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Association between infection with *Helicobacter pylori (H. pylori)* and Platelet Indices Among School-Aged Children in Central Ethiopia: A cross-sectional study

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ABSTRACT

Objective: Previous clinical studies in adults from developed countries have implicated *Helicobacter pylori* infections in the development of thrombocytopenia. However, studies in children, particularly those from low-income countries, are remarkably scarce. We examined the association between *H. pylori* infection and platelet indices in young Ethiopian school children.

Design: Cross sectional study

Setting: This study was conducted in five elementary schools located in central Ethiopia

Participants: Blood and stool samples were collected from 971 children attended in five elementary schools in Ethiopia. *H. pylori* infection was diagnosed using stool antigen and serum antibody tests, and hematological parameters were measured using an automated haematological analyzer. An interviewer-led questionnaire administered to mothers provided information on demographic and lifestyle variables. The independent effects of *H. pylori* infection on platelet indices were determined using multivariate linear and logistic regressions.

Study Outcomes: *H. pylori*-infected children had a lower average platelet counts and Mean Platelet Volume (MPV) than uninfected after adjusting the potential confounders (Adjusted Mean difference: -20.80 x 10^3 /μL; 95% CI; -33.51 – -8.09 x 10^3 /μL, p=0.001, Adjusted Mean difference: -0.236 fl; 95%CI -0.408 – -0.065 fl, p=0.007, respectively). Additionally, *H. pylori* infected children had lower Red Blood cell Counts (RBC) (Adjusted Mean difference: -0.118 million/μl; 95%CI; -0.200 – -0.036, p=0.005) compared to non-infected.

Conclusion: Our study from a developing country provides further support for an association between *H. pylori* infections and reduced platelet indices in young Ethiopian school children,

after controlling for potential confounders. Further research is needed, particularly longitudinal studies to establish causality.

Strength and Limitations

Strengths

- Large sample size
- Exposure to *H. pylori* infection was assessed using a highly sensitive and specific *H. pylori* stool antigen test.
- Key outcomes (platelets indices) was done using a standard automated hematology analyzer and calibrated on a regular basis according to the manufacturer's guidelines.

Limitations

- We could not thoroughly investigate the previous use of anti-platelet agents, which is known to affect platelets indices
- We used EDTA anticoagulant agent that has been associated with time-dependent ultrastructural morphological changes of platelet, thus affecting MPV values.
- The current study is its cross-sectional design, which makes it difficult to attribute causality on the observed association

Introduction

The role of *Helicobacter pylori (H. pylori)* infection as a potential cause of serious upper gastrointestinal diseases has been increasingly appreciated. There is now good evidence that infection with this organism as the principal cause of acute and chronic gastritis, atrophic gastritis (1-3), and widely accepted as the cause of the majority of peptic ulcer diseases and associated complications of bleeding in adults (4, 5). More recently, however, there is growing interest in investigating the effects of *H. pylori* in extra-gastroduodenal diseases (6, 7). Our group's previous work in Ethiopia has found a higher prevalence of anemia and decreased growth trajectory among *H. pylori* infected children compared to non-infected (8, 9). This observation has led us to expand investigations into other extra-gastroduodenal involvement of *H. pylori* in a resource-limited setting. In particular, the effect of *H. pylori* on platelets indices has not been investigated in Ethiopia. Previous studies in various clinical settings from developed countries reported an increased platelet recovery after successful eradication of *H. pylori* infection among patients with idiopathic thrombocytopenic purpura (ITP) (10-13). Additionally, meta-analysis demonstrated that patients receiving treatment had a greater increase in platelet count from baseline compared with untreated controls, regardless of the outcome of eradication therapy (14). However, the possibility of platelet recovery due to the eradication of bacteria other than *H. pylori* or immune modulating effects of the treatment itself is difficult to exclude.

The mechanisms by which *H. pylori* infection can cause low platelet count are still unclear (12, 15), but plausible mechanisms have been proposed. One hypothesis that has attracted attention is that *H. pylori* may hijack the host's immune system through molecular mimicry, where

molecules from the bacteria mimic host antigens and activate T lymphocytes to cause an immune response (12, 15), then the antibody induced by *H. pylori* cross-reacts with platelet glycoprotein antigens and leads to excessive destruction of platelets (15-17).

Whilst the role of *H. pylori* in low platelet counts and ITP disease aetiology is intriguing, most studies to date are conducted in high-income countries on adult populations and lack data in children from low-income countries, where *H. pylori* is a very common bacterial infection, infecting more than 40 % of children (18, 19). Furthermore, most of the available evidence to date comes from retrospective studies of symptomatic ITP patients in clinical settings, which is prone for selection bias and difficult to apply in an apparently healthy population who may have been infected with *H. pylori* sub-clinically prior to ITP. It is therefore important to assess the association between *H. pylori* infection and platelet parameters in apparently healthy populations. This may provide clues for the subclinical link between *H. pylori* and platelet indices prior to ITP diagnosis. The aim of this study was therefore to investigate any possible association between *H. pylori* infection and platelet indices among apparently healthy primary school children in Ethiopia.

Study Setting and Design

A two-part cross-sectional study was conducted in the towns Ziway and Sululta, which are both located in the Oromia region Ethiopia, approximately 160 km South and 30 km North from the capital city, Addis Ababa, respectively. The region surrounding Sululta town has an altitude of 2450m above sea level and average temperatures are in the range of 15°-18° Celsius, while the town of Ziway has an elevation of 1643 m above sea level and is adjacent to Lake Ziway (Lake Dambal). The populations of the two towns are roughly similar: Ziway's population is estimated to be 43,660 and Sululta's population is estimated to be 49,000. The first part of the data collection took place in Ziway town between June and July of 2016, while the second part of the study occurred in Sululta from April through June 2017. Five elementary schools, of three (i.e. Laga dima, Wasarbi and Abdi Boru) from Sululta and two schools (i.e. Sher and Batu) from Ziway town were included in the study. A total of 971 school children aged 4-14 years old participated in this study by providing stool and blood samples.

Measurement and Data Collection

After the parents or the legal guardian of the child signed the written consent form, an interview-administered questionnaire was administered to collect information on selected demographic, life-style, and behavioral factors in both towns. Information was collected but not limited to the student's age, sex, residency, sanitary conditions, hygiene, eating habits, and deworming status. Furthermore, parents' monthly income, educational status, and occupation were collected to determine the student's socioeconomic status. The questionnaire was first designed in English and then translated and pretested in local languages such as Amharic and Oromiffa languages. In addition to the questionnaire data, mothers in both towns were provided with small leak-proof

plastic container and clean wooden applicator sticks to bring sufficient stool sample to ascertain the child's *H. pylori* and intestinal parasite infection. Furthermore a 5 mL blood sample was collected from each child using a vacutainer tube and transported to Sher Ethiopia and St. Paul's Hospital laboratories for haematological analysis.

Laboratory testing

H. pylori Stool Antigen Test

H. pylori antigen rapid test was conducted to detect active *H. pylori* infection (Immunotek, USA). The capture antibody used for this enzyme immunoassay was a mixture of monoclonal anti-*H. pylori* antibodies and the detection antibody was a mixture of peroxidase-conjugated monoclonal anti-*H. pylori* antibodies. A small amount of stool was homogenized with a buffer solution, and 2 drops of the stool/ buffer mixture was added to the test well. After 15 minutes, the test was read. The development of 2 lines, the control (C) line and the test (T) line, indicated an *H. pylori*-positive test result, while the development of only the C line indicated a negative test result. In the instances where the T line was significantly fainter than the C line, the results were interpreted and recorded as positive.

H. pylori Antibody Test

A similar antibody rapid test was conducted to detect any past or current infections, without differentiation between the two. This rapid test was a double antigen chromatographic lateral flow immunoassay, where 1-2 drops of serum were added to the test well and after 15 minutes the test was read. The development of both the C line and the T line indicated a positive test, while the development of only the C line was indicative of a negative test result. As it was with

the stool antigen test, when the T line was significantly fainter than the C line, the results were interpreted and recorded as positive.

Hematological Analyses

A two ml whole blood sample was collected into Ethylene diaminetetraacetic acid (EDTA) tubes between 8:00 and 10:00 am and analyzed on the same day using an automated haematological analyzer (CELL-DYN 800 Hematology Analyzer (Abbott, USA) and Sysmex KX-21N Hematology Analyzer (Sysmex, Japan)) at Sher Ethiopia and St. Paul's Hospitals Hematology laboratory. The analyzers aspirate the blood sample, dilute and count leukocytes, erythrocytes and platelets, measure Mean Platelet Volume (MPV), Mean Cell Volume (MCV) and Haemoglobin (Hb), and calculate Haematocrit, Mean Cell Haemoglobin (MCH), and Mean Cell Haemoglobin Concentration (MCHC). These instruments were monitored daily with normal, high and low controls provided by the manufacturer before running the specimen to ensure quality of haematological analyses

Outcome variables

The primary study outcome was platelets counts (cells per μL) and mean platelets volume (MPV) (continuous variables).

Statistical Analysis

Demographics and laboratory data from both towns were cleaned coded and merged ready for analysis using IBM SPSS Statistics version 24 (SPSS, Inc., Chicago, IL, USA). Mean and standard deviation for continuous variables and proportions for categorical variables are

reported. Prior to investigating the association between *H. pylori* infection and platelet indices, univariate analyses were used to identify the possible confounders. Variables that were associated with both exposure and outcome variables in the crude analysis using statistical significance at p value <0.3 were considered to be possible confounders. These included sex, place of residence, age, family size, maternal education and occupation, water source, toilet type, and waste disposal site. Additionally, we included variables previously shown to be associated with low platelet indices in the literature such as intestinal parasite status (20). The primary outcomes of the current analysis were platelet count (cells per μL) and Mean Platelet Volume (MPV). Our hypothesis that *H. pylori* infection would be associated with lower platelet counts (continuous variable) were assessed using generalized linear models. We first examined the crude mean difference between *H. pylori*-positive and negative individuals, and then we repeated the analysis while adjusting for the possible confounders using backwards elimination. These analyses were repeated for MPV, RBC, and WBC.

Further analyses were carried out to assess the association between *H. pylori* infection and platelet categories (polytomous outcome variable) using multinomial logistic regression. Multinomial regression is the most appropriate technique in a situation where the dependent variables are categorical and have more than two categories. In our multinomial regression analysis, platelet counts were categorized into three as low (platelets counts <150 $x10^3$ per μL), high (>450 $x10^3$ per μL), or normal (150$x10^3$ - 450 $x10^3$ per μL). Platelet count <150$x10^3$/μL or >450$x10^3$/μL was used for classification of thrombocytopenia or thrombocytosis respectively. We also categorized MPV level either low (<7 fL), high (>10.5 fL), or normal (7-10.5 fL) using the cutoffs as described by the British Journal of Haematology, respectively (21). Covariates

were kept in the model if they changed the coefficient of exposure (*H. pylori* infection) by > 10 % or if they were independently associated with the outcome at p < 0.10. Probability values < 0.05 were considered statistically significant for main effects.

Results

Selected demographic characteristics and H. pylori infection status

A total of 971 school children participated in the study. Of these, 55.5% (539/971) were female and a slight majority 56.4 % (546/971) were living in a rural area. The age range of the study participants was 4 to 15 years (mean age, 9.95). Most mothers 57.9 %(565/971) reported using indoor-pipe water as their primary drinking source, and 82.3% (802/971) used traditional pit toilet. Maternal demographic characteristics showed that 56.4% (550/971) of the mothers did not have a formal education, and 32.0% (311/971) were housewives. The prevalence of *H. pylori* infection was 35.9% (343/954) (Table 1).

Univariate analysis for relationships between potential confounders and H. pylori infection

The crude association between demographic variables and infection with *H. pylori* was analyzed using univariate logistic regression. Sex, place of residence, age, maternal education and occupation, water source, family size, toilet type and site of waste disposal were all found to be potential confounders for *H. pylori* infection (Table 2). When compared between males and females, there were no significant differences for most hematological parameters except for MCHC and RBC counts, respectively ($p<0.05$) (Table 3)

Association between *H. pylori* infection and Platelet counts

Linear regression models related platelet counts per μL of blood (continuous outcomes) to the individual estimates of *H. pylori* infection status (exposures). These showed a significant reduction in mean platelet counts among children infected with *H. pylori* compared to non-infected children (Mean difference: -21.95 $x10^3$/ μL; 95%CI; - 34.3 – -9.58, p=0.001). When the

analysis adjusted for potential confounders such as sex, age, family size, and toilet type, the findings did not materially alter the magnitude of the effect estimate (Table 4). In separate multivariate analysis adjusted for a priori confounders, *H. pylori*-infected individuals had a lower average MPV and RBC than uninfected individuals (Adjusted mean difference: -0.236fl; 95%CI; -0.408 – -0.065, p=0.007, and Adjusted mean difference: -0.118 million/μl; 95%CI; -0.200 – -0.036, p=0.005, respectively Table 4). Additionally, participants infected with *H. pylori* had an elevated WBC compared to uninfected individuals (p=0.02) after adjusting for socio-demographic characteristics (Table 4).

