Addis Ababa University, College of Health Sciences, Department of Medical Laboratory Sciences.

PREVALENCE OF *H.PYLORI* INFECTION IN PEDIATRIC PATIENTS WHO IS CLINICALLY DIAGNOSED FOR GASTROENTERITIS IN BEHAM SPECIALIZED CHILDREN'S HIGHER CLINIC, ADDIS ABABA, ETHIOPIA.

By: AHMED KEMAL (BSc)

Advisor: GEBERU MULUGETA (MSc)

A thesis submitted to Addis Ababa University, college of health sciences, department of medical laboratory science in partial fulfilment of the requirements for the degree of masters in clinical laboratory science speciality in Diagnostic and public health microbiology.

MAY, 2014

Addis Ababa

Ethiopia

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Approved by the Examining Board

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Chairman, Dep. Graduate Committee	Signature
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Advisor	Signature
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Examiner	Signature

ACKNOWLEDGEMENT

My heartfelt thanks go to the Almighty God next to my family.

My thanks go to Addis Ababa University, Department of Medical Laboratory Sciences for arranging a program to conduct my MSc Thesis work.

I would like to express my sincere gratitude and deep appreciation to my advisor, Gebru Mulugeta (MSc) whose advice and support made this work fruitful. His guidance was very clear since the beginning of the process and his input always valuable in term of giving direction and helps to solve problems in this thesis.

My very great gratitude also goes to Dr. Mulu Aberha (expert paediatrician) for his unlimited and encouraging moral and material support throughout the research work.

My special thanks also go staffs working in Beham Paediatric higher clinic laboratory; and clients who participated in this study.

ACRONYMS

Ag	Antigen
CD4	Cluster of Differential
GUTH	Gondar University Teaching Hospital
HP	Helicobacter pylori
HpSA	Helicobacter pylori stool Antigen
IL	Interleukin
IFN	Interferon
PCR	Polymerization Chain Reaction
PPIs	Proton Pump Inhibitors
SPSS	Software Package for Social Study
TASRH	Tikur Anbessa Specialized Referral Hospital
TLR	Toll Like Receptor

ABSTRACT

Background: The public health impact of *Helicobacter pylori* (HP) infection is gradually becoming obvious, the bacterium now being implicated as an aetiologic agent in a variety of gastric diseases. In developing countries, Hp infection is markedly more prevalent at younger ages than in developed countries. According to World Gastroenterology Organisation (WGO) 2010 the prevalence of HP in Ethiopia was 48% in age between 2–4, 80% at the age of 6 and 95% in adult's population.

Objective: To determine the prevalence of *H.pylori* infection among paediatric patients who's clinically diagnosed for gastroenteritis.

Method: A cross sectional study was conducted to determine the prevalence of *H.pylori* infection among pediatric patient in private pediatric clinic, Addis Ababa Ethiopia. The study was conduct from March 3rd to May 11th 2014. Non-probability convenient sampling technique was used to collect the data. The stool samples were tested according to the manufacture instruction and procedure. Information from the laboratory analysis and questionnaires were entered into SPSS, version.17 and odd ratio, 95%CI were calculated to measure the degree of relationship between risk factors for *H. pylori* infection.

Result: A total of two hundred twenty one paediatric patients having upper gastrointestinal symptoms because of a suspicion of *H. pylori* infection participated in this prospective study at Beham specialized children's higher clinic between March 2014 and May 2014.Among the study subjects 114(51.6%) were girls and 107 (48.4%) were boys aged from 9months to 15years with the mean age of 6.29 [95% CI 5.9-6.7]. *Helicobacter pylori* antigens were detected in 57 of the 221children giving an overall prevalence of 25.8% (95%CI 18.8-31), 27.2% in female and 24.3% in male children. The frequency of *H. pylori* colonization was higher in females than in males. Of the 114 females enrolled in the study, *H. pylori* antigen was detected in the stools of 31 (27.2%) against 26 (24.3%) of the 107 males (OR = 0.85 95%CI=0.46-1.57). Age of acquisition was one year and five month.

Conclusions:

The prevalence of *H. pylori* infection is 25.8%. This is high among children in the study area. Infection also is acquired at very early age. Household crowding is one of the factors that enhance the infection.

TABLE OF CONTENTS

CONTENTS PAGE

LIST OF TABLES

CHAPTER ONE
INTRODUCTION
1.1 BACKGROUND

The first known reference to microorganisms which have been identified as Helicobacter began before 19th century, with the description of spirochaetes in the gastric mucosa of animals, by Bizzozero in 1893, and Solomon in 1896, in the stomach of cats and dogs. The first observation of spiral-shaped micro organism in the human stomach was made in 1906 by Krienitz. Since that time and until the eighties, several observations of these organisms were reported in gastric tissue either from post-mortem individuals or from patients with peptic ulcer and gastric carcinoma (1).

In the early 1980s, gastroenterologist Barry Marshal and his pathologist colleague, Robert Warren, found spiral-shaped bacteria in about half of the routine biopsies, obtained from patients attending the gastroenterology consultation, and their presence was closely associated with mucosal inflammation (2).

As a result of the accelerating research on both microbiological aspects and the pathogenic role of these newly discovered bacteria, the role of *H. pylori* in duodenal and gastric ulcer disease was established and reported in a consensus statement in 1994. Also, theories of an etiological link between *H. pylori* and gastric cancer and mucosa-associated lymphoid tissue (MALT)-lymphomas prompted a number of epidemiological studies (3).

H pylori cause acute and chronic gastritis, and can cause duodenal and gastric ulcers. There is strong epidemiological evidence to implicate *H.pylori* gastritis in marginal B cell mucosal lymphomas. Although these significant diseases are typically found among adults, there are clear parallels with gastro duodenal disease in children. In particular, Peptic ulceration, abdominal pain in the absence of peptic ulceration and Gastro-oesophageal reflux diseases are paediatric disorders have been associated with *H pylori* (4).

The natural history of *H.pylori* infection in children has not yet been extensively studied, but there are several reports that affected children develop a chronic gastritis, localised especially in

gastric antrum, similar to adult. The majority of infected children remain asymptomatic, but the inflammatory response may result in an ulcerogenic process. Also the prevalence of *H.pylori* associated peptic ulcer in children is not clearly known. It is thought to be low, based on the studies of large paediatric endoscopy unit which report an incidence of 5-9 new peptic ulcer, cases per year. *H.pylori* is crucial factor in the pathogenesis of peptic ulcer, especially duodenal ulcer, since almost all children with the disease were positive for the bacterium (5).

1.2 STATEMENT OF THE PROBLEM

The public health impact of *Helicobacter pylori* (HP) infection is gradually becoming obvious, the bacterium now being implicated as an aetiologic agent in a variety of gastric diseases (6).

The overall prevalence is high in developing countries and lower in developed countries and within areas of different countries. There may be similarly wide variations in the prevalence between more affluent urban populations and rural populations. The principal reasons for these variations involve socioeconomic differences between populations. Transmission of Hp is largely by the oral–oral or fecal–oral routes. A lack of proper sanitation, safe drinking water, and basic hygiene, as well as poor diets and overcrowding, play a role in determining the overall prevalence of infection (6, 7).

In developing countries, Hp infection is markedly more prevalent at younger ages than in developed countries. According to World Gastroenterology Organisation (WGO) 2010 the prevalence of HP in Ethiopia were 48% in age between 2–4, 80% at the age of 6 and 95% in adults population(7).

In many reports it has been estimated that between 15% to 20% of people infected with *H. pylori* will develop ulcers. Some evidence also links *H. pylori* infection to gastric cancer, gastric mucosa-associated lymphoid tissue (MALT) lymphoma, and perhaps pancreatic cancer and cardiovascular disease (8).

