Cervantes, Diana T., <u>Transmission and Establishment of *Helicobacter pylori* Infection in Childhood</u>. Doctor of Public Health (Epidemiology), July 2013, 335 pp., 35 tables, 24 figures, bibliography, 159 titles.

Transmission of *Helicobacter pylori* infection is thought to occur predominately during childhood in both developed and developing countries. Various bacterial, host and environmental factors influence *H. pylori* transmission and establishment of infection. The most likely routes of *H. pylori* transmission include gastro-oral, fecal-oral and oral-oral. Therefore, conditions such as close, personal contact with a child's infected mother and siblings may favor *H. pylori* transmission. The immunological response mounted against *H. pylori* infection by a child, possibly modulated by age and passive immunity, may alter the establishment of infection.

The current research, utilizing data from The Pasitos Cohort Study, aimed to estimate the effect that *H. pylori* infected mothers, *H. pylori* infected siblings and breastfeeding may have in the acquisition and establishment of *H. pylori* infection in children. The Pasitos Cohort Study, a birth cohort established on the U.S.-Mexico border, recruited pregnant women from El Paso, Texas and Juarez, Mexico to identify risk factors for *H. pylori* infection during childhood. Assessment of active *H. pylori* infection for 615 children (472 index children and 143 younger siblings) was performed from April 1998 until December 2005 accounting for an average of 3.8 years of follow-up for the index child and an average of 2.5 years of follow-up for younger siblings of the index. The Pasitos Cohort Study provides advantages such as longitudinal

assessment of *H. pylori* status for determination of directionality of *H. pylori* infection in familial transmission. In addition, differentiation between infection acquisition, short-term and long-term establishment of infection was attempted. The current study found that infected older siblings in the household were positively associated with both the acquisition and long-term infection establishment of *H. pylori* in younger siblings. Mothers and breastfeeding may also be positively associated with *H. pylori* outcomes but due to highly uncertain results obtained, further studies are need to assess their association with infection acquisition, short-term infection and long-term infection in children.

TRANSMISSION AND ESTABLISHMENT OF *HELICOBACTER PYLORI* INFECTION

IN CHILDHOOD

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TRANSMISSION AND ESTABLISHMENT OF *HELICOBACTER PYLORI* INFECTION

IN CHILDHOOD

DISSERTATION

Presented to the School of Public Health

University of North Texas

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By

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This dissertation is dedicated to my late father, Jose L. Cervantes, and my mother, Maria Elena Treviño de Cervantes, who came to this country in order to provide their children with the opportunities they were denied; their hard work and sacrifice is embedded in everything I do and my accomplishments are theirs and could not be achieve without them. This dissertation is also dedicated to my husband Christopher W. Famer and my daughter Malena Cervantes Farmer who give my life balance, happiness and meaning, allowing me to forge ahead with this dissertation. I am forever in debit to fate for surrounding me with people who have exhibited nothing less than extreme patience, love and support in all of my personal and academic endeavors.

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CHAPTER 1

INTRODUCTION

Since the isolation of a spiral shaped bacterium (now designated as *Helicobacter pylori*) in patients with gastritis and peptic ulceration by Marshall and Warren in 1982 (Marshall, 2001), the factors related to its transmission and establishment of infection have been extensively studied and continue to generate questions. Transmission of *H. pylori* is thought to occur primarily during childhood via ingestion of the bacteria from vomitus, feces or saliva of an infected individual but these routes have not yet been confirmed (Khalifa, Sharaf, & Aziz, 2010). It is thought that close family contacts, especially that of the mother and siblings, may play a significant role in *H. pylori* infection in childhood (Khalifa et al., 2010). Once *H. pylori* infection occurs short-term (spontaneous loss of infection) and long-term establishment of infection are possible infection outcomes (J. Wang, Blanchard, & Ernst, 2001; Xia & Talley, 1997). Host and bacterial factors may also influence *H. pylori* infection outcomes. One such host factor which may be pertinent to the course of *H. pylori* infection in childhood is breastfeeding status. Breastfeeding, possibly by influencing host immunological responses, may be important in preventing *H. pylori* acquisition and infection establishment (Chak, Rutherford, & Steinmaus, 2009; M'Rabet, Vos, Boehm, & Garssen, 2008).

Although previous studies have investigated the association between the family and breastfeeding status as they relate to *H. pylori* infection in childhood, gaps exist in the current literature which may hinder identifying relevant causal effects. First, no clearly depicted causal mechanisms in the form of directed acyclic graphs (DAGs) for *H. pylori* causal pathways involving familial transmission or breastfeeding exist in the current

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literature. A lack of clear understanding of underlying causal mechanisms via DAGs may have led to inappropriate adjustments resulting in biased estimates. Secondly, many studies relied on *H. pylori* antibody detection methods that cannot distinguish between current and previous *H. pylori* infection. In addition, these antibody-based diagnostic assays may have led to false negative results due to diminished sensitivity in children less than ten years of age, the age of study populations frequently utilized in familial transmission and breastfeeding studies. In regards to breastfeeding studies, another limitation of some past studies is not taking into account duration of breastfeeding; this may have led to an ill-defined exposure that does not correspond to breastfeeding duration standards utilized worldwide. Lastly, few studies exist that conducted longitudinal observation of study participants. Without follow-up in familial transmission studies, it is not possible to determine which family member was infected first and hence could have subsequently transmitted the infection to other family members. For familial transmission studies and *H. pylori*-breastfeeding studies, follow-up is also necessary in order to differentiate between *H. pylori* infection outcomes. According to a thorough literature review conducted, no familial transmission studies or breastfeeding studies to date have observed *H. pylori* infection beyond acquisition.

In order to address the limitations of the existing literature and contribute to the current body of work, three studies were conducted. One study focused on the association between maternal infection status and *H. pylori* acquisition and establishment of a long-term infection. A second study explored the association between sibling infection status and both the acquisition of *H. pylori* and establishment of a long-term infection by identifying the chronological order of *H. pylori* infection

outcomes in older and younger siblings. A third research study estimated the effect of breastfeeding on the outcomes of initial acquisition of *H. pylori* as well as the establishment of both short-term and long-term *H. pylori* infections during childhood.

Data from the Pasitos Cohort Study was utilized for the studies. The Pasitos Cohort Study, a birth cohort study conducted on the U.S.-Mexico border, followed 472 index children and 143 younger siblings from birth during 1998 through 2005, collecting information regarding potential risk factors for *H. pylori* infection in childhood. The Pasitos Cohort Study assessed active *H. pylori* infection status in children utilizing a ^{13}C-urea breath test, a non-invasive diagnostic method with both high specificity and sensitivity in this population.

The three studies longitudinally examined current, active *H. pylori* infection during childhood, defining *H. pylori* infection at differing outcome states (acquisition of infection, short-term and long-term infection). It is important to make the distinction between infection acquisition and establishment of infection as disease outcomes may be influenced by the duration of *H. pylori* infection. Long-term *H. pylori* infection may result in the development of gastric ulcers and stomach cancer. It has been estimated that long-term *H. pylori* infection accounts for roughly half of all cases of gastric ulcers and up to 65% of all cases of stomach cancer (Nyrén, 2007; Walker, Teare, & McNulty, 2008). Although *H. pylori* infection occurs in both developed and developing countries, the greatest burden of infection occurs in the developing world, with a reported prevalence of 90% or greater in some areas (Ford & Axon, 2010; Nahar et al., 2009; Brown, 2000; Khalifa et al., 2010; Magalhaes, Queiroz & Luzza, 2006a). Due to the significant role *H. pylori* infection plays in disease outcomes, especially in resource-

limited populations, it is of value to investigate feasible interventions such as

breastfeeding which may lead to clearance of infection.

Research Questions and Specific Aims

The aims of the three studies addressed the following main questions:

1. What is the effect of the mother's *H. pylori* infection on the acquisition of a child's *H. pylori* infection? What factors modify this effect?

2. What is the effect of the mother's *H. pylori* infection on the acquisition of a child's long-term *H. pylori* infection? What factors modify this effect?

3. What is the effect of having a sibling in the same household who acquires an *H. pylori* infection on acquiring an *H. pylori* infection? What factors modify this effect?

4. What is the effect of having a sibling in the same household with an established, long-term *H. pylori* infection on acquiring an established, long-term *H. pylori* infection? What factors modify this effect?

5. What is the effect of having a sibling in the same household who acquires an *H. pylori* infection on acquiring and established, long-term *H. pylori* infection? What factors modify this effect?

6. Is there a predominate direction of *H. pylori* transmission among siblings (i.e. Do older siblings transmit to younger siblings or vice versa?).

7. What effect does duration of breastfeeding have on *H. pylori* infection acquisition in children? What factors modify this effect?

8. What effect does duration of breastfeeding have on the establishment of an *H. pylori* infection in children? What factors modify this effect?

Aim A- H. pylori Transmission and Maternal Infection Status

1. To estimate the effect of exposure to a mother who was seropositive for *H. pylori* during pregnancy on the acquisition of an *H. pylori* infection in children from the Pasitos Cohort (using the hazard ratio as the effect measure); determine if this effect measure varies by country of residence, age, antibiotic use, *H. pylori* status of siblings and breastfeeding status.

2. To estimate the effect of exposure to a mother who was seropositive for *H. pylori* during pregnancy on the establishment of a long-term *H. pylori* infection in children from the Pasitos Cohort (using the hazard ratio as the effect measure); determine if this effect measure varies by country of residence, age, antibiotic use *H. pylori* status of siblings and breastfeeding status

Aim B- Helicobacter pylori Transmission and Sibling Infection Status[1]

1. To estimate the effect of having a younger sibling in the same household who has acquired an *H. pylori* infection on the *H. pylori* infection acquisition of the older sibling (index child) from the Pasitos Cohort (using the hazard ratio as the effect measure); determine if this effect measure varies by country of residence and age difference between siblings.

2. To estimate the effect of having a younger sibling in the same household who has a long-term *H. pylori* infection on the *H. pylori* infection acquisition of the

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[1]This study has been conducted and published: Cervantes DT, Fischbach LA, Goodman KJ, Phillips CV, Chen S, Broussard CS. Exposure to *Helicobacter pylori*-positive siblings and persistence of *Helicobacter pylori* infection in early childhood. J Pediatr Gastroenterol Nutr. 2010 May; 50(5):481-5. PubMed PMID: 20639704; PubMed Central PMCID: PMC2907533.

older sibling (index child) from the Pasitos Cohort (using the hazard ratio as the effect measure); determine if this effect measure varies by country of residence and age difference between siblings.

3. To estimate the effect of having a younger sibling in the same household who has acquired an *H. pylori* infection on the establishment of a long-term *H. pylori* infection in the older sibling (index child) from the Pasitos Cohort (using the hazard ratio as the effect measure); determine if this effect measure varies by country of residence and age difference between siblings.

4. To estimate the effect of having a younger sibling in the same household who has a long-term *H. pylori* infection on the establishment of a long-term *H. pylori* infection in the older sibling (index child) from the Pasitos Cohort (using the hazard ratio as the effect measure); determine if this effect measure varies by country of residence and age difference between siblings.

5. To estimate the effect of having an older sibling (index child) in the same household who has acquired an *H. pylori* infection on the *H. pylori* infection acquisition of the younger siblings from the Pasitos Cohort (using the hazard ratio as the effect measure); determine if this effect measure varies by country of residence and age difference between siblings.

6. To estimate the effect of having an older sibling (index child) in the same household who has a long-term *H. pylori* infection on the *H. pylori* infection acquisition of the younger siblings from the Pasitos Cohort (using the hazard ratio as the effect measure); determine if this effect measure varies by country of residence and age difference between siblings.

7. To estimate the effect of having an older sibling (index child) in the same household who has acquired an *H. pylori* infection on the of establishment of a long-term *H. pylori* infection in the younger siblings from the Pasitos Cohort (using the hazard ratio as the effect measure); determine if this effect measure varies by country of residence and age difference between siblings.

8. To estimate the effect of having an older sibling (index child) in the same household who has a long-term *H. pylori* infection on the establishment of a long-term *H. pylori* infection in the younger siblings from the Pasitos Cohort (using the hazard ratio as the effect measure); determine if this effect measure varies by country of residence and age difference between siblings.

Aim C-Breastfeeding and Helicobacter pylori Infection

1. To estimate the effect of being breastfed for any duration compared to not being breastfed on the *H. pylori* infection acquisition in children from the Pasitos Cohort (using the hazard ratio as the effect measure); determine if this effect measure varies by country of residence, antibiotic use, *H. pylori* status of the mother and *H. pylori* status of the siblings.

2. To estimate the effect of being breastfed for any duration compared to not being breastfed on the establishment of a short-term *H. pylori* infection in children from the Pasitos Cohort (using the hazard ratio as the effect measure); determine if this effect measure varies by country of residence, antibiotic use, *H. pylori* status of the mother and *H. pylori* status of the siblings.

3. To estimate the effect of being breastfed for any duration compared to not being breastfed on the establishment of a long-term *H. pylori* infection in children from

the Pasitos Cohort (using the hazard ratio as the effect measure); determine if this effect measure varies by country of residence, antibiotic use, *H. pylori* status of the mother and *H. pylori* status of the siblings.

4. To estimate the effect of being breastfed per Healthy People 2020 MICH-21.2 objectives (at least six months) compared to children who were breastfed for less than six months on the *H. pylori* infection acquisition in children from the Pasitos Cohort (using the hazard ratio as the effect measure); determine if this effect measure varies by country of residence, antibiotic use, *H. pylori* status of the mother and *H. pylori* status of the siblings.

5. To estimate the effect of being breastfed per Healthy People 2020 MICH-21.2 objectives (at least six months) compared to children who were breastfed for less than six months on the establishment of a short-term *H. pylori* infection in children from the Pasitos Cohort (using the hazard ratio as the effect measure); determine if this effect measure varies by country of residence, antibiotic use, *H. pylori* status of the mother and *H. pylori* status of the siblings.

6. To estimate the effect of being breastfed per Healthy People 2020 MICH-21.2 objectives (at least six months) compared to children who were breastfed for less than six months on the establishment of a long-term *H. pylori* infection in children from the Pasitos Cohort (using the hazard ratio as the effect measure); determine if this effect measure varies by country of residence, antibiotic use, *H. pylori* status of the mother and *H. pylori* status of the siblings.

7. To estimate the effect of being breastfed per Healthy People 2020 MICH-21.3 objectives (receiving any breastfeeding at twelve months of age) compared to

children who were not receiving any breastfeeding at twelve months of age on the *H. pylori* infection acquisition in children from the Pasitos Cohort (using the hazard ratio as the effect measure); determine if this effect measure varies by country of residence, antibiotic use, *H. pylori* status of the mother and *H. pylori* status of the siblings.

8. To estimate the effect of being breastfed per Healthy People 2020 MICH-21.3 objectives (receiving any breastfeeding at twelve months of age) compared to children who were not receiving any breastfeeding at twelve months of age on the establishment of a short-term *H. pylori* infection in children from the Pasitos Cohort (using the hazard ratio as the effect measure); determine if this effect measure varies by country of residence, antibiotic use, *H. pylori* status of the mother and *H. pylori* status of the siblings.

9. To estimate the effect of being breastfed per Healthy People 2020 MICH-21.3 objectives (receiving any breastfeeding at twelve months of age) compared to children who were not receiving any breastfeeding at twelve months of age on the establishment of a long-term *H. pylori* infection in children from the Pasitos Cohort (using the hazard ratio as the effect measure); determine if this effect measure varies by country of residence, antibiotic use, *H. pylori* status of the mother and *H. pylori* status of the siblings.

Background

Epidemiology and Transmission

Helicobacter pylori a Gram negative, flagellated, spiral shaped bacterium is one of the most ubiquitous bacterial species known to infect humans (H. Mitchell, 2001). It is

estimated that half of the world's population is infected with *H. pylori* (Khalifa et al., 2010) with prevalence estimates differing greatly between developing and developed countries. The reported prevalence estimates in developing areas of Asia and Africa range from 70-90% while prevalence from developed areas of Europe and North America are estimated to be between 7-30% (Ford & Axon, 2010; Khalifa et al., 2010; Nahar et al., 2009). In developed countries, a cohort effect, in which older segments of the population exhibit a higher *H. pylori* prevalence, is often noted (Khalifa et al., 2010; Kivi &Tindberg, 2006) and the estimated incidence during childhood is drastically lower compared to that of developing countries. This vast divergence in *H. pylori* prevalence and incidence within and among different areas is often attributed to stark differences in risk factors such as sanitation advancements, living conditions and access to medical care (H. Mitchell, 2001).

Direct transmission from person to person, although not yet confirmed, is most likely the most frequent mode of *H. pylori* transmission and specific routes may include fecal-oral, oral-oral and gastro-oral (Vale & Vitor, 2010). *H. pylori* has been detected and isolated from feces and vomitus as well as saliva of those infected (Vale & Vitor, 2010). The role of indirect transmission through environmental reservoirs may be of significance as bacteria have been detected in reservoirs such as food and water (Vale & Vitor, 2010). But unlike human reservoirs, isolation of *H. pylori* has not been successful in these vehicles and it may be possible that a coccoid, non-culturable form of the bacterium may exist which could serve to extend survival of *H. pylori* in the environment (Delport & van der Merwe, 2007; Vale & Vitor, 2010).

Infection Establishment and Host Immune Responses

Once acquired, *H. pylori* may establish an infection. In order for *H. pylori* to establish an infection, i.e. achieve colonization, three critical steps must occur after transmission (Testerman, McGee, & Mobley, 2001): 1) adherence to a niche conducive to bacterial growth and proliferation within the host; 2) avoidance, manipulation or exploitation of host immune response and defenses; and 3) acquisition of nutrients for bacterial replication. With roughly 50% of the world's population infected (H. Mitchell, 2001), this is a testament to *H. pylori's* superior ability to continuously adapt to its host and achieve these critical steps.

If any of the three steps towards colonization are not continuously maintained, a short-term infection leading to spontaneous clearance of *H. pylori* in the absence of antibiotic treatment may occur. Short-term infections have been reported to occur frequently during childhood, (Delport & van der Merwe, 2007) taking place in up to 80% of infected children (Broussard et al., 2009; Delport & van der Merwe, 2007; Goodman et al., 2005). Short-term infections have also been documented in older adults (Leal-Herrera et al., 2003) but studies of short-term infection in adults are needed. One possible explanation for short-term infections may be the inability to mount a robust immune response against *H. pylori* in these age groups. Although this seems counterintuitive, *H. pylori's* evolved ability to successfully adapt to its host has resulted in the utilization of the immune responses to promote the maintenance of an established infection (long-term infection) and hence benefit bacterial survival (J. Wang et al., 2001). In mounting an immune response, a cascade of cytokines including TNF and INF-γ stimulate the production of one of several cell receptors recognized by *H. pylori*, class II

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MHC receptors; these receptors can be recognized by *H. pylori* adhesions (J. Wang et al., 2001). Once adherence to host cells occurs via receptors, disruption of tight junctions between cells may then allow access of *H. pylori* to interstitial fluid which contains nutrients for bacterial proliferation (J. Wang et al., 2001). Therefore, a diminished immune response leading to reduced cytokine release may be one factor in deterring the establishment of a long-term infection and hence resulting in a short-term infection.

