,36-40,41-45,and above 45 years were 17.2%,19.4%,32.1%,14.9%,10.4%,6.0%, respectively. In this study, the prevalence is higher in the age group 31-35 (32.1%) and lower in the age group above 45 (6.0%) (Table.2).

In this study, the prevalence of intestinal parasites from urban participants (55.2%) and Rural participants (44.8%) was higher than the study done by Alemu . et al. (25%)(6). And the study conducted in Bedele prison(26.2%) (26). This is due to the individual variation regarding how to keep personal hygiene, and other healthcare facilities.

According to the present study we have found that there was the different prevalence of intestinal parasites among the study participants who have different educational level. The prevalence of intestinal parasites those who have non-formal educational level (24.0%) is lower than the study conducted by Abate A. et al (65.8%) and among inmates who have certificate and diploma level of education (17.9%) were also had lower IPIs than the previous study (41.7%). (29). This difference in prevalence could be the variation in study area and season of study.

Furthermore in this study the prevalence of intestinal parasites among prison inmates who used washing their hands before meal (97.8%) is higher than the study done by Abate. et al (64.2%)(27). And do not wash their hands after toilet (63.2%) is lower than in previous study (80.9%). This could be due to the quality of water source and other sanitary facilities, and local customs or behavior, differences in the method of stool detection, the climate of the study area and study season.

10. Strength and limitation of the study

10.1 Strength

- The present study was done for the first time at Qilinto prision.
- Positive slides were re-checked by experienced laboratory personel.
- Positive controlled slides were used.

10.2 Limitation

- Limited to do other diagnostic approach like stool antigen immunoassay.

11. Conclusion and recommendation

11.1. Conclusion

Intestinal parasitism, in general, is an important public health problem in Qilinto prison in Addis Ababa. The most commonly encountered parasites in the study have the potential to cause anemia and malabsorption and other complications. Therefore, routine examination of stool samples and treatment of infected individuals on regular basis would significantly contribute towards improving the health situation at the prison.

11.2 Recommendations

Based on the findings of the present study the following recommendations can be made:-

1. Health education related to intestinal parasitic infections prevention and control to the prison community should be given.
2. Boiling and filtering water for drinking could be a recommended method to reduce the existing increased prevalence of Amoebiasis and Giardiasis
3. There is an urgent need to provide safe and clean drinking water to the prision community.
4. Long term control measures to improve inmates' health and treatment for the effective control of intestinal parasitic infections in the study area.
5. Moreover, further in-depth epidemiological studies should be made in the study area.

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13.Annexes

Annex I Patient information Sheet (English and Amharic version)

1. **Purpose**
   - To know the Prevalence of intestinal parasites among diarrhogenic patients in prison inmates using different diagnostic methods
2. **Procedure to be carried out**
   - Collection of stool sample
   - There is no risk or discomfort during collection of stool sample
3. **Expected benefit of the study**
   - The individual can be diagnosed for intestinal parasites
   - The study gives information for Qilinto prison management staff for improving preventive and control strategies for the wellbeing of the inmates particularly relating to intestinal parasitic infections.
   - The assessed information about the prevalence of intestinal parasite and associated factors among prisoners will be used as a base line data for government policy makers
4. **Confidentiality of your information**
   - Records are kept confidential
   - Your name will not be mentioned.
   - Your specimens are used only for the purpose this study.
5. **Voluntary participation**
   - The participation to the study is based on your willingness.
   - The participant can be with draw himself from the study at any time and cannot be asked by the researcher.
   - With drawl from the study does not has any impact in the participants' right of getting the health service.
6. **Contact address**
   - Investigator – Senya Asfir
   - Email – senia26sabir@gmail.com
   - Mobile- +251 910063798,0987278729

ማውጫ

ለተሳታፊዎች መረጃ ቅጽ (በአማርኛ)