Association between *H. pylori* infection and platelet count category

Table 6 presents the results of multinomial logistic regression analysis for association between *H. pylori* infection and platelet count category (i.e. low, high and normal platelet counts)**.** Children infected with *H. pylori* had 1.26-fold higher odds of having low platelet counts (defined platelet counts <150 x10^3 cells per µL) compared to those of non-infected, though failed to reached statistical significant (Adjusted OR; 1.26; 95%CI:0. 53-3.01, P>0.05) (Table 5). Comparison with reference ranges used for classifying thrombocytopenia and thrombocytosis (platelet count <150x10^3/µL or >450x10^3/µL, respectively) is also reported in Table S1. About 3.2% of *H. pylori* infected children were found to be thrombocytopenic (platelet count <150x10^3/µL).

Association between *H. pylori* infection and MPV category

In separate multinomial logistic model adjusted for potential confounders, children infected with *H. pylori* showed a significant decrease odd of having high MPV (defined MPV> 10.5fl) to those

of non-infected (Adjusted OR:0. 27; 95% CI: 0.17-0.44, P<0.05). Whilst, *H. pylori* infected had higher odds of having low MPV (defined MPV< 7.0fl), though not significant (Adjusted OR: 1.60; 95%CI: 0.25-10.12, P>0.05) (Table 6). Comparison with normal ranges used for classifying high and low MPV level (MPV <7fL or MPV>10.5fL, respectively) is also reported in Table S2. A slightly higher proportion of low MPV level (<7fL) was found in *H. pylori* infected than non-infected (1% vs. 0.2%, respectively) Table S2.

Discussion

This study adds to the evidence on the influence of *H. pylori* infection on platelet parameters among apparently healthy school children in Ethiopia. We found that platelet counts and MPV were significantly lowered in children infected with *H. pylori* than non-infected. We also found that children infected with *H. pylori* were more likely to have platelet counts and MPV below the normal lower limit compared to non-infected

Most studies investigating the link between *H. pylori* infection and platelet parameters to date have been retrospective clinical studies aimed to evaluate the effectiveness of *H. pylori* eradication treatment on ITP patients (10-13), and most reported an increased platelet count after successful eradication of *H.pylori* infection among patients diagnosed with ITP. However, studies in apparently healthy populations before the onset of ITP, particularly those from low-income countries, are remarkably scarce. One study by Umit H and Umit EG in Turkey analyzed platelet count as it related to *H. pylori* infection before the onset of ITP (16), and they reported a significant decrease in mean platelet count among *H. pylori*-positive individuals than those who were *H. pylori*-negative ($p<0.001$), which is consistent with the finding of the current study. Our findings are also consistent with those of another cross-sectional study reported by Raza, et al. (22) from Pakistan and Ali et al (23) from Sudan, who found lower platelet counts in *H. pylori* infected than non-infected. In contrast with these findings, no significant difference in platelet counts between *H. pylori* infected (n=108) and *H. pylori* non-infected patients (n=600) was reported in a cross-sectional study from the Netherlands (24). These inconsistent findings could be due to variations in age, outcome ascertainment, and differences in the method used for the

assessment of *H. pylori* status. More importantly, among these studies, there were differences in the distribution of factors that affect platelet counts and differences in study design.

In this study, a significantly lower MPV level along with low platelets counts in children infected with *H. pylori* compared to non-infected can be contrasted with the previous reports. Two cross-sectional studies in Turkey (16) and Sudan (23) reported a significantly higher MPV level in *H. pylori* infected than non-infected. These authors speculated an ongoing and compensated platelet destruction-production process as possible justification for the increase in MPV. Indeed, a high MPV value is related with an increase in the entry of young platelets into circulation from the bone marrow either due to the high destruction of platelets or severe systemic inflammatory conditions such as rheumatoid arthritis and inflammatory bowel disease (25). However, our study population is distinctly different, as all are apparently healthy children, and severe systemic inflammatory conditions would not be expected to occur in the current study. In contrast to this hypothesis, however, a decreased MPV level has been found in studies related with localized inflammatory disease such as gastrointestinal diseases (20, 26). A study by Matowicka-Karna et al (20) reported significantly lower MPV levels in patients infected with *Entamoeba histolytica* than in controls. Similarly, Mete et al (26) showed a lower MPV in children infected with rotavirus gastroenteritis than in healthy controls. Although the pathogenesis of decreased MPV levels in intestinal inflammation has not been fully explained, it seems reasonable to explain this with the sequestration of large active platelets in the vascular segments of the inflamed bowel, which may cause a relative decrease in the circulation. In our study, the finding that children infected with *H. pylori* have decreased MPV may be related to a localized gastrointestinal inflammation. It has been shown that *H. pylori*-related injury in the

gastric mucosal cells led to local inflammation in the gastric mucosa by neutrophils and other inflammatory cells (27).

Our findings should be interpreted in light of the following limitations. First, we did not measure different strains of *H. pylori*, and in previous studies, a more pronounced reduction in mean platelet counts was observed among individuals infected with the more virulent Cag A+ *H. pylori* strains (12, 28). Although we have no data on CagA serology, a previous study in dyspeptic Ethiopian patients detected CagA genes in 79% of the study subjects (29), suggesting this may be the dominant strain in the population. Second, we could not thoroughly investigate the previous use of anti-platelet agents, which is known to affect platelets indices (25). However, our study population is from a low-income area with limited access to standard treatment, making this an unlikely explanation for the observed association between low platelets indices and *H. pylori* infection. Additionally, we used EDTA anticoagulant agent that has been associated with time-dependent ultrastructural morphological changes of platelets (30), thus affecting MPV values. However, our samples were tested within1-2 hours of blood collection making it unlikely that this affected MPV measurement. A further limitation of the current study is its cross-sectional design, which makes it difficult to attribute causality on the observed association since we didn't have hematological parameters prior to infection. Research employing a longitudinal design is required in the future. Finally, *H. pylori* infection might also be a proxy indicator of other infections or socioeconomic conditions (31). To explore such a possibility, the findings were adjusted for markers of socio-economic status and intestinal parasite infections, none of which significantly modified the effect estimates

Despite these limitations, the main strength of this study is a large population-based study sample, unlike most of the previous studies that have used patients in a clinical setting, thereby minimizing selection bias. We have also used a highly sensitive and specific *H. pylori* stool antigen test (32). Additionally, measurement of the key outcomes (platelets indices) was done using a standard automated hematology analyzer and calibrated on a regular basis according to the manufacturer's guidelines.

Several hypotheses have been proposed regarding the mechanism by which *H. pylori* induces the development of thrombocytopenia. One of them is molecular mimicry, according to which *H. pylori* could induce antibody production in response to antigens that cross-react against various platelet glycoprotein antigens (12). Others have proposed the possibility of *H. pylori* induced platelet aggregation resulted from the interaction of *H. pylori*-bound von Willebrand factor and anti-*H. pylori* (IgG) antibodies with platelet surface antigen (GPIb) (33). Furthermore, enhanced platelet activation from the binding of vacuolating cytotoxin (VacA) virulence factor to multimerin 1 on platelets (34), and the down-regulation of FcγRIIB receptors on monocytes, resulting in increased phagocytic activity by *H. pylori* infection have also been proposed as plausible mechanisms.

Conclusion

In conclusion, this cross-sectional study from a developing country provides further support for an association between *H. pylori* infections and reduced platelet counts and MPV in young Ethiopian children, after controlling for potential confounders. Further research is needed, particularly longitudinal studies, to establish causality.

Declarations

Acknowledgments

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Competing interests

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

Authors' contributions

BT conceived and designed the study and collected data in the field and wrote this manuscript. KB participated in design, performed data analysis and interpretation and prepared the preliminary results. ST, MT, MW, DA and AM participated in data collection, assisted with the design, performed analysis, interpretation of data and the critical review of the manuscript. AT, KD and MW participated in study design and interpretation of data, helped in drafting the manuscript and critically reviewed the manuscript. All authors read and approved the final manuscript.

Ethical Approval

The study was approved by the Departmental Research and Ethics Review Committee (DRERC) at Department of Medical Laboratory Sciences, Addis Ababa University College of Health Sciences, Ethiopia. Written, informed consent was obtained from the legal guardians of the children. Children were requested to give assent prior to data collection. Children were also informed about their ability to withdraw from this study at any time without jeopardizing their right to receive health services. Invasive procedures such as collection of blood samples were fully explained to parents and children, and were carried out using sterile disposable materials.

Availability of data and materials

The datasets during and/or analyzed in the current study will be available from the corresponding author on reasonable request.

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TABLE 1. Socio-demographic characteristics and *H. pylori* infection status of school children in Ziway and Sululta towns, Ethiopia, 2016-2017 (N=971)

Variables	Number	Percent
Sex		
Male	432	44.5
Female	539	55.5
Place of residence		
Urban	546	56.4
Rural	422	43.6
Age		
≤ 5	91	9.4
6-10	425	43.9
11-15	453	46.7
Maternal education		
Informal only	550	56.4
Formal	425	43.6
Maternal occupation		
Housewife	311	32.0
Farmer	207	21.1
Office	155	15.9
Other	298	30.7
Water source		
Indoor pipe	565	57.9
Outdoor pipe	301	30.9
Wells	83	8.5
River and Rain	23	2.4
Family Size		
2-5	630	65.4
6-9	313	32.5
10-13	21	2.2
Type of Toilet		
Flush Toilet	42	4.3
Ventilated Pit	49	5
Traditional Pit	802	82.3
Field	77	7.9
Waste Disposal Site		
Pit	155	16.3
Open Field	199	20.9
Burning	409	43
Garbage Bin	184	19.3
Other	4	0.4

H. pylori status		
Positive	343	36
Negative	611	64

TABLE 2. Distribution of potential confounders and associations with *H. pylori* infection among school children Ziway and Sululta towns, Ethiopia.

Variable	*H. pylori*-Positive	*H. pylori*-Negative	OR*	95% CI	p-value
Sex					
Male	161 (46.9%)	264 (43.4%)	0.868	0.665-1.132	0.295
Female	182 (53.1%)	344 (56.6%)	1		
Place of residence					
Urban	292 (85.6%)	247 (40.7%)	1		
Rural	49 (14.4%)	360 (59.3%)	0.115	0.082-0.162	<0.001
Age					
≤ 5	43 (12.6%)	48 (7.9%)	2.063	1.303-3.266	0.002
6-10	166 (48.7%)	257 (42.2%)	1.488	1.121-1.973	0.006
11-15	132 (38.7%)	304 (49.9%)	1		
Maternal education					
Informal only	166 (48.4%)	371 (60.6%)	0.609	0.467-0.795	<0.001
Formal	117 (51.6%)	241 (39.4%)	1		
Maternal occupation					
Housewife	31 (9%)	267 (43.6%)	0.053	0.028-0.100	<0.001
Farmer	138 (40.2%)	69 (11.3%)	0.917	0.512-1.640	0.769
Office	48 (14%)	102 (16.7%)	0.216	0.117-0.397	<0.001
Other	126 (47.9%)	174 (28.4%)	1		
Water source					
Indoor pipe	282 (82.5%)	274 (44.9%)	1		
Outdoor pipe	44 (12.9%)	251 (41.1%)	0.17	0.119-0.244	<0.001
Open Well	8 (2.3%)	42 (6.9%)	0.185	0.185-0.085	<0.001
Closed Well	4 (1.2%)	24 (3.9%)	0.162	0.055-0.473	0.001
River	4 (1.2%)	18 (1.9%)	0.216	0.072-0.646	0.006
Rainwater	0 (0%)	1 (0.2%)	–	–	–
Family Size					
2-5	219 (64.2%)	398 (65.9%)	1		
6-9	118 (34.6%)	190 (31.5%)	1.129	0.851-1.498	0.402
10-13	4 (1.2%)	16 (2.6%)	0.454	0.150-1.376	0.163
Type of Toilet					
Flush Toilet	10 (2.9%)	30 (4.9%)	0.496	0.239-1.030	0.06
Ventilated Pit	6 (1.8%)	40 (6.6%)	0.223	0.094-0.533	0.001
Field	8 (2.3%)	67 (11%)	0.178	0.084-0.375	<0.001
Traditional Pit	317 (93%)	427 (77.5%)	1		
Waste Disposal Site					
Burning	179 (52.6%)	224 (37.8%)	1		
Open Field	24 (7.1%)	169 (28.5%)	0.178	0.111-0.284	<0.001
Pit	56 (16.5%)	94 (15.9%)	0.746	0.507-1.095	0.135
Garbage Bin	80 (23.5%)	102 (17.2%)	0.981	0.690-1.396	0.917
Other	1 (0.3%)	3 (0.5%)	0.417	0.043-4.044	0.451

*Odds ratio (OR) was calculated using univariate logistic regressions analysis

CI: Confidence interval

TABLE 3. Hematological parameters of school children by sex in Ziway and Sululta towns, Ethiopia.