There are studies that show the seroprevalence of *H. pylori* in different countries. These studies found that the prevalence vary with geographic location, ethnicity, and demographic factors of the studied population (9).

There are few studies that were conducted on the seroprevalence of *H. pylori* in adult dyspeptic patient in Ethiopia including those at Tikur Anbessa Hospital (TAH), Addis Ababa (A.A), Felege Hiwot Hospital, Bahir Dar and Gondar University Teaching Hospital (GUTH) (10). Even though these studies are conducted they are not up-to-date and didn't consider the prevalence of *H. pylori* infection in paediatric patients. In addition to this gap, the prevalence of

H.pylori infection is not well understood in the study area and also age of acquisition was not described in previous studies in Ethiopia.

In order to design preventive strategies, the explanation of the mode of spread of the pathogen is essential ; designed to assess its prevalence in children remains poorly understood, therefore this study was assessed the prevalence of *H. pylori* infection in paediatric patients and its associated risk factors.

1.3 SIGNIFICANCE OF THE STUDY

Previous studies have attempted to identify risk factors for *H. pylori* infection in older children or adults whose environment has changed as they grow. Because childhood appears to be the principal period during which *H. pylori* is acquired in developing countries where most children are infected by the age of 10 years, paediatric studies would likely address the period of acquisition of H. pylori and more accurately identify epidemiological risk factors for *H. Pylori* infection(11).

The data that would be generated could give an overview to the prevalence and risk factors of *H. pylori* in the area where published information was limited. The finding be support for to take some public health measure like testing susceptible paediatric patients with dyspeptic symptoms and treating them, and for prevention and control of the infection around the studied area and also for the country.

CHAPTER TWO
2. LITERATURE REVIEW

2.1 TREND IN PREVALENCE

In the Western world a number of studies have also reported a high prevalence of the organism in children (12). The difference in the rate of childhood acquisition of infection is probably responsible for the differences seen, in the prevalence of infection, between developed and developing countries. The prevalence of infection and the incidence of gastric cancer are higher in Asia, South America, and the Caribbean than in Europe and the United States (13).

Retrospective seroepidemiological studies have shown a cohort effect consistent with the hypothesis that infection is mainly acquired in early childhood. In a rural village of Linqu Country, Shandong Province, China, a study of 98 children found that nearly 70% of those aged 5-6 years were infected with the organism, a rate similar to that reported for adults in that area, suggesting that most infection takes place early in childhood (12).

Infection with this organism is relatively common in Africa and the organism is the main cause of at least 90% of duodenal ulcers and 70% of gastric ulcers. Studies conducted in various parts of Africa have revealed high seroprevalence of infection (61-100%) which differs from country to country and between different racial groups within each country (10, 13).
Childhood acquisition is the rule with more than 50% of all children in Africa being infected by the age of 10 years (14).

Other reports also show a wide variation of infection rates, with anti-*H. pylori* IgG antibodies reported in 85.6% in 215 dyspeptic individuals in Ethiopia. Kidd et al. (1999) documented a prevalence of 25 % and 97% in Uganda and Ghana respectively (15). Nabwera et al. (2000) in their study observed a high prevalence among Kenyan children aged between 3 - 5 years, indicating that most children in the study area were infected before they reached their third birthday(16). In Mozambique, Carrilho et al. (2009) reported a high prevalence of 94.5% while in Cameroon Ndip et al. (2008) equally documented a high prevalence of 92.2% in their study population. In Ethiopia, a prevalence of 93% was found in a study by Henriksen et al. (1999) on

patients with peptic ulcer disease (17). In the Democratic Republic of Congo, a seroprevalence of 62.4% was delineated among the study participants (18).

H. pylori infection also appears to be common in South Africa; Pelsar et al. (1997) documented a high prevalence (67 - 84%) of *H. pylori* antibodies in children in Bloemfontein, while Mosane et al. (2004) also reported *H. pylori* IgG antibodies in South African mothers and their children (19,20).

A study conducted in Gonder Universty Hospital showed high seroprevalence of *H .pylori* among the adult dyspeptic patients. The trend of the seroprevalence was varied from year to year in three consecutive years (21).

A study conducted to determine sero prevalence of *H.pylori* infection among health blood donors in Addis Ababa showed that prevalence of *Helicobacter pylori* infection among healthy blood donors of Addis Ababa was very high(22).

2.2 EPIDEMIOLOGY AND TRANSMISSION OF *H. PYLORI* INFECTION

Yilmaz et al. conducted a sero-prevalence study in eastern Turkey examining 346 healthy children between the ages of 6 months and 17 years. Enzyme immunoassay (EIA) for *H. pylori* IgG was used for the diagnosis of the infection. The authors reported 44% sero-prevalence of *H. pylori* infection among the studied children that increased with age from 17% at age 6 months and 2 years to 49% by age 11 years. A strong inverse correlation with the educational level of the mother and a higher prevalence of the infection among children living with infected mothers were reported. The study emphasized the important role of the mother in transmitting *H. pylori* infection to her children (23).

Roma-Giannikou et al. supported the intra familial transmission among 32 members of 11 families.The results confirmed that closely related *H. pylori* strains were involved in the intrafamilial dispersion supporting the intrafamilial transmission of *H. pylori* infection (24).

To the contrary, Mitchell et al. in a study from impoverished population in Brazil, found no correlation between the *H. pylori* sero-positivity of the mothers and the sero-positivity of their children (25).

Reported results of two sero-surveys of *H. pylori* infection conducted in Bolivia. The first survey was conducted in 1996 on 188 children between the ages of 21 months to 6 years. At a follow-up survey in 1997, they found 18% annual incidence with a highest rate between the ages of 2 years (10%) and 3 years (32%) (26).

Ayse et al. reported from eastern Turkey a very high prevalence of *H. pylori* (64.4%) among 300 children. The risk factors for acquiring the infection were the low economic status and larger sibling size of the family. However, no significant difference between children whose parents were from different educational levels was found suggesting that the very high prevalence of *H. pylori* in eastern Turkey depends on environmental factors (27).

Heuberger et al. investigated the influence of demographic and socioeconomic factors on the prevalence of *H. pylori* infection among 196 adolescents between 15 and 16 years of age, living in Switzerland. They found one of the lowest prevalence of *H. pylori* infection among adolescents is in Europe (7.3%) among the native Swiss (28).

2.3 RISK FACTOR ASSOCIATION

Several epidemiological studies have examined risk factors for *H. pylori* infection, with lower socio-economic conditions being the most consistently identified. However, social classifications by occupation, level of education or earning are merely markers for groups of people sharing certain characteristics or practices and not a specific cause of infection.

Studies of adults have revealed a stronger association between *H. pylori* infection and childhood living conditions than for current living conditions, thus supporting acquisition early in life. The risk of introduced recall bias when adults and elderly were asked about living conditions before the age of 5 years should not be ignored. However, studies performed among children have confirmed the finding of an inverse association between socio-economic conditions and *H. pylori* infection (29).

Person-to-person transmission of *H. pylori* has been suggested in a number of studies pointing at domestic overcrowding early in life as an important risk factor for infection (29). A common

exposure to infection could, however, not be excluded. Two studies from the UK (Whitaker et al. 1993, Webb et al. 1994) identified childhood crowding, increasing number of siblings and bed sharing as possible risk factors for transmission of the organism. Although statistical analyses could not separate the relative importance of the three, the findings indicated transmission via close personal contact early in life (30).

CHAPTER THREE

3. OBJECTIVES

3.1 GENERAL OBJECTIVE

- To determine the prevalence of *H.pylori* infection among paediatric patients who were clinically diagnosed for gastroenteritis and associated risk factors among children using stool Antigen test.

