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Prevalence of intestinal parasites and *Helicobacter pylori* coinfection, and contributing factors among patients with gastrointestinal manifestations at Addis Zemen primary hospital, Northwest Ethiopia

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Abstract

Background The urease-producing *Helicobacter pylori* increase the likelihood that pathogenic intestinal protozoa will use the stomach's increased hydrogen potential to propagate the disease. Coinfections exacerbate the onset and severity of gastrointestinal symptoms. This study aimed to assess the prevalence of intestinal parasites/*Helicobacter pylori* coinfection and contributing factors in patients with gastrointestinal symptoms at Addis Zemen Primary Hospital, Northwest Ethiopia.

Methods From April to July 2023, patients with gastrointestinal problems participated in a cross-sectional study carried out in a hospital. To collect the clinical and sociodemographic data, a questionnaire was employed. Intestinal parasites and *Helicobacter pylori* were detected using the saline stool wet mount and *Helicobacter pylori* stool antigen tests, respectively. SPSS version 20 was used to analyze the data and variables with p-values < 0.05 were considered statistically significant.

Result The study included 384 participants in total, of which 47.3% (182/384) were farmers and 50.3% (193/384) were women. Of the study subjects, 69/384 or approximately 18%, had intestinal parasitic infections. In 12% of cases (46/384), *Helicobacter pylori* were detected. A coinfection of *Helicobacter pylori* and intestinal parasites was found in 5.5% (21/384) of the subjects. Multiple logistic regression revealed increased risk of coinfection of *Helicobacter pylori* and intestinal parasites in patients who drink surface water (AOR: 10.7, $p = 0.03$) family history of *Helicobacter pylori* (AOR: 3.3, $p = 0.024$) and those with untrimmed fingers (AOR: 4.9, $p = 0.031$).

Conclusions *Giardia lamblia* and *Entamoeba histolytica/dispar/ moshkovskii/ bangladeshi* complex are the most common protozoans that cause coinfection with *Helicobacter pylori*. Drinking surface water, family history of

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Helicobacter pylori and untrimmed fingers are the contributing factors to intestinal parasites/*Helicobacter pylori* coinfection.

Keywords Coinfection, *Helicobacter Pylori*, Intestinal parasites, Prevalence, Factors, Ethiopia

Background

Helicobacter pylori (H. Pylori) is gram-negative bacteria, which mainly causes chronic gastritis, peptic ulcer disease and gastric carcinoma [1]. It is characterized by unipolar flagella, which gives it corkscrew-like motility, which is unique among bacteria. It finds a niche in the mucosa of the stomach under the mucus gel [2]. It affects 50% of the population in developed countries and 70% of the population in developing countries [3]. How the infection is transmitted is unknown but presumably via the oral-to-oral route. It is difficult to detect from dental plaques, saliva, or facial features [4].

Since its discovery, *H. pylori* infection has been increasingly recognized as the main cause of gastritis and gastritis-associated diseases such as duodenal ulcers, gastric ulcers, gastric carcinomas, and gastric MALT lymphomas [1, 5] The factors stimulating the prevalence of *H. pylori* infection are erratic and related to topography, sex, age, and socioeconomic status, which are mainly high in developing countries and low in developed countries [3].

Intestinal Parasitic infections are among the most prevalent infections causing a significant morbidity and mortality in humans. They are primarily caused by intestinal helminths and protozoa and primarily affect people in developing countries [6]. These parasites are more common in the tropical and subtropical countries than in the other regions. They are correlated with poverty, poor personal hygiene, poor environmental sanitation, limited opportunity to clean water, hot and humid climates [7]. They are among the most important causes of gastrointestinal disease, weight loss, and iron-deficiency anemia [8]. The most common intestinal parasites causing high morbidity and mortality in sub-Saharan Africa are protozoan parasites (*Entamoeba histolytica* and *Giardia lamblia*) and soil-transmitted helminths (*Ascaris lumbricoides*, *Trichuris trichiura*, and Hookworm), which affect almost all people [9]. In Ethiopia, intestinal parasitic infections are widespread and the magnitude of infection varies from place to place. They are the second most common cause of morbidity among outpatients in the nation [10]. The existing high burden of these infections is directly related to the in accessibility of clean drinking water, poverty, poor living conditions, and poor personal and environmental hygiene [11].