.

Hematological Parameters	Overall Mean (SD)	Female Mean (SD)	Male Mean (SD)	Mean Difference (95% CI)*	p-value
Hb (g/dL)	14.1 (1.67)	14.2 (1.9)	14.0(1.2)	0.15 (-0.06 – 0.37)	0.172
MCH (pg)	28.7 (10.4)	29 (13.9)	28.4 (2.1)	0.65 (-0.7 – 2.0)	0.298
MCHC (g/L)	50.8 (70.1)	54.8 (77.0)	46.1 (60.5)	8.7 (-0.6 – 17.9)	<0.001
MCV (fL)	84.5 (6.2)	84.3 (6.7)	84.7 (6.0)	-0.4 (-1.2 – 0.5)	0.436
MPV (fL)	9.5 (1.2)	9.6 (1.1)	9.4 (1.3)	0.2 (0.05 – 0.37)	0.983
PLT (10^3 / μL)	326.1 (90.4)	324.3 (95.8)	328.6 (83.7)	-4.3 (-16.2 – 7.7)	0.59
RBC (10^6 /μL)	5.0 (0.6)	5.0 (0.6)	4.9 (0.4)	0.07 (-0.002 – 0.1)	0.026
WBC (10^3 / μL)	7.2 (2.7)	7.1 (2.6)	7.4 (2.7)	-0.4 (-0.7 – -0.003)	0.135

*Independent t-test was used for each parameter to compare the averages hematological parameters by sex.

	n	Mean	SD	Crude Mean Difference (95% CI)	p-value	Adjusted Mean Difference (95% CI)	p-value
Platelet Count (x10^3/μL)							
H. pylori-positive	313	311.5	88.3	-21.95 (-34.3 – -9.580)	**0.001**	-20.801 (-33.506 – -8.096)*	**0.001**
H. pylori-negative	579	333.4	90.6	0 [reference]		0 [reference]	
MPV (fl)							
H. pylori-positive	303	9.08	0.99	-0.69 (-0.85 – -0.53)	**<0.001**	-0.236 (-0.408 – -0.065)**	**0.007**
H. pylori-negative	529	9.7	1.2	0 [reference]		0 [reference]	
RBC (million/μl)							
H. pylori-positive	312	4.8	0.55	-0.3 (-0.38 – -0.23)	**<0.001**	-0.118 (-0.200 – -0.036)+	**0.005**
H. pylori-negative	579	5.1	0.53	0 [reference]		0 [reference]	
WBC (per μl))							
H. pylori-positive	320	7.81	2.48	0.86 (-0.5 – -1.22)	**<0.001**	0.446 (0.053 – 0.839)++	**0.026**
H. pylori-negative	579	6.95	2.87	0 [reference]		0 [reference]	

* Adjusted for toilet type, sex, age, family size
** Adjusted for maternal education, maternal occupation, sex, age, residence
\+ Adjusted for maternal occupation, sex, age, residence
++ Adjusted for age, sex, residence
SD: Stander deviation

TABLE 4. Multivariate generalized linear model of haematological parameters in association with *Helicobacter pylori* infection in school children, Ethiopia

TABLE 5. Association of platelet counts with *Helicobacter pylori* infection according to traditional cut-offs for thrombocytosis and thrombocytopenia in school children, Ethiopia. Multivariate multinomial regression analysis

Platelet count Classification*				
***H. pylori* status**	**Low platelet count** <150 x10^3 platelets per µL		**High platelet count** >450 x10^3 platelets per µL	
	Crude OR (95%CI)	Adjusted **OR**** (95%CI)	Crude OR (95%CI)	Adjusted **OR**** (95%CI)
H. pylori-positive	1.53(0.65-3.59)	1.26 (0.53-3.01)***	0.77(0.46-1.30)	0.70(0.41- 1.21)***
H. pylori-negative	1	1	1	1

*Platelet counts were categorized to form polytomous outcome variable as either low, high, or normal using the cutoffs as described by the British Journal of Haematology for multinomial regression analysis. Normal platelet counts (150-450 platelets per µL) was used as a reference category in Multinomial regression

** Adjusted for sex, age, hemoglobin, WBC,

*** p>0.05

TABLE 6. Association of Mean Platelet Volume (MPV) with *Helicobacter pylori* infection according to the reference interval in school children, Ethiopia. Multivariate multinomial regression analysis

Mean Platelet Volume (MPV) Classification*				
***H. pylori* status**	**Low MPV** (<7fL)		**High MPV** (>10.5 fL)	
	Crude OR (95%CI)	Adjusted **OR**** (95%CI)	Crude OR (95%CI)	Adjusted **OR**** (95%CI)
***H. pylori*-positive**	2.15 (0.36-12.98)	1.60(0.25-10.12)***	0.26 (0.16- 0.41)	0.27(0.17-0.44)****
***H. pylori*-negative**	1	1	1	1

*MPV was categorized to form polytomous outcome variable as either low, high, or normal using the cutoffs described by the British Journal of Haematology for multinomial regression analysis. Normal MPV (7-10.5fL) was used as a reference category in Multinomial regression

** Adjusted for sex, age, place of residence, hemoglobin, WBC

*** p>0.05

**** P<0.05

Table S1. Proportion of low, high and normal platelets counts according to *Helicobacter pylori* infection in school children, Ethiopia

	Platelets count categories *		
***H. pylori* status**	**Low** (<150 x10^3 platelets per μL) n(%)	**High** (>450 x10^3 platelets per μL) n (%)	**Normal** (150x10^3 - 450 x10^3 platelets per μL) n (%)
***H. pylori*-positive**	10 (3.2%)	22 (7.0%)	281(89.9%)
***H. pylori*-negative**	12 (2.1%)	52(9.0%)	515(88.9%)

*Platelet counts were categorized as either low, high, or normal using the cutoffs as described by the British Journal of Haematology

Table S2. Proportion of low, high and normal MPV levels according to *Helicobacter pylori* infection in school children, Ethiopia

	MPV in Classification*		
***H. pylori* status**	**Low** (<7fL) n(%)	**High** (>10.5 fL) n (%)	**Normal** (7-10.5 fL) n (%)
***H. pylori*-positive**	3 (1.0%)	23 (7.6%)	277(91.4%)
***H. pylori*-negative**	2 (0.4%)	129(24.4%)	398 (75.2%)

*Platelet counts were categorized as either low, high, or normal using the cutoffs as described by the British Journal of Haematology

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Association between infection with *Helicobacter pylori (H. pylori)* and Platelet Indices Among School-Aged Children in Central Ethiopia: A cross-sectional study

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ABSTRACT

Objective: Previous clinical studies in adults from developed countries have implicated *Helicobacter pylori* infections in the development of thrombocytopenia. However, studies in children, particularly those from low-income countries, are unusually scarce. We examined the association between *H. pylori* infection and platelet indices in young Ethiopian school children.

Design: Cross sectional study

Setting: This study was conducted in five elementary schools located in central Ethiopia

Participants: Blood and stool samples were collected from 971 children attended in five elementary schools in Ethiopia. *H. pylori* infection was diagnosed using stool antigen and serum antibody tests, and hematological parameters were measured using an automated hematology analyzer. An interviewer-led questionnaire administered to mothers provided information on demographic and lifestyle variables. The independent effects of *H. pylori* infection on platelet indices were determined using multivariate linear and logistic regressions.

Study Outcomes: *H. pylori*-infected children had a lower average platelet counts and Mean Platelet Volume (MPV) than uninfected after adjusting the potential confounders (Adjusted Mean difference: -20.80 x 10^3 /μL; 95% CI; -33.51 – -8.09 x 10^3 /μL, p=0.001, Adjusted Mean difference: -0.236 fl; 95%CI -0.408 – -0.065 fl, p=0.007, respectively). Additionally, *H. pylori* infected children had lower Red Blood cell Counts (RBC) (Adjusted Mean difference: -0.118 million/μl; 95%CI; -0.200 – -0.036, p=0.005) compared to non-infected.

Conclusion: Our study from a developing country provides further support for an association between *H. pylori* infections and reduced platelet indices in young Ethiopian school children,

after controlling for potential confounders. Further research is needed, particularly longitudinal studies to establish causality.

Strength and Limitations

- Exposure to *H. pylori* infection was assessed using highly sensitive and specific *H. pylori* serological tests.
- Key outcomes (platelets indices) was done using a standard automated hematology
- We could not thoroughly investigate the previous use of anti-platelet agents, which is known to affect platelets indices
- The current study is its cross-sectional design, which makes it difficult to attribute causality on the observed association

Introduction

The role of *Helicobacter pylori (H. pylori)* infection as a potential cause of serious upper gastrointestinal diseases has been increasingly appreciated. There is now good evidence that infection with this organism as the principal cause of acute and chronic gastritis, atrophic gastritis (1-3), and widely accepted as the cause of the majority of peptic ulcer diseases and associated complications of bleeding in adults (4, 5). More recently, however, there is growing interest in investigating the effects of *H. pylori* in extra-gastroduodenal diseases (6, 7). Our group's previous work in Ethiopia has found a higher prevalence of anemia and decreased growth trajectory among *H. pylori* infected children compared to non-infected (8, 9). This observation has led us to expand investigations into other extra-gastroduodenal involvement of *H. pylori* in a resource-limited setting. In particular, the effect of *H. pylori* on platelets indices has not been investigated in Ethiopia. Previous studies in various clinical settings from developed countries reported an increased platelet recovery after successful eradication of *H. pylori* infection among patients with idiopathic thrombocytopenic purpura (ITP) (10-13). Additionally, meta-analysis demonstrated that patients receiving treatment had a greater increase in platelet count from baseline compared with untreated controls, regardless of the outcome of eradication therapy (14). However, the possibility of platelet recovery due to the eradication of bacteria other than *H. pylori* or immune modulating effects of the treatment itself is difficult to exclude.

The mechanisms by which *H. pylori* infection can cause low platelet count are still unclear (12, 15), but plausible mechanisms have been proposed. One hypothesis that has attracted attention is that *H. pylori* may hijack the host's immune system through molecular mimicry, where

molecules from the bacteria mimic host antigens and activate T lymphocytes to cause an immune response (12, 15), then the antibody induced by *H. pylori* cross-reacts with platelet glycoprotein antigens and leads to excessive destruction of platelets (15-17).

Whilst the role of *H. pylori* in low platelet counts and ITP disease aetiology is intriguing, most studies to date are conducted in high-income countries on adult populations and lack data in children from low-income countries, where *H. pylori* is a very common bacterial infection, infecting more than 40 % of children (18, 19). Furthermore, most of the available evidence to date comes from retrospective studies of symptomatic ITP patients in clinical settings, which is prone for selection bias and difficult to apply in an apparently healthy population who may have been infected with *H. pylori* sub-clinically prior to ITP. It is therefore important to assess the association between *H. pylori* infection and platelet parameters in apparently healthy populations. This may provide clues for the subclinical link between *H. pylori* and platelet indices prior to ITP diagnosis. The aim of this study was therefore to investigate any possible association between *H. pylori* infection and platelet indices among apparently healthy primary school children in Ethiopia.

Study Setting and Design

A two-part cross-sectional study was conducted in the towns of Ziway and Sululta, which are both located in the Oromia region Ethiopia, approximately 160 km South and 30 km North from the capital city, Addis Ababa, respectively. The region surrounding Sululta town has an altitude of 2450m above sea level and average temperatures are in the range of 15°-18° Celsius, while the town of Ziway has an elevation of 1643 m above sea level and is adjacent to Lake Ziway (Lake Dambal). The populations of the two towns are roughly similar: Ziway's population is estimated to be 43,660 and Sululta's population is estimated to be 49,000. The first part of the data collection took place in Ziway town between June and July of 2016, while the second part of the study occurred in Sululta from April through June 2017. We used a single-stage cluster sampling to recruit participants from the schools. Out of the possible nine governmental primary schools in Sululta town, three (i.e. Laga dima, Wasarbi and Abdi Boru) were selected randomly. Additionally, two primary schools (i.e. Sher and Batu) were included from Ziway town. In each school, students aged 4–14 years, who were willing to provide demographic information and biological specimens participated in this study.

