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Prevalence of intestinal parasites and associated factors among food handlers in food establishments in Lideta sub city of Addis Ababa

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Prevalence of intestinal parasites and associated factors among food handlers in food establishments in Lideta sub city of Addis Ababa Woinishet Abera¹, Binyam Gintamo^{1,2,3}, Tewoderos Shitemaw⁴, Zelalem Negash Mekuria^{1,2,5}, Zemichael Gizaw^{6*} . í .

5	¹ Yanet Health College, Addis Ababa, Ethiopia.
6	² Addis Ababa Medical and Business College, Addis Ababa, Ethiopia.
7 8	³ Department of Biotechnology, Faculty of Applied Sciences and Biotechnology, Shoolini University, Solan, Bajhol, H.P, India
9	⁴ Kotebe Metropolitan University, Addis Ababa, Ethiopia
10	⁵ Yekatit 12 Medical College, Addis Ababa, Ethiopia
11	⁶ Department of Environmental and Occupational Health and safety, Institute of Public Health,
12	College of Medicine and Health Sciences, University of Gondar, Gondar, Ethiopia
13	Email address of authors: Woinishet Abera (woini27ab@gmail.com), Binyam Gintamo
14	(gintamobinyam@gmail.com), Tewodros Shitemaw (tewoderosshitemaw@gmail.com), Zelalem
15	Negash Mekuria (zolabelay256@gmail.com), and Zemichael Gizaw (zemichael12@gmail.com)
16	*Corresponding author: Zemichael Gizaw, Department of Environmental and Occupational
17	Health and safety, Institute of Public Health, College of Medicine and Health Sciences,
18	University of Gondar, Gondar, Ethiopia, Email: zemichael12@gmail.com
19	Abstract
20 21	Objectives: This study was conducted to assess the prevalence of intestinal parasites and associated factors among food handlers.
22	Setting: This study was conducted in different food establishments in Lideta sub city of the
23	Addis Ababa, Ethiopia.
24	Participants: Four hundred and eleven food handlers were participated in this study.
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Design: An institution-based cross-sectional study design was used. Stool samples were collected from food handlers and examined using direct wet mount technique. Personal and establishment related information was collected using pretested questionnaire with structured observation. Multivariable binary logistic regression was used to identify factors associated with prevalence of intestinal parasites on the basis of adjusted odds ratio and 95% confidence interval with p < 0.05.

Outcome measures: The primary outcome is prevalence of intestinal parasites, which was
 defined as presence of one or more intestinal parasitic species in stool samples.

Results: One or more intestinal parasites were detected in 171 (41.6%) of the stool samples. The
most common intestinal parasites were *E. histolytica* (12.2%), *G. lamblia* (10.5%), and *A. lumbricoides* (6.1%). This high prevalence of intestinal parasites among food handlers was
associated with low monthly income (AOR: 2.83, 95% CI:1.50, 8.84), untrimmed fingernails
(AOR: 4.36, 95% CI: 1.98, 11.90), no food safety training (AOR: 2.51, 95% CI: 1.20, 5.58),
low level of education (AOR: 3.13, 95% CI: 1.34, 7.44), poor handwashing practice (AOR:
2.16, 95% CI: 1.03, 4.22), and lack of medical checkup (AOR: 2.31, 95% CI: 1.18, 6.95).

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40 Conclusion: The prevalence of intestinal parasites among food handlers in the studied region
41 was high. This high prevalence was linked to socio-economic conditions, poor hand hygiene
42 condition, and absence of food safety trainings. It is, therefore, important to promote hand
43 washing practice and providing food hygiene and safety training is critical.

Key words: Intestinal parasites, food handlers, food establishments, hand hygiene, Ethiopia

- 45 Strengths and limitations of this study
- 46 The study has focused on one of the most potential groups, i.e., food handlers that had potential to
 47 spread food borne infections to the consumers.
- 48 The use of sensitive diagnostic techniques and combination of methods with triplicate
 49 examination applied in this study would help to recover greater rate of intestinal parasites
 50 that would indicate the true prevalence.
 - 51 As a limitation, we measured the burden of intestinal parasites in the dry season, so that the

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associations reported in the current study are not adjusted for seasonal variations.

53 Introduction

Foodborne diseases are increasingly becoming a serious global public health problem. The World Health Organization (WHO) estimate indicates that each year worldwide, unsafe food causes 600 million cases of foodborne diseases and 420 000 deaths. WHO estimated that 33 million years of healthy lives are lost due to eating unsafe food globally each year, and this number is likely an underestimation [1]. One of the causes of foodborne diseases is contamination during food preparation; food handlers carrying pathogens might be involved in the origin of this condition. Foods can be contaminated with fecal material at the point of production or during food preparation, in both the home and in commercial premises [2]. Food handlers with poor personal hygiene and inadequate knowledge on food safety could be potential sources of infections. Food handlers who harbor and excrete enteropathogens may contaminate foods from their feces via contaminated hands, then to food or food contact surfaces, and finally to healthy individuals [3-7].

The contribution of the infected food worker (whether symptomatic or not) to food borne disease outbreaks has been difficult to establish. However, reports showed that food workers in many settings have been responsible for foodborne disease outbreaks for decades. For instance, members of the Committee on Control of Foodborne Illnesses of the International Association for Food Protection analyzed 816 foodborne disease outbreaks with 80,682 cases in different countries where food workers were implicated as the source of the contamination. The report also estimated that infected food worker was documented as responsible for 18% of 766 outbreaks occurring in the United States [8, 9]. Moreover, according to the Centers for Disease Control (CDC), as cited in Mathew RR (2019), 20 to 40% of food borne illness associated with the consumption of contaminated food originated in catering establishments [10].

Since the food handling personnel play a role in the transmission of food borne diseases and the health of the food handlers is of great importance for maintaining quality of food prepared and served by them, pre-employment and periodic medical checkups are very important to safeguard the consumers from getting diseases from cross contaminated foods along with other food safety measures [11, 12]. However, pre-employment and periodic medical checkups are not commonly Page 5 of 19

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practiced in Ethiopia. As a result of this, money of food handlers working in different food establishments all over the country harbor one or more enteropathogens. For instance, a systematic review and meta-analysis reported that the overall pooled prevalence estimate of intestinal parasites among food handlers of food service establishments in Ethiopia was 33.6% (95% CI: 27.6, 39.6%) [13] and the common factors associated to high prevalence of intestinal pathogens among food handlers are poor hand hygiene, inadequate access to water and sanitation facilities, and poor socio-economic conditions [13-18]. However, the prevalence and risk factors may be different across various settings. Accordingly, this study was conducted to assess the prevalence of intestinal parasites and associated factors among food handlers working in food establishment in Lideta sub city of the Addis Ababa, Ethiopia.

91 Methods

92 Study design and setting

93 This an institution-based cross-sectional study with laboratory investigations was conducted in 94 Lideta sub city of Addis Ababa from March 20 to April 20, 2021. Lideta sub city is one of the 10 95 sub cities of Addis Ababa, the capital of Ethiopia. The sub city is divided into 10 districts 96 (woredas). In Lideta sub city, there are a total of 281 food establishments and 1124 food handlers 97 working in the food establishments.

98 Sample size calculation and sampling techniques

99 The sample size was calculated using simple population proportion formula with the following 100 assumptions: prevalence of intestinal parasites among food handlers in Addis Ababa University 101 students cafeteria, Addis Ababa, Ethiopia (p) = 45.3% [19], level of significance (α) = 5%, 95% 102 confidence interval (standard normal probability), z: the standard normal tabulated value, and 103 margin of error (d) = 5%.

104
$$n = \frac{(Z_{\alpha/2})^2 P(1-P)}{d^2} = \frac{(1.96)^2 0.453(1-0.453)}{0.05^2} = 381$$

105 The final sample size was 419 after considering 10% non-response rate. Using lists of food 106 handlers working in different food establishments in the sub city obtained from Addis Ababa 107 food, medicines, and healthcare administration authority (AAFMHACA) as a sampling frame,

we used computer generated simple random sampling technique to select food handlers. Food
handlers who treated with anti-helminthes and anti-protozoan drugs in the last four weeks were
excluded from the study.

111 Stool sample collection

Stool sample collectors first explained the purpose of collecting stool to the randomly selected food handlers, and then asked them to urinate first without pooping to avoid urine contamination of the stool. Stool sample collectors then handed out paper to food handlers and instructing them to defecate on it to avoid stool contamination stored feces and dirt. Food handlers were asked to bring approximately 50 g of the last part of the stool, the softest part, into the collection container after defecating on the paper. The stool collectors then immediately stored the stool sample in to a cold box after labeled a code on the outer surface of the plastic cup.

25 119 **Personal and food establishment data collection**

Pretested questionnaire with structured observational checklist was used to collect personal and food establishment related information. The questionnaire was developed by reviewing related published articles [15, 20-24]. The tool was first prepared in English language and translated to the local Amharic language by two native Amharic speakers fluent in English, and back-translated into English by two independent English language experts fluent in Amharic to check consistency. The questionnaire consisted of three parts: i) socio-demographic characteristics of food handlers, ii) food handlers' personal hygiene conditions, and iii) food establishment related factors. Data collectors were trained in the data collection tool as well as ethical issues during interviewing and observing. Supervisors supervised the data collection process and checked the completeness of the data on a daily basis. We gathered handwashing data by assessing food handlers' usual handwashing behavior using self-reports. We also looked at the hands of food handlers to see the general cleanliness and conditions of fingernails. In addition, we asked food handlers to demonstrate how they wash their hands on a regular basis, which we evaluated using checklists for effective handwashing.

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134 Detection of ova of parasites in stool samples

We used direct stool examination (wet mount) and rormol-ether concentration techniques to detect ova of intestinal parasites in stool samples. One drop of physiological saline was placed on a clean slide. Using an applicator stick, a small amount of stool specimen was emulsified in saline solution. The preparation was covered with a cover slip and examined under the microscope for the absence or presence of an intestinal parasites. The entire saline preparation was systematically examined for helminthes eggs, larvae, ciliates, cysts and oocysts using $10\times$ objective with condenser iris closed sufficiently to give good contrast, while 40 × objectives was used to assist in the detection of eggs, cysts, and oocytes [25].

For the formol-ether concentration technique, an estimated 1 g of formed stool sample or 2 ml of watery stool was emulsified in about 4 ml of 10% formol water contained in a screw-cap bottle. A further 3 ml of 10% formol water was added and mixed well by shaking. The emulsified stool samples were sieved and the sieved suspension transferred to a conical (centrifuge). 3 ml of diethyl ether was added and the tube was stoppered mixed for 1 minute with a tissue wrapped around the top of the tube, the stopper was loosen. It was then centrifuge at 3000 rpm for 1 minute. Using a stick, the layer of feces debris from the side of the tube was loosen and the tube inverted to discard the ether, fecal debris, and formol water leaving behind the sediment. The tube was returned to its upright position and the fluid from the sides of the tube allowed draining to the bottom. The bottom of the tube was taped to re-suspend and mix the sediment. The sediment was transferred to a slide and covered with a cover glass and was examined microscopically using the 10x objective for focusing and $40 \times$ objective for proper identification.

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Standard operating procedures were used for every laboratory procedure during lab examination,
stool specimen collection, transportation, and storing. The expiry date of normal saline, ether,
and formol was evaluated before stool sample preparation and examination.

⁸ 158 Outcome variable of the study

Prevalence of intestinal parasites, the primary outcome variable of the study is defined as thepresence of one or more intestinal parasite species in stool samples.

161 Data processing and analysis

Data were entered using EPI-INFO version 3.5.3 statistical package and export into Statistical Package for Social Sciences (SPSS) version 20 for further analysis. For most variables, data were presented by frequencies and percentages. Univariable logistic regression analysis was used to choose variables for the multivariable logistic regression analysis, and variables which had p - value less than 0.25 by the univariable analysis were then analyzed by multivariable analysis for controlling the possible effect of confounders. Finally, variables which had significant association were identified on the basis of adjusted odds ratio (AOR) with 95% confidence interval (CI) and p-values < 0.05. Model fitness was check using Hosmer and Lemeshow goodness-of-fit test.

171 Patient and public involvement

172 No patient involved.

173 Results

174 Socio-demographic characteristics of study participants

We collected personal information and stool samples from a total 411 food handlers with a response rate of 97.62%. The majority, 293 (71.3%) of the study participants were female. About half, 198 (48.18%) of the respondents aged 25 years and below. Half, 207 (50.3%) of the respondents reported that they completed primary school education. Two hundred and seventyeight (67.6%) of the food handlers reported that they had 3 years or less work experience. One hundred and eleven (27.0%) of the food handlers earned <1500 Ethiopian birr (Table 1).

Table 1: Socio-demographic characteristics of food handlers (n = 411) working in different food
establishments in Lideta sub city of Addis Ababa, Ethiopia, March 20 to April 20, 2021

Socio-demographic characteristics	Frequency	Percent
Sex of the respondents		
Female	293	71.3
Male	118	28.7
Age of the respondents in years		
≤25	198	48.2

	26-35	113	27.5	
	36-50	100	24.3	
Edu	ucation status of the respondents			
	Tertiary education	23	5.6	
	Secondary school	163	39.7	
	Primary school	207	50.3	
	Illiterate	18	4.4	
Se	rvice year			
	≤3 years	278	67.6	
	>3 years	133	33.4	
Av	verage monthly income in Ethiopian birr			
	<1500	111	27.0	
	1501-2500	152	37.0	
	2501-3500	94	22.9	
	>3500	54	13.1	

Personal hygiene characteristics of food handlers

Two hundred and forty-two (58.9%) of the food handlers did not keep their fingernails short. One hundred and ninety-four (47.2%) and 206 (50.1%) of the food handlers did not regularly wash hands with soap after visiting toilet and before eating, respectively. Two hundred and eight (50.6%) of the food handlers reported that they regularly worn clean protective clothes. About a quarter, 76 (24%) of the food handlers reported that they received food safety training and 121 (29.4%) of the food handlers had medical checkup in the previous 6 months prior to the survey (Table 2).

Table 2: Personal hygiene characteristics of food handlers (n = 411) working in different food establishments in Lideta sub city of Addis Ababa, Ethiopia, March 20 to April 20, 2021

Variables	Frequency	Percent
Condition of fingernails		
Trimmed	169	41.1

Untrimmed	242	58.9
Regular hand washing with soap after toilet		
Yes	217	52.8
No	194	47.2
Regular hand washing with soap before eating		
Yes	205	49.9
No	206	50.1
Wearing clean protective clothes regularly		
Yes	208	50.6
No	203	49.4
Food safety training		
Yes	76	24
No	335	76
Medical checkup (in last 6 month)		
Yes	121	29.4
No	290	70.6

194 Intestinal parasites in food handlers

A total of 411 food handlers were examined, with 171 (41.6%) of them had ova of one or more
intestinal parasites, of which 14 (3.4%) had mixed parasites (Figure 1). The most common
intestinal parasites were *E. histolytica* [50 (12.2%)], *G. lamblia* [43 (10.5%)], *A. lumbricoides*[25 (6.1%)], Hookworms [15 (3.7%)], *T. trichuria* [14 (3.4%)], and Taenia species [10 (2.4%)]
(Table 3).

Table 3: Common intestinal parasites detected among food handlers (n = 411) working in
different food establishments in Lideta sub city of Addis Ababa, Ethiopia, March 20 to April 20,
202 2021

Parasitic species	Frequency	Percent	
E. histolytica	50	12.2	
G. lamblia	43	10.5	
A. lumbricoides	25	6.1	

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Hookworms	15	3.7	
T. trichuria,	14	3.4	
Taenia species	10	2.4	

Factors associated with intestinal parasites among food handlers

Table 4 shows factors associated with prevalence of intestinal parasites in food handlers. This study depicted that educational status of food handlers was significantly associated with intestinal parasites. Illiterate food handlers had higher odds to have intestinal parasite compared with food handlers who attended tertiary education (AOR: 3.13, 95% CI (1.34, 7.44)). Similarly, the odds of having intestinal parasites was higher among food handlers who attended primary education (AOR: 2.22, 95% (1.10, 6.65)) and secondary education (AOR: 2.16, 95% CI (1.10, (5.20)) compared with food handlers who attended tertiary education. The prevalence of intestinal parasites was statistically associated with monthly income of food handlers. The odds of having intestinal parasites was 2.84 times higher among food handlers who earned below 1500 Ethiopian birr per month (AOR: 2.84, 95% CI (1.50, 8.84)). This study revealed that intestinal parasites was significantly associated with hand hygiene of food handlers. Food handlers who did not keep their fingernails short had 4.36 more odds to have intestinal parasites compared with food handlers who trimmed fingernails (AOR: 4.36, 95% CI (1.98, 11.90)). The odds of having intestinal parasites was also 2.16 times higher among food handlers who did not wash hands with soap before eating (AOR: 2.16, 95% CI (1.03, 4.22)). Furthermore, intestinal parasites among food handlers was significantly associated with food safety training and medical checkup. The odds of having intestinal parasites was high among food handlers who did not take food safety training (AOR: 2.51, 95% CI (1.20, 5.58)) compared with food handlers who took food safety training and the odds of having intestinal parasites was 2.32 times higher among food handlers who did not take medical checkups in the previous six months prior to the survey (AOR: 2.32, 95% CI (1.18, 6.95)).

Table 4: Factors associated with intestinal parasites among food handlers (n = 411) working in different food establishments in Lideta sub city of Addis Ababa, Ethiopia, March 20 to April 20,

Variables

Intestinal parasites Crude odds ratio

	Yes	No	with 95% CI	with 95%
Educational status				
Illiterate	9	9	3.60 (1.81, 10.80)	3.13 (1.34, 7.44
Primary school	92	115	2.88 (1.65, 6.17)	2.22 (1.10, 6.65
Secondary school	68	95	2.58 (1.20, 5.56)	2.16 (1.10, 5.20
Tertiary education	5	18	1.0	1.0
Average monthly income in Ethiopian bi	irr			
<1500	56	55	2.91 (1.43, 7.86)	2.84 (1.50, 8.84
1501-2500	68	84	2.31 (1.14, 6.96)	2.28 (0.63, 8.10
2501-3500	33	61	1.55 (1.05, 4.85)	1.41 (0.60, 5.96
>3500	14	40	1.0	1.0
Condition of fingernails				
Untrimmed	134	108	4.43 (2.12, 12.62)	4.36 (1.98, 11.9
Trimmed	37	132	1.0	1.0
Hand washing with soap after toilet				
No	101	93	2.28 (1.10, 7.51)	2.19 (0.92, 5.62
Yes	70	147	1.0	1.0
Hand washing with soap before eating				
No	112	94	2.95 (1.23, 6.72)	2.16 (1.03, 4.22
Yes	59	146	1.0	1.0
Food safety training				
No	153	182	2.71 (1.34, 8.53)	2.51 (1.20, 5.58
Yes	18	58	1.0	1.0
Medical checkup in the last 6 month				
No	140	150	2.71 (1.27, 7.56)	2.32 (1.18, 6.95
Yes	31	90	1.0	1.0
Note: * statistically significant at $p < 0$.05, ** statis	tically sign	nificant at $p < 0.01$, H	Iosmer and Leme
goodness-of-fit test = =0.483				
	11			

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Discussion

This is an institution-based cross-sectional study assessed intestinal parasites among food handlers working in food establishments in Lideta sub city of Addis Ababa, Ethiopia and found that 41.6% of the food handlers had one or more intestinal parasites in the studied region. The prevalence of intestinal parasites reported in this study was lower than findings of studies conducted among food handlers in Bule Hora (46.3%) [16], Addis Ababa University (45.3%) [19], Nekemte town (52.1%) [26], and Mekele University (49.4%) [15]. Furthermore, the prevalence of intestinal parasites reported in the current study was higher than findings of studies conducted among food handlers in Wolaita Sodo (23.6%) [14], Jimma (33%) [27], Madda Walabu University (25.3%) [28], Motta town (27.6%) [29], Nairobi(15.7%)[30], Iran (9%) [31], Saudi Arabia (23%) [32]. and Thailand (10%) [33]. The high prevalence of intestinal parasites among food handlers in the studied region might be explained by poor socio-economic conditions, poor hand hygiene, and inadequate access to basic sanitation services.

The high prevalence of intestinal parasites among the food handlers in studied region suggests that poor hygiene practices of food handlers and inadequate access to sanitation services. The results may also suggests that there might be transmission of intestinal parasitic infections from food handlers to food users, unless large scale screening and mass drug administration are done. As it is documented in literature, infected food handlers play a significant role in infection transmissions to customers of the food establishments where the infected food handlers are working [4].

This study showed that educational status of food handlers was associated with a high prevalence of intestinal parasites. Prevalence of intestinal parasites was higher among food handlers who were illiterate or attended primary and secondary education compared with food handlers who attended tertiary education. This may be due to the fact that educated food handlers may have awareness about the transmission and prevention methods of infectious diseases. Education encourages changes in healthy behaviors. Other similar studies also reported the relation of education with occurrence of parasitic infections [34-38].

The current study revealed that monthly income of food handlers was associated with intestinal parasites in the studied region. Food handlers with low monthly income had higher odds of

intestinal parasites. This may be due to the fact that food handlers with low economic status
could not afford for services like soap, household water treatment, toilets and other facilities, and
so that will have limited opportunities to practice healthy measures. The effect of low income on
risk of parasites is complex and could be attributed to limited access to sanitary materials,
sources of drinking water and food, environment sanitation, and education [37-40].

The high prevalence of intestinal parasites among food handlers was associated with hand hygiene. Food handlers who did not keep their fingernails short had higher odds to have intestinal parasites and the odds of having intestinal parasites was also higher among food handlers who did not wash hands with soap before eating. This might be due to the fact that the area beneath the fingernails has the highest concentration of microorganisms on the hands and is the most difficult to clean [41-44]. Moreover, food handlers may ingest diseases causing pathogens when they eat without washing hands. Hands are one of the most important mechanisms to transmit pathogenic microorganisms leading to infection [45]. Evidence indicates that hands together with food contact or other environmental surfaces cause 60% of the spread of gastrointestinal infection. Contaminated hands could also be associated with up to 50% of respiratory tract infections [46].

This study depicted that intestinal parasites among food handlers was significantly associated with food safety training. The odds of having intestinal parasites was high among food handlers who did not take food safety training compared with their counterparts. This could be due to the fact those food handlers who did not take food safety training may lack the necessary knowledge and practice towards transmission and prevention of disease causing pathogens. Moreover, food safety training or health education promotes health behaviors toward hygiene and sanitation practices. Health education increases knowledge and acceptability of interventions. It also sustains integrated control of the infection [47-49].

Furthermore, intestinal parasites was significantly associated with medical checkup. The odds of having intestinal parasites was high among food handlers who did not take medical checkups in the previous six months prior to the survey. Other studies are also reported that medical checkup of food handlers is associated with intestinal parasites [15, 31, 50]. This is because food handlers who did not know their health condition before employment and while working in different establishments has less chance to take treatment and mass drugs as a result there may be existed

The prevalence of intestinal parasites among food handlers in the studied region was found to be high. This high prevalence of intestinal parasites was linked to socio-economic conditions of food handlers, poor hand hygiene condition, absence of food safety trainings and regular medical checkup. It is, therefore, important to promote hand washing practice of food handlers, provide food hygiene and safety trainings, and establishing a system to regularly check the health conditions of food handlers.

20 295 Data availability statement21

Individual participant data after deidentification that underline the results reported in this article
 will be made available upon requesting the primary author immediately following publication.

26 298 Authors' contribution
27

WA designed the study, facilitated data collection, and conducted data analysis. BG, TS, ZNM,
and ZG supervised data collection and analysis and contributed to conceptualizing the study. ZG
prepared the manuscript. All authors approved the final version of the manuscript.

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Competing interest

- 306 None of the authors have any competing interests in the manuscript.
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311 Ethical Approval

- 312 The ethical and methodological issues of this protocol was approved by the institutional review
- board (IRB) of Yanet Health College (reference number: YEC/060/21).