3.2 SPECIFIC OBJECTIVES

- To determine the prevalence of *H. pylori* infection among clinically diagnosed for gastroenteritis paediatric patients
- To determine the associated risk factors for *H. pylori* infection among children in the study area

CHAPTER FOUR

4. MATERIAL AND METHOD

4.1 STUDY DESIGN

A cross sectional study was conducted to evaluate the prevalence of *H.pylori infection* among pediatric patient based on stool antigen test in private pediatric clinic, Addis Ababa Ethiopia.

4.2 STUDY AREA

The study was conducted in Beham Specialized Higher Children's clinic, it is a private pediatric clinic located at the center of Addis Ababa, Kirkos sub-city, woreda 4 along Debrezeit Road, the clinic offers In-patient and Out-patient services exclusively to pediatric patients came from different parts of the city.

4.3 STUDY PERIOD:

The study was conducted from March 3rd to May 11th 2014

4.4 SOURCE OF POPULATION**:** all patients who attended Beham specialized children higher clinic in the study period.

*4.4.1 STUDY POPULATION***:** those who were clinically confirmed to have gastroenteritis and dyspepsia during the study period.

4.5 SAMPLE SIZE AND SAMPLING PROCEDURE

4.5.1 SAMPLE SIZE DETERMINATION

According to World Gastroenterology Organisation Global Guidelines, 2010 a previous study of prevalence (p) of *H. pylori* infection in Ethiopian were 48% in age between 2–4, 80% at the age of 6 and 95% in adult (21).Therefore the minimum sample size based on the previous studies was calculated using single proportion formula by assuming that prevalence of *H.pylori* is 0.8 in pediatric who is clinically diagnosed for epigastric tenderness.

$$N = \frac{Z^2 P(1-P)}{D^2}$$

Where Z= 95% confidence interval (1.96)

P = Estimated prevalence rate (80%) = (0.8)

D = Marginal of sampling error

N = minimum sample size

$$= \frac{(1.96)^2 . 0.8(1 - 0.8)}{0.05^2}$$

$$= \frac{3.8x0.8(0.2)}{0.0025}$$

= 243

Therefore by adding 10% non response rates, a total of 267 study subjects participated in the study.

4.5.2 SAMPLING PROCEDURE

Non-probability convenient sampling technique was used. Consecutive patients having a complain of upper gastrointestinal pain because of a suspicion of *H. pylori* infection or any other disease was asked to participate in this prospective study and all guardian and mothers were included in the study.

4.6 SAMPLE COLLECTION AND PROCEDURES

4.6.1 DATA COLLECTION PROCEDURE

Structured questionnaire was translated from English to Amharic and then back to English by another person for cross check. Before the actual data collection time, the questionnaire was pre-tested on 12 of the study subjects (5% of the sample), in another pediatric clinic to check for any missing options, ambiguity and clarity.

A trained physician interviewed each volunteer and completed a detailed questionnaire. The individuals were questioned about their child, regarding the presence and the frequency of symptoms referable to the upper gastrointestinal tract including indigestion, and sour stomach. Demographic data focused on the subject's childhood and included social and economic data (parent's education and family income) as well as living conditions (number of rooms, number of persons residing in the home) and related risk factor questions (Annex I).

Age was coded into four categories ((9mo to 4yr), 5-–9, 10–14, , and 14-18 years of age); monthly income 1000---3000 , 3001-6000,6001-10000or >10000 ETB; number of bed room in the house <3 or >3 ; educational attainment of mother or care giver illiterate ,read and write , secondary school or above secondary school; number of siblings as <4 or >4; number of person

in the house as <5 or >5; hand washing with soap yes or no; flush toilet versus traditional pit latrine; water source pipe, boiled and treated or bottled water; Sucking of thumb/fingers yes or no; Feeding of chewing food for the child yes or no; Reporting abdominal pain more than 3 times/week yes or no.

4.6.2 LABORATORY DATA COLLECTION PROCEDURE

Stool samples requested from each participating child was collected in airtight containers at the time of the encounter, the end of the day, or the following morning. Analysis of *H. pylori* stool antigen test, LINEAR CHEMICALS S.L, is performed as per the manufacturer's instructions. A standard positive control test was run after every 20 tests, all of them being verified as positive. The LINEAR HpSA strip is a rapid lateral flow chromatographic immunoassay that utilizes a monoclonal anti-*H.pylori* antibody as the capture and detector antibody. Approximately 1-2gm of stool was transferred into the sample diluents vial and vortexes for 15 seconds. Three drops of the specimen was applied to the test and the result was read after 15 minutes. The results were reported as positive or negative based on the manufacturer's instruction.

4.7 SELECTION CRITERIA

*4.7.1 INCLUSION CRITERIA***:** In the study documented gastroenteritis patients age less than 15 years and all guardians and mothers of these children's were included in the study.

4.7.2 EXCLUSION CRITERIA: Patients with documented *H. pylori* infection, history of taking antibiotic or proton-pump inhibitor beyond four weeks were excluded from the study.

4.8 STUDY VARIABLES

Dependent: prevalence of *H. pylori* infection

Independent: Socio demographic factors; age, sex, parents educational status, income, hygiene practice like hand washing, environmental conditions (latrine, water source etc.)

4.9 DATA QUALITY CONTROL

The stool samples were tested according to the manufacture's instruction. And all quality issue was maintained by using standard operating procedure in detection of *H.pylori* Ag in stool sample during pre analytical, analytical and post analytical stages. The questionnaires were also pre tested in similar patients which are not part of the study and then the necessary adjustments was made.

In order to ensure quality of the data, proper training was given to data collectors. Each of the questionnaires was checked whether the necessary information was properly filled. All the necessary reagents were checked by known positive and negative samples before sample preparation and examination. The test result was examined independently with two experienced laboratory technologist and finally checked by the principal investigator.

4.10. STATISTICAL ANALYSIS

Information from the laboratory analysis and questionnaires were entered into SPSS, version.17. Statistical test was used to estimate odds ratio (ORs) with 95% confidence interval (CI) of positive responses to the different risk factors. Comparison between groups was compared and a *P*-value of < 0.05 was considered significant.

4.11 ETHICAL CLEARANCE

Ethical clearance letter was obtained from Department of Research and ethics review Committee (DREC) of School of medical laboratory, Addis Ababa University. A written permission was obtained from Beham Specialized Children's higher clinic. Additionally A written consent obtained from each child's guardian,and assent was obtained from a child whose age was greater than 12years old, after providing sufficient information on the purpose of study.

4.12 DISSEMINATION OF RESULTS

The result of this study will be disseminated or communicated to Addis Ababa university school of medical laboratory science, Regional health bureaus, local institutions and other concerned bodies through reports and publication on an appropriate journal.

4.13 OPERATIONAL DEFINITION

- **Gastroenteritis-** means inflammation of the stomach and intestine categorized as vomiting with or without diarrhoea, or diarrhoea alone. Only cases of gastroenteritis identified at the baseline visit were included in the present analysis
- **Paediatric -** a person < 18 years of age

5. RESULT

5.1 SOCIO DEMOGRAPHIC CHARACTERISTICS OF THE STUDY SUBJECTS

A total of two hundred twenty one paediatric patients having upper gastrointestinal symptoms because of a suspicion of *H. pylori* infection participated in this prospective study .Among the study subjects 114(51.6%) were girls and 107 (48.4%) were boys aged from 9month to 15years with the mean age of 6.29 [95% CI 5.9-6.7]. The age distributions of the study population are as shown in table 1, participant within the age group 4-9 comprised, 97(43.9%) and followed by age group 0-4 accounts 82(37.1%), while the >14 years age group was the least represented (0.9%). According to the study, 79(35.7%) had completed primary school, 68(30.8%) had completed secondary school, 60 (27.1%) were able to read and write and 11 (5%) parents / guardians were illiterate.