Measurement and Data Collection

We first approached the local health department in both towns and visited each school prior to the beginning of data collection to explain school principals and teachers about the goal and nature of the study. Students were approached through their school principal and asked them to bring their mothers to school. The investigators then invited mother and their child to participate after the objective of the study was explained using a written information sheet. After mothers or

legal guardian of the child signed the written consent form, an interview-administered questionnaire was administered to collect information on selected demographic, life-style, and behavioral factors in both towns. Information was collected but not limited to the student's age, sex, residency, sanitary conditions, hygiene, eating habits, and deworming status. Furthermore, parents' monthly income, educational status, and occupation were collected to determine the student's socioeconomic status. The questionnaire was first designed in English and then translated and pretested in local languages such as Amharic and Oromiffa languages. In addition to the questionnaire data, mothers in both towns were provided with small leak-proof plastic container and clean wooden applicator sticks to bring sufficient stool sample to ascertain the child's *H. pylori* and intestinal parasite infection. Furthermore a 5 mL blood sample was collected from each child using a vacutainer tube and transported to Sher Ethiopia and St. Paul's Hospital laboratories for haematological analysis.

Laboratory testing

H. pylori Stool Antigen Test

H. pylori antigen rapid test was conducted to detect active *H. pylori* infection (Immunotek, USA). The capture antibody used for this enzyme immunoassay was a mixture of monoclonal anti-*H. pylori* antibodies and the detection antibody was a mixture of peroxidase-conjugated monoclonal anti-*H. pylori* antibodies. A small amount of stool was homogenized with a buffer solution, and 2 drops of the stool/ buffer mixture was added to the test well. After 15 minutes, the test was read. The development of 2 lines, the control (C) line and the test (T) line, indicated an *H. pylori*-positive test result, while the development of only the C line indicated a negative

test result. In the instances where the T line was significantly fainter than the C line, the results were interpreted and recorded as positive.

H. pylori Antibody Test

A similar antibody rapid test was conducted to detect any past or current infections, without differentiation between the two. This rapid test was a double antigen chromatographic lateral flow immunoassay, where 1-2 drops of serum were added to the test well and after 15 minutes the test was read. The development of both the C line and the T line indicated a positive test, while the development of only the C line was indicative of a negative test result. As it was with the stool antigen test, when the T line was significantly fainter than the C line, the results were interpreted and recorded as positive.

Platelet measurements

A 2ml of whole blood samples were drawn from a forearm vein, collected into tubes containing ethylenediaminetetraacetic acid (EDTA) between 9:00 and 10:00 am and analyzed within 2 h after venipuncture using an automated haematological analyzer (CELL-DYN 800 Hematology Analyzer (Abbott, USA) and Sysmex KX-21N Hematology Analyzer (Sysmex, Japan) at Sher Ethiopia and St. Paul's Hospitals Hematology laboratory respectively. The analyzers aspirate the blood sample, dilute and count platelets and measure Mean Platelet Volume (MPV). The instruments were monitored daily with normal, high and low controls provided by the manufacturer before running the specimen to ensure quality of haematological analyses. Additionally, the automated hematology analyzer also provide leukocytes and erythrocytes counts, and measures Mean Cell Volume (MCV) and Haemoglobin (Hb), and calculate

Haematocrit, Mean Cell Haemoglobin (MCH), and Mean Cell Haemoglobin Concentration (MCHC).

Outcome and exposure variables

The primary study outcome was platelets counts (cells per μL) and mean platelets volume (MPV) (continuous variables). While "Exposure to *Helicobacter Pylori* infection " was defined as a positive result of either *H. pylori* stool antigen or serum antibody tests.

Statistical Analysis

Demographics and laboratory data from both towns were cleaned coded and merged ready for analysis using IBM SPSS Statistics version 24 (SPSS, Inc., Chicago, IL, USA). Mean and standard deviation for continuous variables and proportions for categorical variables are reported. Prior to investigating the association between *H. pylori* infection and platelet indices, univariate analyses were used to identify the possible confounders. Variables that were associated with both exposure and outcome variables in the crude analysis using statistical significance at p value <0.3 were considered to be possible confounders. These included sex, place of residence, age, family size, maternal education and occupation, water source, toilet type, and waste disposal site. Additionally, we included variables previously shown to be associated with low platelet indices in the literature such as intestinal parasite status (20). The primary outcomes of the current analysis were platelet count (cells per μL) and Mean Platelet Volume (MPV). Our hypothesis that *H. pylori* infection would be associated with lower platelet counts (continuous variable) were assessed using generalized linear models. We first examined the crude mean difference between *H. pylori*-positive and negative individuals, and then we repeated

the analysis while adjusting for the possible confounders using backwards elimination. These analyses were repeated for MPV, RBC, and WBC.

Further analyses were carried out to assess the association between *H. pylori* infection and platelet categories (polytomous outcome variable) using multinomial logistic regression. Multinomial regression is the most appropriate technique in a situation where the dependent variables are categorical and have more than two categories. In our multinomial regression analysis, platelet counts were categorized into three as low (platelets counts <150 $x10^3$ per μL), high (>450 $x10^3$ per μL), or normal (150$x10^3$ - 450 $x10^3$ per μL). Platelet count <150$x10^3$/μL or >450$x10^3$/μL was used for classification of thrombocytopenia or thrombocytosis respectively. We also categorized MPV level either low (<7 fL), high (>10.5 fL), or normal (7-10.5 fL) using the cutoffs as described by the British Journal of Hematology, respectively (21). Covariates were kept in the model if they changed the coefficient of exposure (*H. pylori* infection) by > 10 % or if they were independently associated with the outcome at $p < 0.10$. Probability values < 0.05 were considered statistically significant for main effects. Similar pattern of demographic and life style distributions was observed among study subject who had complete outcome data and all respondents using sensitivity analysis (Data not shown)

Patient and public involvement

Patients and public were not involved in the development of the research question, the design of the study, the recruitment and the conduct of the research. They were informed regarding the research goals and parameters to be measured before starting the study.

Results

Selected demographic characteristics and H. pylori infection status

A total of 1038 school children were invited to participant in the study, of 971 (93.5%) and 955 (92.0%) provided demographic information and biological specimens respectively. Of these, 55.5% (539/971) were female and a slight majority 56.4 % (546/971) were living in a rural area. The age range of the study participants was 4 to 15 years (mean age, 9.95). Most mothers 57.9 %(565/971) reported using indoor-pipe water as their primary drinking source, and 82.3% (802/971) used traditional pit toilet. Maternal demographic characteristics showed that 56.4% (550/971) of the mothers did not have a formal education, and 32.0% (311/971) were housewives. The prevalence of *H. pylori* infection was 35.9% (343/954) (Table 1).

Univariate analysis for relationships between potential confounders and H. pylori infection

The crude association between demographic variables and infection with *H. pylori* was analyzed using univariate logistic regression. Sex, place of residence, age, maternal education and occupation, water source, family size, toilet type and site of waste disposal were all found to be potential confounders for *H. pylori* infection (Table 2). When compared between males and females, there were no significant differences for most hematological parameters except for MCHC and RBC counts, respectively ($p<0.05$) (Table S1)

Association between *H. pylori* infection and Platelet counts

Linear regression models related platelet counts per μL of blood (continuous outcomes) to the individual estimates of *H. pylori* infection status (exposures). These showed a significant reduction in mean platelet counts among children infected with *H. pylori* compared to non-

infected children (Mean difference: -21.95 x10^3/ µL; 95%CI; - 34.3 – -9.58, p=0.001). When the analysis adjusted for potential confounders such as sex, age, family size, and toilet type, the findings did not materially alter the magnitude of the effect estimate (Table 3). In separate multivariate analysis adjusted for a priori confounders, *H. pylori*-infected individuals had a lower average MPV and RBC than uninfected individuals (Adjusted mean difference: -0.236fl; 95%CI; -0.408 – -0.065, p=0.007, and Adjusted mean difference: -0.118 million/µl; 95%CI; -0.200 – -0.036, p=0.005, respectively Table 3). Additionally, participants infected with *H. pylori* had an elevated WBC compared to uninfected individuals (p=0.02) after adjusting for socio-demographic characteristics (Table 3).

Association between *H. pylori* infection and platelet count category

Table 4 presents the results of multinomial logistic regression analysis for association between *H. pylori* infection and platelet count category (i.e. low, high and normal platelet counts)**.** Children infected with *H. pylori* had 1.26-fold higher odds of having low platelet counts (defined platelet counts <150 x10^3 cells per µL) compared to those of non-infected, though failed to reached statistical significant (Adjusted OR; 1.26; 95%CI:0. 53-3.01, P>0.05) (Table 4). Comparison with reference ranges used for classifying thrombocytopenia and thrombocytosis (platelet count <150x10^3/µL or >450x10^3/µL, respectively) is also reported in Table S2. About 3.2% of *H. pylori* infected children were found to be thrombocytopenic (platelet count <150x10^3/µL).

Association between *H. pylori* infection and MPV category

In separate multinomial logistic model adjusted for potential confounders, children infected with *H. pylori* showed a significant decrease odd of having high MPV (defined MPV> 10.5fl) to those of non-infected (Adjusted OR**:**0. 27; 95% CI: 0.17-0.44, P<0.05). Whilst, *H. pylori* infected had higher odds of having low MPV (defined MPV< 7.0fl), though not significant (Adjusted OR: 1.60; 95%CI: 0.25-10.12, P>0.05) (Table 5). Comparison with normal ranges used for classifying high and low MPV level (MPV <7fL or MPV>10.5fL, respectively) is also reported in Table S3. A slightly higher proportion of low MPV level (<7fL) was found in *H. pylori* infected than non-infected (1% vs. 0.2%, respectively) Table S3.

Discussion

This study adds to the evidence on the influence of *H. pylori* infection on platelet parameters among apparently healthy school children in Ethiopia. We found that platelet counts and MPV were significantly lowered in children infected with *H. pylori* than non-infected. We also found that children infected with *H. pylori* were more likely to have platelet counts and MPV below the normal lower limit compared to non-infected

Most studies investigating the link between *H. pylori* infection and platelet parameters to date have been retrospective clinical studies aimed to evaluate the effectiveness of *H. pylori* eradication treatment on ITP patients (10-13), and most reported an increased platelet count after successful eradication of *H.pylori* infection among patients diagnosed with ITP. However, studies in apparently healthy populations before the onset of ITP, particularly those from low-income countries, are remarkably scarce. One study by Umit H and Umit EG in Turkey analyzed platelet count as it related to *H. pylori* infection before the onset of ITP (16), and they reported a significant decrease in mean platelet count among *H. pylori*-positive individuals than those who were *H. pylori*-negative ($p<0.001$), which is consistent with the finding of the current study. Our findings are also consistent with those of another cross-sectional study reported by Raza, et al. (22) from Pakistan and Ali et al (23) from Sudan, who found lower platelet counts in *H. pylori* infected than non-infected. In contrast with these findings, no significant difference in platelet counts between *H. pylori* infected (n=108) and *H. pylori* non-infected patients (n=600) was reported in a cross-sectional study from the Netherlands (24). These inconsistent findings could be due to variations in age, outcome ascertainment, and differences in the method used for the

assessment of *H. pylori* status. More importantly, among these studies, there were differences in the distribution of factors that affect platelet counts and differences in study design.

In this study, a significantly lower MPV level along with low platelets counts in children infected with *H. pylori* compared to non-infected can be contrasted with the previous reports. Two cross-sectional studies in Turkey (16) and Sudan (23) reported a significantly higher MPV level in *H. pylori* infected than non-infected. These authors speculated an ongoing and compensated platelet destruction-production process as possible justification for the increase in MPV. Indeed, a high MPV value is related with an increase in the entry of young platelets into circulation from the bone marrow either due to the high destruction of platelets or severe systemic inflammatory conditions such as rheumatoid arthritis and inflammatory bowel disease (25). However, our study population is distinctly different, as all are apparently healthy children, and severe systemic inflammatory conditions would not be expected to occur in the current study. In contrast to this hypothesis, however, a decreased MPV level has been found in studies related with localized inflammatory disease such as gastrointestinal diseases (20, 26). A study by Matowicka-Karna et al (20) reported significantly lower MPV levels in patients infected with *Entamoeba histolytica* than in controls. Similarly, Mete et al (26) showed a lower MPV in children infected with rotavirus gastroenteritis than in healthy controls. Although the pathogenesis of decreased MPV levels in intestinal inflammation has not been fully explained, it seems reasonable to explain this with the sequestration of large active platelets in the vascular segments of the inflamed bowel, which may cause a relative decrease in the circulation. In our study, the finding that children infected with *H. pylori* have decreased MPV may be related to a localized gastrointestinal inflammation. It has been shown that *H. pylori*-related injury in the

gastric mucosal cells led to local inflammation in the gastric mucosa by neutrophils and other inflammatory cells (27).