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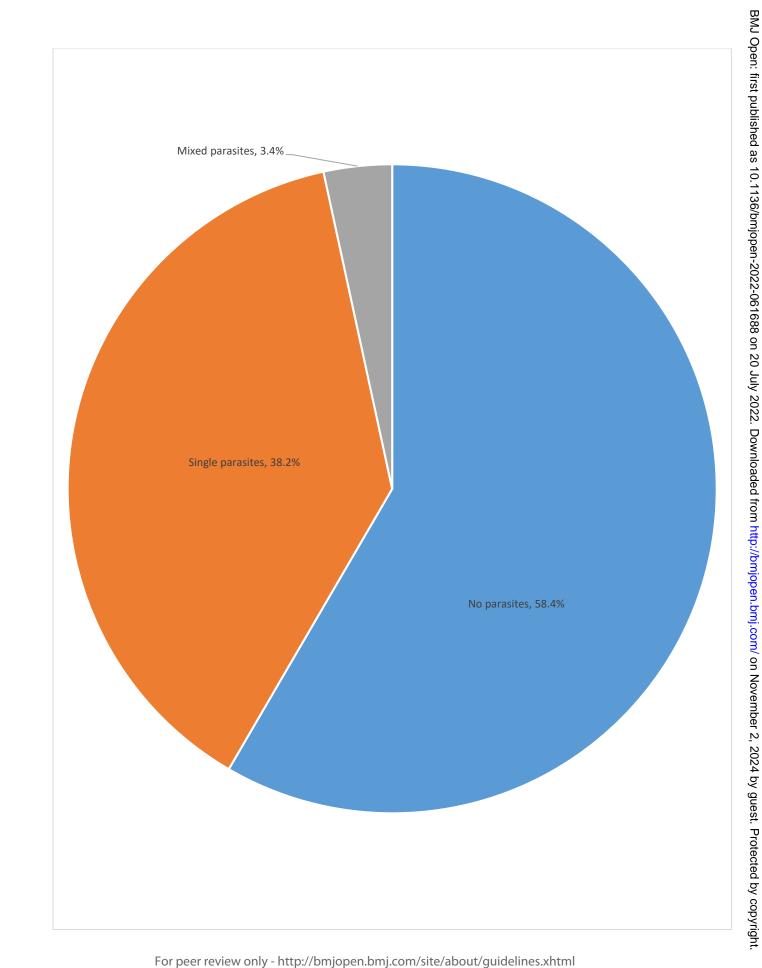
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Prevalence of intestinal parasites and associated factors among food handlers in food establishments in the Lideta sub-city of Addis Ababa, Ethiopia: an institution-based cross-sectional study

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Prevalence of intestinal parasites and associated factors among food handlers in food

establishments in the Lideta sub-city of Addis Ababa, Ethiopia: an institution-based cross-sectional study Woinishet Abera¹, Binyam Gintamo^{1,2,3}, Tewoderos Shitemaw⁴, Zelalem Negash Mekuria^{1,2,5}, Zemichael Gizaw6* ¹Yanet Health College, Addis Ababa, Ethiopia. ²Addis Ababa Medical and Business College, Addis Ababa, Ethiopia. ³ Department of Biotechnology, Faculty of Applied Sciences and Biotechnology, Shoolini University, Solan, Bajhol, H.P., India ⁴ Kotebe Metropolitan University, Addis Ababa, Ethiopia ⁵ Yekatit 12 Medical College, Addis Ababa, Ethiopia ⁶Department of Environmental and Occupational Health and safety, Institute of Public Health, College of Medicine and Health Sciences, University of Gondar, Gondar, Ethiopia Email address of authors: Woinishet Abera (woini27ab@gmail.com), Binyam Gintamo (gintamobinyam@gmail.com), Tewodros Shitemaw (tewoderosshitemaw@gmail.com), Zelalem Negash Mekuria (zolabelay256@gmail.com), and Zemichael Gizaw (zemichael12@gmail.com) *Corresponding author: Zemichael Gizaw, Department of Environmental and Occupational Health and safety, Institute of Public Health, College of Medicine and Health Sciences, University of Gondar, Gondar, Ethiopia, Email: zemichael12@gmail.com Abstract Objectives: This study was conducted to assess the prevalence of intestinal parasites and associated factors among food handlers. Design: An institution-based cross-sectional study design was used. Stool samples were collected from food handlers and examined using direct wet mount and formalin-ether concentration (FEC) techniques. Personal and establishment related information was collected using pretested questionnaire with structured observation. Multivariable binary logistic

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regression was used to identify factors associated with prevalence of intestinal parasites on the basis of adjusted odds ratio and 95% confidence interval with p < 0.05.

Setting: This study was conducted in different food establishments in the Lideta sub-city of
Addis Ababa, Ethiopia.

Participants: Four hundred and eleven food handlers were participated in this study.

Outcome measures: The primary outcome is prevalence of intestinal parasites, which was
 defined as presence of one or more intestinal parasitic species in stool samples.

Results: One or more intestinal parasites were detected in 171 (41.6%) (95% CI: 36.6, 46.4%) of the stool samples. The most common intestinal parasites were E. histolytica/dispar (12.7 %). G. duodenalis (11.2%), and A. lumbricoides (8.3%). This high prevalence of intestinal parasites among food handlers was associated with low monthly income (AOR: 2.83, 95% CI:1.50, 8.84), untrimmed fingernails (AOR: 4.36, 95% CI: 1.98, 11.90), no food safety training (AOR: 2.51, 95% CI: 1.20, 5.58), low level of education (AOR: 3.13, 95% CI: 1.34, 7.44), poor handwashing practice (AOR: 2.16, 95% CI: 1.03, 4.22), and lack of medical checkup (AOR: 2.31, 95% CI: 1.18, 6.95).

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Conclusion: The prevalence of intestinal parasites among food handlers in food 43 establishments in the Lideta sub-city of Addis Ababa was high. This was linked to socio-44 economic conditions, poor hand hygiene condition, and absence of food safety trainings. It is, 45 therefore, important to promote hand washing practice and providing food hygiene and safety 46 training is critical.

47 Key words: Intestinal parasites, food handlers, food establishments, hand hygiene, Ethiopia

8 Strengths and limitations of this study

- The study has focused on one of the most potential groups, i.e., food handlers that had potential to spread food borne infections to the consumers.

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The use of sensitive diagnostic techniques and combination of methods with triplicate
 examinations applied in this study would help to recover greater rate of intestinal parasites
 that would indicate the true prevalence.

- As a limitation, the collection of a single stool sample may affect the result of parasitic examination since the sensitivities of the direct smear examination technique is reduced when a single stool sample is examined and the procedure of FEC technique may also damage the eggs of parasites.

58 Introduction

Foodborne diseases are increasingly becoming a serious global public health problem. The World Health Organization (WHO) estimate indicates that each year worldwide, unsafe food causes 600 million cases of foodborne diseases and 420 000 deaths. WHO estimated that 33 million years of healthy lives are lost due to eating unsafe food globally each year, and this number is likely an underestimation [1]. One of the causes of foodborne diseases is contamination during food preparation; food handlers carrying pathogens might be involved in the origin of this condition. Foods can be contaminated with fecal material at the point of production or during food preparation, in both the home and in commercial premises [2]. Food handlers with poor personal hygiene and inadequate knowledge on food safety could be potential sources of infections. Food handlers who harbor and excrete enteropathogens may contaminate foods from their feces via contaminated hands, then to food or food contact surfaces, and finally to healthy individuals [3-7].

The contribution of the infected food worker (whether symptomatic or not) to food borne disease outbreaks has been difficult to establish. However, reports showed that food workers in many settings have been responsible for foodborne disease outbreaks for decades. For instance, members of the Committee on Control of Foodborne Illnesses of the International Association for Food Protection analyzed 816 foodborne disease outbreaks with 80,682 cases in different countries where food workers were implicated as the source of the contamination. The report also estimated that infected food worker was documented as responsible for 18% of 766 outbreaks occurring in the United States [8, 9]. Moreover, according to the Centers for Disease Control (CDC), as cited in Mathew RR (2019), 20 to 40% of food borne illness associated with the consumption of contaminated food originated in catering establishments [10].

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Food handling personnel play a role in the transmission of food borne diseases. The health of food handlers is of great importance for maintaining quality of food products. Accordingly, pre-employment and periodic medical checkup is very important to safeguard the consumers from getting diseases from contaminated foods along with other food safety measures [11, 12]. However, pre-employment and periodic medical checkups are not commonly practiced in Ethiopia. As a result of this, many of the food handlers working in different food establishments all over the country may harbor one or more enteropathogens. For instance, a systematic review and meta-analysis reported that the overall pooled prevalence estimate of intestinal parasites among food handlers of food service establishments in Ethiopia was 33.6% (95% CI: 27.6, 39.6%) [13] and the common factors associated to high prevalence of intestinal pathogens among food handlers are poor hand hygiene, inadequate access to water and sanitation facilities, and poor socio-economic conditions [13-18]. However, the prevalence and risk factors may be different across various settings. Accordingly, this study was conducted to assess the prevalence of intestinal parasites and associated factors among food handlers working in food establishment in the Lideta sub-city of Addis Ababa, Ethiopia.

96 Methods

97 Study design and setting

This an institution-based cross-sectional study with laboratory investigations was conducted in the Lideta sub-city of Addis Ababa from March 20 to April 20, 2021. Lideta sub-city is one of the 10 sub cities of Addis Ababa, the capital of Ethiopia. The sub-city is located at the global positioning system (GPS) coordinates of 9°0'N and 38°45'E. The sub city is divided into 10 districts (Figure 1). In the Lideta sub-city, there are a total of 281 food establishments and 1124 food handlers working in the food establishments.

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⁶ 104 Sample size calculation and sampling techniques

The sample size was calculated using single population proportion formula with the following assumptions: prevalence of intestinal parasites among food handlers in Addis Ababa University students cafeteria, Addis Ababa, Ethiopia (p) = 45.3% [19], level of significance (α) = 5%, 95% confidence interval (standard normal probability), z: the standard normal tabulated value, and margin of error (d) = 5%.

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$$n = \frac{(Z_{\alpha/2})^2 P(1-P)}{d^2} = \frac{(1.96)^2 0.453(1-0.453)}{0.05^2} = 381$$

The final sample size was 419 after considering 10% non-response rate. The Lideta sub-city was 111 selected at random from a total of 10 sub-cities of Addis Ababa, Ethiopia. Using lists of food 112 handlers working in different food establishments in the sub-city obtained from Addis Ababa 113 food, medicines, and healthcare administration authority (AAFMHACA) as a sampling frame, 114 we used computer generated random number to select food handlers. Food handlers who treated 115 with anti-helminthes and anti-protozoan drugs in the last four weeks were excluded from the 116 117 study.

118 **Stool sample collection**

119 Stool sample collectors first explained the purpose of collecting stool to the randomly selected food handlers, and then asked them to urinate first without pooping to avoid urine contamination 120 121 of the stool. Stool sample collectors then handed out paper to food handlers and instructing them to defecate on it to avoid stool contamination with stored feces and dirt. Food handlers were 122 asked to bring approximately 50 g of the last part of the stool, the softest part, into the collection 123 container after defecating on the paper. Stool sample collectors didn't violate privacy of food 124 125 handlers during stool sample collection. Stool sample collectors then immediately stored the stool sample in to a cold box after labeled a code on the outer surface of the plastic cup. 126

127

Personal and food establishment data collection

128 We used structured questionnaire and observational checklist to collect food handlers' personal data and food establishments' related information. The questionnaire was developed by 129 reviewing related published articles [15, 20-24]. The tool was first prepared in English language 130 and translated to the local Amharic language by two native Amharic speakers fluent in English, 131 and back-translated into English by two independent English language experts fluent in Amharic 132 to check consistency. The questionnaire consisted of three parts: i) socio-demographic 133 characteristics of food handlers, ii) food handlers' personal hygiene conditions, and iii) food 134 establishment related factors. The questionnaire was pretested to evaluate the instructions, 135 response format, and questions work as intended and are understood by those individuals who 54 136 55 are likely to respond to them. Data collectors were trained in the data collection tool as well as 137 56

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ethical issues during interviewing and observing. Supervisors supervised the data collection process and checked the completeness of the data on a daily basis. We gathered handwashing data by assessing food handlers' usual handwashing behavior using self-reports. We also looked at the hands of food handlers to see the general cleanliness and conditions of fingernails. In addition, we asked food handlers to demonstrate how they wash their hands on a regular basis, which we evaluated using checklists for effective handwashing.

5 144

Detection of ova of parasites in stool samples

We used direct stool examination (wet mount) and FEC techniques to detect ova of intestinal parasites in stool samples. One drop of physiological saline was placed on a clean slide. Using an applicator stick, a small amount of stool specimen was emulsified in saline solution. The preparation was covered with a cover slip and examined under the microscope for the absence or presence of an intestinal parasites. The entire saline preparation was systematically examined for helminthes eggs, larvae, ciliates, cysts and oocysts using 10× objective with condenser iris closed sufficiently to give good contrast, while $40 \times$ objectives was used to assist in the detection of eggs, cysts, and oocytes [25].

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For the FEC technique, an estimated 1 g of formed stool sample or 2 ml of watery stool was emulsified in about 4 ml of 10% formol water contained in a screw-cap bottle. A further 3 ml of 10% formol water was added and mixed well by shaking. The emulsified stool samples were sieved and the sieved suspension transferred to a conical (centrifuge). 3 ml of diethyl ether was added and the tube was stoppered mixed for 1 minute with a tissue wrapped around the top of the tube, the stopper was loosen. It was then centrifuge at 3000 rpm for 1 minute. Using a stick, the layer of feces debris from the side of the tube was loosen and the tube inverted to discard the ether, fecal debris, and formol water leaving behind the sediment. The tube was returned to its upright position and the fluid from the sides of the tube allowed draining to the bottom. The bottom of the tube was taped to re-suspend and mix the sediment. The sediment was transferred to a slide and covered with a cover glass and was examined microscopically using the 10x objective for focusing and $40 \times$ objective for proper identification.

Standard operating procedures were used for every laboratory procedure during lab examination,stool specimen collection, transportation, and storing. We used stool sample collection and

167 transportation containers which are leak-proof, dry clean and free from any traces of 168 disinfectants. We ensured correct labelling of stool sample containers with date of sample 169 collection, and code of the study participants. All stool specimens were stored in ice box for 170 transportation and preserved at 4°C in the laboratory until analyzed for ova of parasites. 171 Triplicate examinations of stool samples were applied to improve the recovery rate of intestinal 172 parasites. Moreover, the expiry date of normal saline, ether, and formol was evaluated before 173 stool sample preparation and examination.

Outcome variable of the study

Prevalence of intestinal parasites, the primary outcome variable of the study is defined as thepresence of one or more intestinal parasite species in stool samples.

23 177 Data processing and analysis

Data were entered using EPI-INFO version 3.5.3 statistical package and export into Statistical Package for Social Sciences (SPSS) version 20 for further analysis. For most variables, data were presented by frequencies and percentages. Univariable binary logistic regression analysis was used to choose variables for the multivariable binary logistic regression analysis, and variables which had p - value less than 0.25 by the univariable analysis were then analyzed by multivariable analysis for controlling the possible effect of confounders. In the adjusted model, variables which had significant association were identified on the basis of adjusted odds ratio (AOR) with 95% confidence interval (CI) and p-values < 0.05. Model fitness was check using Hosmer and Lemeshow goodness-of-fit test.

42 187 Ethics approval and consent to participate

Ethical clearance was obtained from the Institutional Review Board of Yanet Health College (reference number: YEC/060/21). There were no risks due to participation and the collected data were used only for this research purpose with complete confidentiality and privacy of food handlers during stool sample collection was assured. Written informed consent was obtained from food handlers. Furthermore, we advised food handlers who had one or more ova of parasites to visit health institutions for treatment. All the methods were carried out in accordance with relevant guidelines and regulations.

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195 Patient and public involvement

196 No patient involved.

197 **Results**

198 Socio-demographic characteristics of study participants

We collected personal information and stool samples from a total 411 food handlers with a response rate of 97.62%. The majority, 293 (71.3%) of the study participants were female. The median age of the respondents was 28 and the interquartile range (IQR) was 20 and 39 years. About half, 198 (48.18%) of the respondents aged 25 years and below. Half, 207 (50.3%) of the respondents reported that they completed primary school education. Two hundred and seventyeight (67.6%) of the food handlers reported that they had 3 years or less work experience. One hundred and eleven (27.0%) of the food handlers earned <1500 Ethiopian birr (Table 1).

Table 1: Socio-demographic characteristics of food handlers (n = 411) working in different food
establishments in the Lideta sub-city of Addis Ababa, Ethiopia, March 20 to April 20, 2021

Socio-demographic characteristics	Frequency	Percent
Sex of the respondents		
Female	293	71.3
Male	118	28.7
Age of the respondents in years		
≤25	198	48.2
26-35	113	27.5
36-50	100	24.3
Education status of the respondents		
Tertiary education	23	5.6
Secondary school	163	39.7
Primary school	207	50.3
Illiterate	18	4.4
Service year		

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≤3 years	278	67.6
>3 years	133	33.4
Average monthly income in Ethiopian birr		
<1500	111	27.0
1501-2500	152	37.0
2501-3500	94	22.9
>3500	54	13.1

Personal hygiene characteristics of food handlers

Two hundred and forty-two (58.9%) of the food handlers did not keep their fingernails short. One hundred and ninety-four (47.2%) and 206 (50.1%) of the food handlers did not regularly wash hands with soap after visiting toilet and before eating, respectively. Two hundred and eight (50.6%) of the food handlers reported that they regularly worn clean protective clothes. About a quarter, 76 (24%) of the food handlers reported that they received food safety training and 121 (29.4%) of the food handlers had medical checkup in the previous 6 months prior to the survey (Table 2).

Table 2: Personal hygiene characteristics of food handlers (n = 411) working in different food
establishments in the Lideta sub-city of Addis Ababa, Ethiopia, March 20 to April 20, 2021

Variables	Frequency	Percent
Condition of fingernails	21	
Trimmed	169	41.1
Untrimmed	242	58.9
Regular hand washing with soap after toilet		
Yes	217	52.8
No	194	47.2
Regular hand washing with soap before eating		
Yes	205	49.9
No	206	50.1
Wearing clean protective clothes regularly		

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Yes	208	50.6	
No	203	49.4	
Food safety training			
Yes	76	24	
No	335	76	
Medical checkup (in last 6 month)			
Yes	121	29.4	
No	290	70.6	
^ `			

219 Intestinal parasites in food handlers

A total of 411 food handlers were examined, with 171 (41.6%) (95% CI: 36.6, 46.4%) of them had ova of one or more intestinal parasites [98 (23.8% were protozoan and 73 (17.8%) were helminth parasites], of which 14 (3.4%) had mixed parasites (Figure 2). The most common intestinal parasites were *E. histolytica/dispar* [52 (12.7%)], *G. duodenalis* [46 (11.2%)], *A. lumbricoides* [34 (8.3%)], Hookworms [15 (3.6%)], *T. trichuria* [14 (3.4%)], and Taenia species [10 (2.4%)] (Table 3).

Table 3: Common intestinal parasites detected among food handlers (n = 411) working in
different food establishments in the Lideta sub-city of Addis Ababa, Ethiopia, March 20 to April
20, 2021

Parasitic species	Frequency	Percent	
E.histolytica/dispar	52	12.7	
G. duodenalis	46	11.2	
A. lumbricoides	34	8.3	
Hookworms	15	3.6	
T. trichuria,	14	3.4	
Taenia species	10	2.4	

229 Factors associated with intestinal parasites among food handlers

Table 4 shows factors associated with prevalence of intestinal parasites in food handlers working
 in different food establishments in the Lideta sub-city of Addis Ababa, Ethiopia. Prevalence of
 intestinal parasites among food handlers was associated with low monthly income (AOR: 2.83,

95% CI:1.50, 8.84), untrimmed fingernails (AOR: 4.36, 95% CI: 1.98, 11.90), no food safety training (AOR: 2.51, 95% CI: 1.20, 5.58), low level of education (AOR: 3.13, 95% CI: 1.34, 7.44), poor handwashing practice (AOR: 2.16, 95% CI: 1.03, 4.22), and lack of medical checkup (AOR: 2.31, 95% CI: 1.18, 6.95).

Table 4: Factors associated with intestinal parasites among food handlers (n = 411) working in different food establishments in the Lideta sub-city of Addis Ababa, Ethiopia, March 20 to April

20, 2021

Variables	Intestinal parasites		COR (95% CI)	AOR (95% CI)
	Yes	No	-	
Educational status				
Illiterate	9	9	3.60 (1.81, 10.80)	3.13 (1.34, 7.44)*
Primary school	92	115	2.88 (1.65, 6.17)	2.22 (1.10, 6.65)*
Secondary school	68	95	2.58 (1.20, 5.56)	2.16 (1.10, 5.20)*
Tertiary education	5	18	1.0	1.0
Average monthly income in Ethiopian birr				
<1500	56	55	2.91 (1.43, 7.86)	2.84 (1.50, 8.84)*
1501-2500	68	84	2.31 (1.14, 6.96)	2.28 (0.63, 8.10)
2501-3500	33	61	1.55 (1.05, 4.85)	1.41 (0.60, 5.96)
>3500	14	40	1.0	1.0
Condition of fingernails				
Untrimmed	134	108	4.43 (2.12, 12.62)	4.36 (1.98, 11.90)**
Trimmed	37	132	1.0	1.0
Hand washing with soap after toilet				
No	101	93	2.28 (1.10, 7.51)	2.19 (0.92, 5.62)
Yes	70	147	1.0	1.0
Hand washing with soap before eating				
No	112	94	2.95 (1.23, 6.72)	2.16 (1.03, 4.22)*
Yes	59	146	1.0	1.0
Food safety training				

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No	153	182	2.71 (1.34, 8.53)	2.51 (1.20, 5.58)*
Yes	18	58	1.0	1.0
Medical checkup in the last 6 month				
No	140	150	2.71 (1.27, 7.56)	2.32 (1.18, 6.95)*
Yes	31	90	1.0	1.0
Note: * statistically significant at $n < 0.05$	** statis	stically sig	nificant at $n < 0.01$	Hosmer and Lemeshov

Note: * statistically significant at p < 0.05, ** statistically significant at p < 0.01, Hosmer and Lemeshow goodness-of-fit test =0.483

Discussion

This is an institution-based cross-sectional study assessed intestinal parasites among food handlers working in food establishments in the Lideta sub-city of Addis Ababa, Ethiopia and found that 41.6% (95% CI: 36.6, 46.4%) of the food handlers had one or more intestinal parasites. The prevalence of intestinal parasites reported in this study was comparable with findings of studies conducted among food handlers in Bule Hora (46.3%) [16] and Addis Ababa University (45.3%) [19]. The prevalence of intestinal parasites reported in this study was lower than findings of studies in Nekemte town (52.1%) [26] and Mekele University (49.4%) [15]. Furthermore, the prevalence of intestinal parasites reported in the current study was higher than findings of studies conducted among food handlers in Wolaita Sodo (23.6%) [14], Jimma (33%) [27], Madda Walabu University (25.3%) [28], Motta town (27.6%) [29], Nairobi(15.7%)[30], Iran (9%) [31], Saudi Arabia (23%) [32], and Thailand (10%) [33]. The high prevalence of intestinal parasites among food handlers among food handlers working in food establishments in the Lideta sub-city of Addis Ababa might be explained by poor socio-economic conditions, poor hand hygiene, and inadequate access to basic sanitation services.

The high prevalence of intestinal parasites among food handlers working in food establishments in the Lideta sub-city suggests that poor hygiene practices of food handlers and inadequate access to sanitation services. The results may also suggests that there might be transmission of intestinal parasitic infections from food handlers to food users, unless large scale screening and mass drug administration are done. As it is documented in literature, infected food handlers play a significant role in infection transmissions to customers of the food establishments where the infected food handlers are working [4].

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This study showed that educational status of food handlers was associated with a high prevalence of intestinal parasites. Prevalence of intestinal parasites was higher among food handlers who were illiterate or attended primary and secondary education compared with food handlers who attended tertiary education. This may be due to the fact that educated food handlers may have awareness about the transmission and prevention methods of infectious diseases. Education encourages changes in healthy behaviors. Other similar studies also reported the relation of education with occurrence of parasitic infections [34-38].

The current study revealed that monthly income of food handlers was associated with intestinal parasites among food handlers. Food handlers with low monthly income had higher odds of intestinal parasites. This may be due to the fact that food handlers with low economic status could not afford for services like soap, household water treatment, toilets and other facilities, and so that will have limited opportunities to practice healthy measures. The effect of low income on risk of parasites is complex and could be attributed to limited access to sanitary materials, sources of drinking water and food, environment sanitation, and education [37-40].

