Parasite Epidemiology and Control 11 (2020) e00177

Contents lists available at ScienceDirect

Parasite Epidemiology and Control

journal homepage: www.elsevier.com/locate/parepi

Concurrent infection of intestinal parasites and *Helicobacter pylori* among school-age children in Central Ethiopia

Hannah Spotts [a], Sosina Walelign [b], Mehret Tesfaye [b], Kassu Desta [b], Aster Tsegaye [b], Bineyam Taye [a,*]

[a] Department of Biology, Colgate University. Hamilton, NY, USA
[b] Department of Medical Laboratory Sciences, Addis Ababa University, Addis Ababa, Ethiopia

ARTICLE INFO

Article history:
Received 1 February 2020
Received in revised form 3 July 2020
Accepted 16 August 2020

Keywords:
Intestinal parasites
H.pylori
Confection
Children
Ethiopia

ABSTRACT

Background: Coinfection of multiple intestinal microbial pathogens plays an important role in individuals harboring these organisms. However, data on magnitude and risk factors are scarce from resource limited settings.

Objective: We examined the prevalence and associated risk factors of intestinal parasites and *Helicobacter pylori* co-infection among young Ethiopian school children.

Method: Data from a total of 434 Ethiopian school children from the Ziway region were analyzed in the study. Stool antigen and blood serum antibody tests were used to detect *H. pylori*, while the presence of any intestinal parasites was detected using direct wet mount microscopy and formol-ether concentration techniques. A structured questionnaire was delivered to mothers and legal guardians of the children by an interviewer to collect data relevant demographic and lifestyle factors. Multivariate logistic regression analysis was performed to assess the association of these sociodemographic characteristics with the coinfection of *H. pylori* and intestinal parasites.

Results: The prevalence of coinfection with any intestinal parasites and *Helicobacter pylori* was 23.0% ($n = 92/400$). Univariate analysis showed an increased risk for co-infection among children whose mothers had non-formal education (COR: 1.917, $p < 0.01$) and those who had no history of child vaccination (COR: 3.455, $p = 0.084$). Children aged 10–14 and those who lived in a house that had a flush or ventilated latrine were found at lower odds of coinfection between intestinal parasites and *Helicobacter pylori* (COR: 0.670, $p = 0.382$; COR: 0.189, $p = 0.108$). Multivariate regression analysis showed increased odds of co-infection among children whose mothers had non-formal education (AOR: 1.978, $p < 0.01$). Maternal education was also associated with a two-fold increase in odds for *H. pylori* and any protozoa co-infection (AOR: 2.047, $p < 0.01$).

Conclusion: Our study shows a moderate prevalence of *H. pylori* and intestinal parasite co-infection and identified maternal education as a significant risk factor among school children.

1. Introduction

In many low-income and resource-limited settings, especially in tropical and rural areas, individuals are co-infected with multiple microorganisms, such as bacteria and parasites (Mcardle et al., 2018; Chard et al., 2019; Ankarklev et al., 2012). The frequency of co-infections adds to the complexity of understanding disease, as different organisms can have potentially synergistic

* Corresponding author at: Colgate University, Department of Biology, 214 Olin Hall, 13 Oak Dr. Hamilton, NY 13346, USA.
E-mail addresses: hspotts@colgate.edu, (H. Spotts), btaye@colgate.edu. (B. Taye).

https://doi.org/10.1016/j.parepi.2020.e00177

or antagonistic interactions, impacting treatment, clinical outcomes, and susceptibility to other diseases (Chard et al., 2019; Mcardle et al., 2018; Queiroz et al., 2013; Krzyzek and Gosciniak, 2017). Studying the magnitude of co-infections can be challenging due to difficulties in obtaining accurate information about multiple organisms in a single host at once (Mcardle et al., 2018; Fuenmayor-Boscán et al., 2016). There can be confounding factors or symptoms and an incomplete understanding of the specific immune complications from interacting parasites and bacteria (Mcardle et al., 2018, Fuenmayor-Boscán et al., 2016). Historically, infectious diseases were studied by isolating the most prominent species, often ignoring the contributions of other microorganisms occupying the same gastrointestinal niche (Tay et al., 2016). As such, there is a growing need to explore the magnitude of concurrent infection and the possible shared risk factors that may contribute to the co-occurrence of microorganisms in a single host (Tay et al., 2016).

Previous studies have shown the rates of co-infection with *Helicobacter pylori* (*H. pylori*) and one or more other intestinal parasites ranging from 22.4% to 44.3% in various populations (Zeyrek et al., 2008; Ankarklev et al., 2012; Seid et al., 2018; Sabet et al., 2009; Ibrahim et al., 2019). *H. pylori* prevalence is estimated to be 79.1% in Africa, and the World Health Organization estimates that 600 million school children live in areas with a high risk of parasite transmission (Sitotaw et al., 2019; Melese et al., 2019). Co-infection with both of these types of organisms has consequences for the development of gastrointestinal diseases, child growth, development, and nutrition through the inflammation, malnutrition, and dehydration from diarrhea associated with these pathogens (Awuku et al., 2017; Smith et al., 2018; Melese et al., 2019; Sabet et al., 2009; Sitotaw et al., 2019; Hernández et al., 2019; Queiroz et al., 2013). Although various risk factors associated with coinfection have been reported in different settings, data are limited in developing countries. We therefore used our data from Ethiopian school children to examine the magnitude and possible risk factors for *H. pylori* and intestinal parasite coinfection.