1. **አላማ**
   - የጥናቱአላማበአዲስአበባቂሊንጦማረሚያቤትውስጥየሚገኙታራሚዎችያለ ውንየሆድውስጥትላትል በሽታ ለማወቅ
2. **የሚካሄደውድርጊት**
   - የሰገራናሙናይሰጣሉ
   - ሰገራበሚሰጥበትጊዜምንምጉዳትየለውም
3. **የጥናቱጥቅም**
   - በተሳትፎዎት የዚህ በሽታ ተጠቂ መሆን ወይምአለመሆንዎያውቃሉ
   - ለማረሚያ ቤቱ አስፈላጊውን መርጃ ለመስጠትና ለመንግስት ፖሊሲ አውጪዎች መነሻ ጥናት እንዲሆን
4. **ሚስጥራዊነት**
   - ውጤቱ በሚስጥር ይቀመጣል
   - ስምዎትአይጠቀስም
   - የሚሰጡትናሙናለዚህጥናትብቻየሚውልነው
5. **በፍቃደኝነትስለመሳተፍ**
   - ተሳታፊነትዎበእርሶፍላጎትላይየተመስረተነው
   - በማንኛውምሰዓትከጥናቱየማቋረጥመብትአሎትምክንያቶትንአይጠየቁም
   - አለመሳተፍዎበሌላየጤናአገልግሎትላይየሚያመጣቦትአሉታዊጎንየለውም
6. **አድራሻ**

ሰንያአስፍርሳቢር

ስልክቁጥር+251 910063798,0987278729

Annex II. Informed Consent form (English and Amharic version)

I the undersigned have been informed and understood that the purpose of this particular research project is to find out Prevalence of intestinal parasites among diarrhogenic patients in prison inmates using different diagnostic methods. I have been told that it is my full right not to participate, to discontinue or to participate in the study. If I do not like to participate, I am not obliged to answer the questionnaire. I have been told that there will be no negative impact on whatever a decision I made. Hence, with this understanding, my honest participation will have contribution to generate information that can be used in the fight against intestinal parasites.

Signature of the participants: _____

Date: _____

የፈቃደኝነትነት መግለጫ ቅፅ

እኔከዚህበታችየፈረምኩት የአንጀት ጥገኛ ተህዋሲያንስርጭትሆዳቸውንከሚያስቀምጣቸውአዲስ አበባ የቂሊንጦ ማረሚያ ከሚገኙ ታራሚዎች ውሰጥ እና የተለያዩየላቦራቶሪመመርመሪያዘዴዎችንበሚልርዕስስለተዘጋጀውየጥናት አላማ ተነግሮኛል ። በጥናቱ ላይ የመሳተፍምሆነያለመሳተፍእነዲሁምበፈለኩ ጊዜ ቃለመጠይቁን የማቋረጥ መብት እንዳለኝተነግሮኛል። የመሳተፍ ፍላጎት ከሌለኝመጠይቁንየመመለስግዴታየለብኝም።ማንኛውንም አይነት ውሳኔብወስን ምንም አይነት ጉዳት እንደማይደርስብኝተነግሮኛል። ስለዚህ ያለውን ነገርተረድቼእኔበሙሉፍቃደኝነትመሳተፌ የአንጀት ጥገኛ ተህዋሲያንስርጭትለማጥፋት መረጃ በመስጠትየበኩሌንአስተዋጽኦአደርጋለው።

የተሳታፊውፊርማ_____

ቀን _____

Annex III. Questionnaires (English Version)

Intestinal parasites and method of detection among diarrhogenic patients at Qilinto prison inmates of Addis Ababa

Identification

1. Name of the prision ……..
2. Adress of the prision …….
3. Date of interview (dd/mm/yyyy) ……/……/…….
4. Name of interviewer ……
5. Participant Code ……

Socio demographic information of Study Participants

6. Region…….Sub city…… Woreda ……. Kebele ……
7. Sex of the respondent 1. Male 2. Female
8. Age of the respondent _____
9. What is the highest level of education you have completed 1. Non-formal education