Table 5.1 Distribution of the study population by age, gender, monthly income and educational level in Beham Specialized Children's Higher clinic, March to May 2014, Addis Ababa.

Variable		Frequency	Percent
Age	0-4	82	37.1%
	4-9	97	43.9%
	9-14	40	18.1%
	>14	2	0.9%
	Total	221	100%
Gender	female	114	51.6%
	Male	107	48.4%
	Total	221	100%
Monthly income of parents/guardians	< 6000 Eth. Birr	174	78.7%
	>6000 Eth. Birr	47	21.3%
	Total	221	100%
Educational level of parents/guardians	Illiterate	11	5%
	Read and write	60	27.1%
	Completed primary school	79	35.7%
	Completed secondary school	68	30.8
	Above secondary school	3	1.4
	Total	221	100%

Table 2 shows the distribution of the study population by household population characteristics, including the number of bed room, the number of siblings, and the number of persons living in the same household. Majority 183(82.8%) study subjects living with in less than three bed room in the household. 165(74.7%) study subjects had less than four siblings. 168 (76%) study participants were living with less than ten persons in the household. Only 44 (19.9%) study children have a history of sucking fingers. 180(81.4%) used tap water for drinking. 135 (61.1%) used flushed water toilet. Majority 173(78.3%) were having experience of washing their hands after toilet use. Among all children who has gastroenteritis only 50(22.6%) were having recurrent abdominal pain. 103(46.6%) children shared bathroom with their parents .33 (14.9%) were having the history of feeding chewing food from their parents.

Table 5.2. Distribution of study population by household population characteristic in Beham specialized Children's higher clinic, Addis Ababa, March to May 2014.

Variable		Frequency	Percent
Number of bed rooms in the house	<3	183	82.8
	>3	38	17.2
Number of sibling in the house	<4	165	74.7
	>4	56	25.3
Sucking fingers	Yes	44	19.9
	No	177	80.1
Number of persons living in the house hold	<5	168	76
	>5	53	24
Type of drinking water in the house	Tap water	180	81.4
	Bottled water	16	7.2
	Boiled/treated tap water	25	1.1
Type of toilet used	Pit latrine	86	38.9
	flush toilet	135	61.1
Washing of hands after toilet use	Yes	173	78.3
	No	48	21.7
Having recurrent abdominal pain	Yes	50	22.6
	No	171	77.4
Share bathroom with parents	Yes	103	46.6
	No	118	53.4
Feeding of chewing foods	Yes No	33 188	14.9 85.1

5.2. PREVALENCE OF *H.PYLORI* AND ASSOCIATION OF RISK FACTORS

Helicobacter pylori antigens were detected in 57 of the 221children giving an overall prevalence of 25.8% (95%CI 18.8-31), 27.2% in female and 24.3% in male children. The frequency of *H. pylori* colonization was higher in females than in males. Of the 114 females enrolled in the study, *H. pylori* antigen was detected in the stools of 31 (27.2%) against 26 (24.3%) of the 107 males (OR = 0.85 95%CI=0.46-1.57). smallest age of stool Ag positivity was one year and five month. According to the study, the subjects were acquiring *H. pylori* infection before they reached the second birthday. The prevalence was 31.7% in the age group 0-4, 21.6% in the age group 5-9 and25% in the age group 9-14. The peak age of the infection was 6year and prevalence was 35.8% at the age of 6years (figure 1). The association of socio economic status and *H.pylori* stool antigen positivity showed, 27.6% in earning < 6,000 Eth. Birr monthly and 19.1% in earning > 6,000 Eth. Birr monthly; (OR=.622; 95%CI .280-1.382, p=0.243), the prevalence of H.pylori infection increased in those earning lower income families compared to those earning more monthly income and low income was a risk factor and statistically significant.

27.3 % of *H.pylori* stool antigen positive children were having illiterate parents, the prevalence increase to 31.7% in parent's complete primary school. Therefore parent's educational level was no significant difference between categories the crowding index, such as number of bed rooms and number of persons in the house hold indicated that, the prevalence was higher in household having less than three bed rooms, 28.8 %(53of 184) than household having more than three bed room 10.8%(4 of 37) (OR= 0.3; 95%CI= 0.101 – 0.887; $p<0.05$).

The prevalence of *H. pylori* infection was higher in persons living more than ten in the household was 47 %(25 of 53) than persons living less than ten in the household 23.2 %(32of 168) (3.795 95%CI 1.956- 7.362; $p<0.05$). Child having more than 4 siblings was associated with *H.pylori* stool Antigen positivity, the prevalence of H. pylori infection is higher in these group it accounts 42.9% than child having less than 4 sibling 20%. (Table 5.3). The relationship of *H. pylori* prevalence to the use of pit latrine or flush water toilet was examined as an indicator of household sanitation practices. However ,*H. pylori* infection didn't show significant difference

between two facilities, 27% in pit latrine users and 25% of flushed water toilet users were infected with *H.pylori* (OR 0.89 95%CI .485-1.664 ; p=0.734).

Table 5.3. Association between epidemiological risk factors and *H. Pylori* infection at Beham specialized Children's higher clinic, March to May 2014, Addis Ababa

Variable and category	N	H .positive (%)	OR (95%CI)	P value
Age				0.380
0-4	82	26(31.7)		
5-9	97	21(21.6)		
10-14	40	10 (25)		
>14	2	0(0)		
Sex			1.164(0.636-2.130)	0.625
Male	107	26(24.3)		
Female	114	31(27.3)		
Guardian /parents educational background				0.992
Illiterate	11	3(27.3)		
Write and read	60	16(26.7)		
Complete primary school	79	19(24.1)		
Complete secondary education	68	18(26.5)		
Above secondary education	3	1(33.3)		
Monthly income			0.622 (0.280-	0.234
<6000 Eth. birr	174	48(27.3)	1.382)	
>6000 Eth. birr	47	9(19.1)		
Number of bed room in the household			0.300 (0.101-.887)	0.022
< 3	184	53(28.8)		
>3	37	4(10.8%)		
Number of person in the house hold			3.795(1.956-7.362	0.000
<5	168	32(23.2)		
>5	53	25(47.2)		
Number of sibling in the household			3.00(1.562-5.760)	0.001
<4	165	33(20)		
>4	56	24(42.9)		
Sucking finger			0.528(0.260-1.069)	0.073
Yes	44	16(36.4)		
No	177	41(23.2)		
Feeding chewing foods			0.218(0.101-0.471)	0.000
Yes	33	18(54.5)		
No	188	39(33.1)		
Bathing the same water			1.055(0.576-1.932)	0.862

Yes No	103 118	26(25.2) 31(26.3)		
Type of toilet Pit latrine toilet Flushed water toilet	 85 136	 23(27.1) 34(25)	0.899(0.485-1.664)	0.736
Practice of hand washing after toilet used Yes No	 178 43	 42(23.6) 15(34.9)	1.735(0.848-3.550)	0.129
Recurrent abdominal pain Yes No	 50 171	 33(66) 24(14.0)	.084(0.041-0.174)	0.000
Total	221	57		

CHAPTER SIX
6. DISCUSSION

The overall prevalence rate of 25.8% obtained in this prospective cross sectional study is high and suggests that *H. pylori* infection is significant in the pediatric age group of the study locality. The prevalence was 31.7% in the age group 0-4, and decreasing to 21.6% in the age group 5-9. This is inconsistent with prevalence rates reported by World Gastroenterology Organisation (WGO) 2010 the prevalence of HP in Ethiopia were 48% in age between 2–4, 80% at the age of 6 (7). The reason for this difference in prevalence within the Ethiopian population is largely unknown, but could be due to the difference in socio-economic factors, and life style. The previous study was done in low socio-economic class of rural population, But this prospective study was conducted in middle income population of Addis Ababa and relatively has improved water supply and good health practice. The reason of decreasing Stool Antigen detection of H. pylori infection has been attributed to an improvement in their general living conditions. In similar developing country, a rural village of China, of 98 children found that nearly 70% of those aged 5-6 years were infected with the organism,(12).