Our findings should be interpreted in light of the following limitations. First, we did not measure different strains of *H. pylori*, and in previous studies, a more pronounced reduction in mean platelet counts was observed among individuals infected with the more virulent Cag A+ *H. pylori* strains (12, 28). Although we have no data on CagA serology, a previous study in dyspeptic Ethiopian patients detected CagA genes in 79% of the study subjects (29), suggesting this may be the dominant strain in the population. Second, we could not thoroughly investigate the previous use of anti-platelet agents, which is known to affect platelets indices (25). However, our study population is from a low-income area with limited access to standard treatment, making this an unlikely explanation for the observed association between low platelets indices and *H. pylori* infection. Additionally, we used EDTA anticoagulant agent that has been associated with time-dependent ultrastructural morphological changes of platelets (30), thus affecting MPV values. However, our samples were tested within1-2 hours of blood collection making it unlikely that this affected MPV measurement. A further limitation of the current study is its cross-sectional design, which makes it difficult to attribute causality on the observed association since we didn't have hematological parameters prior to infection. Research employing a longitudinal design is required in the future. Finally, *H. pylori* infection might also be a proxy indicator of other infections or socioeconomic conditions (31). To explore such a possibility, the findings were adjusted for markers of socio-economic status and intestinal parasite infections, none of which significantly modified the effect estimates. The possibility of reverse causation is difficult to fully eliminate as all, but acquisition of *H. pylori* infection in

developing countries usually occurs during infancy and very early life (32, 33), which limits the possibility that low MPV level preceded *H. pylori* infection.

Studies in clinical settings has implicated that infection other than *H. pylori* such as malaria (34) and viral infection (35) may lead to low platelets level. Although, this remain the possibility, our study was conducted on apparently healthy school children from April to July before the peak season for malaria transmission in Ethiopia, and Sululta town, where the majority of the school located, has an altitude of 2450m above sea level suggesting malaria transmission is expected to be very low and unlikely to be an alternative explanation for our findings. Furthermore, we had data on intestinal parasite status and C-reactive protein (as proxy indicator for overall infections and inflammations) but none of these significantly modified the effect estimates (Data not shown).

Despite these limitations, the main strength of this study is a large population-based study sample, unlike most of the previous studies that have used patients in a clinical setting, thereby minimizing selection bias. We have also used a highly sensitive and specific *H. pylori* stool antigen test (36). Additionally, measurement of the key outcomes (platelets indices) was done using a standard automated hematology analyzer and calibrated on a regular basis according to the manufacturer's guidelines.

Several hypotheses have been proposed regarding the mechanism by which *H. pylori* induces the development of thrombocytopenia. One of them is molecular mimicry, according to which *H.*

pylori could induce antibody production in response to antigens that cross-react against various platelet glycoprotein antigens (12). Others have proposed the possibility of *H. pylori* induced platelet aggregation resulted from the interaction of *H. pylori*-bound von Willebrand factor and anti-*H. pylori* (IgG) antibodies with platelet surface antigen (GPIb) (37). Furthermore, enhanced platelet activation from the binding of vacuolating cytotoxin (VacA) virulence factor to multimerin 1 on platelets (38), and the down-regulation of FcγRIIB receptors on monocytes, resulting in increased phagocytic activity by *H. pylori* infection have also been proposed as plausible mechanisms.

Conclusion

In conclusion, this cross-sectional study from a developing country provides further support for an association between *H. pylori* infections and reduced platelet counts and MPV in young Ethiopian children, after controlling for potential confounders. Further research is needed, particularly longitudinal studies, to establish causality.

Declarations

Acknowledgments

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Competing interests

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

Authors' contributions

BT conceived and designed the study and collected data in the field and wrote this manuscript. KB participated in the design, performed data analysis and interpretation and prepared the preliminary results. SW, MT, MW, DA and AM participated in data collection, assisted with the design, performed analysis, interpretation of data and the critical review of the manuscript. AT, KD and MW participated in study design and interpretation of data, and critically reviewed the manuscript. All authors read and approved the final manuscript.

Ethical Approval

The study was approved by the Departmental Research and Ethics Review Committee (DRERC) at the Department of Medical Laboratory Sciences, Addis Ababa University College of Health Sciences, Ethiopia. Written, informed consent was obtained from the legal guardians of the children. Children were requested to give assent prior to data collection. Children were also informed about their ability to withdraw from this study at any time without jeopardizing their right to receive health services. Invasive procedures such as collection of blood samples were fully explained to parents and children, and were carried out using sterile disposable materials.

Availability of data and materials

The datasets during and/or analyzed in the current study will be available from the corresponding author on reasonable request.

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TABLE 1. Socio-demographic characteristics and *H. pylori* infection status of school children in Ziway and Sululta towns, Ethiopia, 2016-2017 (N=971)

Variables	Number	Percent
Sex		
Male	432	44.5
Female	539	55.5
Place of residence		
Urban	546	56.4
Rural	422	43.6
Age		
≤ 5	91	9.4
6-10	425	43.9
11-15	453	46.7
Maternal education		
Informal only	550	56.4
Formal	425	43.6
Maternal occupation		
Housewife	311	32.0
Farmer	207	21.1
Office	155	15.9
Other	298	30.7
Water source		
Indoor pipe	565	57.9
Outdoor pipe	301	30.9
Wells	83	8.5
River and Rain	23	2.4
Family Size		
2-5	630	65.4
6-9	313	32.5
10-13	21	2.2
Type of Toilet		
Flush Toilet	42	4.3
Ventilated Pit	49	5
Traditional Pit	802	82.3
Field	77	7.9
Waste Disposal Site		
Pit	155	16.3
Open Field	199	20.9
Burning	409	43
Garbage Bin	184	19.3
Other	4	0.4

H. pylori status		
Positive	343	36
Negative	611	64

TABLE 2. Distribution of potential confounders and associations with *H. pylori* infection among school children Ziway and Sululta towns, Ethiopia.

Variable	*H. pylori*-Positive	*H. pylori*-Negative	OR*	95% CI	p-value
Sex					
Male	161 (46.9%)	264 (43.4%)	0.868	0.665-1.132	0.295
Female	182 (53.1%)	344 (56.6%)	1		
Place of residence					
Urban	292 (85.6%)	247 (40.7%)	1		
Rural	49 (14.4%)	360 (59.3%)	0.115	0.082-0.162	<0.001
Age					
≤ 5	43 (12.6%)	48 (7.9%)	2.063	1.303-3.266	0.002
6-10	166 (48.7%)	257 (42.2%)	1.488	1.121-1.973	0.006
11-15	132 (38.7%)	304 (49.9%)	1		
Maternal education					
Informal only	166 (48.4%)	371 (60.6%)	0.609	0.467-0.795	<0.001
Formal	117 (51.6%)	241 (39.4%)	1		
Maternal occupation					
Housewife	31 (9%)	267 (43.6%)	0.053	0.028-0.100	<0.001
Farmer	138 (40.2%)	69 (11.3%)	0.917	0.512-1.640	0.769
Office	48 (14%)	102 (16.7%)	0.216	0.117-0.397	<0.001
Other	126 (47.9%)	174 (28.4%)	1		
Water source					
Indoor pipe	282 (82.5%)	274 (44.9%)	1		
Outdoor pipe	44 (12.9%)	251 (41.1%)	0.17	0.119-0.244	<0.001
Open Well	8 (2.3%)	42 (6.9%)	0.185	0.185-0.085	<0.001
Closed Well	4 (1.2%)	24 (3.9%)	0.162	0.055-0.473	0.001
River	4 (1.2%)	18 (1.9%)	0.216	0.072-0.646	0.006
Rainwater	0 (0%)	1 (0.2%)	–	–	–
Family Size					
2-5	219 (64.2%)	398 (65.9%)	1		
6-9	118 (34.6%)	190 (31.5%)	1.129	0.851-1.498	0.402
10-13	4 (1.2%)	16 (2.6%)	0.454	0.150-1.376	0.163
Type of Toilet					
Flush Toilet	10 (2.9%)	30 (4.9%)	0.496	0.239-1.030	0.06
Ventilated Pit	6 (1.8%)	40 (6.6%)	0.223	0.094-0.533	0.001
Field	8 (2.3%)	67 (11%)	0.178	0.084-0.375	<0.001
Traditional Pit	317 (93%)	427 (77.5%)	1		
Waste Disposal Site					
Burning	179 (52.6%)	224 (37.8%)	1		
Open Field	24 (7.1%)	169 (28.5%)	0.178	0.111-0.284	<0.001
Pit	56 (16.5%)	94 (15.9%)	0.746	0.507-1.095	0.135
Garbage Bin	80 (23.5%)	102 (17.2%)	0.981	0.690-1.396	0.917
Other	1 (0.3%)	3 (0.5%)	0.417	0.043-4.044	0.451

*Odds ratio (OR) was calculated using univariate logistic regressions analysis

CI: Confidence interval

TABLE 3. Multivariate generalized linear model of hematological parameters in association with *Helicobacter pylori* infection in school children, Ethiopia

	n	Mean	SD	Crude Mean Difference (95% CI)	p-value	Adjusted Mean Difference (95% CI)	p-value
Platelet Count (x10^3/μL)							
H. pylori-positive	313	311.5	88.3	-21.95 (-34.3 – -9.580)	**0.001**	-20.801 (-33.506 – -8.096)*	**0.001**
H. pylori-negative	579	333.4	90.6	0 [reference]		0 [reference]	
MPV (fl)							
H. pylori-positive	303	9.08	0.99	-0.69 (-0.85 – -0.53)	**<0.001**	-0.236 (-0.408 – -0.065)**	**0.007**
H. pylori-negative	529	9.7	1.2	0 [reference]		0 [reference]	
RBC (million/μl)							
H. pylori-positive	312	4.8	0.55	-0.3 (-0.38 – -0.23)	**<0.001**	-0.118 (-0.200 – -0.036)+	**0.005**
H. pylori-negative	579	5.1	0.53	0 [reference]		0 [reference]	
WBC (per μl))							
H. pylori-positive	320	7.81	2.48	0.86 (-0.5 – -1.22)	**<0.001**	0.446 (0.053 – 0.839)++	**0.026**
H. pylori-negative	579	6.95	2.87	0 [reference]		0 [reference]	

* Adjusted for toilet type, sex, age, family size
** Adjusted for maternal education, maternal occupation, sex, age, residence
\+ Adjusted for maternal occupation, sex, age, residence
++ Adjusted for age, sex, residence
SD: Stander deviation

TABLE 4. Association of platelet counts with *Helicobacter pylori* infection according to traditional cut-offs for thrombocytosis and thrombocytopenia in school children, Ethiopia. Multivariate multinomial regression analysis

Platelet count Classification*				
***H. pylori* status**	**Low platelet count** <150 x10^3 platelets per µL		**High platelet count** >450 x10^3 platelets per µL	
	Crude OR (95%CI)	Adjusted **OR**** (95%CI)	Crude OR (95%CI)	Adjusted **OR**** (95%CI)
H. pylori-positive	1.53(0.65-3.59)	1.26 (0.53-3.01)***	0.77(0.46-1.30)	0.70(0.41- 1.21)***
H. pylori-negative	1	1	1	1

*Platelet counts were categorized to form polytomous outcome variable as either low, high, or normal using the cutoffs as described by the British Journal of Hematology for multinomial regression analysis. Normal platelet counts (150-450 platelets per µL) was used as a reference category in Multinomial regression
** Adjusted for sex, age, hemoglobin, WBC,
*** p>0.05

TABLE 5. Association of Mean Platelet Volume (MPV) with *Helicobacter pylori* infection according to the reference interval in school children, Ethiopia. Multivariate multinomial regression analysis

Mean Platelet Volume (MPV) Classification*				
***H. pylori* status**	**Low MPV** (<7fL)		**High MPV** (>10.5 fL)	
	Crude OR (95%CI)	Adjusted **OR**** (95%CI)	Crude OR (95%CI)	Adjusted **OR**** (95%CI)
***H. pylori*-positive**	2.15 (0.36-12.98)	1.60(0.25-10.12)***	0.26 (0.16- 0.41)	0.27(0.17-0.44)****
***H. pylori*-negative**	1	1	1	1

*MPV was categorized to form polytomous outcome variable as either low, high, or normal using the cutoffs described by the British Journal of Hematology for multinomial regression analysis. Normal MPV (7-10.5fL) was used as a reference category in Multinomial regression
** Adjusted for sex, age, place of residence, hemoglobin, WBC
*** p>0.05
**** P<0.05

Table S1. Hematological parameters of school children by sex in Ziway and Sululta towns, Ethiopia.