   2. Primary school 3. Secondary school 4. Certificate 5. Diploma & above
10. Marital status?

    1. Single 2, Married 3.Divorced 4. Widowed
11. How long did you stay in prisons? …….

Personal hygiene and environmental sanitation

12. Where is the source of drinking water?

    1. Tap water 2. Bottled water 3.tap and bottled water 4. others _____
13. Is there Latrine: 1. Yes 2. No

14. Do you wash your hands before and after meal?

1. Yes 2. No

15. Do you eat your meal sharing with other inmates?

1. Yes 2. No

16. Do you use food which comes from outside the prison?

1. Yes 2. No

17. Do you eat food sharing (gursha) each other ?

1. Yes 2. No

18. Do you have the following sign and symptom?

1. Fever 2. Vomiting 3.Abdominal pain 4. Others _____

19. Do you have diarrhea 1. Yes 2. No

20. Duration of diarrhea. 1.1-5 days 2. 6-10 days 3. 11-15 days 3, >16 days

21. Consistency of Diarrhea: - 1.Watery 2. Bloody 2.Mucoid 3.Mixed (blood and mucus)

If other, specify _____

22. Have you taken anti parasitic drugs in the last six days?

1. Yes 2. No

23. Do you have chronic diseases such as (TB, HIV, Cancer ...etc)

1. Yes 2. No

24. If the answer to question number 22 is yes, specify type of disease

_____

25. If the answer to question 22 is yes, how long you were diseased?

_____

Annex IV Amharic Questionnaires (Amharic Version)

Intestinal parasites and method of detection among diarrhogenic patients at Qilinto prison inmates of Addis Ababa

መለያ

1. የማረሚያቤቱስም_____
2. የማረሚያቤቱአድራሻ _____
3. ቃለመጠይቁየተደረገበትቀን (ቀን/ወር/ዓ.ም _____/_____/_____
4. ቃለመጠይቁንያደረገውግለሠብስም_____
5. የጥናቱተሳታፊመለያቁጥር _____

የጥናቱ ተሳታፊማህበራዊናግሊዊ መረጃ

6. ክልል _____ክፍለከተማ _____ወረዳ _____ቀበሌ _____
7. ጾታ 1. ወንድ 2. ሴት
8. እድሜ_____
9. የትምህርትደረጃ 1.ያልተማረ2 .የመጀመሪያደረጃ 3. ሁለተኛደረጃ 4. ሰርተፊኬት 5. ዲፕሎማ 6. ዲግሪ

ሌላ _____

10. የትዳርሁኔታ 1. ያላገባ/ች 2. ያገባ/ች 3. ፍቺየፈጸመ/ች 4. የሞተችበት/ባት
11. እዚህማረሚያቤትከገባህምንያህልጊዜይሆንሃል? _____

የራስንንጽህናእናየአካባቢንጽህናበተመለከተ

12. የመጠጥውሃየምታገኙትከየትነው? 1. የቧንቧውሃ 2.የተጣራ ውሃ(የሀይላንድ) 3.የጉድጓድውሃሌላካለይገለጽ ? _____
13. መጸዳጃቤትአላችሁ?1. አዎ 2. አይ

ከምግብበፊትናከምግብበኋላእጅህን/ሽንትታጠቢያለሽ? 1. አዎ 2. አይ

14. ከማረሚያቤቱውጭየሚመጡምግቦችንትጠቀማለህ/ሽ? 1.አዎ 2. አይ

15. ምግብከሌሎችበጋራ(በመጎራረስ)ትመገባላችሁ?. 1. አይ. 2 አዎ

16. □.□ 15 □□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□? 1. አዎ 2 አይ

17. ከሚከተሉትየህመምምልክቶችየትኞቹአሉህ? 1. ትኩሳት 2.ማስመለስ 3. .የሆድህመም

4. ሌሎች (ካሉ) ____

18. .ሆድህን/ሽንያስቀምጥሻል? 1. አዎ 2. አይ

19. ለተራቁጥር 21 መልስአዎክሆነ.የሚያስቀምጦትምንአይነትነው? 1. ውሃ መሰል 2. የሚዝለገለግ

3.ደም የተቀላቀለበትና የሚዝለገለግ 4. ሌላ ካለ ይገለጽ __________________________________

20. ላለፉትስድስትቀናትየአንጀትጥገኛተህዋሲያንመድሃኒትወስደሃል? 1. አዎ 2.

.አይ

22የታወቀ ለረጅም ጊዜ የቆየ ህመም(ቲቢ ኤድስ ካንሰር……የመሳሰሉት) አለብዎትወይ ? 1. አዎ

2. .አይ

23. ለተራቁጥር22መልስዎአዎከሆነየህመሙአይነትይገለፅ ___________________________________

24.ለተራቁጥር22መልስዎአዎከሆነህመሙከጀመርዎምንያህልጊዜ ሆኖዎታል ______________________

Annex V Laboratory standard operating procedures (SOP)

Direct Wet mount Preparation of Fresh Stools

Procedure

1. Place one drop of 0.85% NaCl on the left side of the slide and one drop of iodine (working solution) on the right side of the slide.
2. Take a small amount of fecal specimen and thoroughly emulsify the stool in saline and iodine using an applicator stick. The sample should be spread thinly enough that newsprint can barely be read when the slide is placed on top of text.
3. Slide a 22mm cover slip at an angle into the edge of the emulsified fecal drop. Push the cover slip across the drop before allowing it to fall into place.
4. Systematically scan the entire 22mm cover slip with overlapping fields with the 10x objective.
5. Switch to high dry (40X objective) for more detailed study of any suspect eggs or protozoa.