Detection and Diagnostic Methods

In order to detect and differentiate between short and long-term *H. pylori* infections in children, both follow-up throughout time and laboratory diagnostic methods to distinguish between an active, current *H. pylori* infection and a past *H. pylori* infection are critical. Laboratory diagnosis of *H. pylori* infection is achieved by either detection of the *H. pylori* bacterium (antigen) or detection of antibodies generated in response to infection. *H. pylori* bacterial detection is achieved by stool antigen test (SAT), ^{13}C urea breath test (^{13}C-UBT) to measure *H. pylori's* urease activity or identification or isolation of *H. pylori* from gastric biopsy (Calvet, Lehours, Lario, & Megraud, 2010). Detection of *H. pylori* antigen is indicative of a current, active infection (Guarner, Kalach, Elitsur, & Koletzko, 2010). Antibody-based assays detect IgG anti-*H. pylori* antibodies in serum, saliva or urine (Guarner et al., 2010) and once the infection is eliminated, a drop in antibody levels for determination of elimination may be detected within 6 months (Marchildon, Balaban, Sue et al., 1999). However, detectable IgG antibody levels against *H. pylori* have been documented to persist for months or years after active infection, hence such assays are able to identify both current and previous infections

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(Calvet et al., 2010). A major limitation of antibody based assays, in addition to the inability to differentiate between current and previous infection, is diminished sensitivity and specificity in young children (Rowland et al., 2006; Delport & van der Merwe, 2007; Rothenbacher et al., 1997). Individuals < 10 years of age may not generate adequate antibody levels for detection by commercially available antibody based assays (Khanna et al., 1998). This may therefore lead to false negative results. In adults and adolescents, determination of *H. pylori* active infection status via antibody detection is acceptable if sensitivity and specificity parameters of the assay have been evaluated for the base population (Khanna et al., 1998). Also, as spontaneous elimination in the absence of *H. pylori* treatment is less likely to occur in adults and possibly adolescents (Khanna et al., 1998), detection of anti-*H. pylori* antibodies are likely indicative of a current, active *H. pylori* infection if antibiotic treatment has not been given.

Disease Outcomes

Upon establishment of infection, *H. pylori* leads to chronic active gastritis, a chronic inflammation of the stomach lining, in almost all those infected (Kivi &Tindberg, 2006). Despite inflammation, roughly 70% of infected individuals do not experience signs or symptoms (Vale &Vitor, 2010). In a small percentage of people, chronic active gastritis may then result in the development of peptic ulcers (between 10-15% of infected people) (Mourad-Baars, Hussey, & Jones, 2010) and/or cellular alterations resulting in atrophy and intestinal metaplasia (Dixon, 2001). These cellular alterations may result in the development of stomach cancer, but also may be reversible upon eradication of *H. pylori* (Dixon, 2001). It is believed that 1% of people presenting with long-term *H. pylori* infections will develop some form of stomach cancer (Mourad-Baars et al., 2010); it has

been estimated that this group accounts for approximately 65% (Nyrén, 2007; Walker et al., 2008) of the estimated 989,000 cases of stomach cancers worldwide annually (World Cancer Research Fund International, 2011).

Transmission Factors

Transmission, the first critical step in establishing an infection, is thought to occur primarily during childhood in both developed and developing countries (Delport & van der Merwe, 2007), with incidence in adulthood remaining low (~<0.1-4.5% annually) even in areas of high prevalence (Silva et al., 2010; Weck & Brenner, 2011). In regards to the infectious agent, it is possible that factors which favor *H. pylori* transmission during childhood may include bacterial diversity and adaptability. *H. pylori* has co-evolved with humans, acquiring the ability to rapidly generate strains containing mechanisms that effectively and efficiently transmit from host to host, evade the host's immune response and thrive in the varying gastric conditions (Basso, Plebani, & Kusters, 2010; Delport & van der Merwe, 2007; Vale &Vitor, 2010).

A recent study comparing *H. pylori* strains in symptomatic adults and children in North America found that *H. pylori* strains in children contain on average more genes than adult strains (Talarico et al., 2009). In this study, the genes more commonly found in childhood *H. pylori* strains, yet missing with more frequency in adult strains, encode transport and binding proteins as well as cell envelope proteins. Talarico (Talarico et al., 2009) indicates that these proteins may be essential in transmission. Therefore, childhood may promote transmission efficient strains. Long-term infection establishment common as a person progresses to adulthood may cause selective loss of genes

encoding certain transport and binding proteins (a shift in bacterial strains) resulting in *H. pylori* strains less efficient in transmission.

Contributing to this theory, a study conducted of medium-low income symptomatic adults and children in Mexico (Gonzalez-Valencia et al., 2000) found that mixed infection with multiple *H. pylori* strains is common. Children harbored strains which were less associated with ulceration and atrophic gastritis (CagA-) compared to adults (CagA+). Again, this finding may suggest that *H. pylori* strains found in children differ in virulence markers possibly because immune factors in childhood result in a shift in bacterial strains to those that are more adapt at transmission; a more robust immune response more common as one proceeds to adulthood may lead to long-term infection establishment and loss of transmission efficient bacterial strains and increased proportion of strains with increased virulence.

In regards to transmission routes, gastrointestinal illnesses may provide abundant opportunities for *H. pylori* transmission during childhood. Childhood is marked by increased gastrointestinal disease episodes attributable to emergent hygiene practices, an immunologically naive host, and close person-to-person contact (Perry et al., 2006). It is believed that close, direct, contact facilitates oral-oral, fecal-oral and gastro-oral *H. pylori* transmission routes (Kivi &Tindberg, 2006). In a study conducted by Perry and colleagues (Perry et al., 2006), children who were exposed to an *H. pylori*-infected person with gastrointestinal illness were almost five times more likely to acquire a confirmed or probable *H. pylori* infection (adjusted OR= 4.8, 95% CI 1.4, 17.1) compared to children who were not exposed to an *H. pylori* infected person with gastrointestinal illness; a greater risk for *H. pylori* infection was observed in children

exposed to vomiting in an *H. pylori* infected person (adjusted OR= 6.3, 95% CI 1.6,

24.5) versus those exposed to an *H. pylori* infected person reporting diarrhea alone

(adjusted OR= 3.0, 95% CI 0.5, 17.1). These measures were adjusted for age, sleeping

density and the proportion of household completing both visits during the study.

In elucidating factors integral to *H. pylori* transmission, both environmental and

familial transmission paths have been investigated. Environmental transmission

involves possible environmental reservoirs as well as non-familial human reservoirs

(Vale &Vitor, 2010). Observational and genomic based studies have found evidence of

horizontal transmission especially in areas of high prevalence and societies in which

close contact with individuals outside of the family unit is common (Kivi & Tindberg,

2006; Schwarz et al., 2008). The vast majority of studies favor familial transmission,

which involves transmission among family members, as the primary path of *H. pylori*

transmission (Delport& van der Merwe, 2007;Khalifa et al., 2010;Kivi & Tindberg,

2006;Vale & Vitor, 2010). In particular, infected mother and siblings may promote *H.*

pylori infection (Konno et al., 2005; Magista et al., 2005; K. Muhsen, Athamna, Bialik,

Alpert, & Cohen, 2010; Rowland et al., 1999; Rowland et al., 2006; Taneike, Tamura,

Shimizu, Yamashiro, & Yamamoto, 2001; Weyermann, Adler, Brenner, &

Rothenbacher, 2006). Some studies have described strong positive associations

between maternal infection and *H. pylori* infection in her children (Rowland et al., 2006;

Rowland et al., 1999), while other studies have found strong positive associations

between a siblings and *H. pylori* infection in children or at minimum a significant risk

factor (Rowland et al., 2006; Rowland et al., 1999). The father has also been found to

contribute to *H. pylori* infection of his children albeit to a much lesser degree

(Weyermann, Rothenbacher & Brenner, 2009). Again, these studies are limited as most were not longitudinal in nature and did not distinguish between active and current *H. pylori* infection as well as acquisition and infection establishment.

As the greatest risk for *H. pylori* transmission occurs mainly during childhood, a limited number of observational studies have explored the association between breastfeeding and *H. pylori* infection. Although a few studies have found a positive association between breastfeeding and *H. pylori* acquisition after accounting for maternal antibodies (Gold, Khanna, Huang, Lee, &Banatvala, 1997; Rothenbacher, Bode, & Brenner, 2002), most studies have detailed results consistent with a protective effect against *H. pylori* infection (Ertem et al., 2003; Malaty et al., 2001; McCallion et al., 1996; B. V. Nguyen et al., 2006; Omar et al., 2001; Pearce et al., 2005; Rodrigues et al., 2006; Rowland et al., 2006). Breast milk may protect against *H. pylori* acquisition as it contains antimicrobial components which may not only reduce the incidence of disease during childhood but may also influence immune response and modulation to pathogens later in life (Chirico, Marzollo, Cortinovis, Fonte, &Gasparoni, 2008;M'Rabet et al., 2008).

The passive immunity conferred by breast milk may contribute to selective pressure for *H. pylori* strains which contain enhanced transmissibility factors versus selective pressures of active immunity which may select for strains less efficient at transmission and more adapt to long-term establishment. Breast milk contains secretory IgA antibodies which may block colonization of *H. pylori* without activating the inflammatory response (Lawrence & Pane, 2007); again, the inflammatory response has been found to trigger the complement and cytokine cascades which may favor long-term infection.

Studies of *H. pylori* infected mothers found their breast milk contains high levels of anti-*H. pylori* specific IgA and their children acquired *H. pylori* later compared to breastfed children of *H. pylori* negative mothers (Thomas et al., 1993; Weaver, 1995). Breast milk also contains bioactive factors including proteins such as casein, lactoferrin and lysozyme which act at the mucosal level to prevent bacterial attachment to epithelial cells again, without activating the systemic immune response (Lawrence & Pane, 2007). Lastly, breast milk contains prebiotics which promote the development of the host's gut immunity by stimulating growth of probiotic bacteria such as *Lactobacillus* and *Bifidobacteria* species (Lawrence & Pane, 2007). These commensal bacterial species improve the gastric junction barriers between cells, promote mucous production and secretory IgA levels (Lawrence & Pane, 2007). Again, this interaction with the host's passive immunity may induce short-term infection (spontaneous clearance) and also possibly selectively favor *H. pylori* strains which are more efficient in transmission. It is important to note that current observational breastfeeding studies are limited by lack of follow-up throughout time, differentiation between *H. pylori* acquisition, short-term and long-term infection and little information pertaining to duration of breastfeeding on *H. pylori* outcomes.

Population-based studies which may assist in elucidating the association between breastfeeding and *H. pylori* infection are limited. The role of the immune response in *H. pylori* infection has been well document in laboratory-based studies, but details relating to factors and pathways leading to short or long-term infection which can only be assessed in human populations via observational studies have yet to be elucidated (J. Wang et al., 2001).

Proposed Mechanism

One of the simplest models of infectious disease causation is that of the epidemiologic triad (Dicker, 2002). In the epidemiologic triad, agent, host, and environmental factors must be linked via portals of exit and entry facilitated by appropriate modes of transmission for infection to occur. Therefore, any disruption of these factors will alter the spread of infectious agents such as *Helicobacter pylori*. Directed acyclic graphs (DAGs) presented in Appendices A-C were created using the construct of the epidemiologic triad (via proposed relationships between agent, host and environmental factors) to illustrate the mechanism by which the exposures of interest (*H. pylori* infected mother/siblings or breastfeeding) may promote or prevent the outcome, *H. pylori* infection states, in childhood.

DAGs were created and analyzed with the assistance of DAGitty version 1.1 (Textor, Hardt, &Knuppel, 2011). DAGitty is a software program which is used to draw and analyze DAGs, identifying sufficient adjustment sets. Adjustment sets are a set of covariates within the DAG that if adjusted, stratified or selected via matching or restriction, will reduce bias when estimating the causal effect of the exposure on the outcome. DAGitty analyzes the DAG and identifies sufficient adjustment sets via a six-step process described by Shrier and Platt (2008). Briefly, Shrier and Platt (2008) state that first a potential confounding variable should not be the caused by the exposure. Second, all variables that do not cause the exposure, outcome or the proposed confounding variable(s) either directly or indirectly are removed from the DAG. In the third step, any arrows originating from exposure are removed. In step four, variables that directly cause the same variable are connected. Lastly, all arrowheads are striped

from arrows and all lines connecting the proposed confounder and other variables are removed. This six-step process is repeated by DAGitty until a minimal sufficient set for the total effect is identified and these variables are displayed in an output window. It is important to note this process is highly dependent on determining true causal assumptions by the user who must properly identify and include causal covariates in DAGitty; this process requires extensive search of the literature.

In the epidemiologic triad, the agent, *H. pylori*, evolves with its human host subsequently resulting in diversity in strains harbored by an individual. Differences in *H. pylori* strains isolated from children and adults have been observed, most notably in regards to sequences which may encode proteins involved in transmissibility and virulence (Gonzalez-Valencia et al., 2000; Talarico et al., 2009). Children may harbor a greater proportion of *H. pylori* strains that are efficient in transmission while adults may harbor a greater proportion of strains with enhanced virulence properties (Gonzalez-Valencia et al., 2000; Talarico et al., 2009). It is possible that the immune response mounted during childhood not only promotes short-term infection, but also aids in the selection of transmission efficient strains. Therefore, bacterial transmissibility properties of *H. pylori* may enhance transmission between children. Yet the current literature indicates predominant *H. pylori* transmission from mother to child (Weyermann et al., 2009).This may be due to a higher prevalence of *H. pylori* infection in adult mothers versus children in the household. A transmission pathway from child to mother was not presented; current literature does not support child-to-mother transmission and due to high prevalence of *H. pylori* in Pasitos Cohort mothers at baseline (65%) (O'Rourke, Goodman, Grazioplene, Redlinger, & Day, 2003), this scenario most likely does not

represent a common transmission pathway. DAGs presented in Appendices A and B illustrate pathways which suggest both mother-child and sibling-child transmission, respectively. A DAG considering an infected sibling as a possible intermediate between an *H. pylori* infected mother and her child is presented in Appendix A; in this scenario, the mother is assumed to be the source of infection in the household. Appendix B presents an *H. pylori* infected mother as a possible confounder between the *H. pylori* infection of siblings.

Host factors are "intrinsic factors that influence an individual's exposure, susceptibility, or response to a causative agent" (Dicker, 2002). In regards to *H. pylori*, previous studies have identified age (Khalifa et al., 2010; Vale & Vitor, 2010), breastfeeding (Chak et al., 2009) and antibiotic use (Broussard et al., 2009) as host factors which may influence infection acquisition and long-term infection; DAGs presented in Appendices A-C present the relationships these factors play in the transmission of *H. pylori* infection.

One important mechanism modulated by age which facilitates susceptibility to *H. pylori* infection is the immune response. During some point in childhood, the developing immune system may not mount a sufficient immune response needed to trigger the inflammatory response which may subsequently promote *H. pylori* to establish a long-term infection (J. Wang et al., 2001) and hence spontaneous elimination of infection may occur. Spontaneous elimination of infection is most likely observed during childhood due to the insufficient immune response mounted during this developmental age, leading to the possible selection of transmission efficient strains.

Age is also significant as childhood presents with unique opportunities for exposure to *H. pylori* reservoirs and consequently transmission (Khalifa et al., 2010). Childhood represent a time when close, intimate contact occurs with the mother as well as siblings. If the mother or child is infected with *H. pylori* or any infectious agent, the not yet adequate personal hygiene skills of childhood combined with close contact with family members and other children may facilitate oral-oral, gastro-oral and fecal-oral transmission routes and *H. pylori* infection may ensue (Kivi & Tindberg, 2006; Perry et al., 2006). This combination of developing personal hygiene and close contact with family members, and perhaps in daycare or preschool, may also be a prominent factor for increased gastrointestinal disease experienced in childhood.

Breastfeeding also influences susceptibility to *H. pylori* infection via the immune response. Various components in breast milk may stimulate non-inflammatory immune responses favoring *H. pylori* clearance versus establishment of a long-term infection (Chak et al., 2009; Chirico et al., 2008; Lawrence & Pane, 2007). Due to the role breastfeeding may play in the immune response, lack of breastfeeding may lead to more childhood illnesses and hence increased antibiotic use (M'Rabet et al., 2008). Antibiotic use varies by age, with use being more extensive in early childhood (Bowlware & Stull, 2004; Broussard, Goodman, Nurgalieva, Fischbach, & Gold, 2010). Antibiotics taken for common childhood ailments, especially antibiotics which have been demonstrated to be effective against *H. pylori*, may account for *H. pylori* spontaneous clearance (short-term infection) in some children (Broussard et al., 2009).

For the causal diagram pertaining to the hypothesis of *H. pylori* transmission from mother to child, the above stated intrinsic factors did not result in the potential for

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confounded paths and were therefore not considered as covariates to be controlled in analysis (Appendix A). For causal mechanisms pertaining to hypothesized *H. pylori* transmission between siblings, antibiotic use and immune response were identified by DAGitty version 1.1 as intrinsic factors which may be confounders (Appendix B). As the immune response cannot be controlled directly, a factor that may influence the immune response, child's age, will be controlled for in the analysis. The DAG presented in Appendix C illustrates the possible causal mechanism between breastfeeding and *H. pylori* infection; here the child's age was identified as a potential confounder.

Environment factors represent extrinsic components that influence transmission of an infectious agent to a host (Dicker, 2002). Again, DAGs presented in Appendices A-C present the relationships these factors play in *H. pylori* infection. The physical environment has been implicated in *H. pylori* transmission, with detection of *H. pylori* in both water and food; this most likely reflects conditions which are conducive to contamination of these media including lack of access to modern waste water facilities and crowding (Vale & Vitor, 2010). Again, hygienic practices typically observed in childhood may contribute to this effect. One important extrinsic factor, crowding, is often dictated by household size. Both household size and maternal educational status represent well documented socioeconomic indicators that influence *H. pylori* infection status (Ford & Axon, 2010; Khalifa et al., 2010; Kivi & Tindberg, 2006).

Crowding may also lead to close contact with *H. pylori* infected individuals, a second significant extrinsic factor. As previously stated, children may be more likely to harbor strains which are efficient in transmission. It is possible that familial dynamics, especially those observed in households with a large number of children, may promote

H. pylori transmission by placing children in close contact with other children who harbor transmission efficient *H. pylori* strains. In addition, gastrointestinal illness which occurs most often during childhood may enhance transmission routes favored by *H. pylori*. Studies of families of Mexican descent have found that older siblings often share the responsibility of caring for and entertaining the younger children in the family (Callanan & Perez-Granados, 1997). At approximately 4 years of age, fine and gross motor skills as well as communication skills are well developed, marking a time period in which a child becomes less dependent on the mother and more dependent on the older siblings to define emotional and behavioral development (Callanan & Perez-Granados, 1997). Therefore during childhood, the most common source of *H. pylori* infection may shift from mother to siblings, and as siblings may harbor more transmission efficient *H. pylori* strains, they may represent a more dominant source of *H. pylori* infection than the mother.

For the DAGs presented in Appendices A and B regarding the proposed familial transmission studies, crowding was identified as an extrinsic factor which may confound mother-child association while crowding and having an *H. pylori* infected mother were identified as extrinsic factors which may confound child-child associations. No extrinsic factors were identified which may confound the association between breastfeeding and *H. pylori* infection.

H. pylori infection is perpetuated worldwide by the interplay of agent, host and environment supported by particular modes of transmission. The current studies guided in design by the causal mechanisms depicted via DAGs specifically examined how

maternal and sibling *H. pylori* status as well as breastfeeding are associated with *H. pylori* status in childhood.

CHAPTER 2

LITERATURE REVIEW

A thorough literature search was performed using the MEDLINE database to identify published literature related to *Helicobacter pylori* transmission and establishment of infection. Specifically, searches for studies that evaluated breastfeeding practices or parent-child or sibling-child determinants of *H. pylori* transmission or *H. pylori* associated disease as an outcome were conducted. PubMed was utilized to separately query search terms described in Table 1. Retrieved publications were then reviewed to determine if they met the following inclusionary criteria: English language, publication dates from January 1990-April 2012, peer-reviewed and investigational in nature (not reviews, abstracts or editorials), and studies of human participants.