Nabwera et al. (2000) in their study observed a high prevalence among Kenyan children aged between 3 - 5 years, indicating that most children in the study area were infected before they reached their third birthday (16). Yilmaz et al. reported 44% sero-prevalence of *H. pylori* infection among the studied children that increased with age from 17% at age 6 months and 2 years to 49% by age 11 years in eastern Turkey. In contrast, studies done in the developed countries have reported low prevalence rates in their childhood populations. Heuberger et al. found one of the lowest prevalence of *H. pylori* infection among adolescents in Europe (7.3%) among the native Swiss (28). The higher prevalence rates in developing countries are thought to be a consequence of the poor socioeconomic conditions prevalent in these countries (7)

The data also showed that H. *pylori* infection is acquired early in the study population, age of stool H. *pylori* Ag positive was one year and five month as 23.6% of children less than three year old were HP stool antigen positive. The highest age-specific prevalence of 31.7% was seen in the 0-4 year's age group, while the lowest prevalence was seen in the 5-9 years age group.

There is similarity with the reports of increasing prevalence rate with increasing age from some other studies (7,26)

Previous investigations have shown the importance of age, sex and poor household living conditions in childhood, in the acquisition of *H. pylori* infection and identified household crowding, low socioeconomic status, poor hygiene conditions and bed sharing as risk factors for the infection (29,30) this study found an inverse relationship between *H. pylori* prevalence and childhood socioeconomic status. Colonization rates were higher in children of low socioeconomic class compared with those of high socioeconomic class. This difference was statistically significant ($P = 0.006$). The OR (OR = 2.41, 95% CI: 1.26–4.64) also indicated that low socioeconomic status was associated with a higher prevalence of *H. pylori* colonization in children. This result could reflect the difference in the standards of living conditions between the two groups of children studied. It further supports the consensus that low socioeconomic status is associated with increase in prevalence of *H. pylori* infection (27).

The frequency of infection decrease with age from 31.7% in children <4 years, 21.6% in 5–9 years-old and 25% in those 10-14 years old. This decrease was, however, not statistically significant ($P = 0.380$). children aged <4 years are likely at a higher risk of acquiring infection than children aged 5–9 years and 10–14 years old respectively. It could be due to sampling methods. A previous study has shown that *H. pylori* infection is common from an early age in the developing world where most children are colonized by the age of 10 years but infection is relatively rare in childhood and becomes progressively more common throughout adult life in the developed world (11). However, a previous study involving adults in Ethiopia, Asrat *et al.* (2004) detected anti-*H.pylori* antibodies in 80% of 300 consecutive patients presenting with dyspepsia.

Some studies have found household crowding in childhood to be a risk factor for *H. pylori* infection (29, 30). Examining the number of persons in the households, the study results showed that prevalence was significantly higher ($P = 0.000$) in children having more than ten persons per household (47.1%) than in children having less than ten persons per household (23.2%). The study observed that children living in households with less than three bedroom had a higher prevalence (28.8%) compared with those in households with more than one bedroom (10.8%).

this difference was statistically significant (P = 0.022). Number of sibling in the household also showed a risk factor for *H. pylori* infection, children living with more than four sibling had a higher prevalence(42.9%) compared with those had less than four sibling (20%), this difference was statistically significant (P = 0.001). the results on household crowding as a risk factor for *H. pylori* infection are therefore in agreement with that of Lindkvist *et al.* (1998).

This study found that poor hygienic living conditions in childhood were associated with a higher risk of acquiring *H. pylori* infection. For example, poor practice of hand washing after toilet used showed a higher risk of infection of *H. pylori* compared to good practice of hand washing after toilet used. child feeding chewing foods showed a significantly higher prevalence (54.5%) compared with those who did not (33.1%), this difference was statistically significant (p= 0.000) .children who sucked their thumb/fingers showed a significantly higher prevalence (36.4%) compared with those who did not (23.2%) however this difference didn't show statistically significant (P = 0.073) , because 41 out 57 HP stool antigen positive children hadn't suck their fingers .Use of pit latrine for faecal disposal was not risky than use of water closet toilet system. however several authors have associated high prevalence of *H. pylori* with poor sanitation, especially poor faecal disposal and contact with faecal matter (30).

LIMITATION OF THE STUDY

The limitation of this study was lack of quantitative confirmatory test. The test should be confirmed by enzyme-linked immunosorbant assay (ELISA) stool Antigen test. Because the Linear *Helicobacter Pylori* Ag cassette is limited to the qualitative detection of H. Pylori antigen in human fecal specimen.

This study done in one private paediatric clinic and the data was collected from those clients who came to this clinic during the data collection period. It doesn't include other paediatrics hospitals and clinics in the city of Addis, that may underestimate findings.

CHAPTER SEVEN

7.1 CONCLUSION

In conclusion, the prevalence of *H. pylori* infection is 25.8%. This is high among children in the study area. Infection also is acquired at very early age. Household crowding is one of the factors that enhance the infection. This study confirmed the household living conditions in the acquisition of *H. pylori* infection. It identified age, household crowding, and poor hygienic living conditions as risk factors for infection.

7.2 RECOMMENDATION

In this study overcrowding were positively associated with *H. pylori* prevalence. Thus, minimizing overcrowded condition in the area is mandatory.

As confirmed by many studies, overcrowded living condition facilitates direct contact (person to person) transmission of *H. pylori*. Therefore, educating people, particularly infected mothers and spouses about familial clustering of *H. pylori* infection and the risk of infecting others by direct contact and exchange of saliva is imperative. In addition, sanitary measures among family members (hand and mouth wash, brushing teeth) are essential.

Due to lack of ELISA stool Ag test, the study was not supported by golden standard method to rule out *H.pylori* infection in pediatric population. As a result, further investigation using ELISA stool Ag test is recommended.

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ANNEX I : ENGLISH VERSION QUESTIONNAIRE

STUDY TOOL

The questionnaire was designed to obtain demographic information such as the age and sex of the child, family structure, hygiene, food and feeding practices, history on regular illnesses and antimicrobial treatment in the 3 months prior to sample collection. To describe household characteristics, number of rooms, bedrooms and toilets were described. The concept of domestic crowding was related to the number of people in a household and sharing of a bed and bedroom. Food and feeding habits and practices will be investigated.

I socio-demographic related questions

1. Child age in yr _____
2. What is your child Sex?

A. Male B. Female

3. How much is family monthly income?

A. 1000-3000 C. 4000-6000

B. 7000-9000 D. 10000 and above

4. How many bedrooms do you have in the house?

A. < 3

B. >3

5. How many persons do live in the house?

A. >5

B. <5

6. How many siblings do the child has?

A. 1-2

B. 3-4

C. >4

7. Does the child have a habit of Sucking of thumb/fingers?

A. Yes

B. No

8. Does the mother / care giver has a habit of feeding of chewing food for the child?

A. Yes

B. No

9. Does the family member use the same bathroom with the child?

A. Yes

B. No

10.What is mother's or guardian's level of education?