2. Methods

2.1. Study setting and design

This study was based in Ziway, Ethiopia, which according to 2007 census data has a town population of 43,660 people. Located adjacent to Lake Ziway, it is located 160 km south of Addis Ababa. The town population is comprised of 52.58% males, and has a variety of religious beliefs and practices. There are 51.04% identifying as Orthodox Christian, 24.69% who are Muslim, 22.07% who are Protestant, and a small portion (0.42%) practicing traditional beliefs. Since it was established in 1961, Ziway has continued to experience both population and economic growth. For this study, a majority of data was collected at Sher Ethiopia School and Sher Ethiopia Hospital, with additional data collected at Batu Hospital and Batu Health Clinic, both of which are institutions run by the government. A convenient sampling technique was used in order to enroll participants in the study; a child's school attendance on a particular day determined enrollment. From June 6 to July 30, 2016, data was collected from children 14 years of age or younger living in Ziway who were enrolled in primary school. A cross-sectional design was employed to analyze the prevalence of *Helicobacter pylori* and various intestinal parasites, and the relevant co-infection status among study participants. Various social and demographic risk factors were analyzed in association with the co-infection prevalence rates among participants.

2.2. Measurement and data collection

Written, informed consent was obtained from parents prior to delivering an interviewer led questionnaire to collect demographic and lifestyle information from parents of Ziway children. Questionnaires were translated from English to be administered in local languages. The questions about demographics were related to age, residence, mother's occupation and education level. Information about lifestyle was collected from questions about household size, sanitary and hygiene conditions, and health practices. Participants received leak proof, plastic containers to collect fecal samples with clear instructions. Fecal samples were placed in containers with 10% formalin before transportation and analysis of *H. pylori* antigens and intestinal parasites at Sher-Ethiopia Hospital. Five mL of blood was sterilely collected from each child using disposable syringes. Within two hours of collection, serum samples were separated at Sher-Ethiopia Hospital to determine the presence of antibodies.

2.3. Laboratory testing

2.3.1. H. pylori status

H. pylori was detected using Human *Helicobacter Pylori* Rapid Test (Cassette) Antibody RapiCard InstaTest stool antigen test, (Diagnostic Automation Inc., USA). Monoclonal anti-*H. pylori* antibodies were used to capture antibodies while peroxidase-conjugated monoclonal antibodies were used for detection. A small portion of the stool sample was homogenized with buffer solution and two drops were added to the test well for the immunoassay. Following a 15-min wait period, the test was read for analysis. The presence of both control and test lines was defined as a positive result, even in the case of the control line being much darker than the test. However, if the only line present was the control, this test was deemed negative.

The rapid antibody test (Diagnostic Automation Inc. USA) was used to detect either past or current infection, but cannot distinguish the history of old versus active infection. Approximately 1 to 2 drops of blood serum were added to the test well, in which a double antigen chromatographic lateral flow immunoassay was performed, similarly to the rapid antigen test. After 15 min the test was read and presence of absence of test lines was interpreted the same way as the antigen test.

2.3.2. Direct wet mount microscopy

A wooden applicator was used to mix another portion of the stool sample, approximately 2 mg, with 0.85% NaCl solution to suspend the stool. The uniform suspension was placed under a 22 × 22-mm coverslip for evidence of parasitic infection. At 100× magnification, the sample was examined for parasitic cysts, ova, and/or mobile trophozoites. At 400× magnification, specific parasite species were identified.

2.3.3. Formol-ether concentration technique

Additional stool analysis was performed at Addis Ababa University using the formol-ether concentration technique. In this technique, an applicator was used to homogenize about 1 g of the stool sample in 8 mL of sodium acetate-SAF. The resulting emulsification was sieved before collection in 4 mL of diethyl ether. The tube containing these contents was capped with a rubber stopper before 30 s of inversion, followed by centrifugation for 1 min at 3000 rpm. After decanting off the supernatant (top three layers), the remaining sediment was subjected to microscopic examination for parasitic ova and larvae.

2.4. Outcome variable definition

Co-infection of *H. pylori* and intestinal parasite was defined by a positive result for both *H. pylori* and any intestinal parasites infections.