The high prevalence of intestinal parasites among food handlers was associated with hand hygiene. Food handlers who did not keep their fingernails short had higher odds to have intestinal parasites and the odds of having intestinal parasites was also higher among food handlers who did not wash hands with soap before eating. This might be due to the fact that the area beneath the fingernails has the highest concentration of microorganisms on the hands and is the most difficult to clean [41-44]. Moreover, food handlers may ingest diseases causing pathogens when they eat without washing hands. Hands are one of the most important mechanisms to transmit pathogenic microorganisms leading to infection [45]. Evidence indicates that hands together with food contact or other environmental surfaces cause 60% of the spread of gastrointestinal infection. Contaminated hands could also be associated with up to 50% of respiratory tract infections [46].

This study depicted that intestinal parasites among food handlers was significantly associated with food safety training. The odds of having intestinal parasites was high among food handlers who did not take food safety training compared with their counterparts. This could be due to the fact those food handlers who did not take food safety training may lack the necessary knowledge and practice towards transmission and prevention of disease causing pathogens.

Moreover, food safety training or health education promotes health behaviors toward hygiene and sanitation practices. Health education increases knowledge and acceptability of interventions. It also sustains integrated control of the infection [47-49].

Furthermore, intestinal parasites was significantly associated with medical checkup. The odds of having intestinal parasites was high among food handlers who did not take medical checkups in the previous six months prior to the survey. Other studies are also reported that medical checkup of food handlers is associated with intestinal parasites [15, 31, 50]. This is because food handlers who did not know their health condition before employment and while working in different establishments has less chance to take treatment and mass drugs as a result there may be existed or new or re-infections.

To increase the degree to which inferences from the sample population can be generalized to a larger group of population, we recruited study participants at random or in a manner in which they are representative of the population that we wish to study and we granted that every member of the population had an equal chance to be included in the study. In addition, we calculated adequately powered sample size using sample size determination procedures appropriate to the study objective with appropriate assumptions. Furthermore, our findings could be applicable to other situations and settings which have similar characteristics with the study populations of the current studies.

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As a limitation, even if the use of sensitive diagnostic techniques and combination of methods with triplicate examinations applied in this study would help to recover greater rate of intestinal parasites that would indicate the true prevalence, this study had some limitations. The collection of a single stool sample may affect the result of parasitic examination since the sensitivities of the direct smear examination technique is reduced when a single stool sample is examined. The procedure of FEC technique may also damage the eggs of parasites. Moreover, the handwashing data assessed by self-reports may not be reliable since the study subjects may make the more socially acceptable answer rather than being truthful and they may not be able to assess themselves accurately.

319 Conclusion

The prevalence of intestinal parasites among food handlers working in food establishments in the Lideta sub-city of Addis Ababa, Ethiopia was found to be high. This high prevalence of intestinal parasites was linked to socio-economic conditions of food handlers, poor hand hygiene condition, absence of food safety trainings and regular medical checkup. It is, therefore, important to promote hand washing practice of food handlers, provide food hygiene and safety trainings, and establishing a system to regularly check the health conditions of food handlers.

327 Data availability statement

328 Individual participant data after deidentification that underlie the results reported in this article329 will be made available upon requesting the primary author immediately following publication.

330 Authors' contribution

WA designed the study, facilitated data collection, and conducted data analysis. BG, TS, ZNM,
and ZG supervised data collection and analysis and contributed to conceptualizing the study. ZG
prepared the manuscript. All authors approved the final version of the manuscript.

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The authors of this study didn't receive funds from any funding organization. The cost of data collection tools and data collectors' fee was covered by the principal investigator, i.e., WA.

337 Competing interest

338 None of the authors have any competing interests in the manuscript.

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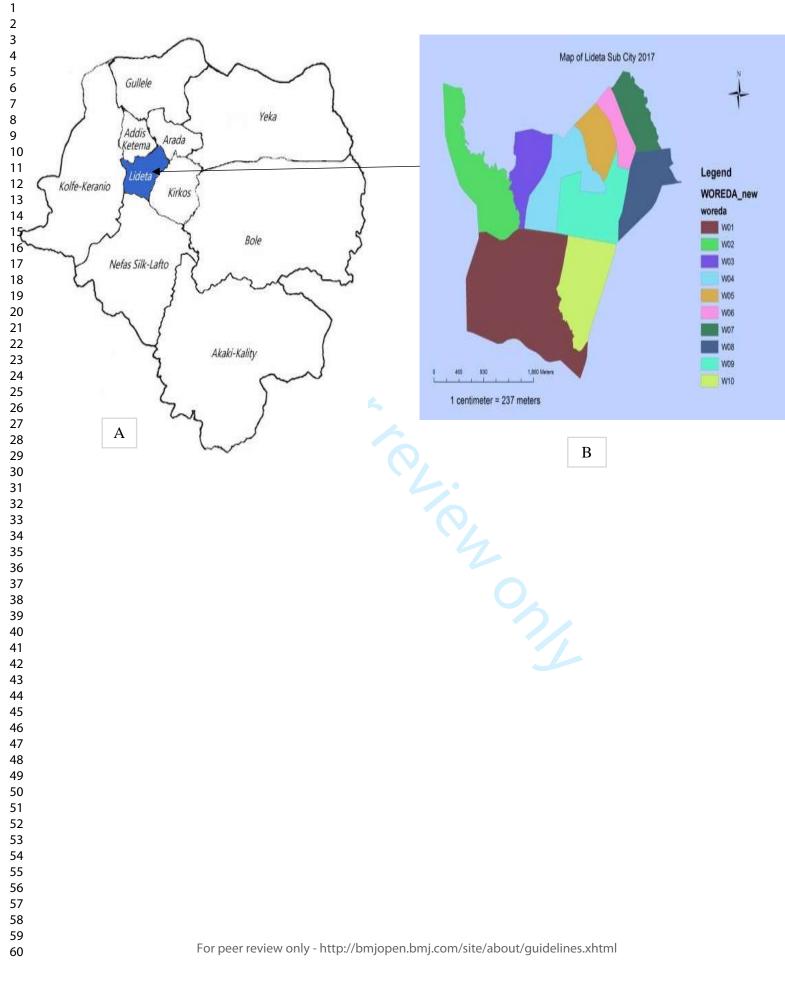
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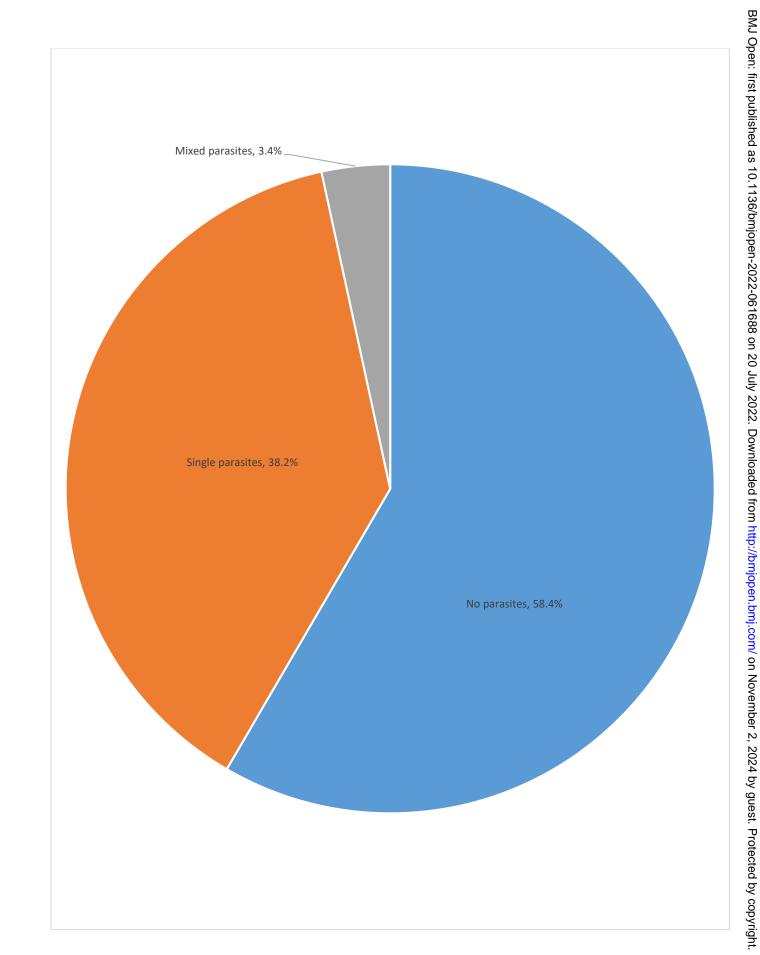
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Figure caption

Figure 1: Map of Addis Ababa city (A) and Lideta sub-city (B) (source: Lideta sub-city administration)

Figure 2: Proportion of food handlers with no, single and mixed parasites (n = 411) working in different food establishments in Lideta sub city of Addis Ababa, Ethiopia, March 20 to April 20, 2021 to occurrent on the second





Prevalence of intestinal parasites and associated factors among food handlers in food establishments in the Lideta sub-city of Addis Ababa, Ethiopia: an institution-based cross-sectional study

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R. O.

Prevalence of intestinal parasites and associated factors among food handlers in food establishments in the Lideta sub-city of Addis Ababa, Ethiopia: an institution-based cross-sectional study Woinishet Abera¹, Binyam Gintamo^{1,2,3}, Tewoderos Shitemaw⁴, Zelalem Negash Mekuria^{1,2,5}, Zemichael Gizaw6* ¹Yanet Health College, Addis Ababa, Ethiopia. ²Addis Ababa Medical and Business College, Addis Ababa, Ethiopia. ³ Department of Biotechnology, Faculty of Applied Sciences and Biotechnology, Shoolini University, Solan, Bajhol, H.P., India ⁴ Kotebe Metropolitan University, Addis Ababa, Ethiopia ⁵ Yekatit 12 Medical College, Addis Ababa, Ethiopia ⁶Department of Environmental and Occupational Health and safety, Institute of Public Health, College of Medicine and Health Sciences, University of Gondar, Gondar, Ethiopia Email address of authors: Woinishet Abera (woini27ab@gmail.com), Binyam Gintamo (gintamobinyam@gmail.com), Tewodros Shitemaw (tewoderosshitemaw@gmail.com), Zelalem Negash Mekuria (zolabelay256@gmail.com), and Zemichael Gizaw (zemichael12@gmail.com) *Corresponding author: Zemichael Gizaw, Department of Environmental and Occupational Health and safety, Institute of Public Health, College of Medicine and Health Sciences, University of Gondar, Gondar, Ethiopia, Email: zemichael12@gmail.com Abstract Objectives: This study was conducted to assess the prevalence of intestinal parasites and associated factors among food handlers. Design: An institution-based cross-sectional study design was used. Stool samples were collected from food handlers and examined using direct wet mount and formalin-ether concentration (FEC) techniques. Personal and establishment related information was collected using pretested questionnaire with structured observation. Multivariable binary logistic

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regression was used to identify factors associated with prevalence of intestinal parasites on the basis of adjusted odds ratio and 95% confidence interval with p < 0.05.

Setting: This study was conducted in different food establishments in the Lideta sub-city of
Addis Ababa, Ethiopia.

Participants: Four hundred and eleven food handlers were participated in this study.

Outcome measures: The primary outcome is prevalence of intestinal parasites, which was
 defined as presence of one or more intestinal parasitic species in stool samples.

Results: One or more intestinal parasites were detected in 171 (41.6%) (95% CI: 36.6, 46.4%) of the stool samples. The most common intestinal parasites were E. histolytica/dispar (12.7 %). G. duodenalis (11.2%), and A. lumbricoides (8.3%). This high prevalence of intestinal parasites among food handlers was associated with low monthly income (AOR: 2.83, 95% CI:1.50, 8.84), untrimmed fingernails (AOR: 4.36, 95% CI: 1.98, 11.90), no food safety training (AOR: 2.51, 95% CI: 1.20, 5.58), low level of education (AOR: 3.13, 95% CI: 1.34, 7.44), poor handwashing practice (AOR: 2.16, 95% CI: 1.03, 4.22), and lack of medical checkup (AOR: 2.31, 95% CI: 1.18, 6.95).

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Conclusion: The prevalence of intestinal parasites among food handlers in food 43 establishments in the Lideta sub-city of Addis Ababa was high. This was linked to socio-44 economic conditions, poor hand hygiene condition, and absence of food safety trainings. It is, 45 therefore, important to promote hand washing practice and providing food hygiene and safety 46 training is critical.

47 Key words: Intestinal parasites, food handlers, food establishments, hand hygiene, Ethiopia

5 48 **St**r

8 Strengths and limitations of this study

- The study has focused on one of the most potential groups, i.e., food handlers that had potential to spread food borne infections to the consumers.

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The use of sensitive diagnostic techniques and combination of methods with triplicate
 examinations applied in this study would help to recover greater rate of intestinal parasites
 that would indicate the true prevalence.

- As a limitation, the collection of a single stool sample may affect the result of parasitic examination since the sensitivities of the direct smear examination technique is reduced when a single stool sample is examined and the procedure of FEC technique may also damage the eggs of parasites.

58 Introduction

Foodborne diseases are increasingly becoming a serious global public health problem. The World Health Organization (WHO) estimate indicates that each year worldwide, unsafe food causes 600 million cases of foodborne diseases and 420 000 deaths. WHO estimated that 33 million years of healthy lives are lost due to eating unsafe food globally each year, and this number is likely an underestimation [1]. One of the causes of foodborne diseases is contamination during food preparation; food handlers carrying pathogens might be involved in the origin of this condition. Foods can be contaminated with fecal material at the point of production or during food preparation, in both the home and in commercial premises [2]. Food handlers with poor personal hygiene and inadequate knowledge on food safety could be potential sources of infections. Food handlers who harbor and excrete enteropathogens may contaminate foods from their feces via contaminated hands, then to food or food contact surfaces, and finally to healthy individuals [3-7].

The contribution of the infected food worker (whether symptomatic or not) to food borne disease outbreaks has been difficult to establish. However, reports showed that food workers in many settings have been responsible for foodborne disease outbreaks for decades. For instance, members of the Committee on Control of Foodborne Illnesses of the International Association for Food Protection analyzed 816 foodborne disease outbreaks with 80,682 cases in different countries where food workers were implicated as the source of the contamination. The report also estimated that infected food worker was documented as responsible for 18% of 766 outbreaks occurring in the United States [8, 9]. Moreover, according to the Centers for Disease Control (CDC), as cited in Mathew RR (2019), 20 to 40% of food borne illness associated with the consumption of contaminated food originated in catering establishments [10].

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Food handling personnel play a role in the transmission of food borne diseases. The health of food handlers is of great importance for maintaining quality of food products. Accordingly, pre-employment and periodic medical checkup is very important to safeguard the consumers from getting diseases from contaminated foods along with other food safety measures [11, 12]. However, pre-employment and periodic medical checkups are not commonly practiced in Ethiopia. As a result of this, many of the food handlers working in different food establishments all over the country may harbor one or more enteropathogens. For instance, a systematic review and meta-analysis reported that the overall pooled prevalence estimate of intestinal parasites among food handlers of food service establishments in Ethiopia was 33.6% (95% CI: 27.6, 39.6%) [13] and the common factors associated to high prevalence of intestinal pathogens among food handlers are poor hand hygiene, inadequate access to water and sanitation facilities, and poor socio-economic conditions [13-18]. However, the prevalence and risk factors may be different across various settings. Accordingly, this study was conducted to assess the prevalence of intestinal parasites and associated factors among food handlers working in food establishment in the Lideta sub-city of Addis Ababa, Ethiopia.

96 Methods

97 Study design and setting

This an institution-based cross-sectional study with laboratory investigations was conducted in the Lideta sub-city of Addis Ababa from March 20 to April 20, 2021. Lideta sub-city is one of the 10 sub cities of Addis Ababa, the capital of Ethiopia. The sub-city is located at the global positioning system (GPS) coordinates of 9°0'N and 38°45'E. The sub city is divided into 10 districts (Figure 1). In the Lideta sub-city, there are a total of 281 food establishments and 1124 food handlers working in the food establishments.

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⁶ 104 Sample size calculation and sampling techniques

⁴⁹ 105 The sample size was calculated using single population proportion formula with the following ⁵⁰ 106 assumptions: prevalence of intestinal parasites among food handlers in Addis Ababa University ⁵² 107 students cafeteria, Addis Ababa, Ethiopia (p) = 45.3% [19], level of significance (α) = 5%, 95% ⁵⁴ 108 confidence interval (standard normal probability), z: the standard normal tabulated value, and ⁵⁵ 109 margin of error (d) = 5%.

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$$n = \frac{(Z_{\alpha/2})^2 P(1-P)}{d^2} = \frac{(1.96)^2 0.453(1-0.453)}{0.05^2} = 381$$

The final sample size was 419 after considering 10% non-response rate. The Lideta sub-city was 111 selected at random from a total of 10 sub-cities of Addis Ababa, Ethiopia. Using lists of food 112 handlers working in different food establishments in the sub-city obtained from Addis Ababa 113 food, medicines, and healthcare administration authority (AAFMHACA) as a sampling frame, 114 we used computer generated random number to select food handlers. Food handlers who treated 115 with anti-helminthes and anti-protozoan drugs in the last four weeks were excluded from the 116 117 study.

118 **Stool sample collection**

119 Stool sample collectors first explained the purpose of collecting stool to the randomly selected food handlers, and then asked them to urinate first without pooping to avoid urine contamination 120 121 of the stool. Stool sample collectors then handed out paper to food handlers and instructing them to defecate on it to avoid stool contamination with stored feces and dirt. Food handlers were 122 asked to bring approximately 50 g of the last part of the stool, the softest part, into the collection 123 container after defecating on the paper. Stool sample collectors didn't violate privacy of food 124 125 handlers during stool sample collection. Stool sample collectors then immediately stored the stool sample in to a cold box after labeled a code on the outer surface of the plastic cup. 126

127

Personal and food establishment data collection

128 We used structured questionnaire and observational checklist to collect food handlers' personal data and food establishments' related information. The questionnaire was developed by 129 reviewing related published articles [15, 20-24]. The tool was first prepared in English language 130 and translated to the local Amharic language by two native Amharic speakers fluent in English, 131 and back-translated into English by two independent English language experts fluent in Amharic 132 to check consistency. The questionnaire consisted of three parts: i) socio-demographic 133 characteristics of food handlers, ii) food handlers' personal hygiene conditions, and iii) food 134 establishment related factors (supplementary file). The questionnaire was pretested to evaluate 135 53 the instructions, response format, and questions work as intended and are understood by those 54 136 55 individuals who are likely to respond to them. Data collectors were trained in the data collection 137 56

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tool as well as ethical issues during interviewing and observing. Supervisors supervised the data collection process and checked the completeness of the data on a daily basis. We gathered handwashing data by assessing food handlers' usual handwashing behavior using self-reports. We also looked at the hands of food handlers to see the general cleanliness and conditions of fingernails. In addition, we asked food handlers to demonstrate how they wash their hands on a regular basis, which we evaluated using checklists for effective handwashing.

₅ 144

Detection of ova of parasites in stool samples

We used direct stool examination (wet mount) and FEC techniques to detect ova of intestinal parasites in stool samples. One drop of physiological saline was placed on a clean slide. Using an applicator stick, a small amount of stool specimen was emulsified in saline solution. The preparation was covered with a cover slip and examined under the microscope for the absence or presence of an intestinal parasites. The entire saline preparation was systematically examined for helminthes eggs, larvae, ciliates, cysts and oocysts using 10× objective with condenser iris closed sufficiently to give good contrast, while $40 \times$ objectives was used to assist in the detection of eggs, cysts, and oocytes [25].

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For the FEC technique, an estimated 1 g of formed stool sample or 2 ml of watery stool was emulsified in about 4 ml of 10% formol water contained in a screw-cap bottle. A further 3 ml of 10% formol water was added and mixed well by shaking. The emulsified stool samples were sieved and the sieved suspension transferred to a conical (centrifuge). 3 ml of diethyl ether was added and the tube was stoppered mixed for 1 minute with a tissue wrapped around the top of the tube, the stopper was loosen. It was then centrifuge at 3000 rpm for 1 minute. Using a stick, the layer of feces debris from the side of the tube was loosen and the tube inverted to discard the ether, fecal debris, and formol water leaving behind the sediment. The tube was returned to its upright position and the fluid from the sides of the tube allowed draining to the bottom. The bottom of the tube was taped to re-suspend and mix the sediment. The sediment was transferred to a slide and covered with a cover glass and was examined microscopically using the 10x objective for focusing and $40 \times$ objective for proper identification.

Standard operating procedures were used for every laboratory procedure during lab examination,stool specimen collection, transportation, and storing. We used stool sample collection and

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167 transportation containers which are leak-proof, dry clean and free from any traces of 168 disinfectants. We ensured correct labelling of stool sample containers with date of sample 169 collection, and code of the study participants. All stool specimens were stored in ice box for 170 transportation and preserved at 4°C in the laboratory until analyzed for ova of parasites. 171 Triplicate examinations of stool samples were applied to improve the recovery rate of intestinal 172 parasites. Moreover, the expiry date of normal saline, ether, and formol was evaluated before 173 stool sample preparation and examination.

Outcome variable of the study

Prevalence of intestinal parasites, the primary outcome variable of the study is defined as thepresence of one or more intestinal parasite species in stool samples.

3 177 Data processing and analysis

Data were entered using EPI-INFO version 3.5.3 statistical package and export into Statistical Package for Social Sciences (SPSS) version 20 for further analysis. For most variables, data were presented by frequencies and percentages. Univariable binary logistic regression analysis was used to choose variables for the multivariable binary logistic regression analysis, and variables which had p-value less than 0.25 by the univariable analysis and other well-known confounders from the literature were then analyzed by multivariable analysis for controlling the possible effect of confounders and to predict the prevalence of intestinal parasites among food handlers based on the predictors. In the adjusted model, variables which had significant association were identified on the basis of adjusted odds ratio (AOR) with 95% confidence interval (CI) and p-values < 0.05. The predictive power of the model was checked using McFadden's pseudo R-squared.

⁵ 189 Ethics approval and consent to participate

Ethical clearance was obtained from the Institutional Review Board of Yanet Health College
(reference number: YEC/060/21). There were no risks due to participation and the collected data
were used only for this research purpose with complete confidentiality and privacy of food
handlers during stool sample collection was assured. Written informed consent was obtained
from food handlers. Furthermore, we advised food handlers who had one or more ova of

26-35

36-50

Education status of the respondents

Tertiary education

Secondary school

Primary school

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27.5

24.3

5.6

39.7

50.3

parasites to visit health institutions for treatment. All the methods were carried out in accordance with relevant guidelines and regulations. Patient and public involvement There was no patient or public involvement in the study. **Results** Socio-demographic characteristics of study participants We collected personal information and stool samples from a total 411 food handlers with a response rate of 97.62%. The majority, 293 (71.3%) of the study participants were female. The median age of the respondents was 28 and the interquartile range (IQR) was 20 and 39 years. About half, 198 (48.18%) of the respondents aged 25 years and below. Half, 207 (50.3 %) of the respondents reported that they completed primary school education. Two hundred and seventy-eight (67.6%) of the food handlers reported that they had 3 years or less work experience. One hundred and eleven (27.0%) of the food handlers earned <1500 Ethiopian birr (Table 1). Table 1: Socio-demographic characteristics of food handlers (n = 411) working in different food establishments in the Lideta sub-city of Addis Ababa, Ethiopia, March 20 to April 20, 2021 Socio-demographic characteristics Frequency Percent Sex of the respondents Female 71.3 Male 28.7 Age of the respondents in years 48.2 <25

		10	
	Illiterate	18	4.4
	Service year		
	≤3 years	278	67.6
	>3 years	133	33.4
	Average monthly income in Ethiopian birr		
	<1500	111	27.0
	1501-2500	152	37.0
	2501-3500	94	22.9
	>3500	54	13.1
210	Personal hygiene characteristics of food handle	rc	

Two hundred and forty-two (58.9%) of the food handlers did not keep their fingernails short. One hundred and ninety-four (47.2%) and 206 (50.1%) of the food handlers did not regularly wash hands with soap after visiting toilet and before eating, respectively. Two hundred and eight (50.6%) of the food handlers reported that they regularly worn clean protective clothes. About a quarter, 76 (24%) of the food handlers reported that they received food safety training and 121 (29.4%) of the food handlers had medical checkup in the previous 6 months prior to the survey (Table 2).