TABLE 1. SEARCH TERMS FOR LITERATURE REVIEW

Breastfeeding-*Helicobacter pylori* Associations[a] (Number of articles retrieved)	Familial *Helicobacter pylori* Transmission[a] (Number of articles retrieved)
Helicobacter pylori Breastfeeding (24)	*Helicobacter pylori* sibling transmission (54)
Helicobacter pylori breast milk (52)	*Helicobacter pylori* family transmission (168)
Helicobacter pylori nursing (94)	*Helicobacter pylori* familial transmission (174)
	Helicobacter pylori intrafamilial transmission (49)
	Helicobacter pylori mother transmission (72)
	Helicobacter pylori maternal transmission (59)
	Helicobacter pylori vertical transmission (40)
	Helicobacter pylori paternal transmission (4)
[a] Use of *campylobacter pylori* or *campylobacter pyloridis* did not result in additional articles	

In order to retrieve additional articles not identified by the MEDLINE search, references cited from retrieved articles were checked for publications which also met the inclusionary criteria. A total of 111 articles met the inclusionary criteria and were included in the review (Appendices D, G, and K).For each publication, data was extracted on publication year, study design, study population, geographic location of study, sample size, *H. pylori* detection methodology, sensitivity and specificity of *H. pylori* detection methods, length of follow-up (if applicable), statistical analysis methods utilized, covariate adjustment, effect measure modifiers and measures of associations (if presented).

Intrafamilial *H. pylori* Transmission

Numerous studies have been conducted to ascertain the associations between specific family members and *H. pylori* infection status in children. Associations of the mother and siblings with *H. pylori* infection acquisition have been described with contradictory conclusions across studies. Differences in study parameters of studies identified may greatly influence the ability to decipher the associations between family members and therefore, must be critically evaluated.

Maternal Associations

Fifty-two articles meeting the inclusionary criteria presented information pertaining to the association of the mother in the transmission of *H. pylori*. The mother was identified as a strong contributor to *H. pylori* infection by the majority of studies examining vertical transmission (Appendices D and E) (Aguemon, Struelens, Massougbodji, & Ouendo, 2005; Braga et al., 2007; Farrell, Doherty, Milliken, Shield, &McCallion, 2005; Fialho et al., 2010; Ito et al., 2006; Kivi, Johansson, Reilly, & Tindberg, 2005; Ma et al., 1998;

Malaty et al., 2000; Miyaji et al., 2000; Miyazaki, Kato, Takata, & Une, 2002; K. Muhsen, Athamna, Athamna, Spungin-Bialik, & Cohen, 2006; B. V. Nguyen et al., 2006; V. B. Nguyen et al., 2006; O'Rourke et al., 2003; Rocha et al., 2003; Rodrigues et al., 2006; Roma et al., 2009; Rothenbacher et al., 1999; Rothenbacher, Winkler, Gonser, Adler, & Brenner, 2002; Rowland et al., 2006; Weyermann et al., 2006; Weyermann et al., 2009; Yang, Sheu, Lee, Yang, & Wu, 2005).

One such cross-sectional study which found a strong positive association between *H. pylori* status of the mother and that of her child conducted by Weyermann and colleagues (Weyermann et al., 2009) in Ulm, Germany found that children were thirteen times more likely to be infected with *H. pylori* if their mother was also infected compared to children whose mother was not infected with *H. pylori* (adjusted OR= 13.0, 95% CI: 3.0, 55.2). This measure of association was estimated after adjusting for *H. pylori* infection of the spouse and siblings, nationality and single-child status. Similarly, in a cross-sectional study conducted in Minas Gerais, Brazil (Rocha et al., 2003), it was found that the odds of an active *H. pylori* infection in children whose mother was seropositive for *H. pylori* was over twenty-two times that of *H. pylori* infected children whose mother was seronegative for *H. pylori* (adjusted OR= 22.7, 95% CI: 2.3, 223.2). This measure of association was estimated after adjusting for age of the child, number of *H. pylori* infected siblings and serostatus of the father.

A strength of the both studies conducted by Weyermann and colleagues as well as by Rocha and colleagues is assessment of *H. pylori* infection in children via methods other than detection of anti-*H. pylori* antibodies, an element missing in many studies examining mother-child *H. pylori* transmission (Appendix D) (Aguemon et al., 2005; Ito

et al., 2006; Kivi et al., 2005; Malaty et al., 2000; Miyaji et al., 2000; Miyazaki et al., 2002; B. V. Nguyen et al., 2006; O'Rourke et al., 2003). The use of anti-*H. pylori* IgG assays is not recommended in children less than 10 years of age; children of this age may not be able to mount a detectable immune response to infection due to lack of immunological maturity, leading to false negative results and non-differential misclassification of *H. pylori* status(Khanna et al., 1998). A recent meta-analysis demonstrated a high degree of variability in both sensitivity and specificity in the performance of immunoassays in children, with reported sensitivities ranging from 79-94% and specificity ranging from 89-96% (Leal, Flores, Garcia-Cortes, Cedillo-Rivera, & Torres, 2008). Non-differential misclassification of *H. pylori* infection resulting from variability in immunoassay test performance in children may lead to a negative bias towards the null. Although studies which did utilize antibody-based assays to infer *H. pylori* infection in children did find positive associations between maternal *H. pylori* seropositive status and *H. pylori* seropositivity of the child, the magnitude of effect as estimated by adjusted measures of association were weaker compared to studies which utilized diagnostic methods for detection of active infection of *H. pylori* (Appendix E). Utilizing a fixed effect model to obtain an overall estimate of effect, it was found that in studies which assessed active *H. pylori* infection (biopsy and histology, ^{13}C-UBT or SAT) a greater magnitude of overall effect was noted (overall OR= 4.4, 95% CI: 3.3, 5.7) versus those studies which assess anti-*H. pylori* IgG (overall OR= 3.8, 95% CI: 2.5, 5.8) (Appendix E).

A small number of studies utilized maternal gastric disease status as a proxy for *H. pylori* infection in assessing the association between the mother and familial *H. pylori*

infection status, with conflicting results (Appendix D) (Brenner, Rothenbacher, Bode, & Adler, 1998; Brenner, Bode, & Boeing, 2000; Chang et al., 2002; Gasbarrini et al., 1995; Kikuchi, Kurosawa, &Sakiyama, 1998; Kurosawa, Kikuchi, Inaba, Ishibashi, & Kobayashi, 2000; B. V. Nguyen et al., 2006). In a cross-sectional study of 6-year old children in Germany, Brenner et al (Brenner et al., 1998) found that the odds of an *H. pylori* infection in children with a mother with peptic ulceration was more than eleven times that of an active *H. pylori* infection in a child whose mother did not have a peptic ulcer (adjusted OR=11.7, 95% CI: 3.8, 36.2). This result was obtained after adjusting for nationality, mother's education, father's education, housing density, birth order, history of breastfeeding, attendance at a nursery, antibiotic treatment and history of peptic ulcer in the father. A much weaker positive association between maternal peptic ulceration and *H. pylori* status of the child was observed prior to adjustment for covariates in the study conducted by Brenner and colleagues (crude OR=2.4, 95% CI: 0.9, 6.4). As many of the covariates adjusted for in this study were only associated with the outcome, (*H. pylori* status of the child) and not the exposure, (peptic ulceration status of the mother), unnecessary adjustment most likely resulted in inflated point estimates (Cummings, 2009; Schisterman, Cole, & Platt, 2009).

A study conducted by Gasbarrini et al (Gasbarrini et al., 1995) found a negative association between maternal peptic ulcer and *H. pylori* status of her adult child. Gasbarrini and colleagues conducted a cross-sectional study in individuals over the age of 18 and found the odds of *H. pylori* seropositivity in individuals over 18 years of age whose mother had a peptic ulcer was 0.7 times that of seropositivity in individuals over 18 years of age whose mother did not have a peptic ulcer (95% CI: 0.5, 1.1). Yet the

same study found that the odds of *H. pylori* seropositivity in individuals whose mother had gastric cancer was 1.4 times that of seropositivity in individuals who mother did not have gastric cancer (95% CI: 0.79, 2.28). This is in concordance with another cross-sectional study conducted by Brenner and colleagues (Brenner et al.,1998) in which the odds of *H. pylori* seropositivity in individuals 30-74 years of age whose mother had gastric cancer was 3.2 times that of seropositivity in individuals 30-74 years of age whose mother did not have gastric cancer (95% CI: 1.0, 10.2).

Gastric disease may serve as a poor proxy for *H. pylori* infection in transmission studies as peptic ulcers and gastric cancer only manifest in a very small percentage of those infected with *H. pylori* (15% and 1%, respectively) (Mourad-Baars et al., 2010) and may result due to other lifestyle factors. For example, although peptic ulcers result primarily from *H. pylori* infection, these ulcers, especially gastric ulcers, can occur for other reasons such as use of non-steroidal anti-inflammatory drugs (Alakkari, Zullo, & O'Connor, 2011). In regards to gastric cancer, *H. pylori* infection has mainly been associated with non-cardia cancer and not cancer of the gastric cardia (Helicobacter and Cancer Collaborative Group, 2001). Compared to studies in which maternal *H. pylori* status was assessed by active infection (Appendix E), measures of associations to estimate the magnitude of effect of *H. pylori* mother-child associations were weaker when maternal gastric disease was used as a proxy for maternal *H pylori* infection (Appendix F). Utilizing a fixed effect model to obtain an overall estimate of effect, it was found that studies which assessed active *H. pylori* infection (biopsy and histology, ^{13}C-UBT or SAT) in both the mother and child had a greater magnitude of overall effect

(overall OR= 5.6, 95% CI: 3.8, 8.2) versus those studies which assess maternal gastric disease as the outcome (overall OR= 1.2, 95% CI: 1.0, 1.5) (Appendix F).

Although most studies found a positive association between maternal gastric disease and *H. pylori* status of the child (Appendix F), the magnitude of the estimated measure of effect may have been affected by non-differential misclassification leading to a negative bias towards or even beyond the null.

Several studies were conducted to determine genetic similarities in *H. pylori* strains between that of the mother and her child (Appendix D) (Bamford et al., 1993; Han et al., 2000; Herrera et al., 2008; Kivi et al., 2003; Kivi et al., 2007; Konno et al., 2005; Konno et al., 2008; Nahar et al., 2009; Taneike et al., 2001). All of these studies found *H. pylori* strains which were genetically similar in both the mother and her child. A study conducted by Herrera et al (Herrera et al., 2008) of 62 families in the shantytowns of Peru examined *H. pylori* strain relatedness via RAPD, random amplified polymorphic DNA, finding that 30% of mother-child *H. pylori* strains matched at 99.5%, with no *H. pylori* strain relatedness detected outside of the family. In another study by Taneike and colleagues (Taneike et al., 2001) of *H. pylori* strains in a Japanese family, one child in a family was initially infected with the same genetic *H. pylori* strain as the father as determined by ribotyping analysis. Thirty-six weeks later, after all family members, with the exception of the mother, underwent eradication therapy, the child was found to be infected with the same genetic *H. pylori* strain as the mother which differed from the father.

One weakness of transmission studies, which utilize ribotyping or RAPD based assays to make mother-child *H. pylori* infection strain concordance determinations, is

the possibility of low sensitivity. Most studies which examined maternal-child *H. pylori* genetic strains relied on either ribotyping or RAPD based assays (Bamford et al., 1993; Han et al., 2000; Herrera et al., 2008; Kivi et al., 2003; Kivi et al., 2007; Konno et al., 2005; Konno et al., 2008; Nahar et al., 2009; Taneike et al., 2001). Ribotyping and RAPD molecular based assays are considered mid-resolution molecular methods which may be sensitive to DNA quality and concentration as well as incomplete digestion by the enzymes utilized resulting in low typability; typability percentages of 13-66% have been reported (Delport& van der Merwe, 2007). Low typability of *H. pylori* isolates may result in non-differential misclassification of both the exposure (*H. pylori* infection strain of the mother) as well as the outcome (*H. pylori* infection strain of the child). This non-differential misclassification can result a bias towards the null. None of the studies examining mother-child *H. pylori* strain relatedness estimated measures of effect. It has also been demonstrated that those infected with *H. pylori* may harbor multiple strains (Schwarz et al., 2008). Biopsied material or gastric fluid from those infected with *H. pylori* may not contain a representation of all *H. pylori* strains a person is infected with, making it difficult to determine genetic relatedness of *H. pylori* strains among mother and child.

In addition to weaknesses related to laboratory methodologies, it is important to note that detection of genetically identical or related *H. pylori* strains in family members does not rule out the possibility of a common environmental source or provide information regarding direction of *H. pylori* transmission. Studies which determined mother-child *H. pylori* strain concordance did not account for or adjust for potential covariates such as environmental factors which would most likely obscure the causal pathway between

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mother-to-child *H. pylori* transmission. These studies (Appendix D), with the exception

of the study conducted by Taneike and colleagues (Taneike et al., 2001), were also

cross-sectional in nature and it could not be determined if the child was infected by the

mother or if the child infected the mother.

A major limitation of most studies examining mother-child *H. pylori* transmission is

lack of directionality. In addition to accurate evaluation of *H. pylori* infection status of the

mother and child, transmission studies rely heavily on the ability to derive direction of *H.

pylori* transmission. Direction of *H. pylori* transmission can only be determined by

assessing *H. pylori* status of the mother and child at the beginning of the study and

determining infection status through time. Temporality is necessary to determine

causality (Rothman, Greenland, Poole, & Lash, 2008). To address temporality, the

outcome must be measured longitudinally, determining incidence. Only five studies

were longitudinal in nature, with observations taken at more than one time during the

study period (Fujimura, Kato, Nagai, Kawamura, & Iinuma, 2004; Konno et al., 2005;

Malaty et al., 2000; Rowland et al., 2006; Sinha et al., 2004; Weyermann et al., 2009)

(Appendix D.). Of these studies, all revealed a positive association between maternal *H.

pylori* status and *H. pylori* infection of her child, and only one (Rowland et al., 2006)

utilized incidence of *H. pylori* infection to assess risk.

The longitudinal study conducted by Rowland et al followed children from Ireland

between the age of 24 to 48 months for four years, assessing risk factors and *H. pylori*

infection status on an annual basis (Rowland et al., 2006). This study found that

children with an *H. pylori* infected mother were roughly seven times more likely to be

infected with *H. pylori* compared to a child with a mother not infected with *H. pylori*

(adjusted OR= 7.2, 95% CI 2.0, 26.4) after adjusting for *H. pylori* infection of the sibling, income, drinking at least two bottles per day and sharing sleeping accommodations with the mother. This study did have several strengths including assessment of active *H. pylori* infection in the index child and mother, and analysis as a nested case-control to estimate relative risk. But the DAG illustrating the causal pathway for mother-child *H. pylori* infection (Appendix A) indicates adjustment for household crowding is sufficient to control for confounding, and therefore unnecessary adjusting for *H. pylori* infection of the sibling and drinking two or more bottles per day may have resulted in a lower magnitude for the estimated effect of the mother's infection on the child acquiring the infection. Also, as many children were infected at baseline, directionality of *H. pylori* infection cannot be determined to be that of mother to child.

Another major weakness present in many mother-child transmission studies is reliance on significant p-values to determine whether an association existed versus actually providing estimates of effect (Appendix D) (Ceylan et al., 2007; Dowsett et al., 1999; Drumm, Perez-Perez, Blaser, & Sherman, 1990; Escobar & Kawakami, 2004; Fujimoto et al., 2007; Fujimura et al., 2004; Malaty et al., 1991; Sinha et al., 2004; Yilmaz, Dogan, Gurgoze, & Unal, 2002; Yucel, Sayan, & Yildiz, 2009; Zhou, Chan, Chu, & Tam, 2000). Effect measure point and interval estimation are needed to provide both a degree of magnitude and direction of the association between exposure and outcome (Rothman, Greenland, & Lash, 2008).

In summary, limitations in current studies evaluating the association between the *H. pylori* status of a mother and the infection status of her children exist. These limitations may have resulted in measures of associations which deviate greatly from the

measures of effect to be estimated. Although some studies assessed active *H. pylori* infection in children, many studies utilized anti-*H. pylori* antibodies to determine the outcome, *H. pylori* status in children (Aguemon et al., 2005; Ito et al., 2006; Kivi et al., 2005; Malaty et al., 2000; Miyaji et al., 2000; Miyazaki et al., 2002; B. V. Nguyen et al., 2006; V. B. Nguyen et al., 2006; O'Rourke et al., 2003). In addition, some studies assessed gastric disease as a proxy for the exposure, *H. pylori* status of the mother (Brenner, Rothenbacher, Bode, & Adler, 1998; Brenner, Bode, & Boeing, 2000; Chang et al., 2002; Gasbarrini et al., 1995; Kikuchi, Kurosawa, & Sakiyama, 1998; Kurosawa, Kikuchi, Inaba, Ishibashi, & Kobayashi, 2000; B. V. Nguyen et al., 2006). Inappropriate adjustment of variables that did not meet the criteria of a confounder or use of DAGs to identify possible confounders may have led to biased estimates in existing studies. Also, although clonal relatedness of *H. pylori* strains between the mother and her children were described by numerous studies (Bamford et al., 1993; Han et al., 2000; Herrera et al., 2008; Kivi et al., 2003; Kivi et al., 2007; Konno et al., 2005; Konno et al., 2008; Nahar et al., 2009; Taneike et al., 2001), few studies were longitudinal in nature (Fujimura, Kato, Nagai, Kawamura, & Iinuma, 2004; Konno et al., 2005; Malaty et al., 2000; Rowland et al., 2006; Sinha et al., 2004; Weyermann et al., 2009) and therefore most were not able to determine direction of infection or differentiate between acquisition and establishment of a long-term *H. pylori* infection. Lastly, many studies relied on p-values versus measure of association (Ceylan et al., 2007; Dowsett et al., 1999; Drumm, Perez-Perez, Blaser, & Sherman, 1990; Escobar & Kawakami, 2004; Fujimoto et al., 2007; Fujimura et al., 2004; Malaty et al., 1991; Sinhaet al., 2004; Yilmaz, Dogan, Gurgoze, & Unal, 2002; Yucel, Sayan, & Yildiz, 2009; Zhou, Chan, Chu,

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& Tam, 2000). The current study addressed these limitations as it is longitudinal and determined active, *H. pylori* infection status of the child, and *H. pylori* serostatus of the mother at baseline. Confounders were identified by a thorough review of *a prior* knowledge which was incorporated into DAGs. Hazard ratios were used to determine magnitude and direction of associations.

Associations with Siblings

Sixty-three articles related to sibling transmission of *H. pylori* met the inclusionary criteria. As with maternal studies, many studies which examined the association between siblings or children within the household and *H. pylori* infection did find a positive association. (Appendix G) (Chi et al., 2009; Ertem, Harmanci, & Pehlivanoglu, 2003; Fall, Goggin, Hawtin, Fine, & Duggleby, 1997; Farrell et al., 2005; Fialho et al., 2010; Ford & Axon, 2010; Garg, Perry, Sanchez, & Parsonnet, 2006; Glynn et al., 2002; Goodman et al., 1996; Goodman & Correa, 2000a; Halitim et al., 2006; Herbarth et al., 2001; Ito et al., 2006; Kivi et al., 2005; Koch et al., 2005; Kurosawa et al., 2000; Lin et al., 1999; Malaty, Logan, Graham, & Ramchatesingh, 2001; Mbulaiteye et al., 2006; McCallion et al., 1996; H. M. Mitchell, Bohane, Hawkes, & Lee, 1993; Miyaji et al., 2000; Moayyedi et al., 2002; K. Muhsen et al., 2010; K. Muhsen et al., 2006; Rocha et al., 2003; Roma et al., 2009; Rothenbacher et al., 1997; Rowland et al., 1999; Rowland et al., 2006; Santos et al., 2005; Sykora et al., 2009; Weyermann et al., 2009; Yang et al., 2005). Such an association was found in a cross-sectional study conducted by Sykora and colleagues (Sykora et al., 2009) of children 0-15 years of age in the Czech Republic. In this study, the odds of an *H. pylori* infection in children living with two or more children within a household was more than four times that of having an *H. pylori*

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infection in children who did not share a household with other children (adjusted OR= 4.3, 95% CI: 1.9, 9.8); this measure of association was obtained after adjusting for the father's educational status and institutional status of the child.

In another cross-sectional study of adults conducted in Southern Brazil, Santos et al found an increased positive association for *H. pylori* infection in individuals with seven or more siblings compared to those with 3-4 siblings (Santos et al., 2005). In this study, the prevalence ratio increased from 1.4 (95% CI: 1.1, 1.9) for 3-4 siblings to 1.55 (95% CI: 1.2, 2.0) for seven or more siblings after adjusting for father's educational status, day care attendance in childhood, ethnicity, dyspeptic symptoms and current family income.