A. Illiterate

B. Read and write

C. Primary school

D. Secondary school

E. Above secondary school

11. What is your family drinking water Source?

A. Tap water

B. Bottled water

C. Boiled tap water

12. What type of toilet used in your house?

B. Pit latrine

C. flush toilet

13. Do you have a habit of washing your hands after using toilet?

A. Yes

B. No

14. Does the child report abdominal pain more than 3 times per week

A. yes

B. No

15. Is there any parent have the infection of *H. pylori*?

A. Yes B. No C. Not known

ANNEX II: AMHARIC VERSION OF QUESTIONNAIRE

የጨህራ ህመሞች ምክንያት የሆነውን የኤች ፓይሎሪ ባክቴሪያ አንቲጅን በልጆች ላይ ስላለው ስርጭትና የመተላለፊያ መንገዶችንና ተዛማጅ ችግሮችን ለይቶ ለማወቅ የተዘጋጀ ቃለ መጠይቅ፡፡

ክፍል አንድ ፡ *መሠረታዊ* መረጃ

1. **የህፃኑ እድሜ** ----------
2. **የህፃኑ ፆታ ፡** 1. **ወንድ** 2. **ሴት**
3. **የቤተሠብ ወርሀዊ ገቢ**
   1. 1000-3000
   2. 4000-6000
   3. 7000-9000
   4. >10,000
4. **ስንት *መኝታ* ክፍል ቤት አለዎት?**
   1. 1-2
   2. 3-5
   3. **ከ**5**በላይ**
5. ***ምን ያህል ሰው በቤት ውስጥ ይኖራል?***
   1. 3-6
   2. 7-10
   3. **ከ**10 **በላይ**
6. **ስንት ወንድምና እህት አሉት/አላት?**
   1. 1-2
   2. 3-4
   3. **ከ**4**በላይ**
7. **ልጆት ጣት የመጥባት ልምድ አለው/አላት?**
   1. **አዎ** 2. **አይ የለውም/የላትም**
8. **ምግብ አኝከሽ *ታበያዋለሽ*/*ታበያታለሽ* ወይ?**
   1. **አዎ** 2. **አይ**
9. **የጋራ የሆነ የገላ *መታጠቢያ* ከህጻናቱ *ጋር* ይጠቀማሉ?**
   1. **አዎ** 2. **አይ**
10. **የአሳዳጊው የትምህርት ደረጃ**
    1. ***ያልተማረ***
    2. ***ማንበብና መጻፍ***
    3. **አንደኛ ደረጃ ትምህርት ያጠናቀቀች**
    4. ***ሁለተኛ ደረጃ ትምህርት ያጠናቀቀች***
11. **ቤተሠቡ ለመጠጥ የሚጠቀመው ውሀ አይነት**

1. የቧንቧ ውሀ
2. የታሸገ የፕላስቲክ ውሀ
3. የፈላ የቧንቧ ውሀ

12. ቤተሠቡ የሚጠቀመው የመጸዳጃ ቤት አይነት
    1. ባለጉርኀድ ሽንት ቤት
    2. ውሃ መልቀቂያ ያለው ዘመናዊ ሽንት ቤት
13. መጸዳጃ ቤት ከተጠቀሙ በዋላ እጆትን የመታጠብ ልምድ አሎት?
    1. አዎ አለኝ 2. የለኝም
14. ልጆት በሳምንት ከ ሶስት ጊዜ በላይ የሆድ ህመም ስሜት ይሰማዋል?
    1. አዎ ይሰማዋል 2. አይ አይሰማውም

15. ከወላጆች መሀል የኤች ፓይሎሪ ተጠቂ አለ ወይ ?
    1. አዎ አለ 2. የለም 3. አናውቅም

ANNEX III: ENGLISH VERSION OF THE INFORMATION SHEET

Addis Ababa University Post graduate School

Principal Investigator: Ahmed Kemal

Name of Organization: AAU, College of Health Sciences, School of Allied Health Sciences, Department of Medical Laboratory Sciences

Information sheet for the prevalence of *H. pylori* infection in pediatric patients who is clinically diagnosed for gastroenteritis in Beham specialized children's higher clinic Addis Ababa, Ethiopia.

Title: The "prevalence of *H. pylori* infection in pediatric patients who is clinically diagnosed for gastroenteritis in Beham specialized children's higher clinic Addis Ababa Ethiopia .

Aim: The aim of this study is to determine prevalence of *H. pylori* infection in pediatric patients who is clinically diagnosed for gastroenteritis . In order to design preventive strategies, the explanation of the mode of spread of this potentially fatal pathogen is crucial; particularly since its prevalence in children is still remain poorly understood, therefore this study was assessed the prevalence of *H. pylori* infection in paediatric patients and associated risk factors.

Duration: The duration of this study depend upon the availability of study subjects. It might take about two months or more.

Procedures to be carried on:For this study to be successful we need your participation. If you are voluntary to let your child participate in this study, you are expected to understand and sign the informed consent. Then Socio demographic and clinical information related to *H. pylori* infection will be filled on the questionnaire. Stool sample will be collected from your child at the time of the encounter, the end of the day, or the following morning by attending laboratory technician. Collected samples will be transported to Beham laboratory as soon as possible and will be analyzed for the presence *H. pylori* stool antigen by using standard operating procedures (SOPs).

Risk: No risk is associated with the specimen collection, because the collection of these specimens will follow the routine procedures for the laboratory investigation.

Expected benefits: It is reasonable to expect the following benefits from this research: knowing the result of this study, however, I can't guarantee that your child will personally experience benefits from participating in this study. Others may benefit in the future from the information we find in this study.

Confidentiality: All your personal information collected for the purpose of this study will be kept confidential.

Payment: No payment will be provided by participating in this study.

Right: Participation in the study is voluntary, and refusal to participate involves no penalty or loss of benefits to which you are otherwise entitled. The study participants have a right to withhold information, decline to cooperate in the study and refuse provision of specimens.

Approval: This research project has got ethical clearance from the Departmental Research and Ethics Review Committee (DRERC) of Addis Ababa University, College of Health Sciences, School of Allied Health Sciences, and Department of Medical Laboratory Science.

Whom to contact: If you have any question or description about this study, you can communicate on the following address:

1. Addis Ababa University, College of Health Sciences, School of Allied Health Sciences, Department of Medical Laboratory Sciences

   Tel: +251-112-75-51-70

   Fax: +251-112-75-46-69

   E-mail:SMLT@ethionet.et

   P.o.Box: 1176, Addis Ababa, Ethiopia

2.Principal Investigator: Ahmed Kemal (candidate of MSc in AAU)

   Tel: +251-911-440-829/+251-921-742-102

   E-mail: ahmkoki@gmail.com

   P.O. Box: 57388, Addis Ababa, Ethiopia

ANNEX IV: AMHARIC VERSION OF THE INFORMATION SHEET

ቅፅ 4፡ ለተሳታፊዉ በቂ መረጃ ለመስጠትየተዘጋጀ ቅጽ (ትርጉም በአማርኛ)

አዲስአበባ ዩኒቨርሲቲ የድህረ ምረቃ ትምህርት

የተመራማሪዉ ስም፡ አህመድ ከማል

የድርጅቱ ስም፡ አአዩ፣ የጤናሳይንስኮሌጅ፣የአላይድጤና ሳይንስ ት/ት፣ ህክምና ላቦራቶሪ ሳይንስ ክፍል

የኤች ፓይሎሪ ባክቴሪያ በልጆች ላይ ስላለው ስርጭትና ተዛማች ችግሮችን ለይቶ ለማወቅ ለማጥናት ለተሳታፊዉ በቂ መረጃ ለመስጠት የተዘጋጀ ቅጽ ነው።