2.5. Exposure definition

Several of the individual and household factors were defined in the context of this study based upon the data that was collected. Individuals who had received a single dose of 500 mg of mebendazole within the past 6 months were considered dewormed as opposed to those not receiving treatment within the specified time frame. The wall materials of the house were defined as wood or mud, brocket, or other materials which included cement, stone, bricks, or corrugated iron. Besides piped, the other sources of water were defined as wells, springs, rivers, ponds, or dams. Maternal occupation was categorized into housewife, farming, trading, and others, which included government or other work. In univariate analyses, maternal occupation was defined as housewife compared to others, encompassing all other options. The variable assessing ownership of farm animals defined ownership of a sheep, goat, pig, hen, cow, ox, horse, mule, or donkey. Electricity use was defined as households using electricity sometimes or every day for cooking or non-cooking purposes. The housing socioeconomic conditions represent a combined variable to measure roof and floor type with whether or not the child had a bed. Lower conditions defined as combinations of no bed and/or mud floor and thatched roof. Higher conditions were defined as children having a bed with cement floors and a corrugated iron roof in the house. The pillow and mattress material were categorized into sponge or others, which included grass, cloth, natural fibers, or lack of a pillow and/or mattress.

2.6. Statistical analysis

All of the data were entered into SPSS Statistics (IBM Corp., Armonk, NY, USA) where further coding and merging was performed for the analysis. The frequencies and distributions of demographic variables were calculated to characterize the representation of study variables among the population. Univariate analysis for logistic regression was performed to identify initial factors associated with co-infection status. Variables associated with co-infection at $p < 0.4$ were entered into multivariate regression analysis. Backwards elimination of confounding variables was performed to determine the potential risk factors for co-infection of *H. pylori* with an extensive selection of intestinal parasites. Crude and adjusted odds ratios were calculated at univariate and multivariate stages, respectively, with 95% confidence intervals. Statistical significance was defined as $p < 0.05$.

3. Results

3.1. Sociodemographic results

In the sample of 434 children for which there were blood and stool samples available, 50.5% (219) were male and 96.3% (418) lived in urban areas. There were 48.4% who were between 5 and 9 years of age (210), followed by 43.1% aged 10 to 14 (187), and 7.8% less than 5 years old (34). Most of the children, 98.2% (426) had a history of some prior vaccination and 72.1% had been dewormed in the past six months (313). There were 58.3% of mothers (253) who had formal education and 31.8% who had breastfed their children (138). Most families had 5 to 7 people in the home (49.1%), with 56.2% of children having 1 to 4 older siblings. Within the home, 50.2% of walls were made of wood or mud (218), 94.7% used electricity in some capacity sometimes or every day (411), and 76.5% of families cooked inside the house (332). Table 1.

3.2. Distribution of H. pylori and intestinal parasite infections

Table 2 provides the distribution of *H. pylori* and intestinal parasites. Overall, 35.7% (136/434) of the children were infected with at least one intestinal parasite. *Giardia lamblia was* the most prevalent parasite (13.1%) followed by *Entamoeba histolytica/dispar (11.3%)*. *H. pylori* was detected in 65% of children (282/400) either by *H. pylori* antigen or antibody test (Table 2).

Table 1
Selected socio-demographic characteristics among Ethiopian schoolchildren ($n = 434$).

Variable	Frequency (%)
Age	
<5	34 (7.8)
5 to 9	210 (48.4)
10 to 14	187 (43.1)
Sex	
Male	219 (50.5)
Female	215 (49.5)
Residence	
Rural	16 (3.7)
Urban	418 (96.3)
Maternal education	
Non-formal	181 (41.7)
Formal	253 (58.3)
History of Child Vaccination	
Yes	426 (98.2)
No	8 (1.8)
Breastfeeding	
Yes	138 (31.8)
No	248 (57.1)
Child dewormed[a]	
Yes	313 (72.1)
No	121 (27.9)
Wall material made of	
Wood and mud	218 (50.2)
Brocket	180 (41.5)
Others[b]	34 (7.8)
Cooking location	
Inside house	332 (76.5)
Outside house	101 (23.3)
Water Source	
Piped	419 (96.5)
Other[c]	15 (3.5)
Toilet Type	
Flush and ventilated pit	22 (5.1)
Traditional pit or none	409 (94.2)
Waste Disposal	
Pit	78 (18.0)
Burning	235 (54.1)
Garbage bin	105 (24.2)
Other	15 (3.5)
Number of people in home	
2 to 4	182 (41.9)
5 to 7	213 (49.1)
>7	37 (8.5)
Number of older siblings (alive)	
0	168 (38.7)
1 to 4	244 (56.2)
>5	21 (4.8)
Maternal occupation	
Housewife	197 (45.4
Farming	78 (18.0)
Trading	70 (16.1)
Others[d]	89 (20.5)
Cat or dog	
Yes	185 (42.6)
No	249 (57.4)
Farm animals[e]	
Yes	150 (34.6)
No	284 (65.4)
Electricity Use[f]	
Yes	411 (94.7)
No	22 (5.1)
Housing socioeconomic conditions[g]	

Table 1 *(continued)*

Variable	Frequency (%)
Higher	316 (72.8)
Lower	118 (27.2)
Child sleeping conditions[h]	
Sponge	321 (74.0%)
Others	113 (26.0%)

[a] Defined as receiving single dose of 500 mg of mebendazole within past 6 months.
[b] Other materials include cement, stone, bricks, corrugated iron.
[c] Other sources of water defined as well, spring, river, pond, dam.
[d] Other jobs include government work or those falling outside of these categories.
[e] Defined as ownership of a sheep, goat, pig, hen, cow, ox, horse, mule, or donkey.
[f] Combined electricity use for cooking and non-cooking; Yes defined as sometimes or everyday.
[g] Defined by roof type (thatched or corrugated iron), floor type (cement, mud, other), and if the child slept on a bed. Lower conditions indicate combinations of no bed and/or lower housing conditions (mud floor and thatched roof). Higher conditions defined by child having a bed with cement floor and corrugated iron roof.
[h] Defined by whether children slept on pillows and mattresses made of sponge or other materials (grass, cloth, natural fibers, or no pillow/mattress).