Table 2: Personal hygiene characteristics of food handlers (n = 411) working in different food establishments in the Lideta sub-city of Addis Ababa, Ethiopia, March 20 to April 20, 2021

Variables	Frequency	Percent
Condition of fingernails		
Trimmed	169	41.1
Untrimmed	242	58.9
Regular hand washing with soap after toilet		
Yes	217	52.8
No	194	47.2
Regular hand washing with soap before eating		
Yes	205	49.9

2				
3 4	No	206	50.1	
5	Wearing clean protective clothes regularly			
6 7	Yes	208	50.6	
8 9	No	203	49.4	
10	Food safety training			
11 12	Yes	76	24	
13 14	No	335	76	
15	Medical checkup (in last 6 month)			
16 17	Yes	121	29.4	
18 19	No	290	70.6	

Intestinal parasites in food handlers

A total of 411 food handlers were examined, with 171 (41.6%) (95% CI: 36.6, 46.4%) of them had ova of one or more intestinal parasites [98 (23.8% were protozoan and 73 (17.8%) were helminth parasites], of which 14 (3.4%) had mixed parasites (Figure 2). The most common intestinal parasites were E. histolytica/dispar [52 (12.7%)], G. duodenalis [46 (11.2%)], A. lumbricoides [34 (8.3%)], Hookworms [15 (3.6%)], T. trichuria [14 (3.4%)], and Taenia species [10 (2.4%)] (Table 3).

Table 3: Common intestinal parasites detected among food handlers (n = 411) working in different food establishments in the Lideta sub-city of Addis Ababa, Ethiopia, March 20 to April 20, 2021

Parasitic species	Frequency	Percent		
E.histolytica/dispar	52	12.7		
G. duodenalis	46	11.2		
A. lumbricoides	34	8.3		
Hookworms	15	3.6		
T. trichuria,	14	3.4		
Taenia species	10	2.4		

Factors associated with intestinal parasites among food handlers

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Educational status, average monthly income, condition of fingernails, hand washing with soap before eating, food safety training, and medical checkup in the last six month were the candidate variables for the final model which were selected based on p < 0.25 in the univariable binary logistic regression analysis and hand washing with soap after visiting toilet was a well-known confounder in the literature included in the final model even if its p value is greater than 0.25 in the univariable binary logistic regression analysis. In the multivariable binary logistic regression analysis, prevalence of intestinal parasites among food handlers was associated with low monthly income (AOR: 2.83, 95% CI:1.50, 8.84), untrimmed fingernails (AOR: 4.36, 95% CI: 1.98, 11.90), no food safety training (AOR: 2.51, 95% CI: 1.20, 5.58), low level of education (AOR: 3.13, 95% CI: 1.34, 7.44), poor handwashing practice (AOR: 2.16, 95% CI: 1.03, 4.22), and lack of medical checkup (AOR: 2.31, 95% CI: 1.18, 6.95) (Table 4).

Table 4: Factors associated with intestinal parasites among food handlers (n = 411) working in
different food establishments in the Lideta sub-city of Addis Ababa, Ethiopia, March 20 to April
20, 2021

28	,					
29	Variables	Intestina	l parasites	COR (95% CI)	AOR (95% CI)	
30 31		Yes	No	-		
32 33	Educational status		6			
34	Illiterate	9	9	3.60 (1.81, 10.80)	3.13 (1.34, 7.44)*	
35 36	Primary school	92	115	2.88 (1.65, 6.17)	2.22 (1.10, 6.65)*	
37 38	Secondary school	68	95	2.58 (1.20, 5.56)	2.16 (1.10, 5.20)*	
39 40	Tertiary education	5	18	1.0	1.0	
41 42	Average monthly income in Ethiopian birr					
43 44	<1500	56	55	2.91 (1.43, 7.86)	2.84 (1.50, 8.84)*	
45	1501-2500	68	84	2.31 (1.14, 6.96)	2.28 (0.63, 8.10)	
46 47	2501-3500	33	61	1.55 (1.05, 4.85)	1.41 (0.60, 5.96)	
48 49	>3500	14	40	1.0	1.0	
50 51	Condition of fingernails					
52 53	Untrimmed	134	108	4.43 (2.12, 12.62)	4.36 (1.98, 11.90)**	
54 55	Trimmed	37	132	1.0	1.0	
55 56						

Hand washing with soap after toilet				
No	101	93	2.28 (1.10, 7.51)	2.19 (0.92, 5.62)
Yes	70	147	1.0	1.0
Hand washing with soap before eating				
No	112	94	2.95 (1.23, 6.72)	2.16 (1.03, 4.22)*
Yes	59	146	1.0	1.0
Food safety training				
No	153	182	2.71 (1.34, 8.53)	2.51 (1.20, 5.58)*
Yes	18	58	1.0	1.0
Medical checkup in the last 6 month				
No	140	150	2.71 (1.27, 7.56)	2.32 (1.18, 6.95)*
Yes	31	90	1.0	1.0

Note: * statistically significant at p < 0.05, ** statistically significant at p < 0.01, and McFadden's pseudo R-squared = 0.492

246 Discussion

This is an institution-based cross-sectional study assessed intestinal parasites among food handlers working in food establishments in the Lideta sub-city of Addis Ababa, Ethiopia and found that 41.6% (95% CI: 36.6, 46.4%) of the food handlers had one or more intestinal parasites. The prevalence of intestinal parasites reported in this study was comparable with findings of studies conducted among food handlers in Bule Hora (46.3%) [16] and Addis Ababa University (45.3%) [19]. The prevalence of intestinal parasites reported in this study was lower than findings of studies in Nekemte town (52.1%) [26] and Mekele University (49.4%) [15]. Furthermore, the prevalence of intestinal parasites reported in the current study was higher than findings of studies conducted among food handlers in Wolaita Sodo (23.6%) [14], Jimma (33%) [27], Madda Walabu University (25.3%) [28], Motta town (27.6%) [29], Nairobi(15.7%)[30], Iran (9%) [31], Saudi Arabia (23%) [32], and Thailand (10%) [33]. The high prevalence of intestinal parasites among food handlers among food handlers working in food establishments in the Lideta sub-city of Addis Ababa might be explained by poor socio-economic conditions, poor hand hygiene, and inadequate access to basic sanitation services.

The high prevalence of intestinal parasites among food handlers working in food establishments in the Lideta sub-city suggests that poor hygiene practices of food handlers and inadequate access to sanitation services. The results may also suggests that there might be transmission of intestinal parasitic infections from food handlers to food users, unless large scale screening and mass drug administration are done. As it is documented in literature, infected food handlers play a significant role in infection transmissions to customers of the food establishments where the infected food handlers are working [4].

This study showed that educational status of food handlers was associated with a high prevalence of intestinal parasites. Prevalence of intestinal parasites was higher among food handlers who were illiterate or attended primary and secondary education compared with food handlers who attended tertiary education. This may be due to the fact that educated food handlers may have awareness about the transmission and prevention methods of infectious diseases. Education encourages changes in healthy behaviors. Other similar studies also reported the relation of education with occurrence of parasitic infections [34-38].

The current study revealed that monthly income of food handlers was associated with intestinal parasites among food handlers. Food handlers with low monthly income had higher odds of intestinal parasites. This may be due to the fact that food handlers with low economic status could not afford for services like soap, household water treatment, toilets and other facilities, and so that will have limited opportunities to practice healthy measures. The effect of low income on risk of parasites is complex and could be attributed to limited access to sanitary materials, sources of drinking water and food, environment sanitation, and education [37-40].

The high prevalence of intestinal parasites among food handlers was associated with hand hygiene. Food handlers who did not keep their fingernails short had higher odds to have intestinal parasites and the odds of having intestinal parasites was also higher among food handlers who did not wash hands with soap before eating. This might be due to the fact that the area beneath the fingernails has the highest concentration of microorganisms on the hands and is the most difficult to clean [41-44]. Moreover, food handlers may ingest diseases causing pathogens when they eat without washing hands. Hands are one of the most important mechanisms to transmit pathogenic microorganisms leading to infection [45]. Evidence indicates

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that hands together with food contact or other environmental surfaces cause 60% of the spread of
gastrointestinal infection. Contaminated hands could also be associated with up to 50% of
respiratory tract infections [46].

This study depicted that intestinal parasites among food handlers was significantly associated with food safety training. The odds of having intestinal parasites was high among food handlers who did not take food safety training compared with their counterparts. This could be due to the fact those food handlers who did not take food safety training may lack the necessary knowledge and practice towards transmission and prevention of disease causing pathogens. Moreover, food safety training or health education promotes health behaviors toward hygiene and sanitation practices. Health education increases knowledge and acceptability of interventions. It also sustains integrated control of the infection [47-49].

Furthermore, intestinal parasites was significantly associated with medical checkup. The odds of having intestinal parasites was high among food handlers who did not take medical checkups in the previous six months prior to the survey. Other studies are also reported that medical checkup of food handlers is associated with intestinal parasites [15, 31, 50]. This is because food handlers who did not know their health condition before employment and while working in different establishments has less chance to take treatment and mass drugs as a result there may be existed or new or re-infections.

To increase the degree to which inferences from the sample population can be generalized to a larger group of population, we recruited study participants at random or in a manner in which they are representative of the population that we wish to study and we granted that every member of the population had an equal chance to be included in the study. In addition, we calculated adequately powered sample size using sample size determination procedures appropriate to the study objective with appropriate assumptions. Furthermore, our findings could be applicable to other situations and settings which have similar characteristics with the study populations of the current studies.

As a limitation, even if the use of sensitive diagnostic techniques and combination of methods
 with triplicate examinations applied in this study would help to recover greater rate of intestinal
 parasites that would indicate the true prevalence, this study had some limitations. The collection

of a single stool sample may affect the result of parasitic examination since the sensitivities of the direct smear examination technique is reduced when a single stool sample is examined. The procedure of FEC technique may also damage the eggs of parasites. Moreover, the handwashing data assessed by self-reports may not be reliable since the study subjects may make the more socially acceptable answer rather than being truthful and they may not be able to assess themselves accurately.

325 Conclusion

The prevalence of intestinal parasites among food handlers working in food establishments in the Lideta sub-city of Addis Ababa, Ethiopia was found to be high. This high prevalence of intestinal parasites was linked to socio-economic conditions of food handlers, poor hand hygiene condition, absence of food safety trainings and regular medical checkup. It is, therefore, important to promote hand washing practice of food handlers, provide food hygiene and safety trainings, and establishing a system to regularly check the health conditions of food handlers.

30 333 Data availability statement 31

Individual participant data after deidentification that underlie the results reported in this articlewill be made available upon requesting the primary author immediately following publication.

37 336 Authors' contribution

WA designed the study, facilitated data collection, and conducted data analysis. BG, TS, ZNM,
and ZG supervised data collection and analysis and contributed to conceptualizing the study. ZG
prepared the manuscript. All authors approved the final version of the manuscript.

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343 Competing interest

344 None of the authors have any competing interests in the manuscript.

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8 9	348	contr	ibution in the study.					
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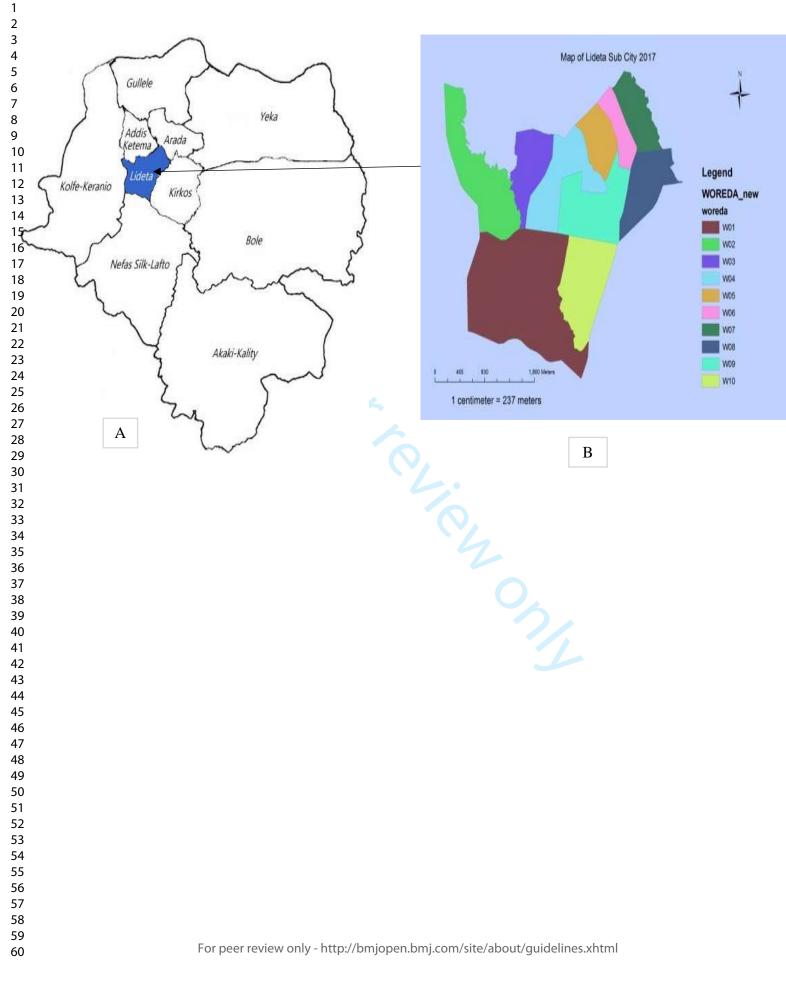
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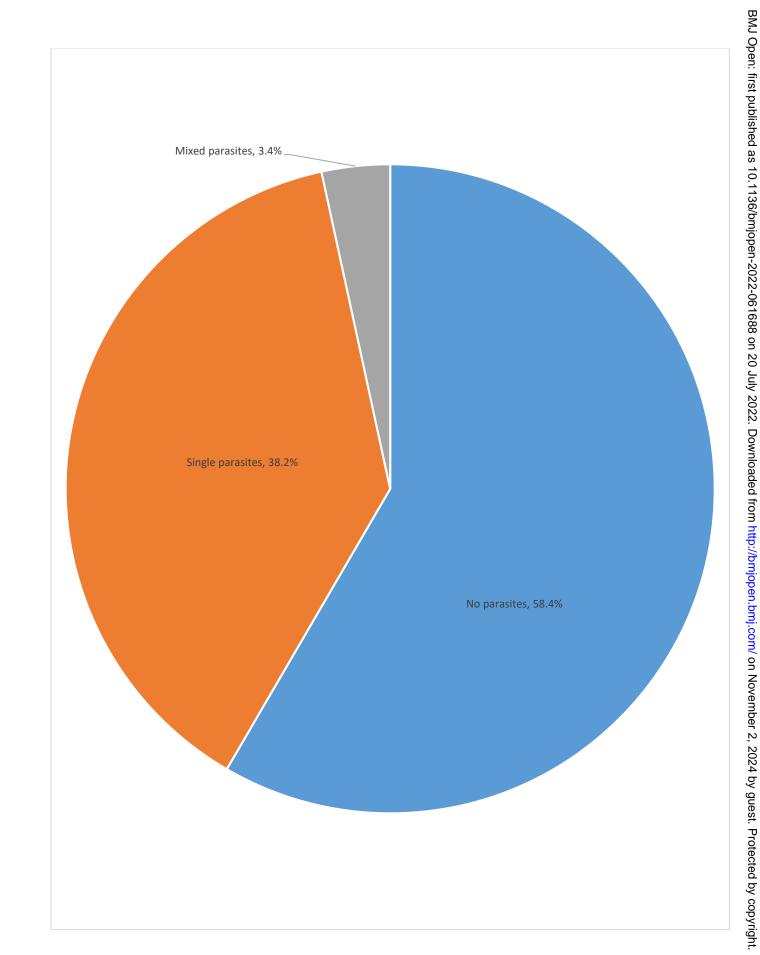
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495	Figu	ire caption
496	U	re 1: Map of Addis Ababa city (A) and Lideta sub-city (B) (source: Lideta sub-city
497	admi	nistration)
498	Figu	re 2: Proportion of food handlers with no, single and mixed parasites ($n = 411$) working
499	in di	fferent food establishments in Lideta sub city of Addis Ababa, Ethiopia, March 20 to April
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Supplementary file: Data collection tool

Questionnaire Identification Code:

Consent Form

Hello, I am ______ working with a research team from Yanet Health Science College. We are contacting you to collect information for a study conducted to assess "Prevalence of intestinal parasites and associated factors among food handlers in food establishments in the Lideta sub-city of Addis Ababa, Ethiopia". This questionnaire is prepared to collect food handlers personal and food establishment related information.

Thank you so much for agreeing to be interviewed for this research work. All of the information we got from you will be completely confidential and coded with unique number. Your name will not be written down and will never be used in connection with any of the information you provided. There will not be any direct benefit that you will get associated with participation in this study. If you do not want to answer all or some of the questions, you have the right to refuse participation at any time. However, we would greatly appreciate your help in responding to this questionnaire. This questionnaire is expected to be completed in 10 minutes.

Do I have your permission to continue?

1. Yes, continue your interview with thanks after signing the consent

2. If no, skip to the next participant by writing reasons for his/ her refusal;

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41		r: Name:	•	Date:	
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Code	Questions	Response	Skip			
Part 1: Socio-demographic information of food handlers						
101	Sex	1. Male 2. Female				
102	Age (in complete years)					
103	Educational status	1. Illiterate				
		2. Primary school				
		3. Secondary				
		School				
	0,	4. Tertiary				
104	What is your monthly income?					
105	How many years of work experience do you have in food handling?					
Part 2: Food handlers' personal hygiene conditions						
201	Condition of fingernails	1. Trimmed				
		2. Untrimmed				
202	Regular hand washing with soap after toilet	1. Yes 2. No				
203	Regular hand washing with soap before eating	1. Yes 2. No				
204	Wearing clean protective clothes regularly	1. Yes 2. No				
Part 3: Food establishment related factors						
301	Food safety training	1. Yes 2. No				
302	Medical checkup (in last 6 month)	1. Yes 2. No				
Intestinal parasites identified (to be filled based on the laboratory results)						
Please carefully read the laboratory reports and write the intestinal parasites						
identified in the space provided. If no ova of parasites detected, write "No ova						
of paras	ites detected".					

Prevalence of intestinal parasites and associated factors among food handlers in food establishments in the Lideta sub-city of Addis Ababa, Ethiopia: an institution-based cross-sectional study

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Prevalence of intestinal parasites and associated factors among food handlers in food establishments in the Lideta sub-city of Addis Ababa, Ethiopia: an institution-based cross-sectional study Woinishet Abera¹, Binyam Gintamo^{1,2,3}, Tewoderos Shitemaw⁴, Zelalem Negash Mekuria^{1,2,5}, Zemichael Gizaw6* ¹Yanet Health College, Addis Ababa, Ethiopia. ²Addis Ababa Medical and Business College, Addis Ababa, Ethiopia. ³ Department of Biotechnology, Faculty of Applied Sciences and Biotechnology, Shoolini University, Solan, Bajhol, H.P., India ⁴ Kotebe Metropolitan University, Addis Ababa, Ethiopia ⁵ Yekatit 12 Medical College, Addis Ababa, Ethiopia ⁶Department of Environmental and Occupational Health and safety, Institute of Public Health, College of Medicine and Health Sciences, University of Gondar, Gondar, Ethiopia Email address of authors: Woinishet Abera (woini27ab@gmail.com), Binyam Gintamo (gintamobinyam@gmail.com), Tewodros Shitemaw (tewoderosshitemaw@gmail.com), Zelalem Negash Mekuria (zolabelay256@gmail.com), and Zemichael Gizaw (zemichael12@gmail.com) *Corresponding author: Zemichael Gizaw, Department of Environmental and Occupational Health and safety, Institute of Public Health, College of Medicine and Health Sciences, University of Gondar, Gondar, Ethiopia, Email: zemichael12@gmail.com Abstract Objectives: This study was conducted to assess the prevalence of intestinal parasites and associated factors among food handlers. Design: An institution-based cross-sectional study design was used. Stool samples were collected from food handlers and examined using direct wet mount and formalin-ether concentration (FEC) techniques. Personal and establishment related information was collected using pretested questionnaire with structured observation. Multivariable binary logistic

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regression was used to identify factors associated with prevalence of intestinal parasites on the basis of adjusted odds ratio and 95% confidence interval with p < 0.05.

Setting: This study was conducted in different food establishments in the Lideta sub-city of
Addis Ababa, Ethiopia.

Participants: Four hundred and eleven food handlers were participated in this study.

Outcome measures: The primary outcome is prevalence of intestinal parasites, which was
 defined as presence of one or more intestinal parasitic species in stool samples.

Results: One or more intestinal parasites were detected in 171 (41.6%) (95% CI: 36.6, 46.4%) of the stool samples. The most common intestinal parasites were E. histolytica/dispar (12.7 %). G. duodenalis (11.2%), and A. lumbricoides (8.3%). This high prevalence of intestinal parasites among food handlers was associated with low monthly income (AOR: 2.83, 95% CI:1.50, 8.84), untrimmed fingernails (AOR: 4.36, 95% CI: 1.98, 11.90), no food safety training (AOR: 2.51, 95% CI: 1.20, 5.58), low level of education (AOR: 3.13, 95% CI: 1.34, 7.44), poor handwashing practice (AOR: 2.16, 95% CI: 1.03, 4.22), and lack of medical checkup (AOR: 2.31, 95% CI: 1.18, 6.95).

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Conclusion: The prevalence of intestinal parasites among food handlers in food 43 establishments in the Lideta sub-city of Addis Ababa was high. This was linked to socio-44 economic conditions, poor hand hygiene condition, and absence of food safety trainings. It is, 45 therefore, important to promote hand washing practice and providing food hygiene and safety 46 training is critical.

47 Key words: Intestinal parasites, food handlers, food establishments, hand hygiene, Ethiopia

5 48 **St**r

8 Strengths and limitations of this study

- The study has focused on one of the most potential groups, i.e., food handlers that had potential to spread food borne infections to the consumers.

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The use of sensitive diagnostic techniques and combination of methods with triplicate
 examinations applied in this study would help to recover greater rate of intestinal parasites
 that would indicate the true prevalence.

- As a limitation, the collection of a single stool sample may affect the result of parasitic examination since the sensitivities of the direct smear examination technique is reduced when a single stool sample is examined and the procedure of FEC technique may also damage the eggs of parasites.

58 Introduction

Foodborne diseases are increasingly becoming a serious global public health problem. The World Health Organization (WHO) estimate indicates that each year worldwide, unsafe food causes 600 million cases of foodborne diseases and 420 000 deaths. WHO estimated that 33 million years of healthy lives are lost due to eating unsafe food globally each year, and this number is likely an underestimation [1]. One of the causes of foodborne diseases is contamination during food preparation; food handlers carrying pathogens might be involved in the origin of this condition. Foods can be contaminated with fecal material at the point of production or during food preparation, in both the home and in commercial premises [2]. Food handlers with poor personal hygiene and inadequate knowledge on food safety could be potential sources of infections. Food handlers who harbor and excrete enteropathogens may contaminate foods from their feces via contaminated hands, then to food or food contact surfaces, and finally to healthy individuals [3-7].

The contribution of the infected food worker (whether symptomatic or not) to food borne disease outbreaks has been difficult to establish. However, reports showed that food workers in many settings have been responsible for foodborne disease outbreaks for decades. For instance, members of the Committee on Control of Foodborne Illnesses of the International Association for Food Protection analyzed 816 foodborne disease outbreaks with 80,682 cases in different countries where food workers were implicated as the source of the contamination. The report also estimated that infected food worker was documented as responsible for 18% of 766 outbreaks occurring in the United States [8, 9]. Moreover, according to the Centers for Disease Control (CDC), as cited in Mathew RR (2019), 20 to 40% of food borne illness associated with the consumption of contaminated food originated in catering establishments [10].