Although both studies conducted by Sykora and Santos assessed current *H. pylori* infection of the index child (*H. pylori* stool antigen and ^{13}C-UBT respectively), avoiding non-differential misclassification of the outcome (*H. pylori* infection), non-differential misclassification of the exposure may still have occurred. These studies, as well as many studies in the current literature (Chi et al., 2009; Ertem et al., 2003; Fall et al., 1997; Ford et al., 2007; Goodman et al., 1996; Herbarth et al., 2001; Ito et al., 2006; Koch et al., 2005; Kurosawa et al., 2000; Lin et al., 1999; Malaty et al., 2001; McCallion et al., 1996; Moayyedi et al., 2002; K. Muhsen et al., 2006; Reshetnikov, Denisova, Zavyalova, Haiva, & Granberg, 2003; Rothenbacher et al., 1997; Tsugane, Tei, Takahashi, Watanabe, & Sugano, 1994; Webb et al., 1994), examined as the exposure, number of siblings or children within the household, without determining their *H. pylori* infection status (Appendix G). Therefore, the index child may have been exposed to other children in the household who were not infected with *H. pylori* resulting in non-

differential misclassification of the exposure. This misclassification of *H. pylori* status of the sibling or child within the home may have resulted in a negative bias towards the null for the estimated measure of effect of a sibling's *H. pylori* infection on a child's *H. pylori* infection. When comparing studies which assessed *H. pylori* status of siblings as the exposure (fixed effect model overall OR= 3.6) versus studies which determined *H. pylori* associations between the index child and having four or more siblings (fixed effect model overall OR=2.1), assessment of *H. pylori* infection status of the sibling resulted in measures of association of greater magnitude (Appendix H.).

A limited number of studies did assess current *H. pylori* infection in both the index child and sibling or other children in the household with a fixed effect model overall odds ratio of 3.6, 95% CI (2.6, 4.9) (Appendices H-J) (Farrell et al., 2005; Fialho et al., 2010; Garg et al., 2006; Goodman & Correa, 2000a; Halitim et al., 2006; K. Muhsen et al., 2010; K. Muhsen et al., 2006; Rocha et al., 2003; Roma et al., 2009; Rowland et al., 1999; Rowland et al., 2006; Weyermann et al., 2009). Roma and colleagues found that having at least one infected sibling (average age 11 years old) was associated with *H. pylori* infection in symptomatic children (unadjusted OR= 19.1, 95% CI: 4.0, 91.7) (Roma et al., 2009). In this study, *H. pylori* infection status of both the index child and siblings was determined by ^{13}C-urea breath test. Although the study reported in this dissertation also assessed active infection of *H. pylori* for both the index child and younger siblings, observation was longitudinal in nature and therefore has the advantage of determining direction of infection as well as incidence over prevalence.

A cross-sectional study by Muhsen et al (K. Muhsen et al., 2010) in children 6-9 years of age found the odds of *H. pylori* infection in children who had at least one *H.*

pylori infected sibling was more than four times that of *H. pylori* infection in children whose sibling was not infected with *H. pylori* (adjusted PR=4.6, 95% CI: 0.8, 28.2); this measure of association was adjusted for maternal education and crowding index. Based on the DAG illustrating the causal pathway for child-sibling *H. pylori* associations, either maternal education or crowding would provide minimal adjustment for possible confounding; in the study by Muhsen and colleagues, unnecessary adjustment may have taken place potentially leading to a bias away from the null. In this study, active infection was assessed in both the index child and siblings utilizing a test which detects *H. pylori* in the stool.

Some studies examined birth order of the child as the main exposure in relation to the outcome of *H. pylori* status (Appendix I) (Fall et al., 1997; Ford et al., 2007; Goodman et al., 1996; Goodman & Correa, 2000a; Mbulaiteye et al., 2006; Rothenbacher et al., 1999; Ueda, Kikuchi, Kasugai, Shunichi, & Miyake, 2003) with conflicting results and an overall fixed model estimate odds ratio of 1.5 (95% CI: 1.3, 1.7). Studies conducted by Goodman and colleagues (Goodman & Correa, 2000a) as well as Mbulaiteye and colleagues (Mbulaiteye et al., 2006) revealed that the magnitude of effect increased as birth order increased. For example Goodman et al (Goodman & Correa, 2000a) found an increasing trend in *H. pylori* status as birth order ascended from first born after adjusting for the number of children in the family (adjusted OR=1.8, 95% CI: 1.0, 3.3 for 2nd birth order, adjusted OR=2.1, 95% CI: 1.0, 4.4 for 3rd birth order and adjusted OR=2.2, 95% CI: 0.9, 5.2 for 4th-9th birth order). In an earlier study conducted by Goodman and colleagues (Goodman et al., 1996) and a study conducted by Ford and colleagues (Ford et al., 2007), no association between increasing birth

order and *H. pylori* status of the child was found (Appendix G).When comparing the magnitude of the measures of associations from studies which assessed current *H. pylori* status of children and their siblings as the exposure to studies which simply examined birth order as the main exposure, stronger associations were found when *H. pylori* status was obtained (Appendix I).

In a related issue to birth order, some studies examined the association between *H. pylori* status of the index child and that his or her younger or older siblings or children within the household (Appendix I) (Goodman & Correa, 2000b; Mbulaiteye et al., 2006; Miyaji et al., 2000; Rowland et al., 1999). All of these studies found that the *H. pylori* status of an older sibling or child within the same household was an important contributor to the *H. pylori* status of the younger index child within the household(fixed effect overall odds ratio=4.0, 95% CI: 2.4, 6.7). For instance, Goodman et al (Goodman & Correa, 2000a) found that the odds of *H. pylori* infection in children with one older *H. pylori* infected sibling was almost twice the odds of an *H. pylori* infection in children who did not have an infected older sibling (adjusted OR=1.9, 95% CI: 1.2, 3.1); when the number of *H. pylori* infected older siblings increased to 2-4, the odds ratio increased to 4.4 (95% CI: 2.0, 9.4). Both of these measures of association were adjusted for the number of siblings within the household.

Sibling gastric disease or symptoms were utilized as a proxy for the exposure of *H. pylori* infection in a sibling or child within the household by a limited number of studies (Appendix G.) (Blaser, Chyou, & Nomura, 1995; Chang et al., 2002; Gasbarrini et al., 1995; Kikuchi et al., 1998; Leung et al., 2006; Luzza et al., 2000). Gastric disease or symptoms were also assessed as a proxy for *H. pylori* infection in maternal

transmission studies aiming to determine the association between maternal *H. pylori* status and that of her child. As seen in those maternal studies, positive associations between gastric disease or symptoms of the sibling and *H. pylori* positive status of the index subject were observed. For instance, in a study conducted by Leung and colleagues of 270 adult, Chinese gastric cancer patients (Leung et al., 2006), it was found that *H. pylori* infection was almost two times more likely in individuals whose sibling had gastric cancer (adjusted OR= 1.9, 95% CI: 1.1, 3.3) compared to individuals whose sibling did not have gastric cancer after adjusting for age greater than 45 years of age and being the highest order of all siblings. In regards to gastric symptoms, a study conducted by Luza et al found that the odds of *H. pylori* infection in an index child whose sibling reported a recent history of vomiting was more than two times the odds of *H. pylori* infection in an index child whose sibling had not reported a recent history of vomiting (adjusted OR= 2.4, 95% CI: 1.3, 4.3). The main limitation of studies which utilized gastric disease or symptoms as a proxy for the exposure, *H. pylori* infection of the sibling, is the potential for non-differential misclassification of the exposure leading to a negative bias towards the null. Non-differential misclassification can reduce the magnitude of the measure of association pertaining to the *H. pylori* status of the index child (Appendix J). The magnitude of the measures of associations obtained from studies which assessed current *H. pylori* status of the sibling as the exposure was stronger than the magnitude of the measure of association obtained from studies which examined gastric disease in the sibling as the exposure (fixed effect model overall OR=3.6, 95% CI: 2.6, 4.9 versus fixed effect model overall OR=1.9, 95% CI: 1.5, 2.4) (Appendix J).

Analogous to laboratory-based vertical transmission studies involving the mother, genetically similar *H. pylori* strains have been detected among siblings or children living within the same household (Han et al., 2000; Herrera et al., 2008; Kivi et al., 2003; Raymond et al., 2008; J. T. Wang, Sheu, Lin, Wang, & Wu, 1993). In one study conducted by Kivi et al utilizing RAPD genetic analysis (Kivi et al., 2003), 81% of sibling harbored concordant *H. pylori* strains; of these sibling-sibling groups in which the mother was also infected, 82% harbored a different strain from the mother. In another study conducted by Wang and colleagues analyzing *H. pylori* strain concordance via ribotyping in six families (J. T. Wang et al., 1993), it was found that in families in which siblings were infected, all siblings harbored identical *H. pylori* strains.

Similar limitations as discussed in maternal-child transmission studies relying on laboratory methods apply to child-child transmission studies; specifically, those include potentially low sensitivity of laboratory methods utilized as well as an inability to rule out a common source for *H. pylori* infection and direction of transmission.

Of studies which investigated the association between *H. pylori* infected siblings or children within the household and *H. pylori* infection in the index child, only six were longitudinal in nature and obtained *H. pylori* infection incidence instead of prevalence (Appendix G.) (Glynn et al., 2002; Halitim et al., 2006; K. Muhsen et al., 2010; Rowland et al., 1999; Rowland et al., 2006; Weyermann et al., 2009). The need to determine directionality of *H. pylori* infection in familial transmission studies is imperative to causality as previously stated. Of these six longitudinal studies, the studies conducted by Rowland et al (Rowland et al., 1999) and Halitim et al (Halitim et al., 2006) evaluated *H. pylori* re-infection in children as the outcome.

Rowland and colleagues conducted a study of 52 children who underwent *H. pylori* eradication therapy according to standard treatment protocols (Rowland et al., 1999). After 6 weeks, children underwent testing for *H. pylori* infection via ^{13}C-urea breath test to confirm eradication of *H. pylori* and at the same visit *H. pylori* infection status of family members was also determined. For this study, annual ^{13}C-urea breath tests of the index child were conducted. Rowland and colleagues found that after adjusting for *H. pylori* infection of both parents, lower socioeconomic status and bed sharing with a parent or sibling, children less than five years of age who had more than two *H. pylori* infected older siblings were sixteen times more likely to become re-infected with *H. pylori* than children less than five years of age who had two or few *H. pylori* infected siblings (adjusted RR=16.1, 95% CI: 2.4, 106.4). Based on the DAGs illustrating the causal pathway between the exposure of *H. pylori* infected sibling and the outcome of *H. pylori* infection in the index child, additional adjustment for covariates related to a child's immune response and development such as age, breastfeeding status and antibiotic use may have been more appropriate and may have led to measures of association more closely approximating the true measures of effect, towards the null (Appendix C).

The study conducted by Rowland et al has one major limitation related to possible misclassification bias due to broadly defined categorical variables. Sibling *H. pylori* status of the siblings were defined dichotomously as "more than two *H. pylori* infected siblings" with a referent group of "two or fewer or no *H. pylori* infected siblings". This categorization may represent extreme misclassification as the referent includes part of the exposure (*H. pylori* infected sibling) hence result in a negative bias towards the null.

In the longitudinal study conducted by Halitim and colleagues in France (Halitim et al., 2006) to determine the association between *H. pylori* re-infection of the index and *H. pylori* status of the siblings, a much weaker magnitude of association is observed. In their study, 32 children from a local pediatric hospital with identified *H. pylori* infection underwent eradication therapy for *H. pylori* infection and were assessed 4-6 weeks after treatment for clearance of infection. Between one year and nine years later (2.8 years median time), *H. pylori* re-infection status was determined once via ^{13}C-urea breath test and risk factors for possible infection were determined by survey. Halitim et al found that *H. pylori* re-infection was 30% more likely in those whose siblings had a history of *H. pylori* infection (unadjusted RR=1.3, 95% CI: 1.1, 1.7). In this study, *H. pylori* status of the sibling was not assessed and instead only possible *H. pylori* infection was obtained once by questionnaire at assessment of *H. pylori* eradication of the index child. As the exposure, *H. pylori* infection of the sibling was not determined, directionality of infection cannot be assessed and misclassification of the exposure may have also occurred.

In the longitudinal study conducted by Muhsen et al, active *H. pylori* infection of the index child and siblings was determined by stool antigen test at two different points in time: in 2004 and again between 2007-2009. In this study, the authors categorized a child as having an early and persistent (long-term) infection if they tested positive for *H. pylori* at both points in time. A child who tested negative for *H. pylori* in 2004 but positive at the second assessment period was defined as late infection acquisition. Muhsen and colleagues found that long-term *H. pylori* infection in children who had at least one infected *H. pylori* sibling was almost three times that of children whose sibling was not infected with *H. pylori* (adjusted PR=2.9, 95% CI: 1.0, 8.3). In children who were

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infected only during the second assessment period (late infection acquisition), the prevalence ratio increased (adjusted PR=4.6, 95% CI: 0.8, 28.2); these measures of association were adjusted for maternal education and crowding index. This study conducted by Muhsen is the only study in which an attempt was made to differentiate between *H. pylori* infection states (persistent versus late infection acquisition). One major limitation of this study as previously mentioned, is a lack of adjustment for the potential confounder, maternal *H. pylori* status. This lack of adjustment may result in a positive bias away from the null and result in inflated magnitudes of association of the outcome, *H. pylori* infection of the index child.

A second, later longitudinal study conducted by Rowland and colleagues (Rowland et al., 2006) did determine *H. pylori* infection of children annually by ^{13}C-urea breath test but due to low incidence of infection, prevalent *H. pylori* infection of index children was included in the final analysis to establish the association between *H. pylori* of the index child and that of *H. pylori* infection of siblings. Hence, no directionality of *H. pylori* infection could be determined.

As seen with some studies focusing on maternal association in *H. pylori* infection status of her children, a major weakness of certain studies attempting to assess the association between siblings and *H. pylori* infection status in children is lack of presentation of measures of association (Appendix G) (Ceylan et al., 2007; Dowsett et al., 1999; Drumm et al., 1990; Yucel et al., 2009; Zhou et al., 2000). Instead, p-values of less than 0.05 were utilized to determine significant associations between siblings or children within the household and *H. pylori* status of the index child. Again, properly

adjusted measures of association provide the best reflection of measures of effect while providing both direction and magnitude of effect.

As seen in maternal transmission studies, limitations in sibling-to-sibling *H. pylori* transmission studies exist, again resulting in measures of associations which may not be reflective of the measures of effect to be estimated. Once again, although some studies utilized methods to determine current, active *H. pylori* infection in children, (Farrell et al., 2005; Fialho et al., 2010; Garg et al., 2006; Goodman & Correa, 2000a; Halitim et al., 2006; K. Muhsen et al., 2010; K. Muhsen et al., 2006; Rocha et al., 2003; Roma et al., 2009; Rowland et al., 1999; Rowland et al., 2006; Weyermann et al., 2009)various studies utilized anti-*H. pylori* antibodies to determine *H. pylori* status of the index child and their sibling. Also, some studies used proxy measures for the exposure, *H. pylori* status of the sibling. For example, as with maternal studies, gastric disease in siblings was assessed to make associations to *H. pylori* status of the index child (Blaser, Chyou, &Nomura, 1995; Chang et al., 2002; Gasbarrini et al., 1995; Kikuchi et al., 1998; Leung et al., 2006; Luzza et al., 2000); other studies used the number of siblings (Chi et al., 2009; Ertem et al., 2003; Fall et al., 1997; Ford et al., 2007; Goodman et al., 1996; Herbarth et al., 2001; Ito et al., 2006; Koch et al., 2005; Kurosawa et al., 2000; Lin et al., 1999; Malaty et al., 2001; McCallion et al., 1996; Moayyedi et al., 2002; K. Muhsen et al., 2006; Reshetnikov, Denisova, Zavyalova, Haiva, &Granberg, 2003; Rothenbacher et al., 1997; Tsugane, Tei, Takahashi, Watanabe, & Sugano, 1994; Webb et al., 1994) or birth order (Fall et al., 1997; Ford et al., 2007; Goodman et al., 1996; Goodman & Correa, 2000a; Mbulaiteye et al., 2006; Rothenbacher et al., 1999; Ueda, Kikuchi, Kasugai, Shunichi, & Miyake, 2003) as proxy

measures for transmission of *H. pylori* among siblings. Use of such proxy measures may have resulted in bias towards the null and therefore result in measures of association with lower magnitudes compared to maternal transmission studies.

Related *H. pylori* strains were detected among siblings, but as in maternal transmission studies, few studies were longitudinal in nature and therefore were not able to determine direction of infection. Only one longitudinal study estimated the effect of a sibling's infection on a subsequent child's infection. Inappropriate adjustment of variables may have led to biased estimates in these studies as well. Lastly, many studies relied on p-values versus measures of association to draw conclusions (Ceylan et al., 2007; Dowsett et al., 1999; Drumm et al., 1990; Yucel et al., 2009; Zhou et al., 2000). The study presented in this dissertation addresses these limitations including the following: 1) it is longitudinal in nature; 2) determined active, *H. pylori* infection status of the index child and younger siblings; 3) confounders were identified by *a prior* knowledge and incorporated into DAGs. Hazard ratios were used to determine magnitude and direction of association, estimating risk ratios.

<center>Breastfeeding and *Helicobacter pylori* Infection</center>

Twenty-five articles relating to *H. pylori* and breastfeeding met the inclusionary criteria. Relative to studies examining familial associations in *H. pylori* infection, few studies have been conducted which examine the association between breastfeeding and *H. pylori* infection acquisition. Of the existing studies there are varying results (Braga et al., 2007; Dore et al., 2002; Ertem et al., 2003; Gold et al., 1997; Ito et al., 2006; Mahalanabis et al., 1996; Malaty et al., 2001; McCallion et al., 1996; Monajemzadeh et al., 2010; Naficy et al., 2000; B. V. Nguyen et al., 2006; Omar,

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Ibrahim, Sarkis, & Ahmed, 2001; Pearce, Thomas, Campbell, & Parker, 2005; Rodrigues et al., 2006; Rothenbacher et al., 2002; Rowland et al., 2006; Suoglu, Gokce, Saglam, Sokucu, & Saner, 2007; Sykora et al., 2009; Ueda et al., 2003). As in vertical transmission studies, study parameters and methods utilized need to be examined as causal inference is dependent on such factors.

Of the studies which evaluated the effect of breastfeeding on *H. pylori* status, most reported results consistent with a protective effect of breastfeeding against *H. pylori* infection (Appendix K). Of the studies which indicated breastfeeding to be consistent with a protective effect against *H. pylori* infection and provided measures of association (Ertem et al., 2003; Malaty et al., 2001; McCallion et al., 1996; B. V. Nguyen et al., 2006; Omar et al., 2001; Pearce et al., 2005; Rodrigues et al., 2006; Rowland et al., 2006), most categorized breastfeeding further by duration although duration periods varied. For example, a cross-sectional study by McCallion et al (McCallion et al., 1996) was conducted in children 3-15 years of age attending a hospital in Ireland for non-gastrointestinal day surgery. In this study, history of breastfeeding for two or more weeks was consistent with a protective effect against *H. pylori* seropositivity (adjusted OR=0.5, 95% CI 0.3, 1.1) after adjusting for age, social class and housing density. Two possible weaknesses exist in the study conducted by McCallion and colleagues. First, it was not clear why the authors chose two weeks as the cut point in categorizing breastfeeding. Determination of cut-points for variables should be based on *a priori* information and possible causal roles, otherwise residual confounding may result. Also, the authors did not assess active *H. pylori* infection in children. As previously stated, assays which detect antibodies against *H. pylori* may lack sensitivity in certain children.