Coinfections involving several pathogens commonly occur in countries with low socioeconomic status. Several studies across the world have reported a possible association between *G. intestinalis* and *H. pylori*. Both of these organisms inhabit the gastrointestinal tract (GI) of their human hosts and infect children at a high level in impoverished countries [12]. In addition, coinfection of *H. pylori* with *Entamoeba histolytica* and other *entamoeba* species has been reported in previous study [13]. Only three species of *Entamoeba* have been proven to cause disease and sometimes death in their hosts: *Entamoeba histolytica*, a parasite of humans, *Entamoeba nuttalli*, a parasite of nonhuman primates and *Entamoeba invadens*, a parasite of reptiles. Other species appear to live as commensals in their hosts and do not cause evident disease. Most species of *entamoeba* including *Entamoeba dispar*, *Entamoeba bangladeshi* and *Entamoeba moshkovskii*, remain asymptomatic, but *E. histolytica* can cause intestinal or disseminated disease [14]. However, in recent studies *Entamoeba moshkovskii* [15, 16] has been considered to cause disease and identified from diarrheal patients.

The rates of coinfections are higher in resource-limited settings and developing nations [12, 17]. Its high prevalence in developing nations has been related with low socioeconomic level, over-crowding, poor personal and environmental hygiene, unclean water supplies, animal fecal matter, and food contamination [18]. The acidic environment in the stomach and the presence of bile and trypsin in the duodenum stimulate infectious cysts to excyst, and infectious cysts in contaminated food or water, or directly through the fecal–oral route, are transferred through ingestion of infectious cysts in contaminated food or drink. Then they change into trophozoites, which use the sticky disc to connect to intestinal epithelial cell [19, 20]. An increment of the stomach's lumen PH facilitates easy passage via the stomach's acidic environment in some protozoa. Changes in the gastric environment are essential for increasing gastric PH and/or intestinal metaplasia of the gastric mucosa. Both conditions are well-known complications of *H. pylori* infections [20]. Moreover, concomitant *H. pylori* and intestinal protozoan infections are common because of their related modes of transmission and strong association with socioeconomic status [18].

Parasites and *H. pylori* can considerably modulate the host's immune response. While infected with *H. pylori*, intense Th1 cell polarization is witnessed. Protozoan infection may contribute to the recruitment of Th1 cells, may aggravate the host immune response, and provoke gastric mucosal damage [21]. On the contrary, intestinal helminth infection is affiliated with the polarization of lymphocytes towards Th2; their presence intensifies regenerative processes within the digestive tract

and lowers excessive immune response [22]. Moreover, coinfections aggravate the development and severity of gastrointestinal symptoms. Therefore, determining the frequency of coinfection and the factors contributing to the coinfection of these organisms are essential for public health. However, data concerning coinfection of *H. pylori* and intestinal parasites in the study area are scarce. This study aimed to determine the prevalence of intestinal parasites and *H. pylori* coinfection and contributing factors among patients with gastrointestinal manifestations at Addis Zemen Primary Hospital, Northwest Ethiopia.

Materials and methods

Study design, area, and period

This hospital-based cross-sectional study was conducted at Addis Zemen Primary Hospital (AZPH) from April to July 2023. AZPH is found in Northwestern Ethiopia, located in the South Gondar zone of the Amhara national regional state, on the road connecting Bahir Dar and Gondar. Addis Zemen is a town located at a latitude and longitude of 12º7'N37º47'E and at an altitude of 1975 m above sea level. It is the administrative center of the Libo Kemkem district and is located 102 km from Bahir Dar and 645 km from Addis Ababa.

Sample size determination and sampling technique

The sample size for this study was determined by the formula for single population proportion formula with 50% prevalence, 95% confidence level, and 5% margin of error, and the sample was collected using a convenient sampling technique until a total of 384 samples were enrolled.

Eligibility criteria

Inclusion criteria

All patients with gastrointestinal complain (Abdominal pain, diarrhea, constipation, nausea, vomiting, bloating etc.) who are voluntary to participate in the study were included.

Exclusion criteria

Patients who have taken anti helminthic and anti-protozoal drugs within the past two weeks before data collection were excluded.

Dependent and independent variables

Dependent variables in this study were intestinal parasite and *H. pylori* coinfection status. Independent variables included age, sex, educational status, occupation, fingernail status, residence, family size, family history of *H. pylori*, and source of drinking water.

Operational definitions

Family size was determined as;

*Small: Family consisting of one to four members.

*Large: Family consisting of five or more members.

Data collection and processing

Sociodemographic data collection The sociodemographic data of patients suspected of intestinal parasitosis and/or gastritis that came to the laboratory upon stool examination request were collected using a structured questionnaire via face-to-face interviews.