<u>ርዕስ፡-</u>የኤች ፓይሎሪ ባክቴሪያ በሆድ ታማሚ ልጆች ላይ ስላለው የስርጭት መጠን እና ተዛማች ችግሮች የሚል ነዉ።

<u>የጥናቱ ዋናአላማ፡-ዋናአላማ፡-</u> የዚህ ጥናት አላማ የተለያዩ የጨጓራ ህመሞች ምክንያት የሆነውን የኤች ፓይሎሪ ባክቴሪያ በልጆች ላይ ስላለው ስርጭትና ተዛማች ችግሮችን ለይቶ ለማወቅ ነው። ይህም የሚረዳው እርስዎ በሚሠጡት መልስ በመንተራስ ለኤች ፓይሎሪ ባክቴሪያን ስርጭት ምክንያት የሆኑትን መተላለፊያ መንገዶችን ለመግታት እንዲያስችል ነው።

<u>የጥናቱ ጊዜ፡</u> በሆድ ታማሚ ልጆች ብዛት የሚወሰን ሲሆን 2 ወር እና ከዛም በላይ ሊወስድ ይችላል።

<u>ሐ. የጥናቱ ሂደት፡-</u>ለዚህ ጥናት እዉን መሆን የእርስዎን ተሳትፎ እንፈልጋለን ። በዚህ ጥናት ለመሳተፍ ፈቃደኛ ከሆኑ የስምምነት ቅጹን መረዳትና መፈረም ይጠበቅብዎታል። ከዛም ህብረተሰብነክ እና የህክምና መረጃዎች መጠይቁ ላይ ይሞላሉ። ናሙና የሚሰበሰበው ከ ልጆት ሠገራ ላይ ነው ፣ ምርመራውም ወዲያሁኑ ወይም በንጋታው በቢሃም ላቦራቶሪ ይከናወናል።

<u>ሊከሰቱስለሚችሉስጋቶችናየምችትመጓደሎች፡-</u>ለጥናቱ በሚወሰደዉ ችግር/ስጋት አይኖረዉም ምክንያቱም የጥናቱ ናሙና አወሳሰድ ከተለመደዉ የአገልግሎት አሰጣጥ የተለየ አይደለም።

<u>የተሳታፊዎችጥቅሞች፡-</u>ባክቴሪያዉ ያለባቸዉን ህፃናት የላቦራቶሪ ዉጤታቸዉን በጤና ተቋም በማገልገል ላይ ላለው ሃኪም በማሳወቅ የተሻለ ክትትል ይደረግላቸዋል።

<u>ሚስራጥዊነት፡-</u> ለጥናቱተብለው የተሰባሰቡ የግልዎ መረጃ ሚስጢርነቱ የተጠበቀ ነው።

<u>ክፍያ፡-</u>በዚህ ጥናት በመሳተፍዎ የሚያገኙት ምንም አይነት ልዩ ክፍያ የለም።

<u>የተሳታፊዉመብት፡-</u>ተሳትፎዎ ሙሉ በሙሉ በፈቃደኝነት ላይ የተመሰረተ ነዉ፡፡ ፈቃደኛ ካልሆኑ በዚህ ጥናት ያለመሳተፍ መብትዎ የተጠበቀነዉ፡፡በዚህም ምክንያት ከኪሊኒኩ በሚያገኙት አገልግሎት ላይ ምንም አይነት ተጽዕኖ አይደርስብዎትም፡፡ ከጥናቱ በማንኛዉም ሰዓት ራስዎን የማግለል መብትዎ የተጠበቀ ነዉ፡፡

<u>የጥናቱ ፈቃድ/ህጋዊነት፡-</u> የዚህ ጥናት ህጋዊነት በዲፓርትመንታል ምርምር እና ስነምግባር ቅኝት ኮሚቴ፣አዲስአበባ ዩኒቨርስቲ፣ኮሌጅ ኦፍ ሄልዝ ሳይንስ ስኩል ኦፍ አላይድ ጤና ሳይንስ ፣ፈቃድ ያገኘ ነው፡፡

<u>መረጃ ስለማግኘት፡-</u> ይህን ጥናት አስመልክቶ ምንም አይነት ጥያቄ ወይም ማብራሪያ ቢያስፈልግዎት፡

1. **አዲስአበባዩኒቨርሲቲ፣የጤና ሳይንስ ኮሌጅ፣ የ አላይድ ጤና ሳይንስ ት/ት፣ ህክምና ላቦራቶሪ ሳይንስ ዲፓርትመንት**

   ስ.ቁ.፡- +251-112-75-51-70

   ፋክስ፡+251-112-75-46-69

   ኢ-ሜል፡:SMLT@ethionet.et

   ፒ.ኦ.ቦክስ፡ 1176፣አዲስአበባ፣ኢትዮጲያ.

2. **የጥናቱ ተመራማሪ፡ አህመድ ከማል (በአአ ዩኒቨርሲቲ የማስተር ተማሪ)**

   ስ.ቁ፡-+251-911-440-829/+251-921-742-102

   ኢሜል:ahmkoki@gmail.com

   ፒ.ኦ.ቦክስ፡ 57388 አዲስአበባ፣ኢትዮጲያ

ANNEX V: ENGLISH VERSION OF THE CONSENT FORM

Serial no…….

Card no…..……

Name of study participant: _____

I have been requested to participate about this study, which plans to determine prevalence of *H. pylori* infection in pediatric patients who is clinically diagnosed for gastroenteritis in Beham specialized children's higher clinic Addis Ababa, Ethiopia. I have been informed this study which involves collecting of stool specimen. During collection of the specimen I have been told that there is no harm and I have also read the information sheet or it has been read to me. I have been also informed that all information contained within the questionnaire is to be kept confidential. Moreover, I have also been well informed of my right to keep hold of information, decline to cooperate and drop out of the study if I want and that none of my actions will have any bearing at all on my child overall health care and clinic access.

It is therefore with full understanding of the situations that I agreed to give the informed consent voluntarily to the researcher to use the stool specimen for the investigation. I agree that I am contributing to the treatment of my fellows by participating in this project. I have asked some questions and clarification has been given to me. I have given my consent freely to participate in the study, and I_____ hereby to approve my agreement with my signature.

Participants' signature: _____Date_____
Principal Investigator's signature: _____Date _____
Witness (Illiterate) _____Date _____

ANNEX VI: AMHARIC VERSION OF THE CONSENT FORM

ቅፅ 6 የስምምነትመግለጫ (ትርጉምበአማርኛ)

ተራቁጥር_____

የካርድቁጥር_____

የተሳታፊውስም_____

የኤች ፓይሎሪ ባክቴሪያ በሆድ ታማሚ ልጆች ላይ ስላለው የስርጭት መጠን እና ተዛማች ችግሮችን ለማጥናትየተዘጋጀ ጥናት ላይእድሳተፍ ተጠይቄ ስለጉዳዩም ለመረዳት በቂ መረጃ አግኝቻለሁ፡፡ ስለሆነም ናሙና የሚሰበሰበው ከልጄ ሠገራ ላይ መሆኑንስለተርዳሁኝ ናሙና ወስዶ መመርመር አስፈላጊ ስለሆነና ሙናዉን በመስጠት ልተባበር ሙሉ ፈቃደኛ መሆኔን ገልጫለሁ፡፡ ናሙና በሚወስድበትጊዜ ምንም ስሜት አይነት ጉዳት እንደሌለው ተነግሮኛል እንዲሁም ከመጠይቁ አንብቢያለሁ ወይም ተነቦልኛል፡፡ከምርመሩ መሳተፍ ወይም አለመሳተፍ መብቴ የተጠበቀ መሆኑን እውቃለሁ መሳተፍ ብወስን በጤና ተቋም በሚደረግልኝ ህክምና ላይ ምንም ተፅዕኖ እንደማይኖረዉ ተረድቻለሁ፡፡