Table 2
Prevalence of *H. pylori, any* intestinal parasites, protozoa, and helminths.

Infection	Frequency (%)
Intestinal Parasites	
Giardia lamblia	57 (13.1)
Entamoeba histolytica/ dispar	49 (11.3)
Hymenolepis nana	18 (4.1)
Giardia lamblia and Entamoeba histolytica/ dispar	10 (2.3)
Enterobius vermicularis	6 (1.4)
Hymenolepis nana and Entamoeba histolytica/ dispar	4 (0.9)
Hymenolepis nana and Giardia lamblia	3 (0.7)
Hookworm	2 (0.5)
Tenia Spps	2 (0.5)
Ascaris lumbricoides	1 (0.2)
Ascaris lumbricoides and Entamoeba histolytica/ dispar	1 (0.2)
Schistosoma and Entamoeba histolytica/ dispar	1 (0.2)
Hymenolepis nana and Enterobius vermicularis	1 (0.2)
Any parasite	
Yes	155 (35.7)
No	279 (64.3)
Any protozoa[a]	
Yes	125 (38.8)
No	309 (71.2)
H. pylori antibody test	
Negative	119 (27.4)
Positive	282 (65)
H. Pylori antigen test	
Negative	410 (94.5)
Positive	21 (4.8)
H. Pylori Both Tests Combined	
Negative	118(27.2)
Positive	282 (65)
Any helminth[b]	
Yes	53 (12.2)
No	381 (87.8)

[a] Protozoa species include: *Giardia lamblia, Entamoeba histolytica/dispar,*
[b] Helminth species include: *Hymenolepsis nana, Enterobius vermicularis, Hookworm, Tenia spp, Ascaris lumbricoides, Schistosoma.*

3.3. Co-infection prevalence

Table 3 shows the prevalence of co-infection between *H. pylori* and intestinal parasites. Of the 400 children with complete data for both *H. pylori* and parasites, 23% (92/400) were coinfected with *H pylori* and any intestinal parasite, while 18.7% (75/400) were co-infected with *H. pylori* and any protozoa.

3.4. Risk factors for H. pylori and any intestinal parasite co-infection

The results in Table 4 show both the crude and adjusted odds ratios for selected social and demographic variables and their relationship to the co-infection of *H. pylori* and any parasite. Univariate analysis showed increased prevalence of co-infection among mothers with non-formal education compared to those with formal education (COR: 1.917, $p < 0.01$). Select other factors

Table 3
Coinfections of *Helicobacter pylori* with intestinal parasites ($N = 400$).

Variables	N (%)
H pylori and any intestinal parasites Co-infections[a]	
Yes	92 (23.0)
No	308(77.0)
H pylori and any protozoan Co-infections[b]	
Yes	75 (18.7)
No	325 (81.3)

[a] *Co*-infections includes: positive for both *H. pylori* and any intestinal parasites.
[b] *Co*-infections includes: positive for both *H. pylori* and any protozoan parasites.

Table 4
Crude and adjusted odds ratios for associations between risk factors and co-infection of *Helicobacter pylori* and any intestinal parasite.