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Food handling personnel play a role in the transmission of food borne diseases. The health of food handlers is of great importance for maintaining quality of food products. Accordingly, pre-employment and periodic medical checkup is very important to safeguard the consumers from getting diseases from contaminated foods along with other food safety measures [11, 12]. However, pre-employment and periodic medical checkups are not commonly practiced in Ethiopia. As a result of this, many of the food handlers working in different food establishments all over the country may harbor one or more enteropathogens. For instance, a systematic review and meta-analysis reported that the overall pooled prevalence estimate of intestinal parasites among food handlers of food service establishments in Ethiopia was 33.6% (95% CI: 27.6, 39.6%) [13] and the common factors associated to high prevalence of intestinal pathogens among food handlers are poor hand hygiene, inadequate access to water and sanitation facilities, and poor socio-economic conditions [13-18]. However, the prevalence and risk factors may be different across various settings. Accordingly, this study was conducted to assess the prevalence of intestinal parasites and associated factors among food handlers working in food establishment in the Lideta sub-city of Addis Ababa, Ethiopia.

96 Methods

97 Study design and setting

This an institution-based cross-sectional study with laboratory investigations was conducted in the Lideta sub-city of Addis Ababa from March 20 to April 20, 2021. Lideta sub-city is one of the 10 sub cities of Addis Ababa, the capital of Ethiopia. The sub-city is located at the global positioning system (GPS) coordinates of 9°0'N and 38°45'E. The sub city is divided into 10 districts (Figure 1). In the Lideta sub-city, there are a total of 281 food establishments and 1124 food handlers working in the food establishments.

2.

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⁶ 104 Sample size calculation and sampling techniques

⁴⁹ 105 The sample size was calculated using single population proportion formula with the following ⁵⁰ 106 assumptions: prevalence of intestinal parasites among food handlers in Addis Ababa University ⁵² 107 students cafeteria, Addis Ababa, Ethiopia (p) = 45.3% [19], level of significance (α) = 5%, 95% ⁵⁴ 108 confidence interval (standard normal probability), z: the standard normal tabulated value, and ⁵⁵ 109 margin of error (d) = 5%.

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$$n = \frac{(Z_{\alpha/2})^2 P(1-P)}{d^2} = \frac{(1.96)^2 0.453(1-0.453)}{0.05^2} = 381$$

The final sample size was 419 after considering 10% non-response rate. The Lideta sub-city was 111 selected at random from a total of 10 sub-cities of Addis Ababa, Ethiopia. Using lists of food 112 handlers working in different food establishments in the sub-city obtained from Addis Ababa 113 food, medicines, and healthcare administration authority (AAFMHACA) as a sampling frame, 114 we used computer generated random number to select food handlers. Food handlers who treated 115 with anti-helminthes and anti-protozoan drugs in the last four weeks were excluded from the 116 117 study.

118 **Stool sample collection**

119 Stool sample collectors first explained the purpose of collecting stool to the randomly selected food handlers, and then asked them to urinate first without pooping to avoid urine contamination 120 121 of the stool. Stool sample collectors then handed out paper to food handlers and instructing them to defecate on it to avoid stool contamination with stored feces and dirt. Food handlers were 122 asked to bring approximately 50 g of the last part of the stool, the softest part, into the collection 123 container after defecating on the paper. Stool sample collectors didn't violate privacy of food 124 125 handlers during stool sample collection. Stool sample collectors then immediately stored the stool sample in to a cold box after labeled a code on the outer surface of the plastic cup. 126

127

Personal and food establishment data collection

128 We used structured questionnaire and observational checklist to collect food handlers' personal data and food establishments' related information. The questionnaire was developed by 129 reviewing related published articles [15, 20-24]. The tool was first prepared in English language 130 and translated to the local Amharic language by two native Amharic speakers fluent in English, 131 and back-translated into English by two independent English language experts fluent in Amharic 132 to check consistency. The questionnaire consisted of three parts: i) socio-demographic 133 characteristics of food handlers, ii) food handlers' personal hygiene conditions, and iii) food 134 establishment related factors (supplementary file). The questionnaire was pretested to evaluate 135 53 the instructions, response format, and questions work as intended and are understood by those 54 136 55 individuals who are likely to respond to them. Data collectors were trained in the data collection 137 56

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tool as well as ethical issues during interviewing and observing. Supervisors supervised the data collection process and checked the completeness of the data on a daily basis. We gathered handwashing data by assessing food handlers' usual handwashing behavior using self-reports. We also looked at the hands of food handlers to see the general cleanliness and conditions of fingernails. In addition, we asked food handlers to demonstrate how they wash their hands on a regular basis, which we evaluated using checklists for effective handwashing.

₅ 144

Detection of ova of parasites in stool samples

We used direct stool examination (wet mount) and FEC techniques to detect ova of intestinal parasites in stool samples. One drop of physiological saline was placed on a clean slide. Using an applicator stick, a small amount of stool specimen was emulsified in saline solution. The preparation was covered with a cover slip and examined under the microscope for the absence or presence of an intestinal parasites. The entire saline preparation was systematically examined for helminthes eggs, larvae, ciliates, cysts and oocysts using 10× objective with condenser iris closed sufficiently to give good contrast, while $40 \times$ objectives was used to assist in the detection of eggs, cysts, and oocytes [25].

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For the FEC technique, an estimated 1 g of formed stool sample or 2 ml of watery stool was emulsified in about 4 ml of 10% formol water contained in a screw-cap bottle. A further 3 ml of 10% formol water was added and mixed well by shaking. The emulsified stool samples were sieved and the sieved suspension transferred to a conical (centrifuge). 3 ml of diethyl ether was added and the tube was stoppered mixed for 1 minute with a tissue wrapped around the top of the tube, the stopper was loosen. It was then centrifuge at 3000 rpm for 1 minute. Using a stick, the layer of feces debris from the side of the tube was loosen and the tube inverted to discard the ether, fecal debris, and formol water leaving behind the sediment. The tube was returned to its upright position and the fluid from the sides of the tube allowed draining to the bottom. The bottom of the tube was taped to re-suspend and mix the sediment. The sediment was transferred to a slide and covered with a cover glass and was examined microscopically using the 10x objective for focusing and $40 \times$ objective for proper identification.

Standard operating procedures were used for every laboratory procedure during lab examination,stool specimen collection, transportation, and storing. We used stool sample collection and

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167 transportation containers which are leak-proof, dry clean and free from any traces of 168 disinfectants. We ensured correct labelling of stool sample containers with date of sample 169 collection, and code of the study participants. All stool specimens were stored in ice box for 170 transportation and preserved at 4°C in the laboratory until analyzed for ova of parasites. 171 Triplicate examinations of stool samples were applied to improve the recovery rate of intestinal 172 parasites. Moreover, the expiry date of normal saline, ether, and formol was evaluated before 173 stool sample preparation and examination.

Outcome variable of the study

Prevalence of intestinal parasites, the primary outcome variable of the study is defined as thepresence of one or more intestinal parasite species in stool samples.

3 177 Data processing and analysis

Data were entered using EPI-INFO version 3.5.3 statistical package and export into Statistical Package for Social Sciences (SPSS) version 20 for further analysis. For most variables, data were presented by frequencies and percentages. Univariable binary logistic regression analysis was used to choose variables for the multivariable binary logistic regression analysis, and variables which had p-value less than 0.25 by the univariable analysis and other well-known confounders from the literature were then analyzed by multivariable analysis for controlling the possible effect of confounders and to predict the prevalence of intestinal parasites among food handlers based on the predictors. In the adjusted model, variables which had significant association were identified on the basis of adjusted odds ratio (AOR) with 95% confidence interval (CI) and p-values < 0.05. The predictive power of the model was checked using McFadden's pseudo R-squared.

⁵ 189 Ethics approval and consent to participate

Ethical clearance was obtained from the Institutional Review Board of Yanet Health College
(reference number: YEC/060/21). There were no risks due to participation and the collected data
were used only for this research purpose with complete confidentiality and privacy of food
handlers during stool sample collection was assured. Written informed consent was obtained
from food handlers. Furthermore, we advised food handlers who had one or more ova of

26-35

36-50

Education status of the respondents

Tertiary education

Secondary school

Primary school

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27.5

24.3

5.6

39.7

50.3

parasites to visit health institutions for treatment. All the methods were carried out in accordance with relevant guidelines and regulations. Patient and public involvement There was no patient or public involvement in the study. **Results** Socio-demographic characteristics of study participants We collected personal information and stool samples from a total 411 food handlers with a response rate of 97.62%. The majority, 293 (71.3%) of the study participants were female. The median age of the respondents was 28 and the interquartile range (IQR) was 20 and 39 years. About half, 198 (48.18%) of the respondents aged 25 years and below. Half, 207 (50.3 %) of the respondents reported that they completed primary school education. Two hundred and seventy-eight (67.6%) of the food handlers reported that they had 3 years or less work experience. One hundred and eleven (27.0%) of the food handlers earned <1500 Ethiopian birr (Table 1). Table 1: Socio-demographic characteristics of food handlers (n = 411) working in different food establishments in the Lideta sub-city of Addis Ababa, Ethiopia, March 20 to April 20, 2021 Socio-demographic characteristics Frequency Percent Sex of the respondents Female 71.3 Male 28.7 Age of the respondents in years 48.2 <25

		10	
	Illiterate	18	4.4
	Service year		
	≤3 years	278	67.6
	>3 years	133	33.4
	Average monthly income in Ethiopian birr		
	<1500	111	27.0
	1501-2500	152	37.0
	2501-3500	94	22.9
	>3500	54	13.1
210	Personal hygiene characteristics of food handle	rc	

Two hundred and forty-two (58.9%) of the food handlers did not keep their fingernails short. One hundred and ninety-four (47.2%) and 206 (50.1%) of the food handlers did not regularly wash hands with soap after visiting toilet and before eating, respectively. Two hundred and eight (50.6%) of the food handlers reported that they regularly worn clean protective clothes. About a quarter, 76 (24%) of the food handlers reported that they received food safety training and 121 (29.4%) of the food handlers had medical checkup in the previous 6 months prior to the survey (Table 2).

Table 2: Personal hygiene characteristics of food handlers (n = 411) working in different food establishments in the Lideta sub-city of Addis Ababa, Ethiopia, March 20 to April 20, 2021

Variables	Frequency	Percent
Condition of fingernails		
Trimmed	169	41.1
Untrimmed	242	58.9
Regular hand washing with soap after toilet		
Yes	217	52.8
No	194	47.2
Regular hand washing with soap before eating		
Yes	205	49.9

2				
3 4	No	206	50.1	
5	Wearing clean protective clothes regularly			
6 7	Yes	208	50.6	
8 9	No	203	49.4	
10	Food safety training			
11 12	Yes	76	24	
13 14	No	335	76	
15	Medical checkup (in last 6 month)			
16 17	Yes	121	29.4	
18 19	No	290	70.6	

Intestinal parasites in food handlers

A total of 411 food handlers were examined, with 171 (41.6%) (95% CI: 36.6, 46.4%) of them had ova of one or more intestinal parasites [98 (23.8% were protozoan and 73 (17.8%) were helminth parasites], of which 14 (3.4%) had mixed parasites (Figure 2). The most common intestinal parasites were E. histolytica/dispar [52 (12.7%)], G. duodenalis [46 (11.2%)], A. lumbricoides [34 (8.3%)], Hookworms [15 (3.6%)], T. trichuria [14 (3.4%)], and Taenia species [10 (2.4%)] (Table 3).

Table 3: Common intestinal parasites detected among food handlers (n = 411) working in different food establishments in the Lideta sub-city of Addis Ababa, Ethiopia, March 20 to April 20, 2021

Parasitic species	Frequency	Percent	
E.histolytica/dispar	52	12.7	
G. duodenalis	46	11.2	
A. lumbricoides	34	8.3	
Hookworms	15	3.6	
T. trichuria,	14	3.4	
Taenia species	10	2.4	

Factors associated with intestinal parasites among food handlers

Sex, age, educational level, work experience, monthly income, wearing clean protective clothes. finger nail status, hand washing after toilet, hand washing before eating, food safety training, and medical checkup were the variables entered in to the univariable binary logistic regression analysis, of which educational status, average monthly income, condition of fingernails, hand washing with soap before eating, food safety training, and medical checkup in the last six month were the candidate variables for the final model which were selected based on p < 0.25 and hand washing with soap after visiting toilet was a well-known confounder in the literature included in the final model even if its p value is greater than 0.25. In the multivariable binary logistic regression analysis, prevalence of intestinal parasites among food handlers was significantly associated with poor handwashing practice (AOR: 2.16, 95% CI: 1.03, 4.22), untrimmed fingernails (AOR: 4.36, 95% CI: 1.98, 11.90), lack of medical checkup (AOR: 2.31, 95% CI: 1.18, 6.95), no food safety training (AOR: 2.51, 95% CI: 1.20, 5.58), low level of education (AOR: 3.13, 95% CI: 1.34, 7.44), and low monthly income (AOR: 2.83, 95% CI:1.50, 8.84) (Table 4). Table 4 included effect estimates from the model with all the seven variables. In that case, one should now that the educational status and monthly income estimates are for direct effects.

Table 4: Factors associated with intestinal parasites among food handlers (n = 411) working in different food establishments in the Lideta sub-city of Addis Ababa, Ethiopia, March 20 to April 20, 2021

Variables	Intestinal parasites		COR (95% CI)	AOR (95% CI)
	Yes	No		
Educational status				
Illiterate	9	9	3.60 (1.81, 10.80)	3.13 (1.34, 7.44)*
Primary school	92	115	2.88 (1.65, 6.17)	2.22 (1.10, 6.65)*
Secondary school	68	95	2.58 (1.20, 5.56)	2.16 (1.10, 5.20)*
Tertiary education	5	18	1.0	1.0
Average monthly income in Ethiopian bir	r			
<1500	56	55	2.91 (1.43, 7.86)	2.84 (1.50, 8.84)*
1501-2500	68	84	2.31 (1.14, 6.96)	2.28 (0.63, 8.10)
2501-3500	33	61	1.55 (1.05, 4.85)	1.41 (0.60, 5.96)

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>3500	14	40	1.0	1.0
Condition of fingernails				
Untrimmed	134	108	4.43 (2.12, 12.62)	4.36 (1.98, 11.90)*
Trimmed	37	132	1.0	1.0
Hand washing with soap after toilet				
No	101	93	2.28 (1.10, 7.51)	2.19 (0.92, 5.62)
Yes	70	147	1.0	1.0
Hand washing with soap before eating				
No	112	94	2.95 (1.23, 6.72)	2.16 (1.03, 4.22)*
Yes	59	146	1.0	1.0
Food safety training				
No	153	182	2.71 (1.34, 8.53)	2.51 (1.20, 5.58)*
Yes	18	58	1.0	1.0
Medical checkup in the last 6 month				
No	140	150	2.71 (1.27, 7.56)	2.32 (1.18, 6.95)*
Yes	31	90	1.0	1.0

squared = 0.492

Discussion

This is an institution-based cross-sectional study assessed intestinal parasites among food handlers working in food establishments in the Lideta sub-city of Addis Ababa, Ethiopia and found that 41.6% (95% CI: 36.6, 46.4%) of the food handlers had one or more intestinal parasites. The prevalence of intestinal parasites reported in this study was comparable with findings of studies conducted among food handlers in Bule Hora (46.3%) [16] and Addis Ababa University (45.3%) [19]. The prevalence of intestinal parasites reported in this study was lower than findings of studies in Nekemte town (52.1%) [26] and Mekele University (49.4%) [15]. Furthermore, the prevalence of intestinal parasites reported in the current study was higher than findings of studies conducted among food handlers in Wolaita Sodo (23.6%) [14], Jimma (33%) [27], Madda Walabu University (25.3%) [28], Motta town (27.6%) [29], Nairobi(15.7%)[30],

Iran (9%) [31], Saudi Arabia (23%) [32], and Thailand (10%) [33]. The high prevalence of intestinal parasites among food handlers among food handlers working in food establishments in the Lideta sub-city of Addis Ababa might be explained by poor socio-economic conditions, poor hand hygiene, and inadequate access to basic sanitation services.

The high prevalence of intestinal parasites among food handlers working in food establishments in the Lideta sub-city suggests that poor hygiene practices of food handlers and inadequate access to sanitation services. The results may also suggests that there might be transmission of intestinal parasitic infections from food handlers to food users, unless large scale screening and mass drug administration are done. As it is documented in literature, infected food handlers play a significant role in infection transmissions to customers of the food establishments where the infected food handlers are working [4].

This study showed that educational status of food handlers was associated with a high prevalence of intestinal parasites. Prevalence of intestinal parasites was higher among food handlers who were illiterate or attended primary and secondary education compared with food handlers who attended tertiary education. This may be due to the fact that educated food handlers may have awareness about the transmission and prevention methods of infectious diseases. Education encourages changes in healthy behaviors. Other similar studies also reported the relation of education with occurrence of parasitic infections [34-38].

The current study revealed that monthly income of food handlers was associated with intestinal parasites among food handlers. Food handlers with low monthly income had higher odds of intestinal parasites. This may be due to the fact that food handlers with low economic status could not afford for services like soap, household water treatment, toilets and other facilities, and so that will have limited opportunities to practice healthy measures. The effect of low income on risk of parasites is complex and could be attributed to limited access to sanitary materials, sources of drinking water and food, environment sanitation, and education [37-40].

The high prevalence of intestinal parasites among food handlers was associated with hand hygiene. Food handlers who did not keep their fingernails short had higher odds to have intestinal parasites and the odds of having intestinal parasites was also higher among food handlers who did not wash hands with soap before eating. This might be due to the fact that the Page 15 of 23

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area beneath the fingernails has the highest concentration of microorganisms on the hands and is the most difficult to clean [41-44]. Moreover, food handlers may ingest diseases causing pathogens when they eat without washing hands. Hands are one of the most important mechanisms to transmit pathogenic microorganisms leading to infection [45]. Evidence indicates that hands together with food contact or other environmental surfaces cause 60% of the spread of gastrointestinal infection. Contaminated hands could also be associated with up to 50% of respiratory tract infections [46].

This study depicted that intestinal parasites among food handlers was significantly associated with food safety training. The odds of having intestinal parasites was high among food handlers who did not take food safety training compared with their counterparts. This could be due to the fact those food handlers who did not take food safety training may lack the necessary knowledge and practice towards transmission and prevention of disease causing pathogens. Moreover, food safety training or health education promotes health behaviors toward hygiene and sanitation practices. Health education increases knowledge and acceptability of interventions. It also sustains integrated control of the infection [47-49].

Furthermore, intestinal parasites was significantly associated with medical checkup. The odds of having intestinal parasites was high among food handlers who did not take medical checkups in the previous six months prior to the survey. Other studies are also reported that medical checkup of food handlers is associated with intestinal parasites [15, 31, 50]. This is because food handlers who did not know their health condition before employment and while working in different establishments has less chance to take treatment and mass drugs as a result there may be existed or new or re-infections.

To increase the degree to which inferences from the sample population can be generalized to a larger group of population, we recruited study participants at random or in a manner in which they are representative of the population that we wish to study and we granted that every member of the population had an equal chance to be included in the study. In addition, we calculated adequately powered sample size using sample size determination procedures appropriate to the study objective with appropriate assumptions. Furthermore, our findings could be applicable to other situations and settings which have similar characteristics with the study populations of the current studies.

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As a limitation, even if the use of sensitive diagnostic techniques and combination of methods with triplicate examinations applied in this study would help to recover greater rate of intestinal parasites that would indicate the true prevalence, this study had some limitations. The collection of a single stool sample may affect the result of parasitic examination since the sensitivities of the direct smear examination technique is reduced when a single stool sample is examined. The procedure of FEC technique may also damage the eggs of parasites. The handwashing data assessed by self-reports may not be reliable since the study subjects may make the more socially acceptable answer rather than being truthful and they may not be able to assess themselves accurately. Moreover, we pre-screened variables using univariable analysis (p < 0.25), even though we retained some well-known confounders from the literature regardless of their univariable p-value. This could lead to the incorrect exclusion of a potential confounder and hence led to an inadequate adjustment for confounding.

333 Conclusion

The prevalence of intestinal parasites among food handlers working in food establishments in the Lideta sub-city of Addis Ababa, Ethiopia was found to be high. This high prevalence of intestinal parasites was linked to socio-economic conditions of food handlers, poor hand hygiene condition, absence of food safety trainings and regular medical checkup. It is, therefore, important to promote hand washing practice of food handlers, provide food hygiene and safety trainings, and establishing a system to regularly check the health conditions of food handlers.

341 Data availability statement

Individual participant data after deidentification that underlie the results reported in this articlewill be made available upon requesting the primary author immediately following publication.

7 344 Authors' contribution

WA designed the study, facilitated data collection, and conducted data analysis. BG, TS, ZNM,
and ZG supervised data collection and analysis and contributed to conceptualizing the study. ZG
prepared the manuscript. All authors approved the final version of the manuscript.

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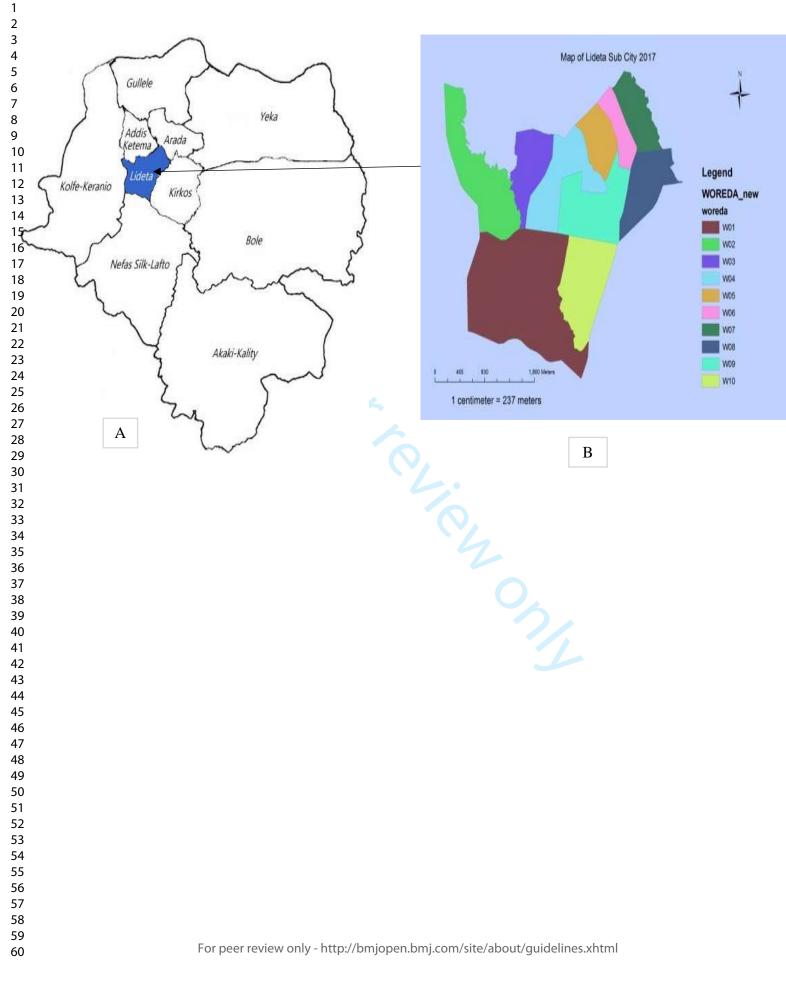
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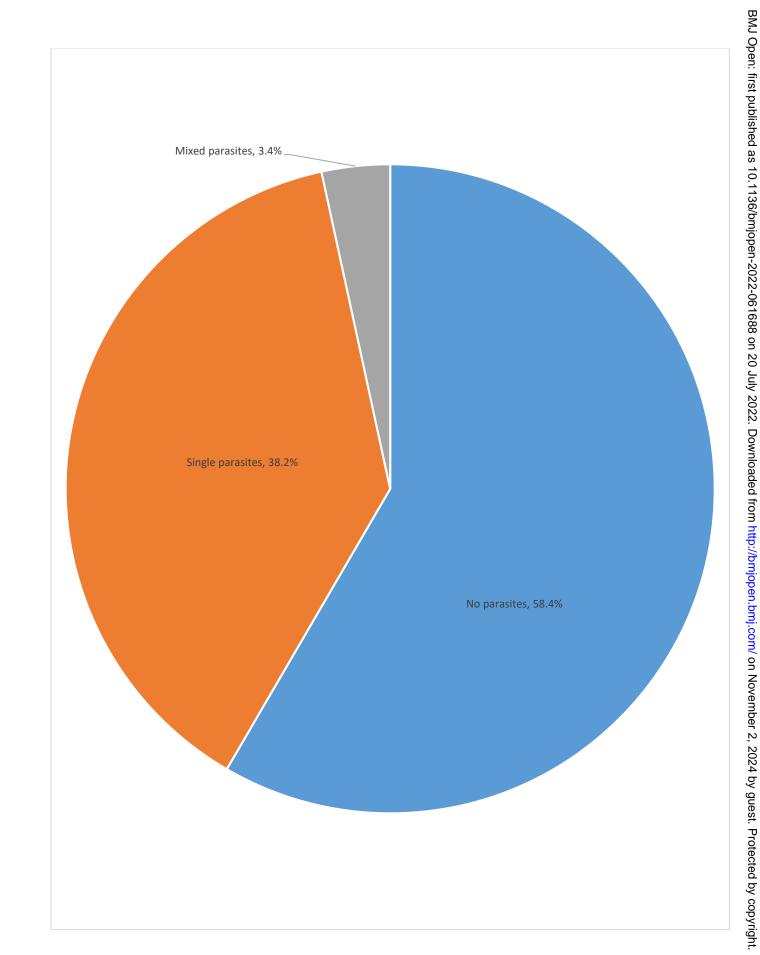
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parasitic infection in food handlers of Iran: A systematic review and meta-analysis. Veterinary
Medicine and Science 2021, 7(6):2450-2462.
- ²⁷ ₂₈ 503 **Figure caption**
 - 504 Figure 1: Map of Addis Ababa city (A) and Lideta sub-city (B) (source: Lideta sub-city 505 administration)
- Figure 2: Proportion of food handlers with no, single and mixed parasites (n = 411) working is 150 to 1
- 507 in different food establishments in Lideta sub city of Addis Ababa, Ethiopia, March 20 to April
- ³⁸ 508 20, 2021
- 41 509





Supplementary file: Data collection tool

Questionnaire Identification Code:

Consent Form

Hello, I am ______ working with a research team from Yanet Health Science College. We are contacting you to collect information for a study conducted to assess "Prevalence of intestinal parasites and associated factors among food handlers in food establishments in the Lideta sub-city of Addis Ababa, Ethiopia". This questionnaire is prepared to collect food handlers personal and food establishment related information.