ስለዚህ የጥናቱን ጠቃሚነት አምኜበት የስምምነት ቃሌን የሰጠሁት በፍፁም ፈቃደኝነትነዉ፡፡ በመጨረሻም ልጄ ከጥናቱ ዉጤት ተጠቃሚ ሊሆን እንደሚችል ተገልፆልኝ በመሳተፌና በመተባበሬ ወገኖቼን ልረዳ በመቻሌ ደስተኛ መሆኔን ገልጨ፤ግለፅ ያልሆኑ ጥያቄዎች ላይማብራርያ እንዲሰጠኝ ጠይቄ መልስ ተሰጥቶኛል ፡፡ እንዲሁም በጥናቱ ሂደት እንድሳተፍ ፍቃደኝነቴን በፊርማየአረጋግጫለሁ፡፡

የተሳታፊ

ፊርማ_____ቀን_____

የጥናቱ አስከያጅፊርማ_____ቀን_____

ምስክር (ማንበብናመፃፍለማይችሉ)

_____ቀን_____

ANNEX VII ASSENT FORM

PROJECT TITLE: PREVALENCE OF *H.PYLORI* INFECTION IN PEDIATRIC PATIENTS WHO IS CLINICALLY DIAGNOSED FOR GASTROENTERITIS IN BEHAM SPECIALIZED CHILDREN'S HIGHER CLINIC, ADDIS ABABA, ETHIOPIA

Your child has been invited to join a research study to look at the prevalence of *H.pylori* infection in children's Please take whatever time you need to discuss the study with your family and friends, or anyone else you wish to. The decision to let you child join, or not to join, is up to you.
In this research study, we are testing feces for *H.pylori* stool antigen .Your child will be asked to give stool sample. We think this will take him/her 20minutes.

The investigators may stop the study or take your child out of the study at any time they judge it is in your child's best interest. They may also remove your child from the study for various other reasons .Your child can stop participating at any time. If your child stops he/she will not lose any benefits. This study involves with no risks.

It is reasonable to expect the following benefits from this research: knowing the result of this study, however, we can't guarantee that your child will personally experience benefits from participating in this study. Others may benefit in the future from the information we find in this study.

Your child's name will not be used when data from this study are published. Every effort will be made to keep clinical records, research records, and other personal information confidential, to protect it from unauthorized disclosure, tampering, or damage .No incentives are arranging for this study.

Participation in this study is voluntary. Your child has the right not to participate at all or to leave the study at any time. Deciding not to participate or choosing to leave the study will not result in any penalty or loss of benefits to which your child is entitled, and it will not harm his/her relationship with the study.
CONTACTS FOR QUESTIONS OR PROBLEMS
Call at 0911440829
Permission for a Child to Participate in Research
As parent or legal guardian, I authorize _____ (child's name) to become a participant in the research study described in this form.

Child's Date of Birth

Parent or Legal Guardian's Signature Date
_____

ANNEX VIII : LABORATORY TEST PROCEDURE

***Helicobacter pylori* Ag is a rapid test for detection *of H.pylori* antigen in feces**

Test principle: the Linier helicobacter pylori Ag strip is a lateral flow chromatography immunoassay for the qualitative detection of H.pylori antigen in human fecal specimen. It is intended to be used by professional as a screening test and as an aid in diagnostic of infection with HP. Any reactive specimen with the Linear Helicobacter pylori Ag strip must be confirmed by clinical findings.

The Linear Helicobacter pylori Ag strip is a sandwich lateral flow chromatographic immunoassay. The test strip consists of

1. A burgundy coloured conjugate pad containing a monoclonal anti H.pylori antibody conjugated with colloid gold (anti-H.P conjugates)
2. A nitrocellulose membrane strip containing a test band (T band) and a control band (C band). The T band is pre-coated with another monoclonal ant-H.P antibody, and the C band is pre-coated with goat anti-mouse IgG antibody.

When an adequate volume of extracted fecal specimen is dispensed into the test strip, the specimen migrates by capillary action along the conjugates. The immuno-complex is then captured on the membrane by the pre-coated antibody test result. Absence of this band suggests that the concentration of H.P in the specimen is below the detectable level,indicating a H.P negative result.

Absence of the T band suggests a negative result. The test contains an internal control(C-band) which should exhibit a burgundy coloured band of the immuno-complex of goat anti-mouse IgG/mouse IgG-gold conjugate regardless of the colour development on the T- band, otherwise , the lest result is invalid and the specimen must be retested with another stip.

Packaging Content: 25X1 test strip, each sealed in a foil pouch. , 25 sample tube with 2ml of extraction buffer.

Storage and stability: store at 2-30^{o}c. The strip is stable through the expiration date printed on the sealed. The test strip must remain in the sealed pouch until use. Don't freeze. Do not used beyond the expiration date.

Specimen collection: consider any material of human orgion as infectious and handle them using standard biosafty procdures.

1. Collect a random sample of feces in a clean, dry receptacle .
2. Unscrew the top of the collection tube and remove the appilicator stick.
3. Randomly pierce the fecal specimen in at least five(5) different site. Do not scoop fecal specimen.
4. Remove excess sample off the shaft and outer grooves. Be sure sample remains on inside grooves.

5. Replace the stick in the tube and tigten securely
6. Shake the extraction tube virology

The specimen is now ready for testing, transportation or storage.

Note: specimen extracted may be stored at 2-8^{0}C for 72 hours. If longer storage is recommended, freezing at -<20^{0}C is recommended.

Material required

General laboratory equipment

A container to hold faecal specimens

Positive and negative control

Procedure

Allow the test, stool samples and buffer to reach to room temperature (15-30^{0}C) prior to testing. Do not open the pack with strip until ready to perform the assay.

1. Place the test strip on a clean, flat surface
2. Shake the sample collection tube vigorously to ensure an effective liquid suspension.
3. Hold the tube upright. Twist off tip. Dispense 3drops of the extracted specimen slowly onto the sample pad drop by drop. do not load all sample at once
4. Start the timer.
5. Result can be read in 15 minutes after adding the specimen. Positive results can be visible in as short as 1minute. Do not read results after 15minute to avoid confusion, discard the test strip after interpreting the result.

Interpretation of the result

Negative Result: if only the C band is developed, the test indicates that no detectable *H.pylori* antigen is presented in the specimen. The result is negative.

Positive result: if both C and T bands are developed, the test indicates for the presence of H.pylori antigen in the specimen. The result is positive.

ANALYTICAL PERFORMANCE

324 fecal samples collected from subjects with symptomatic gastrointestinal disorders and non-gastrointestinal symptoms were tested with linear *Helicobacter pylori* Ag strip with a UBT as reference test. Comparism of all subjects is shown in the following table.

	Linear Helicobacter pylori Ag strip		
UBT	Positive	Negative	Total
Positive	118	7	125
Negative	0	199	199
Total	118	206	324

Relative sensitivity 94.4%

Relative specificity: 100%

Overall agreement : 97.8%

Declaration:

I, the undersigned, declare that this is my original work and has not been presented in this or any other University and all sources of materials used for this thesis have been duly acknowledged.

Name: AHMED KEMAL

Signature: _____

Date: _____

Place: Addis Ababa University, school of medical laboratory science

This thesis has been submitted with my approval as University advisor

GEBRU MULUGETA (BSc, MSc)

Signature: _____

Date: _____

Place: Addis Ababa University, school of medical laboratory science

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