Variable	Coinfected (%)	Not coinfected (%)	COR (95% CI)	P	AOR (95% CI)	P
Age						
<5	8 (28.6%)	20 (71.4%)	1		–	
5 to 9	47 (24.2%)	147 (75.8%)	0.799 (0.330–1.933)	0.619	–	
10 to 14	37 (21.1%)	138 (78.9%)	0.670 (0.273–1.643)	0.382	–	
Sex						
Male	48 (23.6%)	155 (76.4%)	1.077 (0.676–1.716)	0.756	–	
Female	44 (22.3%)	153 (77.8%)	1		–	
Residence						
Rural	3 (27.3%)	8 (72.7%)	1.264 (0.328–4.865)	0.733	–	
Urban	89 (22.9%)	300 (77.1%)	1		–	
Maternal education						
Non-formal	50 (29.8%)	118 (70.2%)	1.917 (1.198–3.068)	0.007	1.978 (1.183–3.307)	0.009
Formal	42 (18.1%)	190 (81.9%)	1		1	
History of child vaccination						
Yes	88 (22.4%)	304 (77.6%)	1		–	
No	4 (50%)	4 (50%)	3.455 (0.847–14.094)	0.084	–	
Breastfeeding mother						
Yes	56 (25.0%)	168 (75.0%)	1.333 (0.788–2.255)	0.283	1.241 (0.726–2.121)	0.43
No	26 (20%)	104 (80%)	1		1	
Child dewormed[a]						
Yes	67 (23.3%)	221 (76.7%	1		–	
No	25 (22.3%)	87 (77.7%)	0.948 (0.562–1.598)	0.841	–	
Wall material						
Wood and mud	49 (24.3%)	153 (75.7%)	1		1	
Brocket	40 (24.1%)	126 (75.9%)	0.991 (0.614–1.601)	0.971	0.425 (0.121–1.496)	0.183
Others[b]	3 (10%)	27 (90%)	0.347 (0.101–1.193)	0.093	0.207 (0.026–1.626)	0.173
Cooking location						
Inside house	69 (22.5%)	237 (77.5%)	0.886 (0.515–1.524)	0.662	–	
Outside house	23 (24.7%)	70 (75.3%)	1		–	
Water source						
Piped	90 (23.2%)	298 (76.8%)	1		–	
Other[c]	2 (16.7%)	10 (83.3%)	0.662 (0.142–3.078)	0.599	–	
Toilet type						
Flush and ventilated pit	1 (5.6%)	17 (94.4%)	0.189 (0.025–1.439)	0.108	–	
Traditional pit or none	90 (23.7%)	289 (76.3%)	1		–	
Waste disposal						
Pit	15 (22.4%)	52 (77.6%)	1		–	
Burning	58 (26.1%)	164 (73.9%)	1.226 (0.641–2.343)	0.538	–	
Garbage bin	17 (17.3%)	81 (82.7%)	0.728 (0.335–1.582)	0.422	–	
Other	2 (16.7%)	10 (83.3%)	0.693 (0.137–3.515)	0.658	–	
Number of people in home						
2 to 4	42 (25.1%)	125 (74.9%)	1.218 (0.517–2.870)	0.652	–	
5 to 7	42 (21.6%)	152 (78.4%)	1.002 (0.426–2.353)	0.997	–	
>7	8 (21.6%)	29 (78.4%)	1		–	
Number of older siblings (alive)						
0	38 (24.8%)	115 (75.2%)	1.322 (0.416–4.197)	0.636	–	
1 to 4	50 (22.1%)	176 (77.9%)	1.136 (0.364–3.552)	0.826	–	
>5	4(20%)	16 (80%)	1		–	
Maternal occupation						
Housewife	46 (25.3%)	136 (74.7%)	1		–	
Others[d]	46 (21.1%)	172 (78.9%	0.791 (0.496–1.261)	0.324	–	
Cat or dog						
Yes	41 (23.0%)	137 (77.0%)	1.003 (0.628–1.603)	0.989	–	
No	51 (23.0%)	171 (77.0%)	1		–	

Table 4 *(continued)*

Variable	Coinfected (%)	Not coinfected (%)	COR (95% CI)	P	AOR (95% CI)	P
Farm animals[e]						
Yes	30 (22.1%)	106 (77.9%)	0.922 (0.562–1.513)	0.748	–	
No	62 (23.5%)	202 (76.5%)	1		–	
Electricity Use[f]						
Yes	90 (23.8%)	288 (76.2%)	1		1	
No	2 (9.5%	19 (90.5%)	0.337 (0.077–1.474	0.149	0.207 (0.026–1.626)	0.134
Housing socioeconomic conditions[g]						
Lower	72 (24.7%)	219 (75.3%)	1.463 (0.841–2.544)	0.178	–	
Higher	20 (18.3%)	89 (81.7%)	1		–	
Child sleeping materials[h]						
Sponge	66 (21.9%)	235 (78.1%)	1		–	
Others	26 (26.3%)	73 (73.7%)	1.268 (0.751–2.142)	0.375	–	

[a] Defined as receiving single dose of 500 mg of mebendazole within past 6 months.
[b] Other materials include cement, stone, bricks, corrugated iron.
[c] Other sources of water defined as well, spring, river, pond, dam.
[d] Other jobs include farming, trading, government work, or others.
[e] Defined as ownership of a sheep, goat, pig, hen, cow, ox, horse, mule, or donkey.
[f] Combined electricity use for cooking and non-cooking; Yes defined as sometimes or everyday.
[g] Defined by roof type (thatched or corrugated iron), floor type (cement, mud, other), and if the child slept on a bed. Lower conditions indicate combinations of no bed and/or lower housing conditions (mud floor and thatched roof). Higher conditions defined by child having a bed with cement floor and corrugated iron roof.
[h] Defined by whether children slept on pillows and mattresses made of sponge or other materials (grass, cloth, natural fibers, or no pillow/mattress).

were borderline significant, including children who had no history of vaccination (COR: 3.455, $p = 0.084$). There was a protective non-significant association between flush and ventilated pit toilets (COR: 0.189, $p = 0.108$) and lack of electricity use (COR: 0.337, $p = 0.149$) with co-infection with any parasite. The children living among lower housing conditions were associated with non-significant increased odds (COR: 1.463, $p = 0.178$). Compared to wood or mud materials, walls made of other materials including cement, stone, bricks, or corrugated iron were found be non-significantly associated with lower co-infection prevalence (COR:0.347, $p = 0.093$). After variables were entered in multivariate regression analysis using backwards elimination to adjust for confounders, the only remaining significant factor was maternal education, with non-formal education being associated with increased odds of co-infected children with both *H. pylori* and any intestinal parasites (AOR: 1.978, $p < 0.01$).