Thank you so much for agreeing to be interviewed for this research work. All of the information we got from you will be completely confidential and coded with unique number. Your name will not be written down and will never be used in connection with any of the information you provided. There will not be any direct benefit that you will get associated with participation in this study. If you do not want to answer all or some of the questions, you have the right to refuse participation at any time. However, we would greatly appreciate your help in responding to this questionnaire. This questionnaire is expected to be completed in 10 minutes.

Do I have your permission to continue?

1. Yes, continue your interview with thanks after signing the consent

2. If no, skip to the next participant by writing reasons for his/ her refusal;

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Time starte	ed:	Time completed	:	_
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	For peer review only - http://bmio	pen.bmi.com/site/ab	out/quidelines.xhtml	
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Code	Questions	Response	Skip
Part 1:	Socio-demographic information of food handlers		
101	Sex	1. Male 2. Female	
102	Age (in complete years)		
103	Educational status	1. Illiterate	
		2. Primary school	
		3. Secondary	
		School	
	0,	4. Tertiary	
104	What is your monthly income?		
105	How many years of work experience do you have in food handling?		
Part 2:	Food handlers' personal hygiene conditions		
201	Condition of fingernails	1. Trimmed	
		2. Untrimmed	
202	Regular hand washing with soap after toilet	1. Yes 2. No	
203	Regular hand washing with soap before eating	1. Yes 2. No	
204	Wearing clean protective clothes regularly	1. Yes 2. No	
Part 3:	Food establishment related factors		1
301	Food safety training	1. Yes 2. No	
302	Medical checkup (in last 6 month)	1. Yes 2. No	
Intestin	al parasites identified (to be filled based on the laboratory results)		
Please c	arefully read the laboratory reports and write the intestinal parasites		
dentifie	ed in the space provided. If no ova of parasites detected, write "No ova		
of paras	ites detected".		

Prevalence of intestinal parasites and associated factors among food handlers in food establishments in the Lideta sub-city of Addis Ababa, Ethiopia: an institution-based cross-sectional study

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Prevalence of intestinal parasites and associated factors among food handlers in food establishments in the Lideta sub-city of Addis Ababa, Ethiopia: an institution-based cross-sectional study Woinishet Abera¹, Binyam Gintamo^{1,2,3}, Tewoderos Shitemaw⁴, Zelalem Negash Mekuria^{1,2,5}, Zemichael Gizaw6* ¹Yanet Health College, Addis Ababa, Ethiopia. ²Addis Ababa Medical and Business College, Addis Ababa, Ethiopia. ³ Department of Biotechnology, Faculty of Applied Sciences and Biotechnology, Shoolini University, Solan, Bajhol, H.P., India ⁴ Kotebe Metropolitan University, Addis Ababa, Ethiopia ⁵ Yekatit 12 Medical College, Addis Ababa, Ethiopia ⁶Department of Environmental and Occupational Health and safety, Institute of Public Health, College of Medicine and Health Sciences, University of Gondar, Gondar, Ethiopia Email address of authors: Woinishet Abera (woini27ab@gmail.com), Binyam Gintamo (gintamobinyam@gmail.com), Tewodros Shitemaw (tewoderosshitemaw@gmail.com), Zelalem Negash Mekuria (zolabelay256@gmail.com), and Zemichael Gizaw (zemichael12@gmail.com) *Corresponding author: Zemichael Gizaw, Department of Environmental and Occupational Health and safety, Institute of Public Health, College of Medicine and Health Sciences, University of Gondar, Gondar, Ethiopia, Email: zemichael12@gmail.com Abstract Objectives: This study was conducted to assess the prevalence of intestinal parasites and associated factors among food handlers. Design: An institution-based cross-sectional study design was used. Stool samples were collected from food handlers and examined using direct wet mount and formalin-ether concentration (FEC) techniques. Personal and establishment related information was collected using pretested questionnaire with structured observation. Multivariable binary logistic

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regression was used to identify factors associated with prevalence of intestinal parasites on the basis of adjusted odds ratio and 95% confidence interval with p < 0.05.

Setting: This study was conducted in different food establishments in the Lideta sub-city of
Addis Ababa, Ethiopia.

Participants: Four hundred and eleven food handlers were participated in this study.

Outcome measures: The primary outcome is prevalence of intestinal parasites, which was
 defined as presence of one or more intestinal parasitic species in stool samples.

Results: One or more intestinal parasites were detected in 171 (41.6%) (95% CI: 36.6, 46.4%) of the stool samples. The most common intestinal parasites were E. histolytica/dispar (12.7 %). G. duodenalis (11.2%), and A. lumbricoides (8.3%). This high prevalence of intestinal parasites among food handlers was associated with low monthly income (AOR: 2.83, 95% CI:1.50, 8.84), untrimmed fingernails (AOR: 4.36, 95% CI: 1.98, 11.90), no food safety training (AOR: 2.51, 95% CI: 1.20, 5.58), low level of education (AOR: 3.13, 95% CI: 1.34, 7.44), poor handwashing practice (AOR: 2.16, 95% CI: 1.03, 4.22), and lack of medical checkup (AOR: 2.31, 95% CI: 1.18, 6.95).

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Conclusion: The prevalence of intestinal parasites among food handlers in food 43 establishments in the Lideta sub-city of Addis Ababa was high. This was linked to socio-44 economic conditions, poor hand hygiene condition, and absence of food safety trainings. It is, 45 therefore, important to promote hand washing practice and providing food hygiene and safety 46 training is critical.

47 Key words: Intestinal parasites, food handlers, food establishments, hand hygiene, Ethiopia

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8 Strengths and limitations of this study

- The study has focused on one of the most potential groups, i.e., food handlers that had potential to spread food borne infections to the consumers.

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The use of sensitive diagnostic techniques and combination of methods with triplicate
 examinations applied in this study would help to recover greater rate of intestinal parasites
 that would indicate the true prevalence.

- As a limitation, the collection of a single stool sample may affect the result of parasitic examination since the sensitivities of the direct smear examination technique is reduced when a single stool sample is examined and the procedure of FEC technique may also damage the eggs of parasites.

58 Introduction

Foodborne diseases are increasingly becoming a serious global public health problem. The World Health Organization (WHO) estimate indicates that each year worldwide, unsafe food causes 600 million cases of foodborne diseases and 420 000 deaths. WHO estimated that 33 million years of healthy lives are lost due to eating unsafe food globally each year, and this number is likely an underestimation [1]. One of the causes of foodborne diseases is contamination during food preparation; food handlers carrying pathogens might be involved in the origin of this condition. Foods can be contaminated with fecal material at the point of production or during food preparation, in both the home and in commercial premises [2]. Food handlers with poor personal hygiene and inadequate knowledge on food safety could be potential sources of infections. Food handlers who harbor and excrete enteropathogens may contaminate foods from their feces via contaminated hands, then to food or food contact surfaces, and finally to healthy individuals [3-7].

The contribution of the infected food worker (whether symptomatic or not) to food borne disease outbreaks has been difficult to establish. However, reports showed that food workers in many settings have been responsible for foodborne disease outbreaks for decades. For instance, members of the Committee on Control of Foodborne Illnesses of the International Association for Food Protection analyzed 816 foodborne disease outbreaks with 80,682 cases in different countries where food workers were implicated as the source of the contamination. The report also estimated that infected food worker was documented as responsible for 18% of 766 outbreaks occurring in the United States [8, 9]. Moreover, according to the Centers for Disease Control (CDC), as cited in Mathew RR (2019), 20 to 40% of food borne illness associated with the consumption of contaminated food originated in catering establishments [10].

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Food handling personnel play a role in the transmission of food borne diseases. The health of food handlers is of great importance for maintaining quality of food products. Accordingly, pre-employment and periodic medical checkup is very important to safeguard the consumers from getting diseases from contaminated foods along with other food safety measures [11, 12]. However, pre-employment and periodic medical checkups are not commonly practiced in Ethiopia. As a result of this, many of the food handlers working in different food establishments all over the country may harbor one or more enteropathogens. For instance, a systematic review and meta-analysis reported that the overall pooled prevalence estimate of intestinal parasites among food handlers of food service establishments in Ethiopia was 33.6% (95% CI: 27.6, 39.6%) [13] and the common factors associated to high prevalence of intestinal pathogens among food handlers are poor hand hygiene, inadequate access to water and sanitation facilities, and poor socio-economic conditions [13-18]. However, the prevalence and risk factors may be different across various settings. Accordingly, this study was conducted to assess the prevalence of intestinal parasites and associated factors among food handlers working in food establishment in the Lideta sub-city of Addis Ababa, Ethiopia.

96 Methods

97 Study design and setting

This an institution-based cross-sectional study with laboratory investigations was conducted in the Lideta sub-city of Addis Ababa from March 20 to April 20, 2021. Lideta sub-city is one of the 10 sub cities of Addis Ababa, the capital of Ethiopia. The sub-city is located at the global positioning system (GPS) coordinates of 9°0'N and 38°45'E. The sub city is divided into 10 districts (Figure 1). In the Lideta sub-city, there are a total of 281 food establishments and 1124 food handlers working in the food establishments.

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⁶ 104 Sample size calculation and sampling techniques

⁴⁹ 105 The sample size was calculated using single population proportion formula with the following ⁵⁰ 106 assumptions: prevalence of intestinal parasites among food handlers in Addis Ababa University ⁵² 107 students cafeteria, Addis Ababa, Ethiopia (p) = 45.3% [19], level of significance (α) = 5%, 95% ⁵⁴ 108 confidence interval (standard normal probability), z: the standard normal tabulated value, and ⁵⁵ 109 margin of error (d) = 5%.

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$$n = \frac{(Z_{\alpha/2})^2 P(1-P)}{d^2} = \frac{(1.96)^2 0.453(1-0.453)}{0.05^2} = 381$$

The final sample size was 419 after considering 10% non-response rate. The Lideta sub-city was 111 selected at random from a total of 10 sub-cities of Addis Ababa, Ethiopia. Using lists of food 112 handlers working in different food establishments in the sub-city obtained from Addis Ababa 113 food, medicines, and healthcare administration authority (AAFMHACA) as a sampling frame, 114 we used computer generated random number to select food handlers. Food handlers who treated 115 with anti-helminthes and anti-protozoan drugs in the last four weeks were excluded from the 116 117 study.

118 **Stool sample collection**

119 Stool sample collectors first explained the purpose of collecting stool to the randomly selected food handlers, and then asked them to urinate first without pooping to avoid urine contamination 120 121 of the stool. Stool sample collectors then handed out paper to food handlers and instructing them to defecate on it to avoid stool contamination with stored feces and dirt. Food handlers were 122 asked to bring approximately 50 g of the last part of the stool, the softest part, into the collection 123 container after defecating on the paper. Stool sample collectors didn't violate privacy of food 124 125 handlers during stool sample collection. Stool sample collectors then immediately stored the stool sample in to a cold box after labeled a code on the outer surface of the plastic cup. 126

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Personal and food establishment data collection

128 We used structured questionnaire and observational checklist to collect food handlers' personal data and food establishments' related information. The questionnaire was developed by 129 reviewing related published articles [15, 20-24]. The tool was first prepared in English language 130 and translated to the local Amharic language by two native Amharic speakers fluent in English, 131 and back-translated into English by two independent English language experts fluent in Amharic 132 to check consistency. The questionnaire consisted of three parts: i) socio-demographic 133 characteristics of food handlers, ii) food handlers' personal hygiene conditions, and iii) food 134 establishment related factors (supplementary file). The questionnaire was pretested to evaluate 135 53 the instructions, response format, and questions work as intended and are understood by those 54 136 55 individuals who are likely to respond to them. Data collectors were trained in the data collection 137 56

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tool as well as ethical issues during interviewing and observing. Supervisors supervised the data collection process and checked the completeness of the data on a daily basis. We gathered handwashing data by assessing food handlers' usual handwashing behavior using self-reports. We also looked at the hands of food handlers to see the general cleanliness and conditions of fingernails. In addition, we asked food handlers to demonstrate how they wash their hands on a regular basis, which we evaluated using checklists for effective handwashing.

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Detection of ova of parasites in stool samples

We used direct stool examination (wet mount) and FEC techniques to detect ova of intestinal parasites in stool samples. One drop of physiological saline was placed on a clean slide. Using an applicator stick, a small amount of stool specimen was emulsified in saline solution. The preparation was covered with a cover slip and examined under the microscope for the absence or presence of an intestinal parasites. The entire saline preparation was systematically examined for helminthes eggs, larvae, ciliates, cysts and oocysts using 10× objective with condenser iris closed sufficiently to give good contrast, while $40 \times$ objectives was used to assist in the detection of eggs, cysts, and oocytes [25].

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For the FEC technique, an estimated 1 g of formed stool sample or 2 ml of watery stool was emulsified in about 4 ml of 10% formol water contained in a screw-cap bottle. A further 3 ml of 10% formol water was added and mixed well by shaking. The emulsified stool samples were sieved and the sieved suspension transferred to a conical (centrifuge). 3 ml of diethyl ether was added and the tube was stoppered mixed for 1 minute with a tissue wrapped around the top of the tube, the stopper was loosen. It was then centrifuge at 3000 rpm for 1 minute. Using a stick, the layer of feces debris from the side of the tube was loosen and the tube inverted to discard the ether, fecal debris, and formol water leaving behind the sediment. The tube was returned to its upright position and the fluid from the sides of the tube allowed draining to the bottom. The bottom of the tube was taped to re-suspend and mix the sediment. The sediment was transferred to a slide and covered with a cover glass and was examined microscopically using the 10x objective for focusing and $40 \times$ objective for proper identification.

Standard operating procedures were used for every laboratory procedure during lab examination,stool specimen collection, transportation, and storing. We used stool sample collection and

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167 transportation containers which are leak-proof, dry clean and free from any traces of 168 disinfectants. We ensured correct labelling of stool sample containers with date of sample 169 collection, and code of the study participants. All stool specimens were stored in ice box for 170 transportation and preserved at 4°C in the laboratory until analyzed for ova of parasites. 171 Triplicate examinations of stool samples were applied to improve the recovery rate of intestinal 172 parasites. Moreover, the expiry date of normal saline, ether, and formol was evaluated before 173 stool sample preparation and examination.

Outcome variable of the study

Prevalence of intestinal parasites, the primary outcome variable of the study is defined as thepresence of one or more intestinal parasite species in stool samples.

3 177 Data processing and analysis

Data were entered using EPI-INFO version 3.5.3 statistical package and export into Statistical Package for Social Sciences (SPSS) version 20 for further analysis. For most variables, data were presented by frequencies and percentages. Univariable binary logistic regression analysis was used to choose variables for the multivariable binary logistic regression analysis, and variables which had p-value less than 0.25 by the univariable analysis and other well-known confounders from the literature were then analyzed by multivariable analysis for controlling the possible effect of confounders and to predict the prevalence of intestinal parasites among food handlers based on the predictors. In the adjusted model, variables which had significant association were identified on the basis of adjusted odds ratio (AOR) with 95% confidence interval (CI) and p-values < 0.05. The predictive power of the model was checked using McFadden's pseudo R-squared.

⁵ 189 Ethics approval and consent to participate

Ethical clearance was obtained from the Institutional Review Board of Yanet Health College
(reference number: YEC/060/21). There were no risks due to participation and the collected data
were used only for this research purpose with complete confidentiality and privacy of food
handlers during stool sample collection was assured. Written informed consent was obtained
from food handlers. Furthermore, we advised food handlers who had one or more ova of

26-35

36-50

Education status of the respondents

Tertiary education

Secondary school

Primary school

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27.5

24.3

5.6

39.7

50.3

parasites to visit health institutions for treatment. All the methods were carried out in accordance with relevant guidelines and regulations. Patient and public involvement There was no patient or public involvement in the study. **Results** Socio-demographic characteristics of study participants We collected personal information and stool samples from a total 411 food handlers with a response rate of 97.62%. The majority, 293 (71.3%) of the study participants were female. The median age of the respondents was 28 and the interquartile range (IQR) was 20 and 39 years. About half, 198 (48.18%) of the respondents aged 25 years and below. Half, 207 (50.3 %) of the respondents reported that they completed primary school education. Two hundred and seventy-eight (67.6%) of the food handlers reported that they had 3 years or less work experience. One hundred and eleven (27.0%) of the food handlers earned <1500 Ethiopian birr (Table 1). Table 1: Socio-demographic characteristics of food handlers (n = 411) working in different food establishments in the Lideta sub-city of Addis Ababa, Ethiopia, March 20 to April 20, 2021 Socio-demographic characteristics Frequency Percent Sex of the respondents Female 71.3 Male 28.7 Age of the respondents in years 48.2 <25

		10	
	Illiterate	18	4.4
	Service year		
	\leq 3 years	278	67.6
	>3 years	133	33.4
	Average monthly income in Ethiopian birr		
	<1500	111	27.0
	1501-2500	152	37.0
	2501-3500	94	22.9
	>3500	54	13.1
210	Personal hygiene characteristics of food handle	rc	

Two hundred and forty-two (58.9%) of the food handlers did not keep their fingernails short. One hundred and ninety-four (47.2%) and 206 (50.1%) of the food handlers did not regularly wash hands with soap after visiting toilet and before eating, respectively. Two hundred and eight (50.6%) of the food handlers reported that they regularly worn clean protective clothes. About a quarter, 76 (24%) of the food handlers reported that they received food safety training and 121 (29.4%) of the food handlers had medical checkup in the previous 6 months prior to the survey (Table 2).

Table 2: Personal hygiene characteristics of food handlers (n = 411) working in different food establishments in the Lideta sub-city of Addis Ababa, Ethiopia, March 20 to April 20, 2021

Variables	Frequency	Percent
Condition of fingernails		
Trimmed	169	41.1
Untrimmed	242	58.9
Regular hand washing with soap after toilet		
Yes	217	52.8
No	194	47.2
Regular hand washing with soap before eating		
Yes	205	49.9

2				
3 4	No	206	50.1	
5	Wearing clean protective clothes regularly			
6 7	Yes	208	50.6	
8 9	No	203	49.4	
10	Food safety training			
11 12	Yes	76	24	
13 14	No	335	76	
15	Medical checkup (in last 6 month)			
16 17	Yes	121	29.4	
18 19	No	290	70.6	
				_

Intestinal parasites in food handlers

A total of 411 food handlers were examined, with 171 (41.6%) (95% CI: 36.6, 46.4%) of them had ova of one or more intestinal parasites [98 (23.8% were protozoan and 73 (17.8%) were helminth parasites], of which 14 (3.4%) had mixed parasites (Figure 2). The most common intestinal parasites were E. histolytica/dispar [52 (12.7%)], G. duodenalis [46 (11.2%)], A. lumbricoides [34 (8.3%)], Hookworms [15 (3.6%)], T. trichuria [14 (3.4%)], and Taenia species [10 (2.4%)] (Table 3).

Table 3: Common intestinal parasites detected among food handlers (n = 411) working in different food establishments in the Lideta sub-city of Addis Ababa, Ethiopia, March 20 to April 20, 2021

Parasitic species	Frequency	Percent	
E.histolytica/dispar	52	12.7	
G. duodenalis	46	11.2	
A. lumbricoides	34	8.3	
Hookworms	15	3.6	
T. trichuria,	14	3.4	
Taenia species	10	2.4	

Factors associated with intestinal parasites among food handlers

Sex, age, educational level, work experience, monthly income, wearing clean protective clothes, finger nail status, hand washing after toilet, hand washing before eating, food safety training, and medical checkup were the variables entered in to the univariable binary logistic regression analysis, of which educational status, average monthly income, condition of fingernails, hand washing with soap before eating, food safety training, and medical checkup in the last six month were the candidate variables for the final model which were selected based on p < 0.25 and hand washing with soap after visiting toilet was a well-known confounder in the literature included in the final model even if its p value is greater than 0.25. In the multivariable binary logistic regression analysis, prevalence of intestinal parasites among food handlers was significantly associated with poor handwashing practice (AOR: 2.16, 95% CI: 1.03, 4.22), untrimmed fingernails (AOR: 4.36, 95% CI: 1.98, 11.90), lack of medical checkup (AOR: 2.31, 95% CI: 1.18, 6.95), no food safety training (AOR: 2.51, 95% CI: 1.20, 5.58), low level of education (AOR: 3.13, 95% CI: 1.34, 7.44), and low monthly income (AOR: 2.83, 95% CI:1.50, 8.84) (Table 4).

Table 4: Factors associated with intestinal parasites among food handlers (n = 411) working in
different food establishments in the Lideta sub-city of Addis Ababa, Ethiopia, March 20 to April
20, 2021

X 7 · 11	T / / 1				
Variables	Intestinal parasites		COR (95% CI)	AOR (95% CI)	
	Yes	No			
Educational status					
Illiterate	9	9	3.60 (1.81, 10.80)	3.13 (1.34, 7.44)*	
Primary school	92	115	2.88 (1.65, 6.17)	2.22 (1.10, 6.65)*	
Secondary school	68	95	2.58 (1.20, 5.56)	2.16 (1.10, 5.20)*	
Tertiary education	5	18	1.0	1.0	
Average monthly income in Ethiopian birr					
<1500	56	55	2.91 (1.43, 7.86)	2.84 (1.50, 8.84)*	
1501-2500	68	84	2.31 (1.14, 6.96)	2.28 (0.63, 8.10)	
2501-3500	33	61	1.55 (1.05, 4.85)	1.41 (0.60, 5.96)	
>3500	14	40	1.0	1.0	
Condition of fingernails					
	11	1			

2					
3 4	Untrimmed	134	108	4.43 (2.12, 12.62)	4.36 (1.98, 11.90)**
5 6	Trimmed	37	132	1.0	1.0
7 8	Hand washing with soap after toilet				
9 10	No	101	93	2.28 (1.10, 7.51)	2.19 (0.92, 5.62)
11	Yes	70	147	1.0	1.0
12 13	Hand washing with soap before eating				
14 15	No	112	94	2.95 (1.23, 6.72)	2.16 (1.03, 4.22)*
16 17	Yes	59	146	1.0	1.0
18 19	Food safety training				
20 21	No	153	182	2.71 (1.34, 8.53)	2.51 (1.20, 5.58)*
22 23	Yes	18	58	1.0	1.0
24	Medical checkup in the last 6 month				
25 26	No	140	150	2.71 (1.27, 7.56)	2.32 (1.18, 6.95)*
27 28	Yes	31	90	1.0	1.0
29 30	Note: * statistically significant at $p < 0.05$,	** statistic	ally signif	ficant at $p < 0.01$, and	McFadden's pseudo R-

squared = 0.492

Discussion

This is an institution-based cross-sectional study assessed intestinal parasites among food handlers working in food establishments in the Lideta sub-city of Addis Ababa, Ethiopia and found that 41.6% (95% CI: 36.6, 46.4%) of the food handlers had one or more intestinal parasites. The prevalence of intestinal parasites reported in this study was comparable with findings of studies conducted among food handlers in Bule Hora (46.3%) [16] and Addis Ababa University (45.3%) [19]. The prevalence of intestinal parasites reported in this study was lower than findings of studies in Nekemte town (52.1%) [26] and Mekele University (49.4%) [15]. Furthermore, the prevalence of intestinal parasites reported in the current study was higher than findings of studies conducted among food handlers in Wolaita Sodo (23.6%) [14], Jimma (33%) [27], Madda Walabu University (25.3%) [28], Motta town (27.6%) [29], Nairobi(15.7%)[30], Iran (9%) [31], Saudi Arabia (23%) [32], and Thailand (10%) [33]. The high prevalence of intestinal parasites among food handlers among food handlers working in food establishments in

the Lideta sub-city of Addis Ababa might be explained by poor socio-economic conditions, poor hand hygiene, and inadequate access to basic sanitation services.