Stool sample collection and laboratory investigation The patients were informed to pass stool samples directly inside a plastic cup with a compacted lid. All specimen containers were labeled appropriately with the patient's name, medical registration number, age, sex, and date of sample collection. About 10–20 g of stools or 5–6 spoons full of watery stools were collected. After the sample was collected in a clean, dry, leak-proof container, intestinal parasites and *H. pylori* were examined using saline wet mount and serological *H. pylori* stool antigen tests (manufactured by Guangzhou Wondfo Biotech Co., Ltd.), respectively.

Direct wet mount A matchstick head-sized stool sample was placed over a glass slide and mixed with one drop of normal saline solution. The slides were shielded with a glass cover slip and examined for the presence of parasite eggs, larvae, trophozoites, and cysts at 10× and 40 × magnifications with Olympus Cx21FS1 Microscope (OLYMPUS CORPORATION, TOKYO JAPAN, made in Philippines).

***H. pylori* stool antigen test** The stool collection apparatus was opened and a collection stick was used to prick the stool sample. Then, the collection stick containing the stool sample was placed back into the sample collection tube and shaken vigorously to mix with the sample solution. The test kit (Wondfo one-step *H. pylori* feces kit, catalog number Wo28, manufactured by Guangzhou Wondfo Biotech Co., Ltd.,) was removed from the foil pouch by tearing at the notch and placed on a level surface. When the sample collector was held upright, the tip of the collector broke at the breakpoint. Three drops of the sample solution were dispensed inside the sample well and allowed to stand for 15 min. The results were considered positive when rose-pink bands were visible in both the control and test regions. The results were reported as negative when a rose-pink band was visible only in the control region.

Data quality assurance

Prior to the start of the data collection, training was provided to the data collectors. Patients were informed accordingly before stool sample collection. Inappropriate samples, such as those with insufficient amounts, were

rejected and recollected to reduce errors in results. The reliability of *H. pylori* stool antigen test was evaluated by implementing daily quality control measures, using known positive and negative samples. About 5% (20) of the samples were randomly checked by experienced microscopists to ensure the consistency of results for intestinal parasites.

Data analysis

The questionnaire data on sociodemographic characteristics and associated factors were checked for completeness and analyzed using the Statistical Package for Social Sciences version 20 (SPSS 20). Descriptive statistics (frequencies and percentages) were used to display the results of the variables. Bivariate logistic regression was used to select variables, and multiple logistic regression was used to determine the association between independent and outcome variables. Variables with p-value < 0.25 in the bivariate logistic regression were selected for multivariate logistic regression analysis. Adjusted Odds ratios (AORs) with 95% confidence intervals were calculated and p-values < 0.05 were considered statistically significant.

Ethical considerations

This study was reviewed by Department of Medical Laboratory Science, Debre Tabor University research review committee with the approval number (157/2023). Written informed consent was obtained from the adult study participants. Written informed assent was obtained from the parents and guardians of the study participants aged below 15 years old. The results were reported to the concerned physicians and patients with positive results were treated accordingly.

Results

Sociodemographic characteristics of study participants

During the study period, a total of 384 study participants were enrolled, of which 80.5% (309/384) were in the age group of 15–64 years old. The majorities, 50.93% (193/384) were females and 60.9% (234/384) were rural residents (Table 1).

Prevalence of intestinal parasites

The overall prevalence of intestinal parasites among gastro intestinal symptomatic patients in the study area was 18% (69/384) [95% CI; 14.45 – 22.26%]. The most prevalent species from the total cases was *G.lamblia*, 40.6% (28/69) [95% CI; 29.8 – 52.6%] followed by *E. histolytica/ dispar/ moshkovskii/ bangladeshi* (32.6% (25/69) [95% CI; 25.9 – 48.02%] (Fig. 1).

Prevalence of *Helicobacter pylori* infection

The prevalence of *H. pylori* among gastro intestinal symptomatic patients in the study area was 12% (46/384) [95% CI; 9.4 – 15.61%] (Table 2).

Prevalence of Intestinal Parasites and *H. pylori* coinfection

The prevalence of intestinal parasites and *H. pylori* coinfection among gastro intestinal symptomatic patients in the study area was 5.5% (21/384) [95% CI; 3.4 – 8.1%] (Table 3).