Therefore, the measures of effect corresponding to the inverse association between breastfeeding and *H. pylori* acquisition may be of greater magnitude than reflected by the resultant measures of association; this is otherwise known as a positive bias towards the null. In addition, young children may experience spontaneous resolution of *H. pylori* infection and antibody based assay will not be able to detect these events. Therefore, the association between breastfeeding and short-term infection cannot be gauged and again the resultant measures of association may be consistent with a less protective effect (a positive bias towards the null).

Of studies which found the effect of breastfeeding to be consistent with a protective effect, only one study evaluated exclusivity of breastfeeding as the main exposure (Pearce et al., 2005). Pearce et al conducted a cohort study of adults 50 years of age who were originally members of a birth cohort in northern England (Pearce et al., 2005). Breastfeeding was defined per 30 days of exclusive breastfeeding during infancy while the outcome was *H. pylori* seropositivity at age 50. In this study, a 10% decrease in *H. pylori* risk in adulthood was found for every additional 30 days of exclusive breastfeeding during infancy after adjusting for housing conditions at birth and social class (adjusted OR=0.9, 95% CI: 0.8, 1.1). A major strength of this study is the longitudinal nature of breastfeeding measurement which may minimize non-differential misclassification of the exposure. One possible limitation of this study is determination of *H. pylori* status in adulthood versus childhood. It is possible that due to a long lapse in time between the exposure, exclusive breastfeeding, and the outcome, *H. pylori* seropositivity, misclassification of the relevant outcome may have occurred. It is possible that as spontaneous resolution of *H. pylori* occurs during childhood, assessing

H. pylori status in adults does not allow for the classification of those who did acquire an *H. pylori* infection but experienced spontaneous resolution due to breastfeeding. This misclassification of the outcome may result in a positive bias towards the null for the measure of association related to breastfeeding and *H. pylori* infection.

Only three studies were longitudinal in nature (Fall et al., 1997;Naficy et al., 2000; Pearce et al., 2005). Of these studies, only two presented measures of association (Naficy et al., 2000; Pearce et al., 2005). Only the study conducted by Naficy and colleagues (2000) assessed *H. pylori* status in childhood but it was assessed via anti-*H. pylori* IgG antibody testing and therefore could not distinguish between differing infection outcomes and may have lacked sensitivity detecting *H. pylori* in children. Hence, bias towards the null may have occurred due to non-differential misclassification of the outcome.

A few studies found a positive association between breastfeeding and *H. pylori* status (Appendix K) (Braga et al., 2007; Gold et al., 1997; Naficy et al., 2000; Rothenbacher et al., 2002; Suoglu et al., 2007); this did not vary by *H. pylori* prevalence. For example, a study conducted by Rothenbacher and colleagues in pre-school aged children in Ulm, Germany (Rothenbacher et al., 2002) found that children who were breastfed were almost two times more likely to be infected with *H. pylori* compared to children who were never breastfed (adjusted OR=1.6, 95% CI: 0.8, 3.1); this positive association became strong when duration of breastfeeding was taken into consideration (< 3 months adjusted OR=1.1 95% CI: 0.5, 2.5, 3-6 months adjusted OR=1.2 95% CI: 0.5, 2.8 and ≥ 6 months adjusted OR=2.6 95% CI: 1.2, 5.6). These measures of association were adjusted for *H. pylori* infection status of the mother, age,

gender, nationality, place of birth, birth weight, education of the father and mother, antibiotic use, number of siblings, housing density, smoking status in the home by mother and father. The results obtained in this study were strengthened by assessment of active *H. pylori* infection in children and further categorizing breastfeeding by duration. Yet over adjustment may have occurred in the final analysis.

Over adjustment by inclusion of unnecessary covariates may result in a complex model which can yield estimates that may still be biased (Greenland, 2008). In the study conducted by Rothenbacher et al, thirteen covariates were included in the final, fully adjusted model. One covariate included in the analysis was mother's *H. pylori* status. The authors indicated that covariates were selected as potential confounder based on significance level obtained from the chi square statistic. The *H. pylori* status of the mother does not meet the definition of a confounder (*H. pylori* status of the mother has not been determined to differ between those who are breastfed and those who are not). Therefore this variable, along with the other covariates, may not have reduced overall bias in the study and instead introduced bias such as a collider bias.

A meta-analysis conducted by Chak and colleagues (Chak et al., 2009) utilizing a random effects model of 14 studies presenting associations between *H. pylori* infection and being breast fed also described most studies to find breastfeeding to protect against *H. pylori* infection (random effects model summary OR=0.8, 95% CI: 0.6, 1.0). In this meta-analysis by Chak (2000) it was also found that studies which utilized ^{13}C-UBT to assess *H. pylori* infection had a greater magnitude of protection (random effects model summary OR=0.7, 95% CI: 0.3, 1.4) compared to studies which assessed seropositivity (random effects model summary OR= 0.9, 95% CI: 0.7, 1.1). In addition, a

greater magnitude of effect was observed in developing versus developed countries (random effects model summary OR=0.6, 95% CI: 0.3, 0.9 and random effects model summary OR=0.9, 95% CI: 0.7, 1.2, respectively). The effect of the duration of breastfeeding was also found to be consistent with a protective effect against *H. pylori* infection (random effects model summary OR=0.8, 95% CI: 0.4, 1.7) but due to few studies which provided duration of breastfeeding, the summary odds ratio may have been slightly higher than overall summary odds ratios.

   A few laboratory based studies examined the role of breastfeeding and *H. pylori* infection by measuring anti-*H. pylori* antibodies in breast milk finding positive associations between low anti-*H. pylori* antibody levels and *H. pylori* infection status of the child (Appendix K.) (Bhuiyan, Saha, Lundgren, Qadri, & Svennerholm, 2010; Thomas et al., 2004; Weaver, 1995). For example, Bhuiyan and colleagues (Bhuiyan et al., 2010) conducted a birth cohort study of 238 children from India; *H. pylori* status was determined the first month of life and every six months afterwards for two years. This study found that children who became infected with *H. pylori* within the first year of life had lower serum anti-*H. pylori* IgG levels prior to infection compared to children who became infected during the second year of life. In addition, the breast milk of children who acquired an *H. pylori* infection within the first month of life had lower concentrations of anti-*H. pylori* IgA antibodies compared with those infected at six months. Lastly, Bhuiyan et al discovered that children who experience spontaneous elimination of *H. pylori* infection developed higher levels of anti-*H. pylori* IgA serum antibodies compared to children who developed a long-term *H. pylori* infection. Similarly, Weaver et al (Weaver, 1995) found that the breast milk provided to children who remained free of an

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H. pylori infection at nine months of life had 2-4 times anti-*H. pylori* levels at 13-39 weeks post-partum compared to children who were infected with *H. pylori* at nine months of life. Thomas and colleagues (Thomas et al., 2004)found that lower anti-*H. pylori* urease IgA antibodies in breast milk were associated with *H. pylori* infection earlier in life.

Some studies examining breastfeeding and *H. pylori* infection status did not present measures of association (Appendix K.) (Fall et al., 1997; Mahalanabis et al., 1996; Monajemzadeh et al., 2010; Okuda et al., 2001; Yucel et al., 2009). As previously stated, measures of association are necessary to determine both magnitude and direction of associations.

In conclusion, studies assessing the association between breastfeeding and *H. pylori* infection are limited in number and of the few existing studies available; methodologies may have resulted in weakened grounds for establishing causality. Of existing longitudinal studies none were able to define and categorized infection acquisition, short-term infection or long-term infection establishment in children.

Once again, although some studies utilized methods to determine current, active *H. pylori* infection in children, the majority of studies utilized anti-*H. pylori* antibodies to determine *H. pylori* status (Chak et al., 2009) possible leading to a bias towards the null. And though some studies did categorize breastfeeding by duration, durations varied greatly (Chak et al., 2009). As in intrafamilial studies, many studies based their conclusions on p-value cut points (Fall et al., 1997; Mahalanabis et al., 1996; Monajemzadeh et al., 2010; Okuda et al., 2001; Yucel et al., 2009).Others inappropriately adjusted for variables that did not meet the criteria of a confounder

(Rothenbacher et al., 2002) and none used DAGs to identify possible confounders that may have led to biased estimates. The longitudinal study presented in this dissertation fills in the current gaps in *H. pylori*-breastfeeding association studies as it determined active, *H. pylori* infection status of children while defining infection acquisition as well as short-term and long-term infection establishment. Further, confounders were identified by *a prior* knowledge and incorporated into DAGs. Again, hazard ratios were used to determine magnitude and direction of association and estimate risk ratios.

CHAPTER 3

METHODOLOGY

Study Design and Data Source

Data for the current research was collected as part of the Pasitos Cohort Study, a

prospective birth cohort study conducted from April 1998 to December 2005 along the

U.S.–Mexico border (Goodman et al., 2003; O'Rourke et al., 2003). Information was

obtained regarding diet, hygiene, household factors and socioeconomic factors to

assess how these factors may affect *H. pylori* infection establishment (Goodman et al.,

2003; O'Rourke et al., 2003). Pregnant women were recruited from maternal child care

centers, including women obtaining assistance through the Women, Infants and

Children (WIC) program in El Paso, Texas as well as women with prenatal benefits from

the Mexican Social Security Institute in Juarez, Mexico (Goodman et al., 2003).

Data Collection

A baseline questionnaire in both English and Spanish and blood samples were

obtained from the mother during pregnancy upon agreement to participate in the

Pasitos Cohort study (Goodman et al., 2003). Follow-up visits were scheduled every 6

months after the birth of the child and information was collected via questionnaire

regarding household environmental factors thought to be associated with *H. pylori*

transmission, dietary intake, illness within the previous 6-month period, medication use;

the^{13}C-urea breath test was used for the detection *of H. pylori* infection (O'Rourke et al.,

2003). In an attempt to collect sufficient data, scheduled visits may have occurred within

three months before or after the scheduled visit and therefore count towards the

scheduled visit. During the course of the study, younger siblings of the index child were

invited to participate in the study and identical information was obtain for the siblings at the same visit as the index child at each scheduled 6 month follow-up visit (O'Rourke et al., 2003).

Data for Research Study

Four-hundred and seventy-two index subjects and 143 younger siblings enrolled in The Pasitos Cohort Study returned for follow-up and had 1 or more ^{13}C-urea breath tests(^{13}C-UBT) during a possible follow-up of 85 months. A total of 3,114 follow-up visits were conducted, with index children attending an average of 5.5 visits and younger siblings attending an average of 3.8 visits. The mean follow-up time was 3.8 years for index children and 2.5 years for younger siblings. Data analyzed was limited to 468 Pasitos Cohort index children (99%) and 140 younger siblings of the index child (98%) enrolled in the study with at least one viable ^{13}C-UBT result. It is important to note no child participating in The Pasitos Cohort Study was given *H. pylori* eradication therapy.

Defining the Exposures

For aims A1-2 investigating the association between maternal infection and *H. pylori* infection establishment, a child was deemed exposed if his/her mother was *H. pylori* seropositive for IgG indicating a present or past infection at the time of enrollment.

For aims B1-8 relating to the association between sibling infection and *H. pylori* infection establishment, a child was considered exposed to an *H. pylori* infected older or younger sibling if there was another child living in the same household who was enrolled in The Pasitos Cohort Study (index child or younger sibling) who had one or more positive ^{13}C-UBT results; this includes index children and younger siblings with short and long-term infections. An index child or younger sibling with a long-term *H.*

pylori infection was defined as a child living within the same household with 3 or more consecutive positive ^{13}C-UBT results and no subsequent negative results during the follow-up period.

In regards to the research investigating the association between breastfeeding and *H. pylori* infection acquisition, short and long-term infection establishment for aims C1-3, any breastfeeding was defined as having been breastfed for any length of time. For aims C4-6, six month breastfeeding was defined as an index child or younger sibling who was breastfed for six months or longer. For aimsC7-9, twelve month breastfeeding was defined as receiving breastfeeding at twelve months of age or older.

<div align="center">Defining the Outcomes</div>

For all study aims A-C, *H. pylori* infection acquisition was defined as the first positive ^{13}C-UBT after a previous negative result. In order to account for missed follow-up visits by cohort participants, time-to-event for all infection outcomes was derived by calculating a mid-point between key dates. The time to first *H. pylori* infection was defined as the number of months from birth to the mid-point between a negative breath test result and the first positive ^{13}C-UBTresult. If the first ^{13}C-UBTwas positive, the time to infection was defined as the number of months from the date of birth to the first positive^{13}C-UBT. Only time to the first *H. pylori* incident infection was determined. This included children with short and long-term infections. For those not infected, time at risk of infection was defined as the cumulative time in months from birth until the last viable^{13}C-UBTresult.

A long-term infection was defined as three consecutive positive ^{13}C-UBT results with no subsequent negative results during the follow-up period for all study aims A-C.

As long-term *H. pylori* infection is not well defined in the current literature, the current study explored three time-to-event scenarios. In the first scenario, time to long-term infection establishment was defined as the number of months from birth until the first of three consecutive, positive[13]C-UBT results. In the second scenario, time to long-term infection establishment was defined as the number of months from birth until the mid-point between the first and second positive ^{13}C-UBT results. In the third scenario, time to long-term infection establishment was defined as the number of months from birth until the mid-point between the first and the third positive[13]C-UBTresults. In all scenarios, for those not experiencing a long-term *H. pylori* infection which included children with an incident infection and those experiencing a short-term infection, the time at risk of a long-term infection was defined as the cumulative time in months from birth until the last viable[13]C-UBT result.

For investigating the association between breastfeeding and short-term establishment of *H. pylori* infection (aims C2, C5 and C8), the outcome was defined as the first positive ^{13}C-UBT result followed by at least one negative urea breath test result without receiving *H. pylori* eradication therapy. Time to short-term infection establishment was defined as the number of months from date of birth to the mid-point between the first positive ^{13}C-UBT result and a subsequent negative test result. Only time to the first *H. pylori* short-term infection event was determined. This included children with long-term infections. For those who only acquired a long-term *H. pylori* infection, time at risk of a short-term infection event was defined as the time to long-term infection establishment. For those not infected, time at risk of a short-term *H. pylori*

infection event was defined as the cumulative time in months from birth until the last viable[13]C-UBTresult.

<div align="center">Defining Confounders and Effect Measure Modifiers</div>

Identification of possible confounders was based on *a priori* information and selection of confounders to be controlled was determined based on identification by DAGitty version 1.1 (Textor et al., 2011); DAGs are presented in Appendices A-C, illustrating the manner in which potential confounders are associated with the exposure and outcome and not intermediates in the causal pathway. Effect measure modifiers were selected based on *a priori* information.

For the study examining the association between maternal *H. pylori* status and child's *H. pylori* status (aims A1-2), crowding (continuous variable defined as the ratio of people residing within a household to the number of rooms within a house) was adjusted for as a possible confounder (Appendix A). Crowding was identified as a potential confounder because it is a socioeconomic factor previously found to be associated with *H. pylori* infection (Magalhaes, Queiroz & Luzza, 2006b).

Country of residence, age difference between siblings, antibiotic use, *H. pylori* status of siblings and breastfeeding status were evaluated as possible effect measure modifiers for aims A1-2. Previous studies of the Pasitos Cohort have demonstrated that effect measure estimates pertaining to risk of *H. pylori* infection differ by country of residence (U.S. versus Mexico) as well as age difference between siblings (Goodman et al., 2003; O'Rourke et al., 2003) and hence infection may also be modified. Breastfeeding and antibiotic use were also considered as possible effect measure modifiers as these factors may both be associated with the immune response mounted

by the host and therefore may influence *H. pylori* acquisition and establishment of a long-term infection (J. Wang et al., 2001). Antibiotic use has also been demonstrated to modify risk relating to *H. pylori* infection establishment (Broussard et al., 2009). For aims A1-2, age difference as an effect measure modifier was categorized as ≤3 years of age and > 3 years of age. This cut point was selected as a previous study of the Pasitos Cohort demonstrated that prevalence of *H. pylori* infection peaked at age three years (O'Rourke et al., 2003). Breastfeeding was defined as binary for being breastfed for any length of time versus not being breastfed at all. Antibiotic use was defined as binary, indicating whether or not the child took at least one course of antibiotics belonging to an antibiotic class previously determined to be effective against *H. pylori*.

For aims B1-8, crowding, antibiotic use, age and *H. pylori* seropositivity status of the mother were adjusted for as possible confounders.

Socioeconomic status was controlled for based on *a priori* knowledge of variables associated with *H. pylori* frequency, and include crowding (Magalhaes, Queiroz & Luzza, 2006b); again, crowding was defined as the ratio of people residing in a household to the number of rooms within a household. Age, also a possible immune response proxy, is accounted for in the use of Cox proportional hazards regression model and therefore not included as a separate covariate. The immune response mounted by the host may influence *H. pylori* acquisition and establishment of a long-term infection (J. Wang et al., 2001).The mother's *H. pylori* status (seropositivity) has been found to be a risk factor for *H. pylori* infection status (Weyermann et al., 2006). The number of potential *H. pylori* effective antibiotic courses taken (Magalhaes, Queiroz

& Luzza, 2006b) was also included in DAGs based on *a priori* information which indicate these factors may both be associated with *H. pylori* infection.

The difference in age between the siblings (≤ 3-year age difference and > 3-year age difference) and the country of residence (United States or Mexico) (Goodman et al., 2003; O'Rourke et al., 2003) were treated as effect measure modifiers for aims B1-8 as both of these factors have been demonstrated to modify effect measures relating to the risk of *H. pylori* infection acquisition and establishment.

For the study estimating the association between breastfeeding and *H. pylori* status of the child (aims C1-9), age was identified by DAGitty as the minimal sufficient adjustment set. As previously mentioned, use of the Cox proportional hazards regression model accounts for age and therefore age is not added to the model as a separate covariate. Country of residence, *H. pylori* status of the mother, *H. pylori* status of the siblings and antibiotic use were investigated as potential effect measure modifiers for aims C1-9. Again country of residence (U.S. versus Mexico) has been demonstrated to potentially modify effect measures relating to *H. pylori* transmission and infection establishment (Goodman et al., 2003; O'Rourke et al., 2003) as has the use of *H. pylori* effective antibiotics (Broussard et al., 2009). As in aims A1-2, antibiotic use was defined as binary, indicating whether or not the child took at least one course of antibiotics belonging to an antibiotic class previously determined to be effective against *H. pylori.* Numerous studies have detailed the positive associations between maternal *H. pylori* status as well as the infection status of the siblings and infection outcomes (Weyermann et al., 2009) and therefore these variables were considered as possible effect measure modifiers for aims C1-9. For aims C 1-9 maternal *H. pylori* status was

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defined as *H. pylori* seropositivity or seronegativity while infection status of siblings was defined binary as having an *H. pylori* positive sibling within the same household (either seropositivity for older, non-index siblings and positive ^{13}C-UBT for index and younger siblings).

<div align="center">Data Management and Quality Assurance</div>

All members of the Pasitos Cohort research team were responsible for data quality. Core research personnel of the Pasitos Cohort were responsible for specific areas of data quality; core research personnel included: interviewers, follow-up coordinator, field study director, laboratory coordinator, data manager, data collection director, project manager and scientific director. The data manager, project manager and scientific director were the only persons designated to perform data verification, cleaning and editing of all cohort study files. All data users including the author are required to submit a Data User Agreement agreeing to adhere to the data management plan as detailed in the procedure manual; any errors noted by the data user are to be reported to the designated individuals stated above.

For the current studies, descriptive analysis was performed on all variables provided and utilized for the creation of exposure, outcome, confounder and effect measure modifying variables to ensure concordance with cohort results previously presented (Broussard et al., 2010; Nurgalieva et al., 2008) and identification of unusual or outlier data points. All variables and code created for the proposed studies will be submitted to the project manager upon completion of analysis and finalization of any resulting manuscripts.