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Hematological Parameters	Overall Mean (SD)	Female Mean (SD)	Male Mean (SD)	Mean Difference (95% CI)*	p-value
Hb (g/dL)	14.1 (1.67)	14.2 (1.9)	14.0(1.2)	0.15 (-0.06 – 0.37)	0.172
MCH (pg)	28.7 (10.4)	29 (13.9)	28.4 (2.1)	0.65 (-0.7 – 2.0)	0.298
MCHC (g/L)	50.8 (70.1)	54.8 (77.0)	46.1 (60.5)	8.7 (-0.6 – 17.9)	<0.001
MCV (fL)	84.5 (6.2)	84.3 (6.7)	84.7 (6.0)	-0.4 (-1.2 – 0.5)	0.436
MPV (fL)	9.5 (1.2)	9.6 (1.1)	9.4 (1.3)	0.2 (0.05 – 0.37)	0.983
PLT (10^3 / μL)	326.1 (90.4)	324.3 (95.8)	328.6 (83.7)	-4.3 (-16.2 – 7.7)	0.59
RBC (10^6 /μL)	5.0 (0.6)	5.0 (0.6)	4.9 (0.4)	0.07 (-0.002 – 0.1)	0.026
WBC (10^3 / μL)	7.2 (2.7)	7.1 (2.6)	7.4 (2.7)	-0.4 (-0.7 – -0.003)	0.135

*Independent t-test was used for each parameter to compare the averages hematological parameters by sex.

Table S2. Proportion of low, high and normal platelets counts according to *Helicobacter pylori* infection in school children, Ethiopia

	Platelets count categories *		
***H. pylori* status**	**Low** (<150 x10^3 platelets per μL) n(%)	**High** (>450 x10^3 platelets per μL) n (%)	**Normal** (150x10^3 - 450 x10^3 platelets per μL) n (%)
***H. pylori*-positive**	10 (3.2%)	22 (7.0%)	281(89.9%)
***H. pylori*-negative**	12 (2.1%)	52(9.0%)	515(88.9%)

*Platelet counts were categorized as either low, high, or normal using the cutoffs as described by the British Journal of Haematology

Table S3. Proportion of low, high and normal MPV levels according to *Helicobacter pylori* infection in school children, Ethiopia

	MPV in Classification*		
***H. pylori* status**	**Low** (<7fL) n(%)	**High** (>10.5 fL) n (%)	**Normal** (7-10.5 fL) n (%)
***H. pylori*-positive**	3 (1.0%)	23 (7.6%)	277(91.4%)
***H. pylori*-negative**	2 (0.4%)	129(24.4%)	398 (75.2%)

*Platelet counts were categorized as either low, high, or normal using the cutoffs as described by the British Journal of Haematology

STROBE Statement—Checklist of items that should be included in reports of ***cross-sectional studies***

	Item No	Recommendation	Page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	5-6
Objectives	3	State specific objectives, including any prespecified hypotheses	6
Methods			
Study design	4	Present key elements of study design early in the paper	7
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	7
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	7
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	10
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	8-9
Bias	9	Describe any efforts to address potential sources of bias	11, lines 12-14
Study size	10	Explain how the study size was arrived at	7, line 10-15
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	10-11
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	10-11
		(*b*) Describe any methods used to examine subgroups and interactions	
		(*c*) Explain how missing data were addressed	
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	11, lines 12-14
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	12, line 3-5
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	12, line 1-8
		(b) Indicate number of participants with missing data for each variable of interest	
Outcome data	15*	Report numbers of outcome events or summary measures	12. line 12
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	12-14

		(*b*) Report category boundaries when continuous variables were categorized	
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	
Discussion			
Key results	18	Summarise key results with reference to study objectives	15, line 1-5
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	17, lines 3-22
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	18-19
Generalisability	21	Discuss the generalisability (external validity) of the study results	18-19
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	19, line 15-20

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Association between infection with Helicobacter pylori (H. pylori) and Platelet Indices Among School-Aged Children in Central Ethiopia: A cross-sectional study

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Association between infection with *Helicobacter pylori (H. pylori)* and Platelet Indices Among School-Aged Children in Central Ethiopia: A cross-sectional study

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ABSTRACT

Objective: Previous clinical studies in adults from developed countries have implicated *Helicobacter pylori* infections in the development of thrombocytopenia. However, studies in children, particularly those from low-income countries, are unusually scarce. We examined the association between *H. pylori* infection and platelet indices in young Ethiopian school children.

Design: Cross-sectional study

Setting: This study was conducted in five elementary schools located in central Ethiopia

Participants: Blood and stool samples were collected from 971 children across five elementary schools in Ethiopia. *H. pylori* infection was diagnosed using stool antigen and serum antibody tests, and hematological parameters were measured using an automated hematological analyzer. An interviewer-led questionnaire administered to mothers provided information on demographic and lifestyle variables. The independent effects of *H. pylori* infection on platelet indices were determined using multivariate linear and logistic regressions.

Study Outcomes: *H. pylori*-infected children had a lower average platelet count and Mean Platelet Volume (MPV) than uninfected after adjusting the potential confounders (Adjusted Mean difference: -20.80 x 10^3 /μL; 95% CI; -33.51 – -8.09 x 10^3 /μL, p=0.001, Adjusted Mean difference: -0.236 fl; 95%CI -0.408 – -0.065 fl, p=0.007, respectively). Additionally, *H. pylori* infected children had lower Red Blood cell Counts (RBC) (Adjusted Mean difference: -0.118 million/μl; 95%CI; -0.200 – -0.036, p=0.005) compared to non-infected.

Conclusion: Our study from a developing country provides further support for an association between *H. pylori* infections and reduced platelet indices in young Ethiopian school children,

after controlling for potential confounders. Further research is needed, particularly longitudinal studies, to establish causality.

Strength and Limitations

- Exposure to *H. pylori* infection was assessed using highly sensitive and specific *H. pylori* serological tests.
- Key outcomes (platelets indices) was done using a standard automated hematology
- We could not thoroughly investigate the previous use of anti-platelet agents, which is known to affect platelets indices
- The current study is its cross-sectional design, which makes it difficult to attribute causality on the observed association

Introduction

The role of *Helicobacter pylori (H. pylori)* infection as a potential cause of serious upper gastrointestinal diseases has been increasingly appreciated. There is now good evidence that infection with this organism is the principal cause of acute and chronic gastritis, atrophic gastritis (1-3), and is widely accepted as the cause of the majority of peptic ulcer diseases and associated complications of bleeding in adults (4, 5). More recently, however, there is growing interest in investigating the effects of *H. pylori* in extra-gastroduodenal diseases (6, 7). Our group's previous work in Ethiopia has found a higher prevalence of anemia and decreased growth trajectory among *H. pylori* infected children compared to non-infected (8, 9). This observation has led us to expand investigations into other extra-gastroduodenal involvement of *H. pylori* in a resource-limited setting. In particular, the effect of *H. pylori* on platelet indices has not been investigated in Ethiopia. Previous studies in various clinical settings from developed countries reported an increased platelet recovery after successful eradication of *H. pylori* infection among patients with idiopathic thrombocytopenic purpura (ITP) (10-13). Additionally, meta-analysis demonstrated that patients receiving treatment had a greater increase in platelet count from baseline compared with untreated controls, regardless of the outcome of eradication therapy (14). However, the possibility of platelet recovery due to the eradication of bacteria other than *H. pylori* or immune modulating effects of the treatment itself is difficult to exclude.

The mechanisms by which *H. pylori* infection can cause low platelet count are still unclear (12, 15), but plausible mechanisms have been proposed. One hypothesis that has attracted attention is that *H. pylori* may hijack the host's immune system through molecular mimicry, where molecules from the bacteria mimic host antigens and activate T lymphocytes to cause an immune

response (12, 15). The antibody induced by *H. pylori* then cross-reacts with platelet glycoprotein antigens and leads to excessive destruction of platelets (15-17).

While the role of *H. pylori* in low platelet counts and ITP disease etiology is intriguing, most studies to date are conducted in high-income countries on adult populations and lack data in children from low-income countries, where *H. pylori* is a very common bacterial infection, infecting more than 40% of children (18, 19). Furthermore, most of the available evidence to date comes from retrospective studies of symptomatic ITP patients in clinical settings, which is prone for selection bias and is difficult to apply in an apparently healthy population who may have been infected with *H. pylori* sub-clinically prior to ITP. It is therefore important to assess the association between *H. pylori* infection and platelet parameters in apparently healthy populations. This may provide clues for the subclinical link between *H. pylori* and platelet indices prior to ITP diagnosis. Therefore, the aim of this study was to investigate any possible association between *H. pylori* infection and platelet indices among apparently healthy primary school children in Ethiopia.

Study Setting and Design

A two-part cross-sectional study was conducted in the towns Ziway and Sululta, which are both located in the Oromia region of Ethiopia, approximately 160 km South and 30 km North from the capital city, Addis Ababa, respectively. The region surrounding Sululta town has an altitude of 2450m above sea level, with average temperatures ranging from 15°-18°C, while the town of Ziway has an elevation of 1643m above sea level and is adjacent to Lake Ziway (Lake Dambal). The populations of the two towns are roughly similar: Ziway's population is estimated to be 43,660 and Sululta's population is estimated to be 49,000. The first part of the data collection took place in Ziway town between June and July of 2016, while the second part of the study occurred in Sululta from April through June 2017. We used a single-stage cluster sampling to recruit participants from the schools. Out of the possible nine governmental primary schools in Sululta town, three (Laga dima, Wasarbi and Abdi Boru) were selected randomly. Additionally, two primary schools (Sher and Batu) were included from Ziway town. In each school, students aged 4–14 years, who were willing to provide demographic information and biological specimens, participated in this study.

Measurement and Data Collection

We first approached the local health department in both towns and visited each school prior to the beginning of data collection to explain to school principals and teachers about the goal and nature of the study. Students were approached through their school principal and asked to bring their mothers to school. The investigators then invited mothers and their children to participate after the objective of the study was explained using a written information sheet. After the mother

or legal guardian of the child signed the written consent form, an interviewer-led questionnaire was administered to collect information on selected demographic, life-style, and behavioral factors in both towns. Information that was collected included, but was not limited to, the student's age, sex, residency, sanitary conditions, hygiene, eating habits, and deworming status. Furthermore, parents' monthly income, educational status, and occupation were collected to determine the student's socioeconomic status. The questionnaire was first designed in English and then translated and pretested in local languages, such as Amharic and Oromiffa languages. In addition to the questionnaire data, mothers in both towns were provided with small leak-proof plastic container and clean wooden applicator sticks to bring a sufficient stool sample to ascertain the child's *H. pylori* and intestinal parasite infection status. Furthermore, a 5 mL blood sample was collected from each child using a vacutainer tube and transported to Sher Ethiopia and St. Paul's Hospital laboratories for hematological analysis.

Laboratory testing

H. pylori Stool Antigen Test

H. pylori antigen rapid test was conducted to detect active *H. pylori* infection (Immunotek, USA). The capture antibody used for this enzyme immunoassay was a mixture of monoclonal anti-*H. pylori* antibodies and the detection antibody was a mixture of peroxidase-conjugated monoclonal anti-*H. pylori* antibodies. A small amount of stool was homogenized with a buffer solution, and 2 drops of the stool/ buffer mixture was added to the test well. After 15 minutes, the test was read. The development of 2 lines, the control (C) line and the test (T) line, indicated an *H. pylori*-positive test result, while the development of only the C line indicated a negative

test result. In the instances where the T line was significantly fainter than the C line, the results were interpreted and recorded as positive.

H. pylori Antibody Test

A similar antibody rapid test was conducted to detect any past or current infections, without differentiation between the two. This rapid test was a double antigen chromatographic lateral flow immunoassay, where 1-2 drops of serum were added to the test well and after 15 minutes the test was read. The development of both the C line and the T line indicated a positive test result, while the development of only the C line was indicative of a negative test result. As it was with the stool antigen test, when the T line was significantly fainter than the C line, the results were interpreted and recorded as positive.

Platelet measurements

2mL of whole blood samples were drawn from a forearm vein, collected into tubes containing ethylenediaminetetraacetic acid (EDTA) between 9:00 and 10:00 am and analyzed within 2 hours after venipuncture using an automated hematological analyzer: CELL-DYN 800 Hematology Analyzer (Abbott, USA) and Sysmex KX-21N Hematology Analyzer (Sysmex, Japan) at Sher Ethiopia and St. Paul's Hospitals Hematology laboratory, respectively. The analyzers aspirate the blood sample, dilute, and count platelets and measure Mean Platelet Volume (MPV). The instruments were monitored daily with normal, high and low controls provided by the manufacturer before running the specimens to ensure quality of hematological analyses. Additionally, the automated hematology analyzers also provided leukocyte and erythrocyte counts, and measured Mean Cell Volume (MCV) and hemoglobin (Hb), and

calculated hematocrit, Mean Cell Hemoglobin (MCH), and Mean Cell Hemoglobin Concentration (MCHC).