Factors associated with intestinal parasites and *H. pylori* coinfection

Logistic regression analysis was performed to identify the factors associated with intestinal parasites and *H. pylori* coinfection. In bivariate logistic regression analysis, the study variables with p-value ≤ 0.25 were selected and imported to the multivariate logistic regression analysis. Finally, source of drinking water [(p=0.03, COR (95%

Table 1 Socio-demographic characteristics of study participants at AZPH, Northwest Ethiopia

Variables	Category	Frequency	Percentage (%)
Age	≤ 14 (Pediatric group)	48	12.5
	15–64 (Young group)	309	80.5
	≥ 65 (Elderly)	27	7
Sex	Male	191	49.7
	Female	193	50.3
Residence	Urban	160	41.7
	Rural	224	58.3
Educational status	Illiterate	130	33.9
	Primary school	97	25.3
	Secondary & above	157	40.9
Occupation:	Farmer	134	34.9
	Government employed	142	37
	Private workers	108	28.1
Family size	Small	127	33.1
	Large	257	66.9

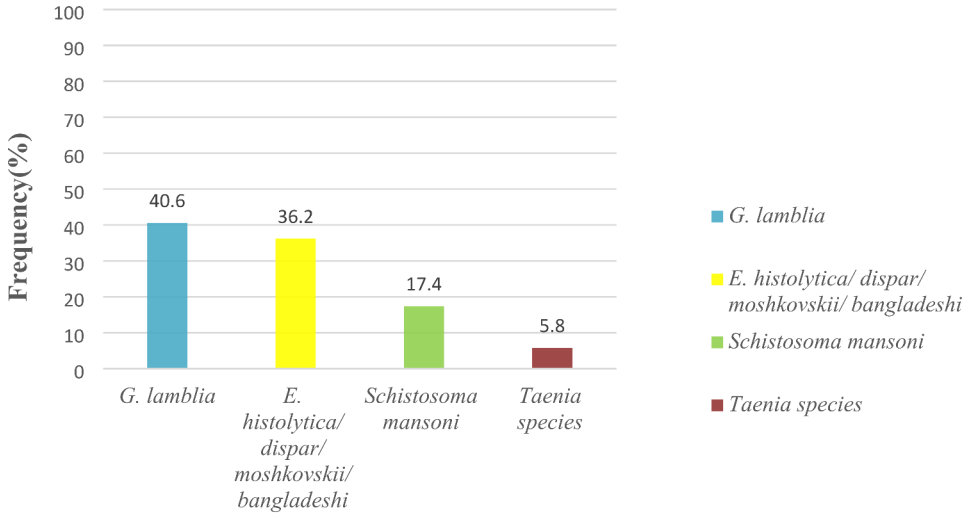

Fig. 1 Distribution of Intestinal parasites among Gastro intestinal symptomatic patients

Table 2 Prevalence of *H. Pylori* infection among gastrointestinal symptomatic patients at AZPH

H. pylori Stool antigen test result	Frequency (*N*)	Percentage (%)
Negative	338	88
Positive	46	12 [95% CI; 9.4 – 15.61%]
Total	384	100

Table 3 Prevalence of intestinal parasites and *H. pylori* coinfection among gastro intestinal symptomatic patients at AZPH

Intestinal parasites	H. pylori		Total
	Yes	No	
G. lamblia	12	16	28
E. histolytica/ dispar/ moshkovskii/ bangladeshi	9	16	25
S. mansoni	0	12	12
Taenia species	0	4	4
Total	21	48	69

CI); 10.7(2.4, 27.2)], family history of *H. pylori* [(p=0.024, COR (95% CI); 3.3(1.2, 9.2)], Fingernail status [(p=0.031, COR (95% CI); 4.9(1.2, 12.7)] were the factors significantly associated with intestinal parasites/ *H. pylori* coinfection (Table 4).

Discussion

Coinfections involving different gastrointestinal organisms frequently cause public health problems in impoverished countries [23].

The prevalence of intestinal parasites and *H. pylori* coinfection in this study (5.2% [95% CI; 3.4 – 8.1%]) is lower than the study results reported in Iran (26.9%) [23], Sudan (23%, 10%) [24, 25], and Ethiopia (31.13%) [26]. This discrepancy may be due to the differences in the study design, sample size, and the study method used for the detection of intestinal parasites and *H. pylori.* For instance, the sample sizes in Iran [23] and Sudan [24, 25] were 130 and 100, respectively, which were too small compared to the sample size in the present study. In addition, enzyme-linked immunosorbent assay (ELISA), a more specific and sensitive technique, compared to serological stool antigen for determination of *H. pylori* and wet mount, sedimentation, and acid-fast staining approaches for the detection of intestinal parasites were employed in a study conducted in Iran [23]. Moreover, studies in Sudan [24, 25] were case-control based studies. In contrast, a study conducted in Ethiopia [26] used a serological antibody technique for the detection of *H. pylori* which may detect past infections as positive and increase the prevalence. Moreover, the variation in the existing lower prevalence of intestinal parasite/ *H. pylori* coinfection in this study may be due to the improvement of prevention and intervention measures for these infections.