3.5. Risk factors for H. pylori and any protozoa co-infection

Table 5 shows the association between any protozoa and *H. pylori* co-infection and the selected social and demographic variables. After univariate analysis of all variables, non-formal maternal education was found to be a risk factor for co-infection among the cohort of children (COR: 2.01, $p < 0.01$). Other variables were found to be borderline or close to significant, including

Table 5
Crude and adjusted odds ratios for associations between risk factors and co-infection of *Helicobacter pylori* with any protozoa.

Variable	Co-infected (%)	Not co-infected (%)	COR (95% CI)	*P*-value	AOR (95% CI)	P-value
Age						
<5	8 (28.6%)	20 (71.4%)	1		–	
5 to 9	37 (19.1%)	157 (80.9%)	0.589 (0.241–1.442)	0.247	–	
10 to 14	30 (17.1%)	145 (82.9%)	0.517 (0.208–1.284)	0.155	–	
Sex						
Male	39 (19.2%)	164 (80.8%)	1.064 (0.643–1.758)	0.81	–	
Female	36 (18.3%)	161 (81.7%)	1		–	
Residence						
Rural	3 (27.3%)	8 (72.7%)	1.651 (0.427–6.377)	0.467	–	
Urban	72 (18.5%)	317 (81.5%)	1		–	
Maternal education						
Non-formal	42 (25%)	126 (75%)	2.010 (1.210–3.339)	0.007	2.047 (1.210–3.463)	0.008
Formal	33 (14.2%)	199 (85.8%)	1		1	
History of child vaccination						
Yes	72 (18.4%)	320 (81.6%)	1		–	
No	3 (37.5%)	5 (62.5%)	2.667 (0.623–11.414)	0.186	–	
Breastfeeding mother						
Yes	23 (17.7%)	107 (82.3%)	1		–	
No	44 (19.6%)	180 (80.4%)	1.137 (0.651–1.987)	0.652	–	
Child dewormed[a]						
Yes	52 (18.1%)	236 (81.9%)	1		1	
No	23 (20.5%)	89 (79.5%)	1.173 (0.678–2.029)	0.569	1.240 (0.689–2.233)	0.472

(continued on next page)

Table 5 *(continued)*

Variable	Co-infected (%)	Not co-infected (%)	COR (95% CI)	*P*-value	AOR (95% CI)	P-value
Wall material						
Wood and mud	41 (20.3%)	161 (79.7%)	1		–	
Brocket	31 (18.7%)	135 (81.3%)	0.902 (0.536–1.516)	0.696	–	
Others[b]	3 (10%)	27 (90%)	0.436 (0.126–1.509)	0.19	–	
Cooking location						
Inside house	55 (18%)	251 (82%)	0.800 (0.450–1.420)	0.446	–	
Outside house	20 (21.5%)	73 (78.5%)	1		–	
Water source						
Piped	74 (19.1%)	314 (80.9%)	1		–	
Other[c]	1 (8.3%)	11 (91.7%)	0.386 (0.049–3.035)	0.365	–	
Toilet type						
Flush and ventilated pit	1 (5.6%)	17 (94.4%)	0.247 (0.032–1.883)	0.177	0.198 (0.025–1.551)	0.123
Traditional pit or none	73 (19.3%)	306 (80.7%)	1		1	
Waste disposal						
Pit	13 (19.4%)	54 (80.6%)	1		–	
Burning	44 (19.8%)	178 (80.2%)	1.027 (0.515–2.046)	0.94	–	
Garbage bin	16 (16.3%)	82 (83.7%)	0.811 (0.361–1.819)	0.611	–	
Other	2 (16.7%)	10 (83.3%)	0.831 (0.162–4.259)	0.824	–	
Number of people in home						
2 to 4	32 (19.2%)	135 (80.8%)	1.016 (0.410–2.520)	0.973	–	
5 to 7	36 (18.6%)	158 (81.4%)	0.976 (0.397–2.399)	0.959	–	
>7	7 (18.9%)	30 (81.1%)	1		–	
Number of older siblings (alive)						
0	31 (20.3%)	122 (79.7%)	1.440 (0.397–5.226)	0.579	–	
1 to 4	41 (18.1%)	185 (79.7%)	1.256 (0.352–4.486)	0.726	–	
>5	3 (15%)	17 (85.0%)	1		–	
Maternal occupation						
Housewife	39 (21.4%)	143 (78.6%)	1		1	
Others[d]	36 (16.5%)	182 (83.5%)	0.725 (0.439–1.2)	0.211	1.210 (0.719–2.035)	0.473
Cat or dog						
Yes	31 (17.4%)	147 (82.6%)	0.853 (0.513–1.419)	0.541	0.828 (0.488–1.404)	0.484
No	44 (19.8%)	178 (80.2%)	1		1	
Farm animals[e]						
Yes	24 (17.6%)	112 (82.4%)	0.895 (0.523–1.530)	0.685	–	
No	51 (19.3%)	213 (80.7%)	1		–	
Electricity Use[f]						
Yes	73 (19.3%)	305 (80.7%)	1		1	
No	2 (9.5%)	19 (90.5%)	0.440 (0.100–1.931)	0.276	0.309 (0.066–1.434)	0.134
Housing socioeconomic conditions[g]						
Lower	58 (19.9%	233 (80.1%)	0.742 (0.411–1.342)	0.324	–	
Higher	17 (15.6%)	92 (84.4%)	1		–	
Child sleeping materials[h]						
Sponge	55 (18.3%)	246 (81.7%)	1		–	
Others	20 (20.2%)	79 (79.8%)	1.132 (0.640–2.004)	0.67	–	