Quality control

For direct smear:

1. Check the working iodine solution each time it is used
2. Iodine should be the color of strong Orange Peko tea, discard if it is too light.
3. Protozoan stained with iodine should contain yellow gold cytoplasm, brown glycogen material and paler refractile nuclei. The chromatoidal bodies may not be as clearly visible as in a saline mount.
4. The microscope should be calibrated (within the last 12 months)

5. All QC results should be appropriately recorded and any "out- of-control" results referred to the laboratory director for action.
6. Ensure that reagents and chemicals used are not expired. Safety note: Universal precautions should be observed.

Zeihl-Neelsen **stain, modified (hot method)**

This staining method highlights the acid resistance of intestinal coccidian.

Procedure

1. The fecal sample is spread on the slide to produce a thin smear.
2. For this procedure, use feces fixed in formalin or SAF or feces that have previously been concentrated (in the last case, to recover *Cryptosporidium* oocysts, all centrifugation phases must be lengthened by at least 10 min).Allow the slide to air dry.
3. Fix in 100% methanol for 1 minute. Allow the slide to air dry.
4. Flood slide with Carbol-fuchsin working solution for 5 minutes,
5. Gently heating on a Bunsen flame or ethanol lamp. Do not boil or dry the staining solution; if it dries, add more staining solution to the slide.
6. Decolorize with acid solution no longer drips from the slide.
7. Wash slide well with tap water.
8. Flood slide with a ethylene blue solution for 30 s.
9. Wash well with tap water, and allow the slide to air dry.

Quality Control

A control slide of *Cryptosporidium* spp. from a 10% formalin preserved specimen should be included with each staining run. *Cryptosporidium* spp. stains a pinkish-red color. The background should stain uniformly green. Examine slides at low (40x objective) and high (100x objective) power. Oocysts stain

pink to red or deep purple. Isospora belli in mature Oocysts, two red sporocysts are visible, surrounded by a clear halo separating them from the Oocyst wall.

Cyclospora: on average only one oocyst out of four becomes stained, taking on a red to pale pink color; the other oocysts can be seen as roundish and colorless.

Phenol- auramine stain

This stain can be used as an alternative to the modified Ziehl-Neelsen stain for staining oocysts of *Cryptosporidium parvum.*

Method

a. Make faecal smears as for ZN and fix in methanol.
b. Stain with phenol-auramine (Lemperts) for 10-15 minutes.
c. Rinse thoroughly in tap water.
d. Decolorize in acid alcohol. (As for ZN)
e. Rinse thoroughly in tap water.
f. Counterstain with 0.1% potassium permanganate for 30 sec.
g. Rinse thoroughly in tap water, allow to air dry. Do not blot dry, many brands of blotting paper will fluoresce!
h. Observe the films with blue light under an incident light fluorescent microscope and low power followed by oil immersion x100 objective if oocysts are suspected.

Quality Control

A control slide of C. parvum from a 10% formalin preserved specimen was included with each staining batch and both staining techniques. If the Cryptosporidia stains well, it was assumed that any other coccidia would also take up the stain.The oocysts of *Isospora belli* and *Cyclospora cayetanensis* do not fluoresce well using the phenol-auramine stain.

Specimen

Concentrated sediment of fresh or formalin-preserved stool may be used. Other types of clinical specimens such as duodenal fluid may also be stained.

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Declaration

Assurance of Principal Investigator

The undersigned agrees to accept responsibility for the scientific ethical and technical conduct of the research project and for provision of required progress reports as per terms and conditions of the research publications office in effect at the time of grant is forwarded as the result of this application.

Name of the student: Senya Asfir

Date _____ Signature _____

Approval of Advisors:

Kassu Desta (MSc,PhD fellow)

Date_____ Signature _____

Adugna Abera (MSc,)

Date_____ Signature _____