The prevalence of *G. lamblia*/*H. pylori* coinfection in this study (3.1% [95% CI; 1.8 – 5.4%]), is in line with the two study results reported in Sudan (4%, 5%), respectively [24, 25]. However, this result is lower than the study findings reported in Iran (20.8) [23] and Ethiopia [24]. Possible explanations for this variation are as previously mentioned. The prevalence of *E. histolytica/ dispar/ moshkovskii/ bangladeshi* and *H. pylori* coinfection in this study (2.3% [95%CI; 1.2 – 4.4%]) is lower than study findings in Iran [23], Sudan [24, 25], and Ethiopia [26]. This variation may be attributed to the aforementioned factors.

Table 4 Factors associated with intestinal parasites and *H. pylori* coinfection among gastrointestinal symptomatic patients at AZPH

Variables	Category	Coinfection		COR (95% CI)	P-value	AOR(95% CI)	P-value
		Yes	No				
Occupation	Farmer	12	122	3.4(1.10, 10.80)	0.04*	5.3(0.91, 13.42)	0.083
	Private worker	5	103	1.7(0.44, 6.40)	0.09	1.3(0.2, 3.1)	
	Government employed	4	138	1			
Residence	Rural	16	208	2.4(0.90, 6.64)	0.097*	1.2(0.40, 4.00)	0.75
	Urban	5	155	1			
Educational status	Illiterate	14	116	4.6(1.50, 14.40)	0.008*	3.1(0.76,6.2)	0.113
	Primary	3	94	1.2 (0.30, 5.60)	0.10	1.2(1.0, 3.1)	0.129
	≥Secondary	4	153	1			
Family size	Large	17	240	2.2(0.72, 6.61)	0.17*	1.2(0.34, 3.7	0.854
	Small	4	123	1			
Source of drinking water	Surface	19	171	3(1.08, 8.40)	0.002*	10.7(2.4, 27.2)	0.03*
	Piped	2	192	1			
Family history of *H. pylori*	Yes	15	184	2.4(0.92, 6.41)	0.072*	3.3(1.2, 9.2)	0.024*
	No	6	179	1			
Finger nail status	Untrimmed	18	166	7.1(2.06, 24.6)	0.002*	4.9(1.2, 12.7)	0.031*
	Trimmed	3	197	1			

*Indicates a statistically significant association at $p<0.05$.

Co-infections with several types of gastrointestinal parasites, is often observed in developing countries, and simultaneous human colonization by *H. pylori* and intestinal parasites is a common phenomenon [27]. Colonization of the human gastrointestinal tract by intestinal parasites and *H. pylori* is very common because of similarities in predisposing factors and fecal-oral mode of transmission. In the present study, the prevalence of intestinal parasites and *H. pylori* is significantly influenced by drinking surface water, family history of *H. pylori* and untrimmed fingers. This finding has been supported by different studies [26, 28–31]. Different factors including socioeconomic status, hygiene, and potential environmental contaminations, larger household sizes, non-piped and potentially contaminated water sources, open air defecation, living in rural settings, and poor hygiene, have been identified so far to be significant contributors in the prevalence of coinfections [26, 28–31]. For example, a study by Seid et al. [26] found that drinking river or spring water is significantly associated with *Giardia* and *H. pylori* coinfection which supports our study finding.

Finally, identifying the coinfection between intestinal parasites and *H. pylori* and significant contributing factors in gastrointestinal symptomatic patients is essential in public health for appropriate treatment and prevention of the cases.

Conclusion

Intestinal parasites; mainly intestinal protozoa, and *H. pylori* coinfections are common findings among patients with gastrointestinal symptoms. The most common protozoans that cause coinfection with *H. pylori* are *G. lamblia* and *E. histolytica*/ *dispar*/ *moshkovskii*/ *bangladeshi.* Drinking surface water, a family history of *H. pylori*, and untrimmed fingers are significant contributing factors for development of intestinal parasites and *H. pylori* coinfection.

Limitation

This is a cross-sectional study without involving more specific and sensitive molecular techniques for identification of species and conducting genetic analysis. Year based studies involving molecular techniques are recommended to address the variations in prevalence rates in different seasons and confirming species.

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Author contributions
"A.A, A.A and T.K" wrote the main manuscript text, involved in examination and data analysis"A.B, B.M, A.A, A.F, and Y.S" participated data collection and examination"T.E, ME and B.G" participated in revision, design and prepared tables.

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Data availability
Data will be available up on reasonable request.

Declarations

Competing interests
The authors declare no competing interests.

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