Data Analysis Plan

For all aims A-C, descriptive analysis was performed on exposure and outcome variables. Descriptive analysis includes tabulation of frequencies, cross-tabulation of frequencies, and display of percentages. The frequency of older, non-index, index children and younger siblings who became infected with *H. pylori*, achieved spontaneous clearance (short-term infection), and/or obtained an established long-term infected with *H. pylori* are included in the descriptive analysis. *H. pylori* serostatus of the mother and breastfeeding duration was also be included in the descriptive analysis. Descriptive analysis of all potential confounding and effect measure modifying variables was performed including, crowding, antibiotic use of the child, age difference, country of residence and the number of total *H. pylori* infected siblings in the household. Contingency tables of key variables were also displayed. Lastly, frequencies regarding follow-up time patterns and ^{13}C-UBT testing and results per visit were conducted.

For aims A1, B1, B2, B5, B6, C1, C4 and C7, Cox proportional hazards regression analysis was utilized to calculate hazard ratios to estimate the effect of the exposure on the first incident *H. pylori* infection. For aims A2, A4, B3, B4, B7, B8, C3, C6 and C9, Cox proportional hazards regression analysis was utilized to calculate hazard ratios to estimate the effect of the exposure on the establishment of a long-term *H. pylori* infection. For aims C2, C5 and C8, Cox proportional hazards regression analysis was utilized to calculate hazard ratios to estimate the effect of the exposure on *H. pylori* spontaneous clearance (short-term infection). For all aims A-C, Due to the difficulty in determining proportionality of hazards by visual methods alone when sample sizes are small, the proportional hazards assumption was verified prior to calculation of hazard

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ratios utilizing three methods (Garson, 2012): 1) the log minus log plot 2) Partial residual plot and 3) time interaction test. If the proportionality hazards assumption was violated in two of three tests, Cox proportional hazards regression with time dependent covariates analysis was performed.

For all aims A-C, the rate ratio was the estimated measure of effect via both crude and multivariable models. Potential confounders were controlled for by inclusion in multivariable models while stratification by potential factors which may modify estimates of effect was performed; both potential confounders and effect measure modifiers have been previously described. Confidence intervals at the 95% level will be calculated.

Cox proportional hazards regression was selected to estimate measures of effect for all aims A-C of the proposed studies as it is best suited to account for censored time (missed or skipped follow up visits) while assessing time to acquiring a first, long-term or short-term *H. pylori* infection.

<div align="center">Research Ethics</div>

The Pasitos Cohort study was approved by institutional review boards at multiple academic and governing institutions: the University of Texas Health El Paso, the University of Texas Health Science Center at Houston, the Texas Department of State Health Services and the Mexican Social Security Institute. Additionally, the proposed research and analysis was approved by the institutional review board at the University of North Texas Health Science Center (IRB# 2010-088 and IRB# 2010-089).

Information pertaining to possible outcomes relating to *H. pylori* infection and the possible need for medical follow-up was provided to parents in the informed consent documentation. All test result obtained from study participants were released to a parent

and contact information for a physician involved in the study were provided in case the

participants' parents desired additional information or consultation.

CHAPTER 4

RESULTS

Aim A- *Helicobacter pylori* Transmission and Maternal Infection Status

Descriptive Analysis

In the Pasitos Cohort Study of 608 children with one or more classifiable[13]C-UBT

results, 265 (44%) acquired *H. pylori* at least once during follow-up while 343 (56%)

children remained uninfected; time to event minimum, maximum and median are shown

in Table 2. The earliest documented infection occurred roughly 6 weeks after birth while

the median time to infection was roughly 17 months after birth. For children not infected,

the shortest follow-up time was 3 months with a median follow-up of 23 months.

TABLE 2. TIME TO *HELICOBACTER PYLORI* INCIDENT INFECTION, THE PASITOS
COHORT

Incident Infection (n, %[a])	Time to Event or Last Follow-up (Months)		
	Minimum	Maximum	Median
Yes (265, 43.6)	1.5	81.1	16.6
No (343, 56.4)	3.0	85.4	23.1
[a] Percentage of 608 children with one or more classifiable[13]C-UBT results.			

Only 45 (7%) of children in the cohort acquired a long-term *H. pylori* infection, while

the majority of the cohort did not achieve a long-term infection (93%). The time-to-event

minimum, maximum and medians are shown in Table 3.The time to a long-term

infection event was defined by three different scenarios. In scenario one, time to the

establishment of a long-term *H. pylori* infection was defined as the time in months from

birth until the date of the first positive ^{13}C-UBT of three consecutive positive tests. In

scenario two, time to the establishment of a long-term infection was defined as the time

in months from birth until the midpoint between the first and second positive ^{13}C-UBT of three consecutive positive tests. Lastly, in scenario three, time to the establishment of a long-term infection was defined as the time in months from birth until the midpoint between the first and third positive ^{13}C-UBT of three consecutive positive tests. In all scenarios, for children not achieving a long-term *H. pylori* infection including children with an incident infection and short-term infection, time-to-event was defined as birth until the date of the last classifiable[13]C-UBT. Time to the establishment of a long-term infection increased in each scenario but the earliest documented establishment of an *H. pylori* infection ranged from 5 months to 11 months after birth while the median time to achieve a long-term infection ranged from 32 months to 43 months after birth. In those who did not achieve a long-term infection, a shorter median follow-up was observed compared to those who achieved a long-term infection in scenarios 2 and 3 but this was due to the manner in which time to event was defined for these scenarios.

TABLE 3. TIME TO *HELICOBACTER PYLORI* LONG-TERM INFECTION ESTABLISHMENT, THE PASITOS COHORT

Long-Term Infection (n, %[a])		Time to Event or Last Follow-up (Months)		
		Minimum	Maximum	Median
Yes (45, 7.4)				
	Scenario 1	5.0	65.5	31.8
	Scenario 2	8.8	68.1	37.4
	Scenario 3	11.0	71.3	43.1
No (563, 92.6)		3.0	85.4	31.3
[a] Percentage of 608 children with one or more classifiable[13]C-UBT results.				

Cohort characteristics by infection status are shown in Table 4. Inclusion of female (51%) and male (49%) children was comparable as was their infection status (44% of

female children and 43% of male children). More children from the United States were included in the final analysis (64%) while more children from Mexico became infected with *H. pylori* during the study period.

More than half of children in the study took at least one course of an *H. pylori* active antibiotic and 50% of these children became infected with *H. pylori* either prior to or after the antibiotic course; this is in contrast to only 37% of children who did not take *H. pylori* active antibiotics who became infected with *H. pylori* either prior to or after the antibiotic course. A slightly higher percentage of children living in a household with greater than one person per room acquired an *H. pylori* infection (48% versus 41%) while the majority of children in the study lived in households with no more than one person per room (68%).

Three hundred and seventy-five children lived with one or more siblings and in the majority of these households, *H. pylori* was not detected in the children (58%). In those households with one or more *H. pylori* infected siblings, a slightly higher percentage of children acquired an infection (48%) compared to children who did not live in a household with an infected sibling (44%). Among index and siblings of the Pasitos Cohort Study utilized for this analysis, 59% were no more than three years apart in age and a slightly higher percentage of these children (39%) became infected with *H. pylori* compared to children who were more than three years apart in age from their siblings (36%).

In regards to the socioeconomic factor of maternal education, 45% of mothers had 7-11 years of education yet households in which the mother obtained six or fewer years of education had the highest percentage of children who became infected with *H. pylori*

(56%).*H. pylori* seropositivity status at baseline was obtained for 581 (95%) of mothers.

Of these mothers, antibodies against *H. pylori* were detected in 363 (62%). A greater

percentage of children living in the same household with a seropositive mother (46%)

became infected with *H. pylori* compared to children who lived in a household with a

seronegative mother (38%).

TABLE 4. COHORT CHARACTERISTICS BY *HELICOBACTER PYLORI* INFECTION STATUS, THE PASITOS COHORT

Characteristics	N	N	%[a]	Infected[b]	%[c]
Sex	608				
F		309	51	135	44
M		299	49	130	43
Country	608				
United States		387	64	163	42
Mexico		221	36	102	46
Mother's education (years)	608				
0-6		100	16	56	56
7-11		275	45	119	43
12-17		233	38	90	39
Mother's *H. pylori* status at baseline	581				
Positive		363	62	167	46
Negative		218	38	83	38
Crowding[d]	608				
≤1		416	68	172	41
>1-2		150	25	73	49
>2		42	7	20	48
***H. pylori* active antibiotic courses taken**	602				
Yes		319	53	161	50
No		283	47	104	37
Age difference >3 years	140				
Yes		58	41	21	36
No		82	59	32	39
***H. pylori* infected sibling in household**	375				
Yes		157	42	75	48
No		218	58	96	44

[a] Percentage within group (N)
[b] Denotes children with at least one positive ^{13}C-UBT result.
[c] Percentage within subgroup (n)
[d]Crowding is defined as the ratio of people living within the household to number of people living in the household.

Participation in The Pasitos Cohort resulted in the potential for a maximum of 85 months of follow-up per participant. The number of visits attended by the cohort is shown in Table 5. Of the possible maximum 14 follow-up visits scheduled in 6 month intervals, no children attended all visits. Less than 1% of children attended 13 follow-up visits while 18% of children attended only one follow-up visit.

TABLE 5. NUMBER AND PERCENTAGE OF COMPLETED FOLLOW-UP VISITS, THE PASITOS COHORT

Visits Completed	Number of Children	%
1	108	17.6
2	77	12.5
3	65	10.6
4	68	11.1
5	47	7.6
6	49	8.0
7	42	6.8
8	38	6.2
9	38	6.2
10	30	4.9
11	25	4.1
12	23	3.7
13	5	0.8
14	0	0.0
Total	**615**	**100.0**

The number of classifiable ^{13}C-UBT results obtained during the possible maximum 85 months of follow-up is shown in Table 6. At least one classifiable ^{13}C-UBT result was obtained for 99% of The Pasitos Cohort and therefore determination of *H. pylori* infection status was made for 608 participants. In order to determine a long-term infection, three or more classifiable ^{13}C-UBT results were necessary; three or more classifiable results were obtained for 419 (68%) of The Pasitos Cohort.

TABLE 6. NUMBER AND PECENTAGE OF CLASSIFIABLE[13]C-UBT RESULTS, THE PASITOS COHORT

# of Classifiable[13]C-UBT Results	Frequency	%
0	7	1.1
1	112	18.2
2	77	12.5
3	65	10.6
4	76	12.4
5	50	8.1
6	45	7.3
7	43	7.0
8	38	6.2
9	34	5.5
10	27	4.4
11	25	4.1
12	14	2.3
13	2	0.3
14	0	0.0
Total	**615**	**100.0**

Participation by cohort members at each scheduled visit as well as information pertaining to ^{13}C-UBT results and *H. pylori* prevalence at each scheduled visit is shown in Table 7. The first visit scheduled at 6 months of age had the highest percentage of attendance (77%) while the fourteenth visit had the lowest percentage of attendance (17%). Testing of children by ^{13}C-UBT was conducted for the majority of children who attended the schedule visit number and classifiable results were obtained in 89%-99% of children. The prevalence of *H. pylori* was the lowest at the first scheduled visit (7.8%) and increased steadily, peaking at 37% in the last scheduled visit.

TABLE 7. VISIT ATTENDANCE, ^{13}C-UREA BREATH TESTING AND *HELICOBACTER PYLORI* INFECTION BY TARGET AGE, THE PASITOS COHORT

Target Age (mos)[a]	Visit Number	Attended[b]	% Attended[c]	^{13}C-UBT[b,d]	% ^{13}C-UBT[e]	Classifiable ^{13}C-UBT[b]	% Classifiable ^{13}C-UBT[f]	Infected	% Infected
6	1	469	77.1	443	94.5	434	98.0	34	7.8
12	2	435	71.5	427	98.2	420	98.4	63	15.0
18	3	361	59.4	356	98.6	347	97.5	59	17.0
24	4	292	48.0	284	97.3	275	96.8	50	18.2
30	5	239	39.3	236	98.7	227	96.2	47	20.7
36	6	197	32.4	197	100.0	186	94.4	41	22.0
42	7	153	25.2	151	98.7	142	94.0	30	21.1
48	8	133	21.9	128	96.2	124	96.9	32	25.8
54	9	108	17.8	107	99.1	104	97.2	21	20.2
60	10	145	23.8	143	98.6	141	98.6	38	27.0
66	11	168	27.6	165	98.2	162	98.2	39	24.1
72	12	169	27.8	168	99.4	156	92.9	41	26.3
78	13	133	21.9	132	99.2	128	97.0	36	28.1
84	14	103	16.9	100	97.1	89	89.0	33	37.1

[a] Actual age of participant was +/- 3 months of target age

[b] Limited to participants with one or more classifiable ^{13}C-UBT (n=608)

[c] Proportion of participants who attended scheduled visit of those with one or more classifiable ^{13}C-UBT results

[d] Number of ^{13}C-urea breath tests performed for scheduled visit number

[e] Proportion of ^{13}C-UBT performed of those who attended the scheduled visit number

[f] Proportion of classifiable ^{13}C-UBT results obtained of total number of ^{13}C-UBT performed

Visit number attendance and *H. pylori* prevalence stratified by *H. pylori* serostatus of the mother are shown in Figures 1 and 2. Attendance was slightly higher in children whose mother was seropositive (n=218) ranging from 18-80% while for children whose mother was seronegative (n=363), attendance ranged from 14-75%. Higher attendance by children whose mother was *H. pylori* seropositive was greatest after visit nine, corresponding to targeted visit ages 60-84 months.

FIGURE 1. VISIT ATTENDANCE AND *HELICOBACTER PYLORI* INFECTION STATUS BY VISIT NUMBER- *HELICOBACTER PYLORI* SERONEGATIVE MOTHER
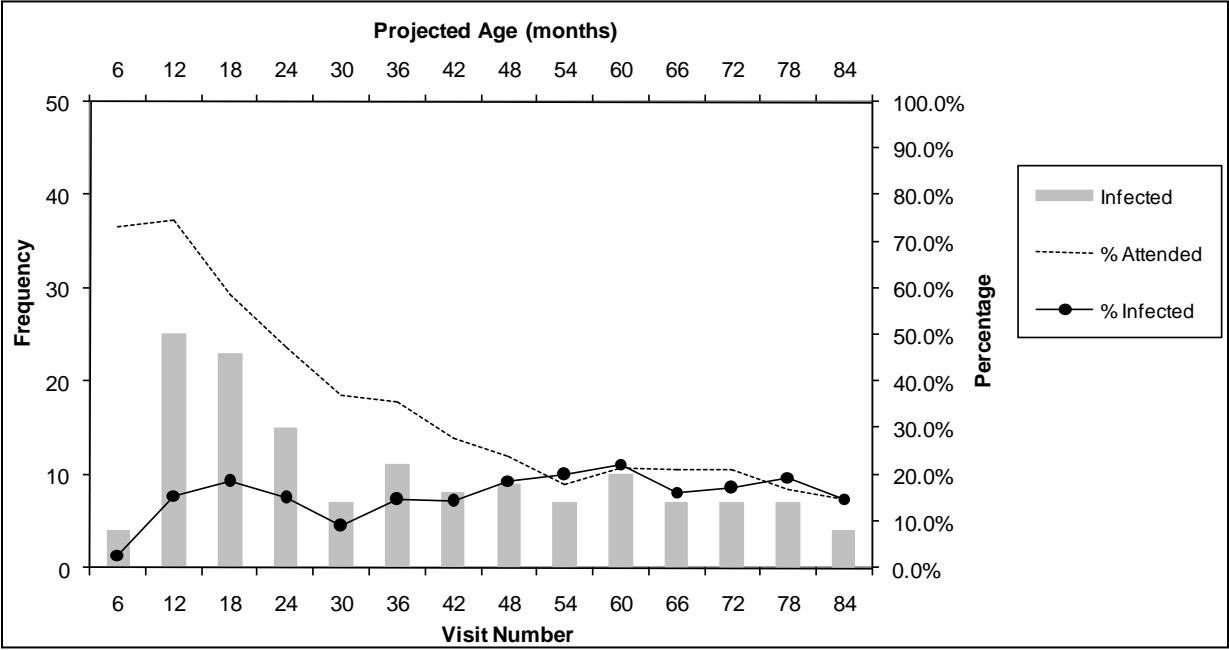
FIGURE 2. VISIT ATTENDANCE AND *HELICOBACTER PYLORI* INFECTION STATUS BY VISIT NUMBER- *HELICOBACTER PYLORI* SEROPOSITIVE MOTHER
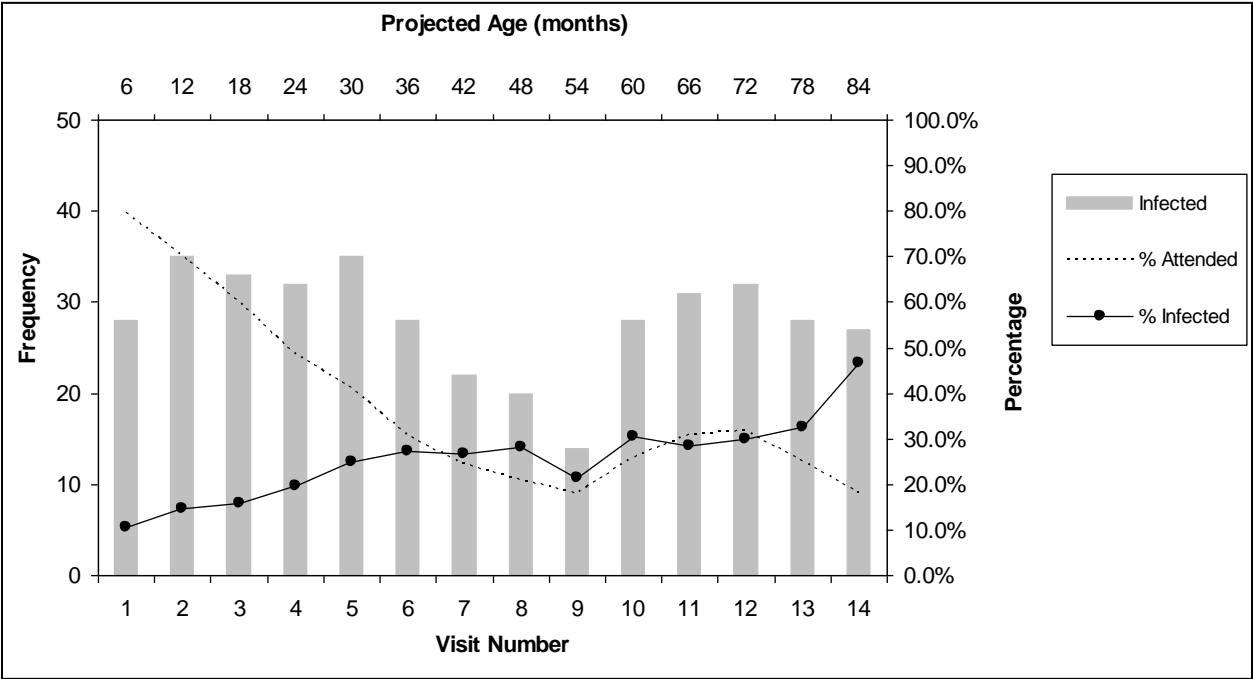
The incidence proportion of *H. pylori* infection in children of seropositive mothers was 46% (Table 4). Of these children, 38 proceeded to a long-term infection (23%). The incidence proportion was lower in children whose mother was seronegative (38%; Table 4), with only five children developing a long-term infection (6%). When *H. pylori* incidence proportion was restricted to household with no *H. pylori* seropositive or infected siblings but a seropositive mother, 54 of 117 children (46%) acquired an *H. pylori* infection; of these children, nine (17%) developed a long-term infection. *H. pylori* incidence proportion was lower in household with neither exposure: no *H. pylori* seropositive or infected siblings and seronegative mothers; 38 of 93 children (41%) acquired an *H. pylori* infection and two (5%) developed a long-term infection.