The high prevalence of intestinal parasites among food handlers working in food establishments in the Lideta sub-city suggests that poor hygiene practices of food handlers and inadequate access to sanitation services. The results may also suggests that there might be transmission of intestinal parasitic infections from food handlers to food users, unless large scale screening and mass drug administration are done. As it is documented in literature, infected food handlers play a significant role in infection transmissions to customers of the food establishments where the infected food handlers are working [4].

This study showed that educational status of food handlers was associated with a high prevalence of intestinal parasites. Prevalence of intestinal parasites was higher among food handlers who were illiterate or attended primary and secondary education compared with food handlers who attended tertiary education. This may be due to the fact that educated food handlers may have awareness about the transmission and prevention methods of infectious diseases. Education encourages changes in healthy behaviors. Other similar studies also reported the relation of education with occurrence of parasitic infections [34-38].

The current study revealed that monthly income of food handlers was associated with intestinal parasites among food handlers. Food handlers with low monthly income had higher odds of intestinal parasites. This may be due to the fact that food handlers with low economic status could not afford for services like soap, household water treatment, toilets and other facilities, and so that will have limited opportunities to practice healthy measures. The effect of low income on risk of parasites is complex and could be attributed to limited access to sanitary materials, sources of drinking water and food, environment sanitation, and education [37-40].

The high prevalence of intestinal parasites among food handlers was associated with hand hygiene. Food handlers who did not keep their fingernails short had higher odds to have intestinal parasites and the odds of having intestinal parasites was also higher among food handlers who did not wash hands with soap before eating. This might be due to the fact that the area beneath the fingernails has the highest concentration of microorganisms on the hands and is the most difficult to clean [41-44]. Moreover, food handlers may ingest diseases causing

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pathogens when they eat without washing hands. Hands are one of the most important mechanisms to transmit pathogenic microorganisms leading to infection [45]. Evidence indicates that hands together with food contact or other environmental surfaces cause 60% of the spread of gastrointestinal infection. Contaminated hands could also be associated with up to 50% of respiratory tract infections [46].

This study depicted that intestinal parasites among food handlers was significantly associated with food safety training. The odds of having intestinal parasites was high among food handlers who did not take food safety training compared with their counterparts. This could be due to the fact those food handlers who did not take food safety training may lack the necessary knowledge and practice towards transmission and prevention of disease causing pathogens. Moreover, food safety training or health education promotes health behaviors toward hygiene and sanitation practices. Health education increases knowledge and acceptability of interventions. It also sustains integrated control of the infection [47-49].

Furthermore, intestinal parasites was significantly associated with medical checkup. The odds of having intestinal parasites was high among food handlers who did not take medical checkups in the previous six months prior to the survey. Other studies are also reported that medical checkup of food handlers is associated with intestinal parasites [15, 31, 50]. This is because food handlers who did not know their health condition before employment and while working in different establishments has less chance to take treatment and mass drugs as a result there may be existed or new or re-infections.

To increase the degree to which inferences from the sample population can be generalized to a larger group of population, we recruited study participants at random or in a manner in which they are representative of the population that we wish to study and we granted that every member of the population had an equal chance to be included in the study. In addition, we calculated adequately powered sample size using sample size determination procedures appropriate to the study objective with appropriate assumptions. Furthermore, our findings could be applicable to other situations and settings which have similar characteristics with the study populations of the current studies.

As a limitation, even if the use of sensitive diagnostic techniques and combination of methods with triplicate examinations applied in this study would help to recover greater rate of intestinal

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parasites that would indicate the true prevalence, this study had some limitations. The collection of a single stool sample may affect the result of parasitic examination since the sensitivities of the direct smear examination technique is reduced when a single stool sample is examined. The procedure of FEC technique may also damage the eggs of parasites. The handwashing data assessed by self-reports may not be reliable since the study subjects may make the more socially acceptable answer rather than being truthful and they may not be able to assess themselves accurately. Moreover, we pre-screened variables using univariable analysis (p < 0.25), even though we retained some well-known confounders from the literature regardless of their univariable p-value. This could lead to the incorrect exclusion of a potential confounder and hence led to an inadequate adjustment for confounding.

331 Conclusion

The prevalence of intestinal parasites among food handlers working in food establishments in the Lideta sub-city of Addis Ababa, Ethiopia was found to be high. This high prevalence of intestinal parasites was linked to socio-economic conditions of food handlers, poor hand hygiene condition, absence of food safety trainings and regular medical checkup. It is, therefore, important to promote hand washing practice of food handlers, provide food hygiene and safety trainings, and establishing a system to regularly check the health conditions of food handlers.

7 339 **Data availability statement**

Individual participant data after deidentification that underlie the results reported in this article
 will be made available upon requesting the primary author immediately following publication.

342 Authors' contribution

WA designed the study, facilitated data collection, and conducted data analysis. BG, TS, ZNM,
and ZG supervised data collection and analysis and contributed to conceptualizing the study. ZG
prepared the manuscript. All authors approved the final version of the manuscript.

Funding information

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3 4	347	The au	thors of this study didn't receive funds from any funding organization. The cost of data
4 5 6	348	collect	ion tools and data collectors' fee was covered by the principal investigator, i.e., WA.
7		~	
8	349	Comp	eting interest
9			
10 11	350	None c	of the authors have any competing interests in the manuscript.
12			
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14			
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18	353	1000 63	stabilishinents in Lideta sub-city of Addis Ababa, data conectors, and supervisors for then
19	354	contrib	ution in the study.
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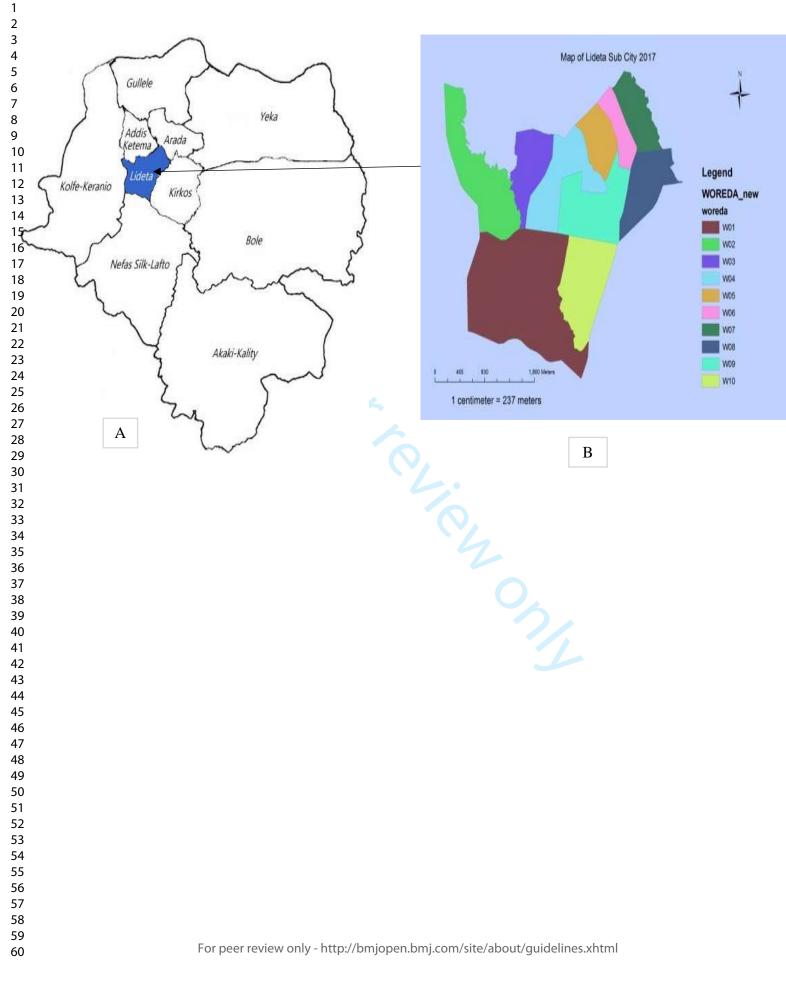
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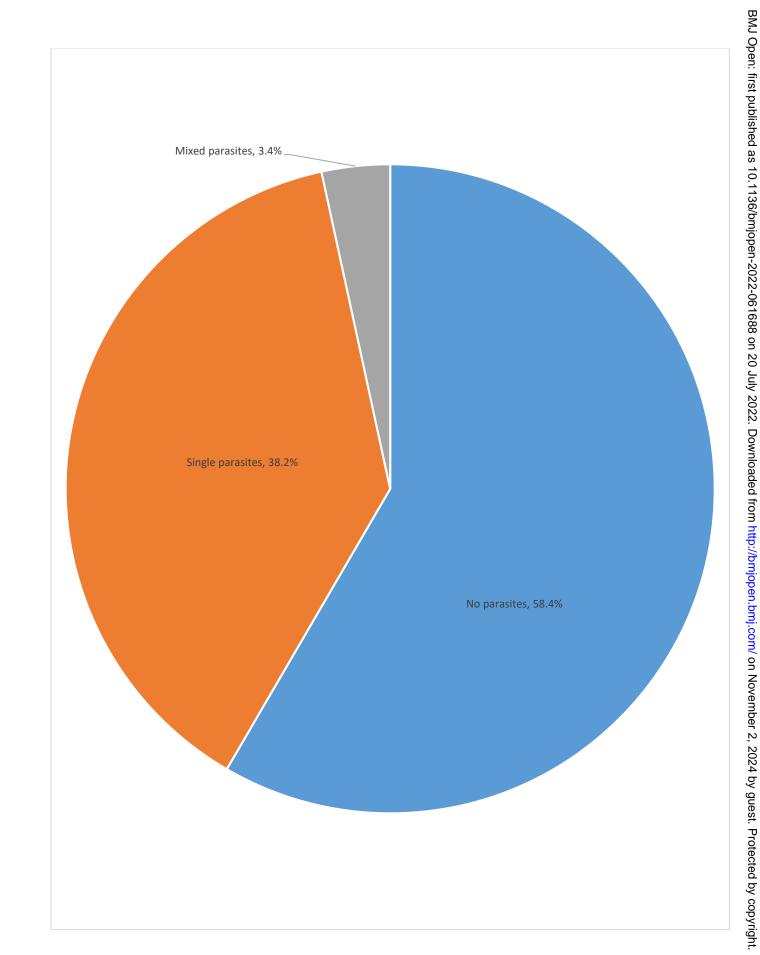
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parasitic infection in food handlers of Iran: A systematic review and meta-analysis. Veterinary
Medicine and Science 2021, 7(6):2450-2462.
- ²⁷ ₂₈ 501 **Figure caption**
 - 502 Figure 1: Map of Addis Ababa city (A) and Lideta sub-city (B) (source: Lideta sub-city 503 administration)
- 32 503 administration) 33
- Figure 2: Proportion of food handlers with no, single and mixed parasites (n = 411) working in different food establishments in Lideta sub city of Addis Ababa, Ethiopia, March 20 to April
- ³⁸ 506 20, 2021 39
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Supplementary file: Data collection tool

Questionnaire Identification Code:

Consent Form

Hello, I am ______ working with a research team from Yanet Health Science College. We are contacting you to collect information for a study conducted to assess "Prevalence of intestinal parasites and associated factors among food handlers in food establishments in the Lideta sub-city of Addis Ababa, Ethiopia". This questionnaire is prepared to collect food handlers personal and food establishment related information.

Thank you so much for agreeing to be interviewed for this research work. All of the information we got from you will be completely confidential and coded with unique number. Your name will not be written down and will never be used in connection with any of the information you provided. There will not be any direct benefit that you will get associated with participation in this study. If you do not want to answer all or some of the questions, you have the right to refuse participation at any time. However, we would greatly appreciate your help in responding to this questionnaire. This questionnaire is expected to be completed in 10 minutes.

Do I have your permission to continue?

1. Yes, continue your interview with thanks after signing the consent

2. If no, skip to the next participant by writing reasons for his/ her refusal;

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Time starte	ed:	Time completed	:	_
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	2 Not completed	2 The man and a	at does not complete the	whole questions
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Code	Questions	Response	Skip
Part 1:	Socio-demographic information of food handlers		
101	Sex	1. Male 2. Female	
102	Age (in complete years)		
103	Educational status	1. Illiterate	
		2. Primary school	
		3. Secondary	
		School	
	0,	4. Tertiary	
104	What is your monthly income?		
105	How many years of work experience do you have in food handling?		
Part 2:	Food handlers' personal hygiene conditions		
201	Condition of fingernails	1. Trimmed	
		2. Untrimmed	
202	Regular hand washing with soap after toilet	1. Yes 2. No	
203	Regular hand washing with soap before eating	1. Yes 2. No	
204	Wearing clean protective clothes regularly	1. Yes 2. No	
Part 3:	Food establishment related factors		1
301	Food safety training	1. Yes 2. No	
302	Medical checkup (in last 6 month)	1. Yes 2. No	
Intestin	al parasites identified (to be filled based on the laboratory results)		
Please c	arefully read the laboratory reports and write the intestinal parasites		
dentifie	ed in the space provided. If no ova of parasites detected, write "No ova		
of paras	ites detected".		

Prevalence of intestinal parasites and associated factors among food handlers in food establishments in the Lideta sub-city of Addis Ababa, Ethiopia: an institution-based cross-sectional study

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Prevalence of intestinal parasites and associated factors among food handlers in food establishments in the Lideta sub-city of Addis Ababa, Ethiopia: an institution-based cross-

Woinishet Abera¹, Binyam Gintamo^{1,2,3}, Tewoderos Shitemaw⁴, Zelalem Negash Mekuria^{1,2,5},

- ¹Yanet Health College, Addis Ababa, Ethiopia.
- ²Addis Ababa Medical and Business College, Addis Ababa, Ethiopia.

³ Department of Biotechnology, Faculty of Applied Sciences and Biotechnology, Shoolini

- University, Solan, Bajhol, H.P., India
- ⁴ Kotebe Metropolitan University, Addis Ababa, Ethiopia
- ⁵ Yekatit 12 Medical College, Addis Ababa, Ethiopia

⁶Department of Environmental and Occupational Health and safety, Institute of Public Health,

College of Medicine and Health Sciences, University of Gondar, Gondar, Ethiopia

Email addresses of authors: Woinishet Abera (woini27ab@gmail.com), Binyam Gintamo (gintamobinyam@gmail.com), Tewodros Shitemaw (tewoderosshitemaw@gmail.com), Zelalem Negash Mekuria (zolabelay256@gmail.com), and Zemichael Gizaw (zemichael12@gmail.com)

*Correspondence to: Zemichael Gizaw, Department of Environmental and Occupational Health and safety, Institute of Public Health, College of Medicine and Health Sciences, University of Gondar, Gondar, Ethiopia, Email: zemichael12@gmail.com

Objectives: This study was conducted to assess the prevalence of intestinal parasites and associated factors among food handlers in the Lideta sub-city of Addis Ababa, Ethiopia.

Design: An institution-based, cross-sectional study design was used. Stool samples were collected from food handlers and examined using direct wet mount and formalin-ether concentration (FEC) techniques. Personal and establishment related information was collected using a pretested questionnaire with structured observation. Multivariable binary logistic

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27 regression was used to identify factors associated with prevalence of intestinal parasites on the

basis of adjusted odds ratios and 95% confidence intervals and p values <0.05.

29 Setting: Food establishments in the Lideta sub-city of Addis Ababa, Ethiopia.

Participants: 411 food handlers participated in the study.

Outcome measures: The primary outcome was prevalence of intestinal parasites, which was
defined as presence of one or more intestinal parasitic species in stool samples.

Results: One or more intestinal parasites were detected in 171 (41.6%; 95% CI: 36.6, 46.4%) of
the stool samples. The most common intestinal parasites were *E. histolytica/dispar* (12.7%), *G. duodenalis* (11.2%), and *A. lumbricoides* (8.3%). Presence of intestinal parasites among food
handlers was associated with low monthly income (AOR: 2.83, 95% CI: 1.50, 8.84),
untrimmed fingernails (4.36, 1.98, 11.90), no food safety training (2.51, 1.20, 5.58), low level
of education (3.13, 1.34, 7.44), poor handwashing practice (2.16, 1.03, 4.22), and lack of
medical checkup (2.31, 1.18, 6.95).

Conclusion: The prevalence of intestinal parasites among food handlers in food 41 establishments in the Lideta sub-city of Addis Ababa was high. The presence of intestinal 42 parasites was linked to socioeconomic conditions, poor hand hygiene condition, and absence 43 of food safety trainings. It is crucially important to promote hand washing practice and 44 providing food hygiene and safety training in these settings.

45 Keywords: Intestinal parasites, food handlers, food establishments, hand hygiene, Ethiopia

47 Strengths and limitations of this study

The study focused on a key group (food handlers) that has potential to spread foodborneinfections to consumers.

The use of sensitive diagnostic techniques and a combination of methods with triplicate
 examinations will have led to an enhanced recovery greater rate of intestinal parasites, to
 better indicate the true prevalence.

- The use of a single stool sample might have affected the results of parasitic examination since the sensitivity of the direct smear examination technique is reduced when a single stool sample is examined, and the procedure of formalin-ether concentration technique might damage the parasite eggs.

58 Introduction

Foodborne diseases are increasingly becoming a serious global public health problem. The World Health Organization (WHO) estimate indicates that each year worldwide, unsafe food causes 600 million cases of foodborne diseases and 420 000 deaths. WHO estimated that 33 million years of healthy lives are lost due to eating unsafe food globally each year, and this number is likely an underestimation [1]. One of the causes of foodborne diseases is contamination during food preparation; food handlers carrying pathogens might be involved in the origin of this condition. Foods can be contaminated with fecal material at the point of production or during food preparation, in both the home and in commercial premises [2]. Food handlers with poor personal hygiene and inadequate knowledge on food safety could be potential sources of infections. Food handlers who harbor and excrete enteropathogens may contaminate foods from their feces via contaminated hands, then to food or food contact surfaces, and finally to healthy individuals [3-7].

The contribution of the infected food worker (whether symptomatic or not) to food borne disease outbreaks has been difficult to establish. However, reports showed that food workers in many settings have been responsible for foodborne disease outbreaks for decades. For instance, members of the Committee on Control of Foodborne Illnesses of the International Association for Food Protection analyzed 816 foodborne disease outbreaks with 80,682 cases in different countries where food workers were implicated as the source of the contamination. The report also estimated that infected food worker was documented as responsible for 18% of 766 outbreaks occurring in the United States [8, 9]. Moreover, according to the Centers for Disease Control (CDC), as cited in Mathew RR (2019), 20 to 40% of food borne illness associated with the consumption of contaminated food originated in catering establishments [10].

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Food handling personnel play a role in the transmission of food borne diseases. The health of food handlers is of great importance for maintaining quality of food products. Accordingly, pre-employment and periodic medical checkup is very important to safeguard the consumers from getting diseases from contaminated foods along with other food safety measures [11, 12]. However, pre-employment and periodic medical checkups are not commonly practiced in Ethiopia. As a result of this, many of the food handlers working in different food establishments all over the country may harbor one or more enteropathogens. For instance, a systematic review and meta-analysis reported that the overall pooled prevalence estimate of intestinal parasites among food handlers of food service establishments in Ethiopia was 33.6% (95% CI: 27.6, 39.6%) [13] and the common factors associated to high prevalence of intestinal pathogens among food handlers are poor hand hygiene, inadequate access to water and sanitation facilities, and poor socioeconomic conditions [13-18]. However, the prevalence and risk factors may be different across various settings. Accordingly, this study was conducted to assess the prevalence of intestinal parasites and associated factors among food handlers working in food establishment in the Lideta sub-city of Addis Ababa, Ethiopia.

96 Methods

97 Study design and setting

This an institution-based cross-sectional study with laboratory investigations was conducted in the Lideta sub-city of Addis Ababa from March 20 to April 20, 2021. Lideta sub-city is one of the 10 sub cities of Addis Ababa, the capital of Ethiopia. The sub-city is located at the global positioning system (GPS) coordinates of 9°0'N and 38°45'E. The sub city is divided into 10 districts (Figure 1). In the Lideta sub-city, there are a total of 281 food establishments and 1124 food handlers working in the food establishments.

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⁶ 104 Sample size calculation and sampling techniques

⁴⁹ 105 The sample size was calculated using single population proportion formula with the following ⁵⁰ 106 assumptions: prevalence of intestinal parasites among food handlers in Addis Ababa University ⁵² 107 students cafeteria, Addis Ababa, Ethiopia (p) = 45.3% [19], level of significance (α) = 5%, 95% ⁵⁴ 108 confidence interval (standard normal probability), z: the standard normal tabulated value, and ⁵⁵ 109 margin of error (d) = 5%.

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110 $n = \frac{(Z_{\alpha/2})^2 P(1-P)}{d^2} = \frac{(1.96)^2 0.453(1-0.453)}{0.05^2} = 381$

The final sample size was 419 after considering 10% non-response rate. The Lideta sub-city was selected at random from a total of 10 sub-cities of Addis Ababa, Ethiopia. Using lists of food handlers working in different food establishments in the sub-city obtained from Addis Ababa food, medicines, and healthcare administration authority (AAFMHACA) as a sampling frame, we used computer generated random number to select food handlers. Food handlers who treated with anti-helminth and anti-protozoan drugs in the last 4 weeks were excluded from the study.

117 Stool sample collection

Stool sample collectors first explained the purpose of collecting stool to the randomly selected 118 119 food handlers, and then asked them to urinate first without pooping to avoid urine contamination of the stool. Stool sample collectors then handed out paper to food handlers and instructing them 120 121 to defecate on it to avoid stool contamination with stored feces and dirt. Food handlers were asked to bring approximately 50 g of the last part of the stool, the softest part, into the collection 122 container after defecating on the paper. Stool sample collectors didn't violate privacy of food 123 handlers during stool sample collection. Stool sample collectors then immediately stored the 124 stool sample into a cold box after labeled a code on the outer surface of the plastic cup. 125

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Personal and food establishment data collection

We used structured questionnaire and observational checklist to collect food handlers' personal 127 128 data and food establishments' related information. The questionnaire was developed by reviewing related published articles [15, 20-24]. The tool was first prepared in English language 129 and translated to the local Amharic language by two native Amharic speakers fluent in English, 130 and back-translated into English by two independent English language experts fluent in Amharic 131 to check consistency. The questionnaire consisted of three parts: i) sociodemographic 132 characteristics of food handlers, ii) food handlers' personal hygiene conditions, and iii) food 133 establishment related factors (supplementary file). The questionnaire was pretested to evaluate 134 the instructions, response format, and questions work as intended and are understood by those 135 individuals who are likely to respond to them. Data collectors were trained in the data collection 136 54 55 tool as well as ethical issues during interviewing and observing. Supervisors supervised the data 137 56

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collection process and checked the completeness of the data on a daily basis. We gathered
handwashing data by assessing food handlers' usual handwashing behavior using self-reports.
We also looked at the hands of food handlers to see the general cleanliness and conditions of
fingernails. In addition, we asked food handlers to demonstrate how they wash their hands on a
regular basis, which we evaluated using checklists for effective handwashing.