[a] Defined as receiving single dose of 500 mg of mebendazole within past 6 months.
[b] Other materials include cement, stone, bricks, corrugated iron.
[c] Other sources of water defined as well, spring, river, pond, dam.
[d] Other jobs include government work or those falling outside of these categories.
[e] Defined as ownership of a sheep, goat, pig, hen, cow, ox, horse, mule, or donkey.
[f] Combined electricity use for cooking and non-cooking; Yes defined as sometimes or everyday.
[g] Defined by roof type (thatched or corrugated iron), floor type (cement, mud, other), and if the child slept on a bed. Lower conditions indicate combinations of no bed and/or lower housing conditions (mud floor and thatched roof). Higher conditions defined by child having a bed with cement floor and corrugated iron roof.
[h] Defined by whether children slept on pillows and mattresses made of sponge or other materials (grass, cloth, natural fibers, or no pillow/mattress.

history of child vaccination, with increased odds of co-infection among children not vaccinated (COR: 2.667, $p = 0.186$). Older children were found to be protected slightly against co-infection for both 5 to 9 and 10 to 14 age groups, however these were not statistically significant (COR: 0.589, $p = 0.247$; COR: 0.517, $p = 0.155$, respectively). After adjustment for confounding variables, non-formal maternal education remained to be significant in the multivariate regression analysis (AOR: 2.047, $p < 0.01$). Other variables entered into this analysis were not found to be significant following adjustment.

4. Discussion

Our study provides a comprehensive analysis of the co-infection between *H. pylori* and intestinal parasites in a resource-limited setting. When a pathogenic species enters a host, the presence of other microorganisms can impact the ability of the entering species to colonize and the immune system's ability to effectively rid the body of the pathogen (Chard et al., 2019). Here,

we report the prevalence of co-infection between *H. pylori* and intestinal parasites among school children and their potential shared risk factors. The most commonly significant risk factor after adjusting for confounders was maternal education, with non-formal education among mothers increasing the odds of co-infection in their children. Several other variables were shown to be borderline significant and different measures of socioeconomic status and sanitary conditions varied in their respective associations studied in this analysis.

The prevalence of co-infection among the Ethiopian school children in this study mirrors some of the results observed in other studies of *H. pylori* and parasitic co-infections. Several studies in the literature have been case-control based examinations of co-infection related to dyspeptic symptoms, abdominal pain, or diarrhea (Zeyrek et al., 2008; Ibrahim et al., 2019; Seid et al., 2018; Sabet et al., 2009). In one study, 22.4% of patients with recurrent abdominal pain were co-infected with both *H. pylori* and *Giardia* (Zeyrek et al., 2008). This rate is similar to the 23% prevalence observed in our study of Ethiopian school children. In another study, there was a coinfection prevalence of 43.9% among all patients with *H. pylori*, focused on a sample of children with or without diarrheal conditions (Ibrahim et al., 2019). Several studies have reported on this relationship, showing that there was a three times higher frequency of *Giardia lamblia* infection in children who also had *H. pylori,* and in another, that 22.3% of co-infecting parasites found among *H. pylori* infected participants were attributed to *Giardia* species (Ankarklev et al., 2012; Seid et al., 2018). This positively associated relationship is not always seen, as one study reported a decreased risk for *H. pylori* infection among children infected with *Giardia lamblia* (Fuenmayor-Boscán et al., 2016). The temporal ambiguity of which infection occurs first might play a role in the discrepancies seen between these different studies.

Other studies have reported on associations with various species of protozoa and soil-transmitted helminths (Krzyzek and Gosciniak, 2017; Yakoob et al., 2018; Ek et al., 2012; Fuenmayor-Boscán et al., 2016). One small sample study found 27 of 33*H. pylori* infected individuals to have one or more parasites, most commonly *Blastocystis* and *Entamoeba* species (Yakoob et al., 2018). Another study showed significant associations between *H. pylori* and helminth polyparasitism, with the strength of association increasing as the number of parasites increased (Fuenmayor-Boscán et al., 2016). Variations in endemic parasite prevalence have been documented in studies that show how different prevalence rates of species in different regions may contribute to discrepancies in the clinical outcome of gastric cancer, possibly due to the ways in which specific species of microorganisms interact (Ek et al., 2012; Whary et al., 2005).