Regression Analysis

Prior to performing Cox proportional hazards regression analysis, the proportionality of hazards assumption pertaining to *H. pylori* infection acquisition of the child based on the mother's *H. pylori* serostatus at baseline was verified by three methods previously detailed. The log minus log plot of time refers to months to *H. pylori* infection acquisition of the child based on the mother's *H. pylori* serostatus at baseline (Figure 3). Visual inspection of the curves indicates that the proportional hazards assumption was likely not violated: the rate for *H. pylori* infection acquisition was roughly proportional across follow-up for children with both *H. pylori* seropositive and seronegative mothers. The time to interaction test also found that there was no significant interaction between mother's *H. pylori* status and time to infection of her child (β coefficient=0.004, p-value=0.438). This result was confirmed via a partial residual plot (Appendix L).

FIGURE 3. LOGMINUSLOG PLOT: TESTING OF PROPORTIONALITY ASSUMPTION OF *HELICOBACTER PYLORI* INFECTION ACQUISITION BASED ON *HELICOBACTER PYLORI* SEROSTATUS OF THE MOTHER
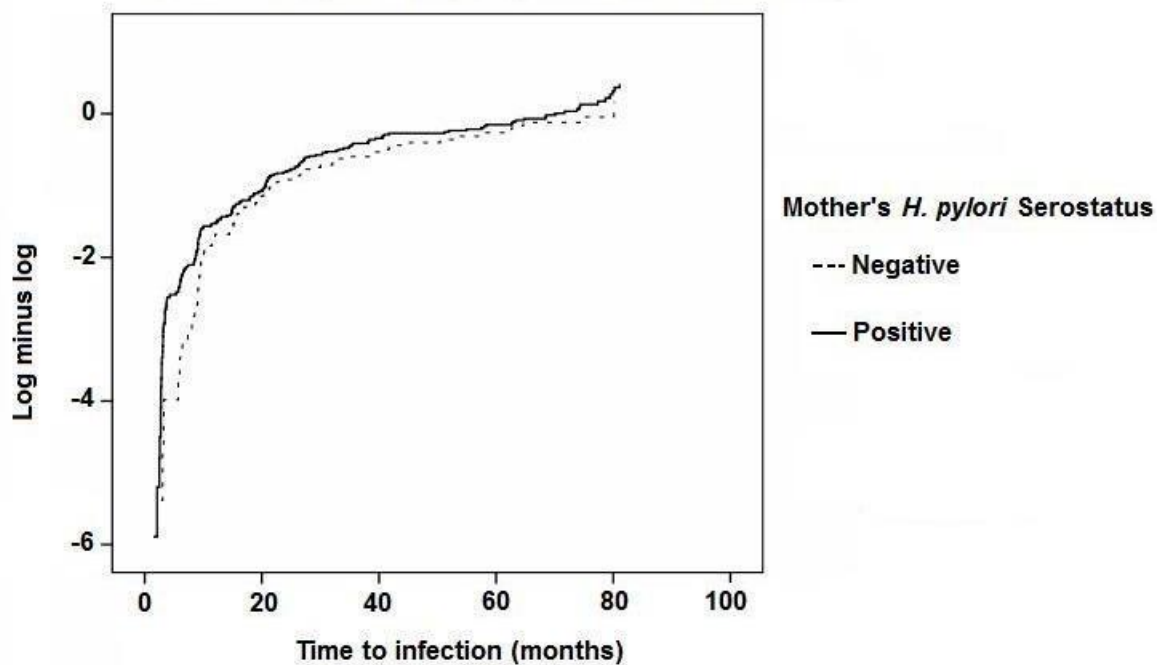
Due to meeting the assumption of proportional hazards, Cox regression without time dependent covariates analysis was performed to obtain hazards ratios for the association between infection acquisition and maternal *H. pylori* seropositivity. Results of regression analysis are shown in Appendix M. Crude analysis revealed that the rate of *H. pylori* incident infection was roughly 20% greater in children whose mother was seropositive for *H. pylori* compared to children whose mother was seronegative for *H. pylori* (crude HR=1.2, 95% CI: 0.9, 1.6). Adjustment for crowding did not change the estimates (adjusted HR=1.2, 95% CI: 0.9, 1.6). Both crude and adjusted measures are somewhat uncertain in the direction of effect and a strong association in either direction can be ruled out.

When evaluating the modification of the estimated effect of maternal *H. pylori* seropositive status on the *H. pylori* infection acquisition of her child by several factors,

measures of association obtained were weak and imprecise. In Mexico, the rate of *H. pylori* incident infection acquisition was slightly greater in children whose mother was seropositive for *H. pylori* relative to children whose mother was seronegative for *H. pylori* (adjusted HR=1.5, 95% CI: 0.9, 2.5). In the United States, the rate of infection acquisition was also just slightly greater in children whose mother was seropositive relative to children whose mother was seronegative (adjusted HR= 1.1, 95% CI: 0.8, 1.6).

Additionally, in children who received at least one course of *H. pylori* active antibiotics the rate of infection acquisition was slightly greater in children whose mother was seropositive for *H. pylori* relative to children whose mother was seronegative for *H. pylori* (adjusted HR= 1.3, 95% CI: 0.9, 1.8). In children who did not receive any *H. pylori* active antibiotics the rate of *H. pylori* incident infection in children whose mother was seropositive relative to those whose mother was seronegative for *H. pylori* was neither lower or greater (adjusted HR= 1.0, 95% CI: 0.7, 1.6).

Among children who were breastfed, the rate of infection acquisition in children whose mother was seropositive for *H. pylori* was just slightly greater relative to children whose mother was seronegative (adjusted HR= 1.2, 95% CI: 0.6, 1.7). Among children who were not breastfed, the rate of infection acquisition was just slightly lower in children whose mother was seropositive for *H. pylori* relative to children whose mother was seronegative for *H. pylori* (adjusted HR= 0.9, 95% CI: 0.6, 1.7).

In children living with a sibling in which there was no more than three years age difference, the rate of *H. pylori* infection acquisition in children whose mother was seropositive for *H. pylori* was slightly greater relative to children whose mother was

seronegative for *H. pylori* (adjusted HR= 1.4, 95% CI: 0.7, 3.0). In children living with a sibling in which there was more than three years age difference, the rate of *H. pylori* infection acquisition in children whose mother was seropositive was still just slightly greater relative to children whose mother was seronegative (adjusted HR= 1.2, 95% CI: 0.4, 3.5, respectively).

In children living in the same household as a sibling who was either seropositive or infected with *H. pylori*, the rate of infection acquisition was 1.6 times greater in children whose mother was seropositive compared to children whose mother was seronegative (adjusted HR: 1.6, 95% CI: 0.9, 2.9). In children living in the same household as a sibling who was either seronegative or not infected with *H. pylori,* the rate of infection acquisition was 1.2 times greater in children whose mother was seropositive for *H. pylori* compared to children whose mother was seropositive for *H. pylori* (adjusted HR= 1.2, 95% CI: 0.8, 1.8).

Overall, effect modification by country of residence, *H. pylori* effective antibiotic use, breastfeeding status, age difference between sibling and *H. pylori* status of siblings within the household was seen on the *H. pylori* infection acquisition by maternal *H. pylori* status. But, all estimate effects regardless of strata do not suggest a strong inverse or a strong positive association between maternal *H. pylori* status and *H. pylori* infection acquisition of her child.

As with *H. pylori* infection acquisition, the proportionality of hazards assumption of the establishment of a long-term *H. pylori* infection of the child based on the mother's *H. pylori* serostatus at baseline was also verified. The log minus log plots refers to months to *H. pylori* long-term infection establishment of the child based on the mother's *H. pylori*

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serostatus at baseline as defined in three different time-to-event scenarios previously described (Figures 4-6).

For the establishment of long-term *H. pylori* infection in a child, visual inspection of the three curves for all time to long-term infection scenarios indicate that the proportional hazards assumption was likely violated (Figures 4-6): the rate for *H. pylori* long-term infection was not proportional across follow-up for children with both *H. pylori* seropositive and seronegative mothers. The time to interaction test also found that there was significant interaction between mother's *H. pylori* status and time to infection of her child (scenario one: β coefficient=0.039, p-value=0.009, scenario two: β coefficient=0.031, p-value=0.011, scenario three: β coefficient=0.029, p-value=0.009). Partial residual plots (Appendices N-P) revealed that the average value of residuals did deviate from zero for scenario one, did not deviate from zero for scenario two and exhibited minor deviations from zero for scenarios three. Therefore based on partial residual plots, scenarios two and three met the proportional hazards assumption. But as two of three tests did not indicated meeting the proportional hazards assumption, a violation of the assumption was determined for all three time to event scenarios.

FIGURE 4. LOG MINUS LOG PLOT: TESTING OF PROPORTIONALITY ASSUMPTION OF *HELICOBACTER PYLORI* INFECTION ESTABLISHMENT BASED ON *HELICOBACTER PYLORI* SEROSTATUS OF THE MOTHER, LONG-TERM INFECTION SCENARIO 1
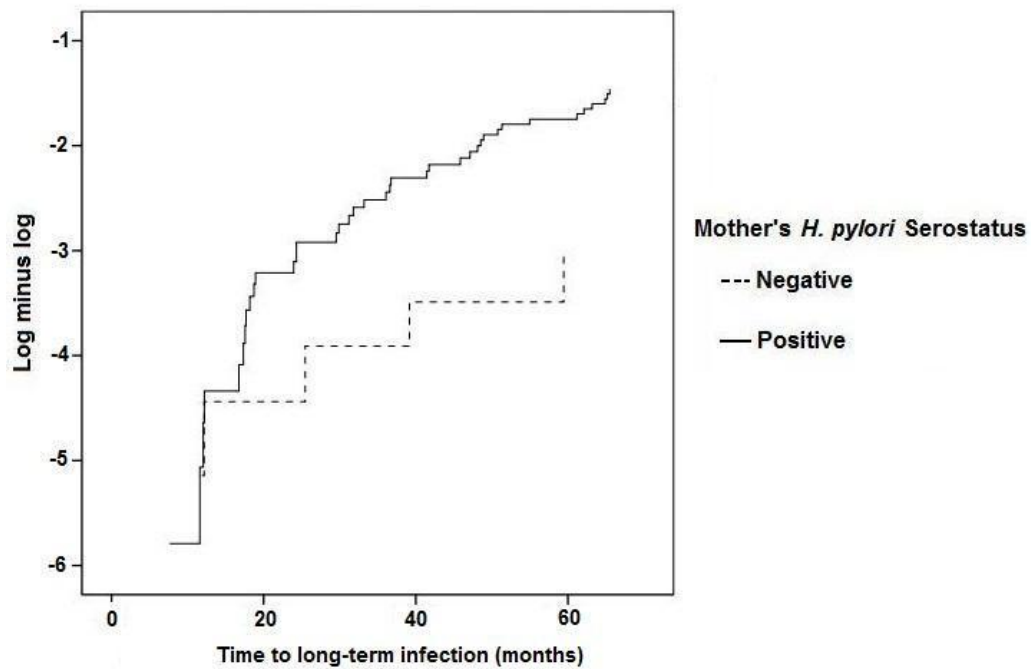
FIGURE 5. LOG MINUS LOG PLOT: TESTING OF PROPORTIONALITY ASSUMPTION OF *HELICOBACTER PYLORI* INFECTION ESTABLISHMENT BASED ON *HELICOBACTER PYLORI* SEROSTATUS OF THE MOTHER, LONG-TERM INFECTION SCENARIO 2
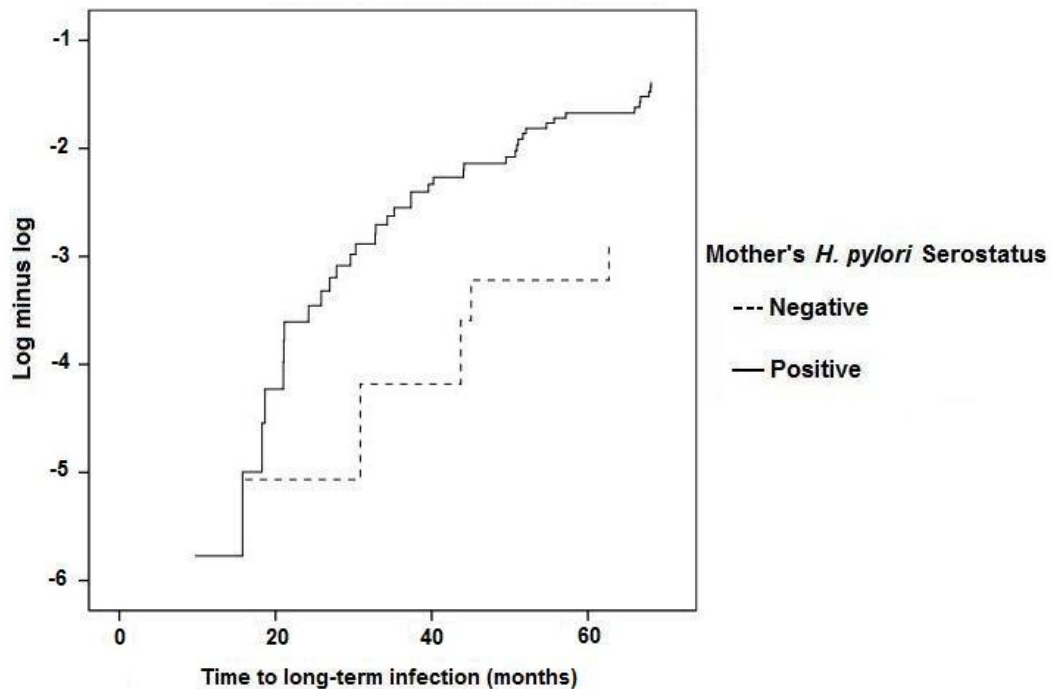
FIGURE 6. LOG MINUS LOG PLOT: TESTING OF PROPORTIONALITY ASSUMPTION OF *HELICOBACTER PYLORI* INFECTION ESTABLISHMENT BASED ON *HELICOBACTER PYLORI* SEROSTATUS OF THE MOTHER, LONG-TERM INFECTION SCENARIO 3
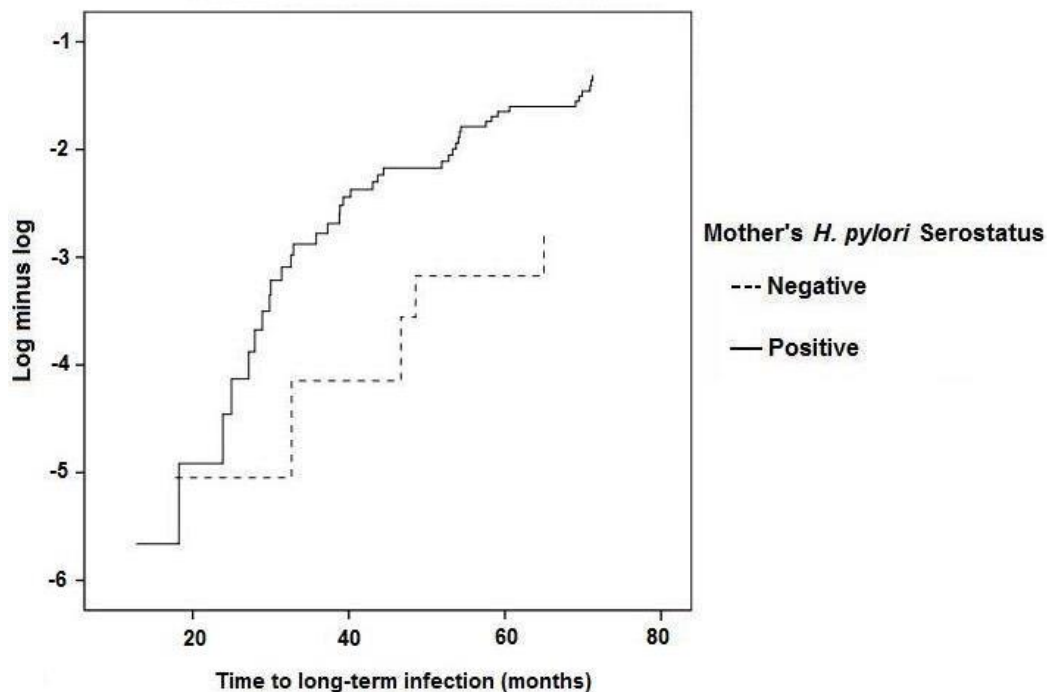
As the proportional hazards assumption was not met, Cox regression with time dependent covariates analysis was performed to obtain hazards ratios for all three time-to-event scenarios. Results of regression analysis are shown in Appendices Q-S. In scenario one, crude analysis revealed that the rate of long-term *H. pylori* infection establishment was more than two times greater in children whose mother was seropositive for *H. pylori* relative to children whose mother was seronegative for *H. pylori* (crude HR= 2.3, 95% CI: 0.3, 16.6). After adjusting for crowding, the rate of long-term *H. pylori* infection establishment was still more than two times greater in children whose mother was seropositive relative to children whose mother was seronegative (adjusted HR= 2.1, 95% CI: 0.3, 15.0). Both crude and adjusted measures are very

uncertain in the direction of effect as well as magnitude of effect with values of strong association in both directions included in the confidence intervals.

As in scenario one, scenarios two and three crude and adjusted estimated effects of maternal *H. pylori* seropositive status on the *H. pylori* long-term infection establishment of her child are strong and in a positive direction but confidence intervals are very wide with estimated values in both directions. In scenarios two and three, crude measures of association revealed that the rate of long-term *H. pylori* infection establishment in children whose mother was seropositive for *H. pylori* was much greater relative to children whose mother was seronegative for *H. pylori* (scenario two crude HR= 4.4, 95% CI: 0.4, 50.9; scenario three crude HR= 3.0, 95% CI: 0.2, 43.5). After adjusting for crowding, estimated measures of effect showed that the rate of long-term *H. pylori* infection establishment in children whose mother was seropositive for *H. pylori* was still much greater relative to children whose mother was seronegative for *H. pylori* (scenario two adjusted HR= 4.0, 95% CI: 0.3, 46.5; scenario three adjusted HR= 2.7, 95% CI: 0.2, 39.7).

Upon stratifying the data on *H. pylori* status of household siblings and country of residence, all point estimates are bounded by very wide confidence intervals and therefore display a high level of uncertainty in the magnitude and direction of estimated effects with values of strong association in both directions in confidence intervals. In all three time-to-event scenarios the rate of *H. pylori* long-term infection establishment was just slightly greater in children whose mother was seropositive for *H. pylori* relative to children whose mother was seronegative for *H. pylori* (scenario one adjusted HR=1.1, 95% CI: 0.1, 30.9; scenario two adjusted HR= 2.4, 95% CI: 0.1, 128.8; scenario three

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adjusted HR= 1.4, 95% CI: 0.0, 107.7) if children were living in the same household with an *H. pylori* seropositive or infected sibling. In children who were living in the same household with an *H. pylori* seronegative or uninfected sibling, the rate of the establishment of a long-term infection in children whose mother was seropositive for *H. pylori* was much greater relative to children whose mother was seronegative for *H. pylori* (scenario one adjusted HR=5.5, 95% CI: 0.2, 137.1; scenario two adjusted HR= 5.9, 95% CI: 0.2, 224.8; scenario three adjusted HR= 4.9, 95% CI: 0.1, 239.8).

It was also found in two of three time-to long-term infection establishment scenarios that in Mexico, the rate of long-term infection in children whose mother was seropositive for *H. pylori* was somewhat greater relative to children whose mother was seronegative for *H. pylori* (scenario one adjusted HR=0.7, 95% CI: 0.1, 9.2; scenario two adjusted HR= 2.4, 95% CI: 0.8, 72.4; scenario three adjusted HR= 1.7, 95% CI: 0.0, 82.6). In the United States, the rate of the establishment of a long-term infection in children whose mother was seropositive for *H. pylori* was much greater relative to children whose mother was seronegative for *H. pylori* (scenario one adjusted HR= 5.4, 95% CI: 0.2, 157.1; scenario two adjusted HR= 5.5, 95% CI: 0.2, 199.7; scenario three adjusted HR= 3.4, 95% CI: 0.8, 143.9) Again, in all three scenarios and strata, a high degree of uncertainty is noted in both the direction and magnitude of effect with values in the confidence intervals which include strong associations in both directions.