Outcome and exposure variables

The primary study outcome was platelet counts (cells per μL) and mean platelet volume (MPV) (continuous variables). "Exposure to *Helicobacter pylori* infection" was defined as a positive result of either *H. pylori* stool antigen or serum antibody tests.

Statistical Analysis

Demographics and laboratory data from both towns were cleaned, coded, and merged for proper analysis using IBM SPSS Statistics version 24 (SPSS, Inc., Chicago, IL, USA). Mean and standard deviation for continuous variables and proportions for categorical variables are reported. Prior to investigating the association between *H. pylori* infection and platelet indices, univariate analyses were used to identify the possible confounders. Variables that were associated with both exposure and outcome variables in the crude analysis using statistical significance at $p < 0.3$ were considered to be possible confounders. These included sex, place of residence, age, family size, maternal education and occupation, water source, toilet type, and waste disposal site. Additionally, we included variables previously shown in the literature to be associated with low platelet indices, such as intestinal parasite status (20). The primary outcomes of the current analysis were platelet count (cells per μL) and Mean Platelet Volume (MPV). Our hypothesis that *H. pylori* infection would be associated with lower platelet counts (continuous variable) was assessed using generalized linear models. We first examined the crude mean difference between *H. pylori*-positive and negative individuals, and then we repeated the analysis

while adjusting for the possible confounders using backwards elimination. These analyses were repeated for MPV, RBC, and WBC.

Further analyses were carried out to assess the association between *H. pylori* infection and platelet categories (polytomous outcome variable) using multinomial logistic regression. Multinomial regression is the most appropriate technique in a situation where the dependent variables are categorical and have more than two categories. In our multinomial regression analysis, platelet counts were sorted into three categories: low (<150 $x10^3$ per μL), high (>450 $x10^3$ per μL), or normal (150$x10^3$ - 450 $x10^3$ per μL). Platelet counts less than 150$x10^3$/μL or greater than 450$x10^3$/μL were used for classification of thrombocytopenia or thrombocytosis, respectively. We also categorized MPV level as low (<7 fL), high (>10.5 fL), or normal (7-10.5 fL), respectively. The cutoffs for both the platelet count classifications and the MPV classifications were set as described by the British Journal of Haematology (21). Covariates were kept in the model if they changed the coefficient of exposure (*H. pylori* infection) by > 10 % or if they were independently associated with the outcome at $p < 0.10$. Probability values < 0.05 were considered statistically significant for main effects. A similar pattern of demographic and life style distributions was observed among study subjects who had complete outcome data and all respondents using sensitivity analysis (Data not shown).

Patient and public involvement

Patients and public were not involved in the development of the research question, the design of the study, the recruitment and the conduct of the research. They were informed regarding the research goals and parameters to be measured before starting the study.

Results

Selected demographic characteristics and H. pylori infection status

A total of 1038 school children were invited to participate in the study, of which 971 (93.5%) and 955 (92.0%) provided demographic information and biological specimens, respectively. Of these, 55.5% (539/971) were female and a slight majority 56.4 % (546/971) were living in a rural area. The age range of the study participants was 4 to 14 years (mean age, 9.95). Most mothers 57.9% (565/971) reported using indoor-pipe water as their primary drinking source, and 82.3% (802/971) used a traditional pit toilet. Maternal demographic characteristics showed that 56.4% (550/971) of the mothers did not have a formal education, and 32.0% (311/971) were housewives. The prevalence of *H. pylori* infection was 35.9% (343/954) (Table 1).

Univariate analysis for relationships between potential confounders and H. pylori infection

The crude association between demographic variables and infection with *H. pylori* was analyzed using univariate logistic regression. Sex, place of residence, age, maternal education and occupation, water source, family size, toilet type and site of waste disposal were all found to be potential confounders for *H. pylori* infection (Table S1). When compared between males and females, there were no significant differences for most hematological parameters except for MCHC and RBC counts ($p<0.05$) (Table S2).

Association between *H. pylori* infection and Platelet counts

Linear regression models related platelet counts per μL of blood (continuous outcomes) to the individual estimates of *H. pylori* infection status (exposures). These showed a significant reduction in mean platelet counts among children infected with *H. pylori* compared to non-

infected children (Mean difference: -21.95 x10^3/ µL; 95%CI; - 34.3 – -9.58, p=0.001). When the analysis was adjusted for potential confounders such as sex, age, family size, and toilet type, the findings did not materially alter the magnitude of the effect estimate (Table 2). In separate multivariate analysis adjusted for a priori confounders, *H. pylori*-infected individuals had a lower average MPV and RBC than uninfected individuals (Adjusted mean difference: -0.236fl; 95%CI; -0.408 – -0.065, p=0.007, and Adjusted mean difference: -0.118 million/µl; 95%CI; -0.200 – -0.036, p=0.005, respectively) (Table 2). Additionally, participants infected with *H. pylori* had an elevated WBC compared to uninfected individuals (p=0.02) after adjusting for socio-demographic characteristics (Table 2).

Association between *H. pylori* infection and platelet count category

Table 3 presents the results of multinomial logistic regression analysis for association between *H. pylori* infection and platelet count category (i.e. low, high and normal platelet counts)**.** Children infected with *H. pylori* had 1.26-fold higher odds of having low platelet counts (defined platelet counts <150 x10^3 cells per µL) compared to those of non-infected, though we failed to reach statistical significance (Adjusted OR; 1.26; 95%CI: 0.53-3.01, P>0.05) (Table 3). Comparison with reference ranges used for classifying thrombocytopenia and thrombocytosis (platelet count <150x10^3/µL or >450x10^3/µL, respectively) is also reported in Table S3. About 3.2% of *H. pylori* infected children were found to be thrombocytopenic (platelet count <150x10^3/µL).

Association between *H. pylori* infection and MPV category

In a separate multinomial logistic model adjusted for potential confounders, children infected with *H. pylori* showed a significantly decreased odds of having high MPV (defined MPV> 10.5fl) compared to those of non-infected (Adjusted OR**:** 0.27; 95% CI: 0.17-0.44, P<0.05). Further, *H. pylori* infected children had higher odds of having low MPV (defined MPV< 7.0fl), though not significant (Adjusted OR: 1.60; 95%CI: 0.25-10.12, P>0.05) (Table 4). Comparison with normal ranges used for classifying high and low MPV level (MPV <7fL or MPV>10.5fL, respectively) is also reported in Table S4. A slightly higher proportion of low MPV level (<7fL) was found in *H. pylori* infected children than non-infected (1% vs. 0.4%, respectively) (Table S4).

Discussion

This study adds to the evidence on the influence of *H. pylori* infection on platelet parameters among apparently healthy school children in Ethiopia. We found that platelet counts and MPV were significantly lowered in children infected with *H. pylori* compared to non-infected children. We also found that children infected with *H. pylori* were more likely to have platelet counts and MPV below the normal lower limit compared to those not infected.

Most studies investigating the link between *H. pylori* infection and platelet parameters to date have been retrospective clinical studies aimed to evaluate the effectiveness of *H. pylori* eradication treatment on ITP patients (10-13), and most reported an increased platelet count after successful eradication of *H. pylori* infection among those patients diagnosed with ITP. However, studies in apparently healthy populations, particularly those from low-income countries, are remarkably scarce. One study by Umit H and Umit EG in Turkey analyzed platelet count as it related to *H. pylori* infection before the onset of ITP (16), and they reported a significant decrease in mean platelet count among *H. pylori*-positive individuals than those who were *H. pylori*-negative ($p<0.001$), which is consistent with the finding of the current study. Our findings are also consistent with those of another cross-sectional study reported by Raza, et al. (22) from Pakistan and Ali et al (23) from Sudan, who found lower platelet counts in *H. pylori* infected individuals compared to non-infected. In contrast with these findings, no significant difference in platelet counts between *H. pylori* infected (n=108) and *H. pylori* non-infected patients (n=600) was reported in a cross-sectional study from the Netherlands (24). These inconsistent findings could be due to variations in age, outcome ascertainment, and differences in the method used for

the assessment of *H. pylori* status. More importantly, among these studies, there were differences in the distribution of factors that affect platelet counts and differences in study design.

In this study, a significantly lower MPV along with low platelet counts in children infected with *H. pylori* compared to non-infected can be contrasted with previous reports. Two cross-sectional studies in Turkey (16) and Sudan (23) reported a significantly higher MPV level in *H. pylori* infected individuals than non-infected. These authors speculated an ongoing and compensated platelet destruction-production process as possible justification for the increase in MPV. Indeed, a high MPV value is related with an increase in the entry of young platelets into circulation from the bone marrow either due to the high destruction of platelets or severe systemic inflammatory conditions such as rheumatoid arthritis and inflammatory bowel disease (25). However, our study population is distinctly different, as all are apparently healthy children, and severe systemic inflammatory conditions would not be expected to occur in the current study. In contrast to this hypothesis, however, a decreased MPV level has been found in studies related with localized inflammatory disease such as gastrointestinal diseases (20, 26). A study by Matowicka-Karna et al (20) reported significantly lower MPV levels in patients infected with *Entamoeba histolytica* than in controls. Similarly, Mete et al (26) showed a lower MPV in children infected with rotavirus gastroenteritis than in healthy controls. Although the pathogenesis of decreased MPV levels in intestinal inflammation has not been fully explained, it seems reasonable to explain this with the sequestration of large active platelets in the vascular segments of the inflamed bowel, which may cause a relative decrease in the circulation. In our study, the finding that children infected with *H. pylori* have decreased MPV may be related to a localized gastrointestinal inflammation. It has been shown that *H. pylori*-related injury in the

gastric mucosal cells led to local inflammation in the gastric mucosa by neutrophils and other inflammatory cells (27).

Our findings should be interpreted in light of the following limitations. First, we did not measure different strains of *H. pylori*, and in previous studies, a more pronounced reduction in mean platelet counts was observed among individuals infected with the more virulent CagA+ *H. pylori* strains (12, 28). Although we have no data on CagA serology, a previous study in dyspeptic Ethiopian patients detected CagA genes in 79% of the study subjects (29), suggesting that this may be the dominant strain in the population. Second, we could not thoroughly investigate the previous use of anti-platelet agents, which is known to affect platelets indices (25). However, our study population is from a low-income area with limited access to standard treatment, making this an unlikely explanation for the observed association between low platelets indices and *H. pylori* infection. Additionally, we used EDTA anticoagulant agent that has been associated with time-dependent ultrastructural morphological changes of platelets (30), thus affecting MPV values. However, our samples were tested within 1-2 hours of blood collection making it unlikely that this affected MPV measurement. A further limitation of the current study is its cross-sectional design, which makes it difficult to attribute causality (i.e. *H. Pylori* infection lead to low platelet indices directly) since we did not have information on ITP or hematological parameters prior to infection. Research employing a longitudinal design is required in the future. Finally, *H. pylori* infection might also be a proxy indicator of other infections or socioeconomic conditions (31). To explore such a possibility, the findings were adjusted for markers of socioeconomic status and intestinal parasite infections, none of which significantly modified the effect estimates. The possibility of reverse causation is difficult to fully eliminate, but acquisition

of *H. pylori* infection in developing countries usually occurs during infancy and very early life (32, 33), which limits the possibility that low MPV level preceded *H. pylori* infection.

Studies in clinical settings have implicated that infections other than *H. pylori,* such as malaria (34) and viral infections (35), may lead to low platelet levels. Although this remains a possibility, our study was conducted on apparently healthy school children from April to July before the peak season for malaria transmission in Ethiopia. Moreover, Sululta town, where the majority of the schools were located, has an altitude of 2450m above sea level, so with temporal and spatial considerations, malaria transmission is expected to be very low and unlikely to be an alternative explanation for our findings. Furthermore, we had data on intestinal parasite status and C-reactive protein (as proxy indicator for overall infections and inflammations), and none of these significantly modified the effect estimates (Data not shown).

Despite these limitations, the main strength of this study is the large, population-based study sample, unlike most of the previous studies that have used patients in a clinical setting, thereby minimizing our selection bias. We have also used a highly sensitive and specific *H. pylori* stool antigen test (36). Additionally, measurement of the key outcomes (platelet indices) was done using a standard automated hematology analyzer, which was calibrated on a regular basis according to the manufacturer's guidelines.

Several hypotheses have been proposed regarding the mechanism by which *H. pylori* induces the development of thrombocytopenia. One of them is molecular mimicry, according to which *H.*

pylori could induce antibody production in response to antigens that cross-react against various platelet glycoprotein antigens (12). Others have proposed the possibility of *H. pylori*-induced platelet aggregation resulting from the interaction of *H. pylori*-bound von Willebrand factor and anti-*H. pylori* (IgG) antibodies with platelet surface antigen (GPIb) (37). Furthermore, enhanced platelet activation from the binding of vacuolating cytotoxin (VacA) virulence factor to multimerin 1 on platelets (38), and the down-regulation of FcγRIIB receptors on monocytes, resulting in increased phagocytic activity by *H. pylori* infection have also been proposed as plausible mechanisms.