The risk factors for both *H. pylori* and intestinal parasites have been studied, showing similarities in terms of factors associated with socioeconomic status, hygiene, and potential environmental contaminations. While an exact transmission mechanism of *H. pylori* is unknown, it is postulated to be transmitted via person to person or oral-fecal routes similar to many intestinal parasites (Ankarklev et al., 2012; Sabet et al., 2009; Fuenmayor-Boscán et al., 2016). Common risk factors shown to have some significant relationship in previous reports include larger household sizes, non-piped and potentially contaminated water sources, open air defecation, living in rural settings, and poor hygiene as it relates to food consumption and hand washing (Awuku et al., 2017; Hernández et al., 2019; Sitotaw et al., 2019). These sociodemographic and environmental factors guided the selection of risk factors we chose to analyze in association with co-infection status.

The lack of formal maternal education was the most consistently significant variable that was associated with an increased chance of the child being co-infected with *H. pylori* and some intestinal parasite. A previous study focused on *H. pylori* risk also found a relationship between non-formal education and infection, however that was not found to be significant after adjustment for confounding factors (Smith et al., 2018). Non-formal education is a potential indicator of lower socio-economic status, which is often associated with increased risks for poorer health habits and outcomes (Smith et al., 2018; Melese et al., 2019). A study conducted in northeast Ethiopia focused on the *Giardia* and *H. pylori* co-infection found significant associations for increased odds of co-infection linked to river water consumption and ground or spring water consumption (Seid et al., 2018). The potential for such a relationship to occur could be due to the transmission of these microbes via contaminated water sources. Regional variations in water quality could help to explain the discrepancy seen in the lack of statistical significance in our study linked to water.

There are some plausible immunological mechanisms to describe some of the ways that microorganisms may interact when concurrently colonizing or how modulation of the immune system allows for opportunistic infections. In the case of *Giardia lamblia*, the organism prefers an elevated pH in the gastric environment to allow for colonization; the pathology of *H. pylori* infection includes the secretion of urease to counteract the acidic stomach environment (Sabet et al., 2009). The destruction of the gastric acid innate immune defense could also allow opportunistic enteric pathogens to co-infect (Queiroz et al., 2013). In addition, different pathogens will skew the immune system's adaptive response towards Th1 or Th2 cell dominant cascades, altering the secretion of cytokines and other regulatory T-cells (Krzyzek and Gosciniak, 2017; Chard et al., 2019).

The results of this study should be interpreted within the context of the limitations which include the cross-sectional design and sampling method. There is temporal ambiguity in the assessment of when infections occur relative to the exposure or multiple risk factors that were assessed by the questionnaire. As the sample was collected conveniently, there is a possibility that the sample over or under-represents certain social or demographic factors, leading towards a biased understanding of the odds ratio calculations. Furthermore, the study is limited by only using a single stool sample to determine intestinal parasite infection, which may underestimate the true prevalence. However, we used multiple diagnostic approaches (i.e., direct microscopy and formol-ether concentration technique) as recommended by the literature to increase the sensitivity of parasite detection.

5. Conclusions

Our data from a resource-limited setting provides evidence of moderate prevalence of *H. pylori* and intestinal parasite co-infections among school children. This work could be supplemented by research into understanding possible immune system

modulations and modifications that alter susceptibility and ability to effectively fight off pathogen infections. Co-infection remains a complex and nuanced biological problem that must be approached from broad epidemiological as well as molecular-level studies to maximally decrease the impact.

Funding

Colgate University Research Council funded the study. Connor Gilleland '19, presents a poster using preliminary data analysis of this project at the National Conference on Undergraduate Research (NCUR), Kennesaw State University in Georgia.

Ethical approval

Departmental Research and Ethics Review Committee (DRERC) of Addis Ababa University College of Health Sciences, Department of Medical Laboratory Sciences, approved the study. We obtained written or fingerprint consent from children's parents or their legal guardians after informing them of the study procedures. To ensure participant privacy, confidential numerical identifiers were assigned to each child and all participant information remains password protected in electronic files. The children were also informed about their ability to withdraw from this study at any time without jeopardizing their right to receive any services at their school. Children who were found to have intestinal parasites were treated with anti-parasitic drugs in local health centers.

Authors' contributions

BT conceived and designed the study and participated in field data collection and wrote this manuscript. HS performed data analysis and interpretation, prepared the preliminary results and draft the manuscript. SW and MT participated in data collection, interpretation of data and the critical review of the manuscript. AT and KD participated in data analysis and interpretation, and critically reviewed the manuscript. All authors read and approved the final manuscript.

Declaration of Competing Interest

We declare that we do not have any conflicts of interest.

Acknowledgments

We gratefully thank the parents/guardians and children at each school, who generously provided their information and thank the school administrators for facilitating stool collection and their commitment during the fieldwork. The views expressed are those of the author(s) and not necessarily those of Colgate University or the Department of Medical Laboratory Sciences, College of Health Sciences, Addis Ababa University.

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