143 Detection of ova of parasites in stool samples

We used direct stool examination (wet mount) and formalin-ether concentration (FEC) techniques to detect ova of intestinal parasites in stool samples. One drop of physiological saline was placed on a clean slide. Using an applicator stick, a small amount of stool specimen was emulsified in saline solution. The preparation was covered with a cover slip and examined under the microscope for the absence or presence of an intestinal parasites. The entire saline preparation was systematically examined for helminth eggs, larvae, ciliates, cysts and oocysts using 10× objective with condenser iris closed sufficiently to give good contrast, while 40 \times objectives was used to assist in the detection of eggs, cysts, and oocytes [25].

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For the FEC technique, an estimated 1 g of formed stool sample or 2 ml of watery stool was emulsified in about 4 ml of 10% formol water contained in a screw-cap bottle. A further 3 ml of 10% formol water was added and mixed well by shaking. The emulsified stool samples were sieved, and the sieved suspension transferred to a conical (centrifuge). 3 ml of diethyl ether was added, and the tube was stoppered-mixed for 1 minute with a tissue wrapped around the top of the tube, with the stopper loosened. It was then centrifuge at 3000 rpm for 1 minute. Using a stick, the layer of feces debris from the side of the tube was loosen and the tube inverted to discard the ether, fecal debris, and formol water leaving behind the sediment. The tube was returned to its upright position and the fluid from the sides of the tube allowed draining to the bottom. The bottom of the tube was taped to re-suspend and mix the sediment. The sediment was transferred to a slide and covered with a cover glass and was examined microscopically using the 10x objective for focusing and $40 \times$ objective for proper identification.

Standard operating procedures were used for every laboratory procedure during lab examination,
 stool specimen collection, transportation, and storing. We used stool sample collection and
 transportation containers which are leak-proof, dry clean and free from any traces of

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disinfectants. We ensured correct labelling of stool sample containers with date of sample collection, and code of the study participants. All stool specimens were stored in ice box for transportation and preserved at 4°C in the laboratory until analyzed for ova of parasites. Triplicate examinations of stool samples were applied to improve the recovery rate of intestinal parasites. Moreover, the expiry date of normal saline, ether, and formol was evaluated before stool sample preparation and examination.

Outcome variable of the study

Prevalence of intestinal parasites, the primary outcome variable of the study is defined as the presence of one or more intestinal parasite species in stool samples.

Data processing and analysis

Data were entered using EPI-INFO version 3.5.3 statistical package and export into Statistical Package for Social Sciences (SPSS) version 20 for further analysis. For most variables, data were presented by frequencies and percentages. Univariable binary logistic regression analysis was used to choose variables for the multivariable binary logistic regression analysis, and variables which had p-value less than 0.25 by the univariable analysis and other well-known confounders from the literature were then analyzed by multivariable analysis for controlling the possible effect of confounders and to predict the prevalence of intestinal parasites among food handlers based on the predictors. The adjusted analysis for the primary exposure (hand hygiene, food safety training, and medical checkup) and secondary risk factors (education status and monthly income) focus on direct effects. In the adjusted model, variables which had significant association were identified on the basis of adjusted odds ratios (AORs) with 95% confidence intervals (CIs) and p-values <0.05. The predictive power of the model was checked using McFadden's pseudo R-squared.

Ethics approval and consent to participate

Ethical clearance was obtained from the Institutional Review Board of Yanet Health College (reference number: YEC/060/21). There were no risks due to participation and the collected data were used only for this research purpose with complete confidentiality and privacy of food handlers during stool sample collection was assured. Written informed consent was obtained from food handlers. Furthermore, we advised food handlers who had one or more ova of

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parasites to visit health institutions for treatment. All the methods were carried out in accordance with relevant guidelines and regulations. Patient and public involvement There was no patient or public involvement in the study. Results Sociodemographic characteristics of study participants We collected personal information and stool samples from a total 411 food handlers with a response rate of 97.62%. The majority, 293 (71.3%) of the study participants were female. The median age of the respondents was 28 and the interquartile range (IQR) was 20–39 years. About half, 198 (48.18%) of the respondents aged 25 years and below. Half (207 [50.3%]) of the respondents reported that they completed primary school education. 278 (67.6%) of the food handlers reported that they had 3 years or less work experience. 111 (27.0%) of the food handlers

earned <1500 Ethiopian birr (Table 1).

Table 1: Sociodemographic characteristics of food handlers (n=411) working in different food establishments in the Lideta sub-city of Addis Ababa, Ethiopia, March 20 to April 20,

Sociodemographic characteristics	Frequency	Percent	
Sex of the respondents	2/		
Female	293	71.3	
Male	118	28.7	
Age of the respondents in years			
≤25	198	48.2	
26-35	113	27.5	
36-50	100	24.3	
Education status of the respondents			
Tertiary education	23	5.6	
Secondary school	163	39.7	

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3 1	Primary school	207	50.3
5	Illiterate	18	4.4
) 7		-	
	Service year		
	\leq 3 years	278	67.6
0	-	122	22.4
1 2	>3 years	133	33.4
-	Average monthly income in Ethiopian birr		
	<1500	111	27.0
	<1300		
	1501-2500	152	37.0
5	2501-3500	94	22.9
)		54	12 1
1	>3500	34	13.1

212 Personal hygiene characteristics of food handlers

213 242 (58.9%) of the food handlers did not keep their fingernails short. 194 (47.2%) and 206 214 (50.1%) of the food handlers did not regularly wash hands with soap after visiting toilet and 215 before eating, respectively. 208 (50.6%) of the food handlers reported that they regularly worn 216 clean protective clothes. About a quarter (76 [24%]) of the food handlers reported that they 217 received food safety training and 121 (29.4%) of the food handlers had medical checkup in the 218 previous 6 months prior to the survey (Table 2).

Table 2: Personal hygiene characteristics of food handlers (n=411) working in different food establishments in the Lideta sub-city of Addis Ababa, Ethiopia, March 20 to April 20, 2021

Freq	luency	Percen
169		41.1
242		58.9
217		52.8
194		47.2

2				
3 4		Yes	205	49.9
5		No	206	50.1
6 7		Wearing clean protective clothes regularly		
8 9		Yes	208	50.6
10		No	203	49.4
11 12		Food safety training		
13 14		Yes	76	24
15		No	335	76
16 17		Medical checkup (in last 6 month)		
18 19		Yes	121	29.4
20		No	290	70.6
21 22	222			

Intestinal parasites in food handlers

A total of 411 food handlers were examined, with 171 (41.6%) (95% CI: 36.6, 46.4%) of them had ova of one or more intestinal parasites [98 (23.8% were protozoan and 73 (17.8%) were helminth parasites], of which 14 (3.4%) had mixed parasites (Figure 2). The most common intestinal parasites were E. histolytica/dispar [52 (12.7%)], G. duodenalis [46 (11.2%)], A. lumbricoides [34 (8.3%)], Hookworms [15 (3.6%)], T. trichuria [14 (3.4%)], and Taenia species [10 (2.4%)] (Table 3).

Table 3: Common intestinal parasites detected among food handlers (n=411) working in different food establishments in the Lideta sub-city of Addis Ababa, Ethiopia, March 20 to April 20, 2021

Parasitic species	Frequency	Percent	
E.histolytica/dispar	52	12.7	
G. duodenalis	46	11.2	
A. lumbricoides	34	8.3	
Hookworms	15	3.6	
T. trichuria,	14	3.4	
Taenia species	10	2.4	

Factors associated with intestinal parasites among food handlers

Sex, age, educational level, work experience, monthly income, wearing clean protective clothes, finger nail status, hand washing after toilet, hand washing before eating, food safety training, and medical checkup were the variables entered in to the univariable binary logistic regression analysis, of which educational status, average monthly income, condition of fingernails, hand washing with soap before eating, food safety training, and medical checkup in the last 6 month were the candidate variables for the final model which were selected based on p < 0.25 and hand washing with soap after visiting toilet was a well-known confounder in the literature included in the final model even if its p value is greater than 0.25. In the multivariable binary logistic regression analysis, prevalence of intestinal parasites among food handlers was significantly associated with poor handwashing practice (AOR: 2.16, 95% CI: 1.03, 4.22), untrimmed fingernails (AOR: 4.36, 95% CI: 1.98, 11.90), lack of medical checkup (AOR: 2.31, 95% CI: 1.18, 6.95), no food safety training (AOR: 2.51, 95% CI: 1.20, 5.58), low level of education (AOR: 3.13, 95% CI: 1.34, 7.44), and low monthly income (AOR: 2.83, 95% CI:1.50, 8.84) (Table 4). Table 4 included effect estimates from the model with all the seven variables. In that case, one should know that the educational status and monthly income estimates are for direct effects.

Table 4: Factors associated with intestinal parasites among food handlers (n=411) working in different food establishments in the Lideta sub-city of Addis Ababa, Ethiopia, March 20 to April 20, 2021

Variables	Intesti	nal parasites	COR (95% CI)	AOR (95% CI)	
	Yes	No			
Educational status					
Illiterate	9	9	3.60 (1.81, 10.80)	3.13 (1.34, 7.44)*	
Primary school	92	115	2.88 (1.65, 6.17)	2.22 (1.10, 6.65)*	
Secondary school	68	95	2.58 (1.20, 5.56)	2.16 (1.10, 5.20)*	
Tertiary education	5	18	1.0	1.0	
Average monthly income in Ethiopian birr					
<1500	56	55	2.91 (1.43, 7.86)	2.84 (1.50, 8.84)*	
1501-2500	68	84	2.31 (1.14, 6.96)	2.28 (0.63, 8.10)	
2501-3500	33	61	1.55 (1.05, 4.85)	1.41 (0.60, 5.96)	

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>3500	14	40	1.0	1.0
Condition of fingernails				
Untrimmed	134	108	4.43 (2.12, 12.62)	4.36 (1.98, 11.90)*
Trimmed	37	132	1.0	1.0
Hand washing with soap after toilet				
No	101	93	2.28 (1.10, 7.51)	2.19 (0.92, 5.62)
Yes	70	147	1.0	1.0
Hand washing with soap before eating				
No	112	94	2.95 (1.23, 6.72)	2.16 (1.03, 4.22)*
Yes	59	146	1.0	1.0
Food safety training				
No	153	182	2.71 (1.34, 8.53)	2.51 (1.20, 5.58)*
Yes	18	58	1.0	1.0
Medical checkup in the last 6 month				
No	140	150	2.71 (1.27, 7.56)	2.32 (1.18, 6.95)*
Yes	31	90	1.0	1.0

squared = 0.492

Discussion

This is an institution-based cross-sectional study assessed intestinal parasites among food handlers working in food establishments in the Lideta sub-city of Addis Ababa, Ethiopia and found that 41.6% (95% CI: 36.6, 46.4%) of the food handlers had one or more intestinal parasites. The prevalence of intestinal parasites reported in this study was comparable with findings of studies conducted among food handlers in Bule Hora (46.3%) [16] and Addis Ababa University (45.3%) [19]. The prevalence of intestinal parasites reported in this study was lower than findings of studies in Nekemte town (52.1%) [26] and Mekele University (49.4%) [15]. Furthermore, the prevalence of intestinal parasites reported in the current study was higher than findings of studies conducted among food handlers in Wolaita Sodo (23.6%) [14], Jimma (33%) [27], Madda Walabu University (25.3%) [28], Motta town (27.6%) [29], Nairobi(15.7%)[30],

Iran (9%) [31], Saudi Arabia (23%) [32], and Thailand (10%) [33]. The high prevalence of intestinal parasites among food handlers among food handlers working in food establishments in the Lideta sub-city of Addis Ababa might be explained by poor socioeconomic conditions, poor hand hygiene, and inadequate access to basic sanitation services.

The high prevalence of intestinal parasites among food handlers working in food establishments in the Lideta sub-city suggests that poor hygiene practices of food handlers and inadequate access to sanitation services. The results may also suggest that there might be transmission of intestinal parasitic infections from food handlers to food users, unless large scale screening and mass drug administration are done. As it is documented in literature, infected food handlers play a significant role in infection transmissions to customers of the food establishments where the infected food handlers are working [4].

This study showed that educational status of food handlers was associated with a high prevalence of intestinal parasites. Prevalence of intestinal parasites was higher among food handlers who were illiterate or attended primary and secondary education compared with food handlers who attended tertiary education. This may be due to the fact that educated food handlers may have awareness about the transmission and prevention methods of infectious diseases. Education encourages changes in healthy behaviors. Other similar studies also reported the relation of education with occurrence of parasitic infections [34-38].

The current study revealed that monthly income of food handlers was associated with intestinal parasites among food handlers. Food handlers with low monthly income had higher odds of intestinal parasites. This may be due to the fact that food handlers with low economic status could not afford for services like soap, household water treatment, toilets and other facilities, and so that will have limited opportunities to practice healthy measures. The effect of low income on risk of parasites is complex and could be attributed to limited access to sanitary materials, sources of drinking water and food, environment sanitation, and education [37-40].

The high prevalence of intestinal parasites among food handlers was associated with hand hygiene. Food handlers who did not keep their fingernails short had higher odds to have intestinal parasites and the odds of having intestinal parasites was also higher among food handlers who did not wash hands with soap before eating. This might be due to the fact that the Page 15 of 23

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area beneath the fingernails has the highest concentration of microorganisms on the hands and is the most difficult to clean [41-44]. Moreover, food handlers may ingest diseases causing pathogens when they eat without washing hands. Hands are one of the most important mechanisms to transmit pathogenic microorganisms leading to infection [45]. Evidence indicates that hands together with food contact or other environmental surfaces cause 60% of the spread of gastrointestinal infection. Contaminated hands could also be associated with up to 50% of respiratory tract infections [46].

This study showed that the presence of intestinal parasites among food handlers was significantly associated with food safety training. The odds of having intestinal parasites were high among food handlers who did not take food safety training compared with their counterparts. This could be due to the fact those food handlers who did not take food safety training may lack the necessary knowledge and practice towards transmission and prevention of disease-causing pathogens. Moreover, food safety training or health education promotes health behaviors toward hygiene and sanitation practices. Health education increases knowledge and acceptability of interventions. It also sustains integrated control of the infection [47-49].

Furthermore, the presence of intestinal parasites was significantly associated with medical checkup. The odds of having intestinal parasites were high among food handlers who did not take medical checkups in the 6 months prior to the survey. Other studies are also reported that medical checkup of food handlers is associated with intestinal parasites [15, 31, 50]. This is because food handlers who did not know their health condition before employment and while working in different establishments has less chance to take treatment and mass drugs as a result there may be existed or new or re-infections.

To increase the degree to which inferences from the sample population can be generalized to a larger group of population, we recruited study participants at random or in a manner in which they are representative of the population that we wished to study, ensuring that every member of the population had an equal chance to be included in the study. In addition, we calculated adequately powered sample size using sample size determination procedures appropriate to the study objective with appropriate assumptions. Furthermore, our findings could be applicable to other situations and settings which have similar characteristics with the study populations of the current studies.

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As a limitation, even if the use of sensitive diagnostic techniques and combination of methods with triplicate examinations applied in this study would help to recover greater rate of intestinal parasites that would indicate the true prevalence, this study had some limitations. The collection of a single stool sample may affect the result of parasitic examination since the sensitivities of the direct smear examination technique is reduced when a single stool sample is examined. The procedure of FEC technique may also damage the eggs of parasites. The handwashing data assessed by self-reports may not be reliable since the study subjects may make the more socially acceptable answer rather than being truthful and they may not be able to assess themselves accurately. Moreover, we pre-screened variables using univariable analysis (p < 0.25), even though we retained some well-known confounders from the literature regardless of their univariable p-value. This could lead to the incorrect exclusion of a potential confounder and hence led to an inadequate adjustment for confounding.

335 Conclusion

The prevalence of intestinal parasites among food handlers working in food establishments in the Lideta sub-city of Addis Ababa, Ethiopia was found to be high. This high prevalence of intestinal parasites was linked to socioeconomic conditions of food handlers, poor hand hygiene condition, absence of food safety trainings and regular medical checkup. It is, therefore, important to promote hand washing practice of food handlers, provide food hygiene and safety trainings, and establishing a system to regularly check the health conditions of food handlers.

⁰ 343

3 344 Data availability statement

Individual participant data after deidentification that underlie the results reported in this article will be made available upon requesting the corresponding author immediately following publication.

348 Contributors

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4	349	WA designed the study, facilitated data collection, and conducted data analysis. BG, TS, ZNM,
5	350	and ZG supervised data collection and analysis and contributed to conceptualizing the study. ZG
6 7 8	351	prepared the manuscript. All authors approved the final version of the manuscript.
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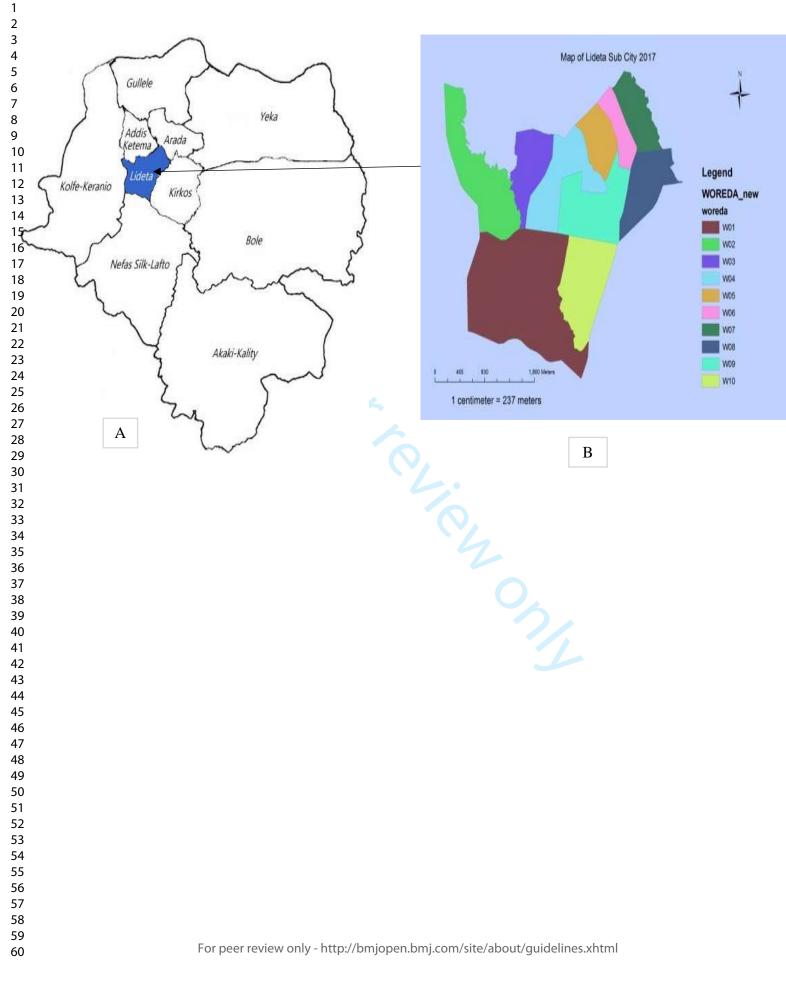
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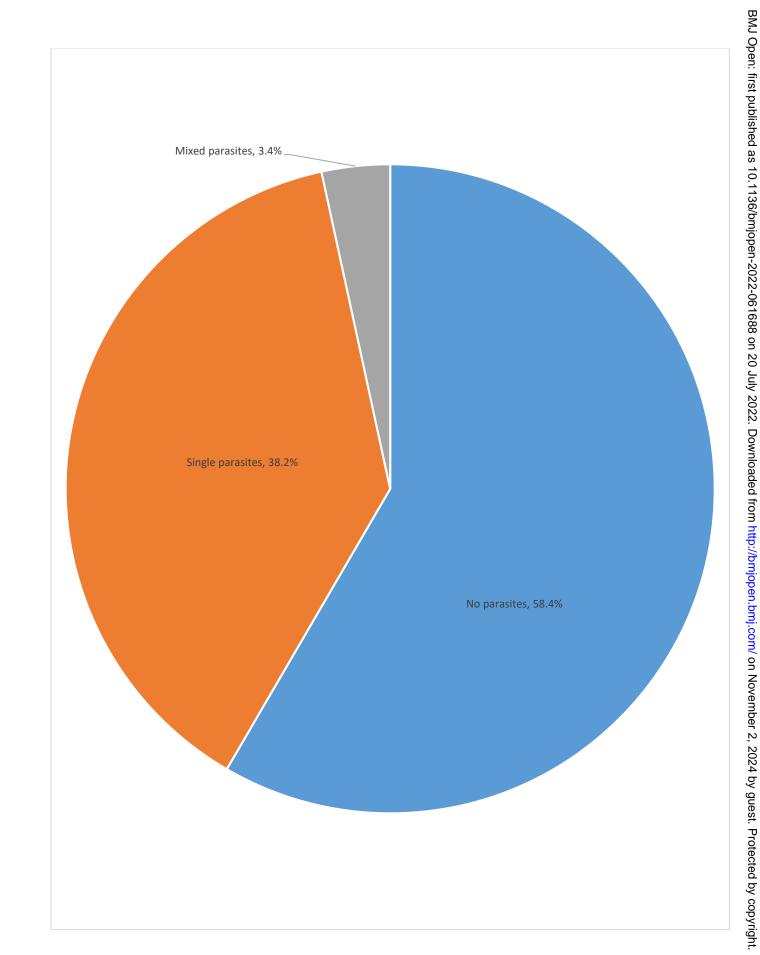
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- ³⁷
 ³⁸ 508 Figure titles and legends
- ⁴⁰ 509 Figure 1: Map of Addis Ababa city (A) and Lideta sub-city (B)
- ⁴³ 510 Source: Lideta sub-city administration.
- Figure 2: Proportion of food handlers with no, single and mixed parasites (n=411)
- ⁴⁷ 512 working in different food establishments in Lideta sub city of Addis Ababa, Ethiopia,
 ⁴⁹ 513 March 20 to April 20, 2021





Supplementary file: Data collection tool

Questionnaire Identification Code:

Consent Form

Hello, I am ______ working with a research team from Yanet Health Science College. We are contacting you to collect information for a study conducted to assess "Prevalence of intestinal parasites and associated factors among food handlers in food establishments in the Lideta sub-city of Addis Ababa, Ethiopia". This questionnaire is prepared to collect food handlers personal and food establishment related information.

Thank you so much for agreeing to be interviewed for this research work. All of the information we got from you will be completely confidential and coded with unique number. Your name will not be written down and will never be used in connection with any of the information you provided. There will not be any direct benefit that you will get associated with participation in this study. If you do not want to answer all or some of the questions, you have the right to refuse participation at any time. However, we would greatly appreciate your help in responding to this questionnaire. This questionnaire is expected to be completed in 10 minutes.

Do I have your permission to continue?

1. Yes, continue your interview with thanks after signing the consent

2. If no, skip to the next participant by writing reasons for his/ her refusal;

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40		. Nomo	Ci ara atauna i	Datas	the whole questions
41		r: Name:	•	Date:	
42	Time starte	ed:	Time completed	:	
43					
44					
45 46	Filled by field supervi	sors			
40 47	Result of interview:				
48		2 Nationarilated	2 The man and a		whale averations
49	1. Completed	2. Not completed	5. The responder	nt does not complete the	whole questions
50					
51	Name of Supervisor: _	Signature: _	Dat	e:	
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58 59					1
59 60		For peer review only - http://bmjo	pen.bmj.com/site/ab	out/quidelines.xhtml	

Code	Questions	Response	Skip			
Part 1:	Socio-demographic information of food handlers					
101	Sex	1. Male 2. Female				
102	Age (in complete years)					
103	Educational status	1. Illiterate				
		2. Primary school				
		3. Secondary				
		School				
	0,	4. Tertiary				
104	What is your monthly income?					
105	How many years of work experience do you have in food handling?					
Part 2:	Food handlers' personal hygiene conditions					
201	Condition of fingernails	1. Trimmed				
		2. Untrimmed				
202	Regular hand washing with soap after toilet	1. Yes 2. No				
203	Regular hand washing with soap before eating	1. Yes 2. No				
204	Wearing clean protective clothes regularly	1. Yes 2. No				
Part 3:	Food establishment related factors					
301	Food safety training	1. Yes 2. No				
302	Medical checkup (in last 6 month)	1. Yes 2. No				
Intestinal parasites identified (to be filled based on the laboratory results)						
Please c	arefully read the laboratory reports and write the intestinal parasites					
dentifie	ed in the space provided. If no ova of parasites detected, write "No ova					
of paras	ites detected".					