Conclusion

In conclusion, this cross-sectional study from a developing country provides further support for an association between *H. pylori* infections and reduced platelet counts and MPV in young Ethiopian children, after controlling for potential confounders. Further research is needed, particularly longitudinal studies, to establish causality.

Declarations

Acknowledgments

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Competing interests

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

Authors' contributions

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

Ethical Approval

The study was approved by the Departmental Research and Ethics Review Committee (DRERC) at Department of Medical Laboratory Sciences, Addis Ababa University College of Health Sciences, Ethiopia. Written, informed consent was obtained from the legal guardians of the children. Children were requested to give assent prior to data collection. Children were also informed about their ability to withdraw from this study at any time without jeopardizing their right to receive health services. Invasive procedures, such as collection of blood samples, were fully explained to parents and children and were carried out using sterile disposable materials.

Availability of data and materials

The datasets during and/or analyzed in the current study will be available from the corresponding author on reasonable request.

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TABLE 1. Socio-demographic characteristics and *H. pylori* infection status of school children in Ziway and Sululta towns, Ethiopia, 2016-2017 (N=971)

Variables	Number	Percent
Sex		
Male	432	44.5
Female	539	55.5
Place of residence		
Urban	546	56.4
Rural	422	43.6
Age		
≤ 5	91	9.4
6-10	425	43.9
11-14	453	46.7
Maternal education		
Informal only	550	56.4
Formal	425	43.6
Maternal occupation		
Housewife	311	32.0
Farmer	207	21.1
Office	155	15.9
Other	298	30.7
Water source		
Indoor pipe	565	57.9
Outdoor pipe	301	30.9
Wells	83	8.5
River and Rain	23	2.4
Family Size		
2-5	630	65.4
6-9	313	32.5
10-13	21	2.2
Type of Toilet		
Flush Toilet	42	4.3
Ventilated Pit	49	5
Traditional Pit	802	82.3
Field	77	7.9
H. pylori status		
Positive	343	36
Negative	611	64

TABLE 2. Multivariate generalized linear model of hematological parameters in association with *Helicobacter pylori* infection in school children, Ethiopia

	n	Mean	SD	Crude Mean Difference (95% CI)	p-value	Adjusted Mean Difference (95% CI)	p-value
Platelet Count (x10^3/μL)							
H. pylori-positive	313	311.5	88.3	-21.95 (-34.3 – -9.580)	**0.001**	-20.801 (-33.506 – -8.096)*	**0.001**
H. pylori-negative	579	333.4	90.6	0 [reference]		0 [reference]	
MPV (fl)							
H. pylori-positive	303	9.08	0.99	-0.69 (-0.85 – -0.53)	**<0.001**	-0.236 (-0.408 – -0.065)**	**0.007**
H. pylori-negative	529	9.7	1.2	0 [reference]		0 [reference]	
RBC (million/μl)							
H. pylori-positive	312	4.8	0.55	-0.3 (-0.38 – -0.23)	**<0.001**	-0.118 (-0.200 – -0.036)+	**0.005**
H. pylori-negative	579	5.1	0.53	0 [reference]		0 [reference]	
WBC (per μl))							
H. pylori-positive	320	7.81	2.48	0.86 (-0.5 – -1.22)	**<0.001**	0.446 (0.053 – 0.839)++	**0.026**
H. pylori-negative	579	6.95	2.87	0 [reference]		0 [reference]	

* Adjusted for toilet type, sex, age, family size
** Adjusted for maternal education, maternal occupation, sex, age, residence
\+ Adjusted for maternal occupation, sex, age, residence
++ Adjusted for age, sex, residence
SD: Stander deviation

TABLE 3. Association of platelet counts with *Helicobacter pylori* infection according to traditional cut-offs for thrombocytosis and thrombocytopenia in school children, Ethiopia. Multivariate multinomial regression analysis

Platelet count Classification*				
***H. pylori* status**	**Low platelet count** <150 x10^3 platelets per μL		**High platelet count** >450 x10^3 platelets per μL	
	Crude OR (95%CI)	Adjusted **OR**** (95%CI)	Crude OR (95%CI)	Adjusted **OR**** (95%CI)
H. pylori-positive	1.53(0.65-3.59)	1.26 (0.53-3.01)***	0.77(0.46-1.30)	0.70(0.41- 1.21)***
H. pylori-negative	1	1	1	1

*Platelet counts were categorized to form polytomous outcome variable as either low, high, or normal using the cutoffs as described by the British Journal of Haematology for multinomial regression analysis. Normal platelet counts (150-450 platelets per μL) was used as a reference category in Multinomial regression

** Adjusted for sex, age, hemoglobin, WBC,

*** p>0.05

TABLE 4. Association of Mean Platelet Volume (MPV) with *Helicobacter pylori* infection according to the reference interval in school children, Ethiopia. Multivariate multinomial regression analysis

Mean Platelet Volume (MPV) Classification*				
***H. pylori* status**	**Low MPV** (<7fL)		**High MPV** (>10.5 fL)	
	Crude OR (95%CI)	Adjusted **OR**** (95%CI)	Crude OR (95%CI)	Adjusted **OR**** (95%CI)
***H. pylori*-positive**	2.15 (0.36-12.98)	1.60(0.25-10.12)***	0.26 (0.16- 0.41)	0.27(0.17-0.44)****
***H. pylori*-negative**	1	1	1	1

*MPV was categorized to form polytomous outcome variable as either low, high, or normal using the cutoffs described by the British Journal of Haematology for multinomial regression analysis. Normal MPV (7-10.5fL) was used as a reference category in Multinomial regression

** Adjusted for sex, age, place of residence, hemoglobin, WBC

*** p>0.05

**** P<0.05

TABLE S1. Distribution of potential confounders and associations with *H. pylori* infection among school children Ziway and Sululta towns, Ethiopia.

Variable	*H. pylori*-Positive	*H. pylori*-Negative	OR*	95% CI	p-value
Sex					
Male	161 (46.9%)	264 (43.4%)	0.868	0.665-1.132	0.295
Female	182 (53.1%)	344 (56.6%)	1		
Place of residence					
Urban	292 (85.6%)	247 (40.7%)	1		
Rural	49 (14.4%)	360 (59.3%)	0.115	0.082-0.162	<0.001
Age					
≤5	43 (12.6%)	48 (7.9%)	2.063	1.303-3.266	0.002
6-10	166 (48.7%)	257 (42.2%)	1.488	1.121-1.973	0.006
11-15	132 (38.7%)	304 (49.9%)	1		
Maternal education					
Informal only	166 (48.4%)	371 (60.6%)	0.609	0.467-0.795	<0.001
Formal	117 (51.6%)	241 (39.4%)	1		
Maternal occupation					
Housewife	31 (9%)	267 (43.6%)	0.053	0.028-0.100	<0.001
Farmer	138 (40.2%)	69 (11.3%)	0.917	0.512-1.640	0.769
Office	48 (14%)	102 (16.7%)	0.216	0.117-0.397	<0.001
Other	126 (47.9%)	174 (28.4%)	1		
Water source					
Indoor pipe	282 (82.5%)	274 (44.9%)	1		
Outdoor pipe	44 (12.9%)	251 (41.1%)	0.17	0.119-0.244	<0.001
Open Well	8 (2.3%)	42 (6.9%)	0.185	0.185-0.085	<0.001
Closed Well	4 (1.2%)	24 (3.9%)	0.162	0.055-0.473	0.001
River	4 (1.2%)	18 (1.9%)	0.216	0.072-0.646	0.006
Rainwater	0 (0%)	1 (0.2%)	–	–	–
Family Size					
2-5	219 (64.2%)	398 (65.9%)	1		
6-9	118 (34.6%)	190 (31.5%)	1.129	0.851-1.498	0.402
10-13	4 (1.2%)	16 (2.6%)	0.454	0.150-1.376	0.163
Type of Toilet					
Flush Toilet	10 (2.9%)	30 (4.9%)	0.496	0.239-1.030	0.06
Ventilated Pit	6 (1.8%)	40 (6.6%)	0.223	0.094-0.533	0.001
Field	8 (2.3%)	67 (11%)	0.178	0.084-0.375	<0.001
Traditional Pit	317 (93%)	427 (77.5%)	1		
Waste Disposal Site					
Burning	179 (52.6%)	224 (37.8%)	1		
Open Field	24 (7.1%)	169 (28.5%)	0.178	0.111-0.284	<0.001
Pit	56 (16.5%)	94 (15.9%)	0.746	0.507-1.095	0.135
Garbage Bin	80 (23.5%)	102 (17.2%)	0.981	0.690-1.396	0.917
Other	1 (0.3%)	3 (0.5%)	0.417	0.043-4.044	0.451

*Odds ratio (OR) was calculated using univariate logistic regressions analysis
CI: Confidence interval

TABLE S2. Hematological parameters of school children by sex in Ziway and Sululta towns, Ethiopia.

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Hematological Parameters	Overall Mean (SD)	Female Mean (SD)	Male Mean (SD)	Mean Difference (95% CI)*	p-value
Hb (g/dL)	14.1 (1.67)	14.2 (1.9)	14.0(1.2)	0.15 (-0.06 – 0.37)	0.172
MCH (pg)	28.7 (10.4)	29 (13.9)	28.4 (2.1)	0.65 (-0.7 – 2.0)	0.298
MCHC (g/L)	50.8 (70.1)	54.8 (77.0)	46.1 (60.5)	8.7 (-0.6 – 17.9)	<0.001
MCV (fL)	84.5 (6.2)	84.3 (6.7)	84.7 (6.0)	-0.4 (-1.2 – 0.5)	0.436
MPV (fL)	9.5 (1.2)	9.6 (1.1)	9.4 (1.3)	0.2 (0.05 – 0.37)	0.983
PLT (10^3 / μL)	326.1 (90.4)	324.3 (95.8)	328.6 (83.7)	-4.3 (-16.2 – 7.7)	0.59
RBC (10^6 /μL)	5.0 (0.6)	5.0 (0.6)	4.9 (0.4)	0.07 (-0.002 – 0.1)	0.026
WBC (10^3 / μL)	7.2 (2.7)	7.1 (2.6)	7.4 (2.7)	-0.4 (-0.7 – -0.003)	0.135

*Independent t-test was used for each parameter to compare the averages hematological parameters by sex.

Table S3. Proportion of low, high and normal platelets counts according to *Helicobacter pylori* infection in school children, Ethiopia

	Platelets count categories *		
***H. pylori* status**	**Low** (<150 x10^3 platelets per μL) n(%)	**High** (>450 x10^3 platelets per μL) n (%)	**Normal** (150x10^3 - 450 x10^3 platelets per μL) n (%)
***H. pylori*-positive**	10 (3.2%)	22 (7.0%)	281(89.9%)
***H. pylori*-negative**	12 (2.1%)	52(9.0%)	515(88.9%)

*Platelet counts were categorized as either low, high, or normal using the cutoffs as described by the British Journal of Haematology

Table S4. Proportion of low, high and normal MPV levels according to *Helicobacter pylori* infection in school children, Ethiopia

	MPV in Classification*		
***H. pylori* status**	**Low** (<7fL) n(%)	**High** (>10.5 fL) n (%)	**Normal** (7-10.5 fL) n (%)
***H. pylori*-positive**	3 (1.0%)	23 (7.6%)	277(91.4%)
***H. pylori*-negative**	2 (0.4%)	129(24.4%)	398 (75.2%)

*Platelet counts were categorized as either low, high, or normal using the cutoffs as described by the British Journal of Haematology

STROBE Statement—Checklist of items that should be included in reports of ***cross-sectional studies***

	Item No	Recommendation	Page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	5-6
Objectives	3	State specific objectives, including any prespecified hypotheses	6
Methods			
Study design	4	Present key elements of study design early in the paper	7
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	7
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	7
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	10
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	8-9
Bias	9	Describe any efforts to address potential sources of bias	11, lines 12-14
Study size	10	Explain how the study size was arrived at	7, line 10-15
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	10-11
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	10-11
		(*b*) Describe any methods used to examine subgroups and interactions	
		(*c*) Explain how missing data were addressed	
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	11, lines 12-14
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	12, line 3-5
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	12, line 1-8
		(b) Indicate number of participants with missing data for each variable of interest	
Outcome data	15*	Report numbers of outcome events or summary measures	12. line 12
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	12-14

		(*b*) Report category boundaries when continuous variables were categorized	
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	
Discussion			
Key results	18	Summarise key results with reference to study objectives	15, line 1-5
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	17, lines 3-22
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	18-19
Generalisability	21	Discuss the generalisability (external validity) of the study results	18-19
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	19, line 15-20

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.