Effect modification on the *H. pylori* long-term infection establishment based on *H. pylori* status of a household sibling and country of residence may have occurred but highly imprecise estimates do not allow for this to be determined.

Due to limited number of observations upon stratifying or model instability, hazard ratios could not be obtained to estimate the effect of exposure to a seropositive mother on the establishment of a long-term infection of her child based on age difference, *H. pylori* active antibiotic use or breastfeeding status.

Aim B- *Helicobacter pylori* Transmission and Sibling Infection Status

Descriptive Analysis

In The Pasitos Cohort Study, there were 608 participating children with one or more classifiable[13]C-UBT results from 468 households: 468 index children and 140 younger siblings. Household participant composition of these children is shown in Table 8. The majority of households did not have younger siblings who participated in The Pasitos Cohort Study (74%). In households that contained index and younger siblings that participated in The Pasitos Cohort Study (n= 122), roughly 87% were composed of the index child and one younger sibling.

TABLE 8. PARTICIPANT HOUSEHOLD COMPOSITION, THE PASITOS COHORT

Household Composition	N	%
Index only households	346	73.9[a]
Index and siblings[b] in household	122	26.1[a]
Index and 1 sibling	106	86.9[c]
Index and 2 siblings	14	11.5[c]
Index and 3 siblings	2	1.6[c]
[a]Percentage of total number of households (n=468).		
[b]Total number of index children (n=468) and younger siblings (n=140) who participated in The Pasitos Cohort and had at least one classifiable[13]C-UBT result. Does not include older or younger siblings that did not participate in The Pasitos Cohort Study.		
[c] Percentage of total number of households with index and younger siblings (n=122).		

Almost half of index children with one or more classifiable[13]C-UBT results, 212 (45%), acquired *H. pylori* at least once during follow-up and 34 (7%) developed a long-term infection. When restricted to 122 index children with one or more Pasitos Cohort younger siblings, 76 (62%) became infected with *H. pylori* at least once during follow-up

and 17 (14%) developed a long-term infection. Of 329 index only homes in which the mother's *H. pylori* status at baseline was known, 210 (64%) lived with a mother who was seropositive at baseline. Of 140 siblings with one or more classifiable[13]C-UBT results, 53 (38%) acquired *H. pylori* and 11(8%) acquired a long-term *H. pylori* infection.

Information pertaining to index-younger sibling *H. pylori* infection status as well as maternal *H. pylori* seropositivity at baseline is shown in Table 9. Of 140 siblings included in the final analysis, 24% who remained uninfected throughout follow-up lived with an index sibling that also remained uninfected while 38% lived with an index sibling who became infected at least once during follow-up; greater than half of these younger siblings had a mother who was seropositive at baseline. Thirty-five younger siblings (25%) became infected with *H. pylori* throughout follow-up while living with an index sibling who also became infected at least once during follow-up and 65% of these children had a mother who was seropositive at baseline. There were only two cases in which a younger sibling developed a long-term infection while the index child's infection did not progress into a long-term infection; both lived with a mother who was seropositive for *H. pylori*. Eighteen younger siblings (13%) became infected with *H. pylori* and two of these siblings achieved a long-term infection while living with an index child who did not become infected with *H. pylori* during follow-up; 50% of the former and 100% of the latter lived with a mother who was seropositive at baseline.

TABLE 9. *HELICOBACTER PYLORI* INFECTION STATUS BY SIBLING AND MATERNAL INFECTION STATUS, THE PASITOS COHORT

Index-Sibling Infection Status	N	%	Mother *H. pylori* Seropositive	%[e]
Index Infected[a]=0[b]/ Sibling Infected=0	34	24.3[c]	18	52.9
Index Infected=1[b]/ Sibling Infected=0	53	37.9[c]	31	58.5
Index LT[d]=1/ Sibling Infected=0	6	11.3[e]	4	66.7
Index Infected=1/ Sibling Infected=1	35	25.0[c]	23	65.7
Index LT=1/ Sibling LT=0	7	20.0[e]	6	85.7
Sibling LT=1/ Index LT= 0	2	5.7[e]	2	100
Sibling LT=1/ Index LT=1	7	20.6[e]	5	71.4
Sibling Infected=1/ Index Infected=0	18	12.9[c]	9	50
Sibling LT=1/ Index Infected= 0	2	11.1[e]	2	100

[a]infected refers to the acquisition of *H. pylori* and includes short-term and long-term infections.

[b]0 indicates absence of *H. pylori* infection or long-term infection. 1 indicates presence of *H. pylori* infection or long-term infection.

[c]Percentage of total number of younger siblings in The Pasitos Cohort with one or more classifiable ^{13}C-UBT (n=140).

[d]LT refers to a long-term *H. pylori* infection.

[e]Percentage within group (n).

Follow-up time information for both index children and younger siblings by *H. pylori* infection status is shown in Table 10. Among index subjects who became infected with *H. pylori* and who had younger siblings, the median age at last follow-up was 1.3 while among those index children who did not become infected, median age at last follow-up was 5.4. For younger siblings with an incident *H. pylori* infection, the median age at last follow-up was also 1.3 but for younger siblings not infected the media age at last follow-up was 1.7. The earliest *H. pylori* infection was detected approximately 3 months after birth among index children and 1.5 months after birth among their younger siblings.

TABLE 10. TIME TO *HELICOBACTER PYLORI* INCIDENT INFECTION BY SIBING AND INFECTIONS STATUS, THE PASITOS COHORT

Incident Infection (n, %)	Time to Event or Follow-up (Months)		
	Minimum	Maximum	Median
Index[a]			
Yes (76, 62.3)	2.7	80.2	16.3
No (46, 37.7)	22.1	84.4	65.2
Younger Sibling[b]			
Yes (53, 37.9)	1.5	57.4	16.4
No (87, 62.1)	3.0	76.7	20.8

[a] Percentage of index children with one or more classifiable[13]C-UBT results living with one or more younger Pasitos Cohort siblings (n=122).

[b] Percentage of younger siblings with one or more classifiable[13]C-UBT results (n=140).

Information pertaining to follow-up time to long-term *H. pylori* infection is presented in Table 11. Three different time-to-event scenarios for long-term infection establishment as described previously indicate that the median age at long-term infection establishment ranged from 3 to 3.7 for index children; for younger siblings, median age ranged from 2.6 to 3.1. The earliest age at which a long-term infection was detected ranged from roughly 7 months after birth to slightly greater than 1 year after birth among index children with one or more siblings. In younger siblings, the first long-term infection was detected from 5 to 11 months after birth. Among index children who did not develop a long-term infection, the median age at follow-up was roughly six while for their younger siblings it was approximately one. Cohort characteristics based on sibling status were not performed as they were presented in Table 4 previously by infection status.

TABLE 11. TIME TO *HELICOBACTER PYLORI* LONG-TERM INFECTION BY INDEX AND
SIBLING, THE PASITOS COHORT

Long-Term Infection (n, %)	Time to Event or Follow-up(Months)		
	Minimum	Maximum	Median
Index[a]			
Yes (17, 13.9)			
Scenario 1	7.6	65.5	36.7
Scenario 2	9.7	68.1	39.6
Scenario 3	12.8	71.3	44.4
No (105, 86.1)	12.1	84.4	70.8
Younger Sibling[b]			
Yes (11, 7.9)			
Scenario 1	5.0	50.8	31.2
Scenario 2	8.8	54.7	34.3
Scenario 3	11.0	57.6	37.3
No (129, 92.1)	3.0	76.7	25.4

[a] Percentage of index children with one or more classifiable[13]C-UBT results living with one or more younger Pasitos Cohort siblings (n=122).

[b] Percentage of younger siblings with one or more classifiable[13]C-UBT results (n=140).

As shown in Table 12, when restricted to index children with one or more classifiable[13]C-UBT results living with one or more Pasitos Cohort younger siblings, the most visits attended was 13; only two index children (1.6%) attended 13 visits. No index child attended the maximum number of visits, fourteen. The greatest number of visits attended by index children was six; 21 index children (17%) attended at least 6 visits. Among younger siblings, one child (0.7%) attended up to ten visits while 22% of younger siblings attended 4 or fewer visits (Table 12). Again, no younger siblings attended all fourteen possible visits.

TABLE 12. NUMBER AND PERCENTAGE OF COMPLETED FOLLOW-UP VISITS BY
SIBLING STATUS, THE PASITOS COHORT

Visits Completed	Index-Number of Children	%	Younger Sibling-Number of Children	%
1	1	0.8	19	13.6
2	3	2.5	20	14.3
3	6	4.9	28	20.0
4	6	4.9	31	22.1
5	4	3.3	20	14.3
6	21	17.2	11	7.9
7	16	13.1	5	3.6
8	15	12.3	3	2.1
9	18	14.8	2	1.4
10	10	8.2	1	0.7
11	9	7.4	0	0.0
12	11	9.0	0	0.0
13	2	1.6	0	0.0
14	0	0.0	0	0.0
Total	**122**	**100.0**	**140**	**100.0**

In regards to classifiable ^{13}C-UBT results (Table 13), all index children with one or more Pasitos Cohort younger siblings had one or more classifiable ^{13}C-UBT results. Therefore, incident *H. pylori* infection was determined for all of these children. *H. pylori* infection was determined for approximately 98% of younger siblings as classifiable ^{13}C-UBT results were not obtained for three Pasitos Cohort younger siblings. In regards to determination of a long-term infection, three or more classifiable ^{13}C-UBT results were obtained from the majority of both index and younger siblings; long-term infection status was determined for 98% of index children and 85% of younger siblings.

TABLE 13. NUMBER AND PERCENTAGE OF CLASSIFIABLE UREA BREATH TEST
RESULTS, THE PASITOS COHORT

Classifiable ^{13}C-UBT Result	Index-		Younger Sibling-	
	Frequency	%	Frequency	%
0	0	0.0	3	2.1
1	1	0.8	23	16.1
2	3	2.5	21	14.7
3	7	5.7	32	22.4
4	10	8.2	30	21.0
5	5	4.1	17	11.9
6	24	19.7	8	5.6
7	17	13.9	5	3.5
8	16	13.1	2	1.4
9	13	10.7	2	1.4
10	10	8.2	0	0.0
11	10	8.2	0	0.0
12	6	4.9	0	0.0
13	0	0.0	0	0.0
14	0	0.0	0	0.0
Total	**122**	**100.0**	**143**	**100.0**

Participation at each scheduled visit as well as information pertaining to ^{13}C-UBT

results and *H. pylori* prevalence at each scheduled visit is shown in Table 14 for index

children with one or more Pasitos Cohort younger siblings and in Table 15 for younger

siblings of The Pasitos Cohort. For index children, attendance was highest at visit

number three (83%) when the index child was approximately one and a half years of

age. For younger siblings, attendance was highest for visit number two (65%) when the

child was approximately one year of age. For both index children and their younger

siblings, visit number 14 had the lowest attendance; only 31% of index children and less

than 1% of younger siblings attended visit 14. Only index children, but not younger

siblings (unless they were twins or other multiples of the index child) had the potential to

attend all 14 visits.

^{13}C-UBT was done on the majority of children who attended each visit and classifiable results were also obtained for most children tested. For index children, 93%-100% of children were tested and classifiable results obtained for each visit. In younger siblings, after the fourth visit,^{13}C-UBT was performed on 100% of children who attended each visit; classifiable results were obtained from 92%-100% of these children except for visit 14 in which classifiable results could not be obtained from the only younger sibling who attended that visit.

TABLE 14. VISIT ATTENDANCE, ^{13}C-UREA BREATH TESTING AND *HELICOBACTER PYLORI* INFECTION BY TARGET AGE FOR INDEX CHILD- THE PASITOS COHORT

Target Age (mos)[a]	Visit Number	Attended[b]	% Attended[c]	^{13}C-UBT[b,d]	% ^{13}C-UBT[e]	Classifiable ^{13}C-UBT[b]	% Classifiable ^{13}C-UBT[f]	^{13}C- Infected	% Infected
6	1	98	80.3	91	92.9	90	98.9	7	7.8
12	2	96	78.7	95	99.0	94	98.9	25	26.6
18	3	102	83.6	101	99.0	95	94.1	18	18.9
24	4	84	68.9	81	96.4	77	95.1	18	23.4
30	5	71	58.2	69	97.2	65	94.2	12	18.5
36	6	54	44.3	54	100.0	51	94.4	10	19.6
42	7	47	38.5	46	97.9	44	95.7	12	27.3
48	8	47	38.5	45	95.7	44	97.8	10	22.7
54	9	45	36.9	44	97.8	44	100.0	7	15.9
60	10	62	50.8	62	100.0	61	98.4	16	26.2
66	11	71	58.2	70	98.6	68	97.1	17	25.0
72	12	70	57.4	70	100.0	63	90.0	19	30.2
78	13	55	45.1	54	98.2	50	92.6	18	36.0
84	14	38	31.1	38	100.0	31	81.6	13	41.9

[a] Actual age of participant was +/- 3 months of target age
[b] Limited to index children with one or more classifiable ^{13}C-UBT living with one or more younger Pasitos Cohort siblings (n=122).
[c] Proportion of participants who attended scheduled visit of those with one or more classifiable ^{13}C-UBT results
[d] Number of ^{13}C-urea breath tests performed for scheduled visit number
[e] Proportion of ^{13}C-UBT performed of those who attended the scheduled visit number
[f] Proportion of classifiable ^{13}C-UBT results obtained of total number of ^{13}C-UBT performed

TABLE 15. VISIT ATTENDANCE, ^{13}C-UREA BREATH TESTING AND *HELICOBACTER PYLORI* INFECTION BY TARGET AGE OF YOUNGER SIBLING- THE PASITOS COHORT

Target Age (mos)[a]	Visit Number	Attended[b]	% Attended[c]	^{13}C-UBT[b,d]	% ^{13}C-UBT[e]	Classifiable ^{13}C-UBT[b]	% Classifiable ^{13}C-UBT[f]	Infected	% Infected
6	1	87	62.1	82	94.3	80	97.6	9	11.3
12	2	91	65.0	89	97.8	86	96.6	8	9.3
18	3	82	58.6	81	98.8	78	96.3	16	20.5
24	4	61	43.6	59	96.7	56	94.9	8	14.3
30	5	51	36.4	51	100.0	49	96.1	15	30.6
36	6	42	30.0	42	100.0	39	92.9	13	33.3
42	7	36	25.7	36	100.0	33	91.7	8	24.2
48	8	26	18.6	26	100.0	25	96.2	8	32.0
54	9	20	14.3	20	100.0	17	85.0	5	29.4
60	10	12	8.6	12	100.0	11	91.7	5	45.5
66	11	7	5.0	7	100.0	6	85.7	3	50.0
72	12	3	2.1	3	100.0	2	66.7	0	0.0%
78	13	1	0.7	1	100.0	1	100.0	0	0.0%
84	14	1	0.7	1	100.0	0	0.0	0	NA

[a] Actual age of participant was +/- 3 months of target age
[b] Limited to younger siblings with one or more classifiable ^{13}C-UBT (n=140)
[c] Proportion of participants who attended scheduled visit of those with one or more classifiable ^{13}C-UBT results
[d] Number of ^{13}C-urea breath tests performed for scheduled visit number
[e] Proportion of ^{13}C-UBT performed of those who attended the scheduled visit number
[f] Proportion of classifiable ^{13}C-UBT results obtained of total number of ^{13}C-UBT performed

In regards to prevalence of *H. pylori* infection, 8% of index children and 11% of their younger siblings attendees were infected by visit one, approximately 6 months of age (Tables 14 and 15). Prevalence of *H. pylori* ranged from 8%-42% in index children and 0%-50% in Pasitos younger siblings. Peak prevalence for index children (42%) was documented at visit 14 when the child was about seven years of age. For younger siblings, peak prevalence of 50% was documented at visit 11, when the child was approximately five and a half years of age.

Figures 7 and 8 display visit attendance and *H. pylori* infection status by visit for index children with one or more younger Pasitos siblings; Figure 7 is limited to index children for whom the *H. pylori* was not detected in the younger sibling during follow-up (n=72)

and Figure 8 is limited to index children for whom *H. pylori* was detected in the younger

sibling at least once during follow-up (n=49). Visit attendance was slightly higher among

index children with *H. pylori* infected younger siblings (37-86%) than in index children

whose younger sibling was not infected with *H. pylori* (23-83%). Prevalence of *H. pylori*

was again slightly higher among index children living with younger sibling for whom *H.

pylori* was detected once during follow-up (8%-43%) versus those living with younger

siblings who were not infected with *H. pylori* during follow-up (5%-41%).

When restricted to index children living in households in which the mother was

seronegative at baseline (n=30), 57% of index children with younger siblings who did

not become infected with *H. pylori* during follow-up became infected with *H. pylori*; 6%

of these children proceeded to develop a long-term infection. Half (50%) of index

children living with a mother who was seronegative for *H. pylori* at baseline (n=16) as

well as younger siblings that did become infected with *H. pylori* during follow-up also

became infected and 13% of these index children developed a long-term infection.

FIGURE 7. VISIT ATTENDANCE AND *HELICOBACTER PYLORI* INFECTION STATUS BY VISIT NUMBER FOR INDEX CHILD- YOUNGER SIBLING NOT *HELICOBACTER PYLORI* INFECTED
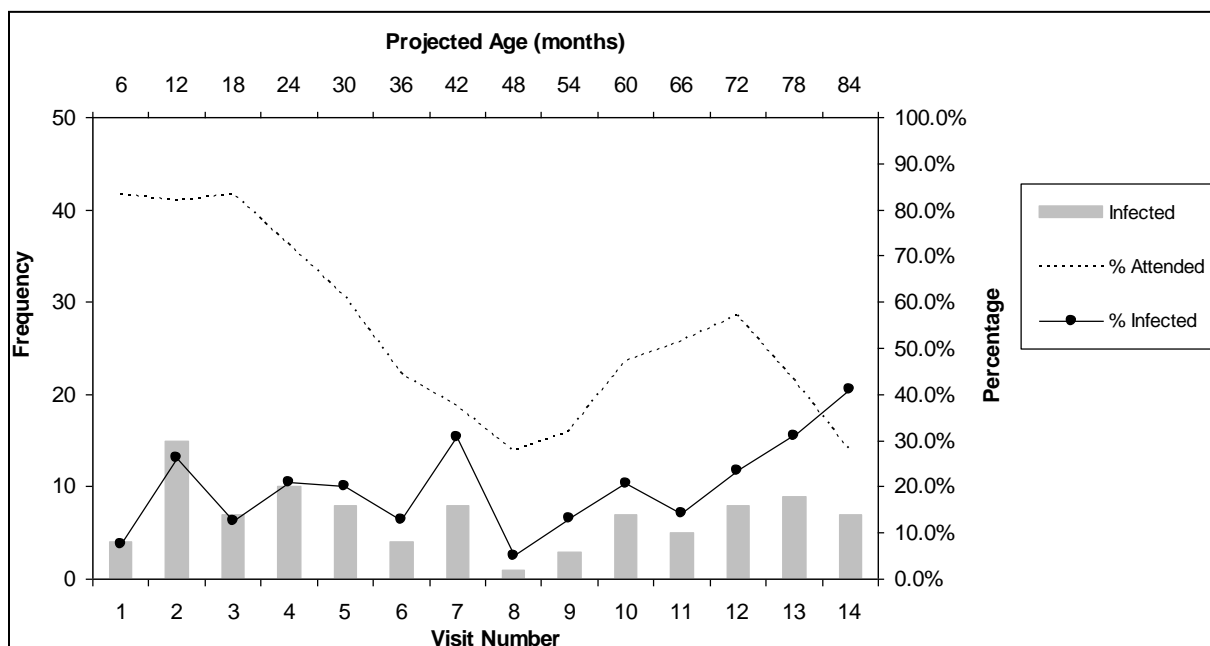
FIGURE 8. VISIT ATTENDANCE AND *HELICOBACTER PYLORI* INFECTION STATUS BY VISIT NUMBER FOR INDEX CHILD- YOUNGER SIBLING *HELICOBACTER PYLORI* INFECTED
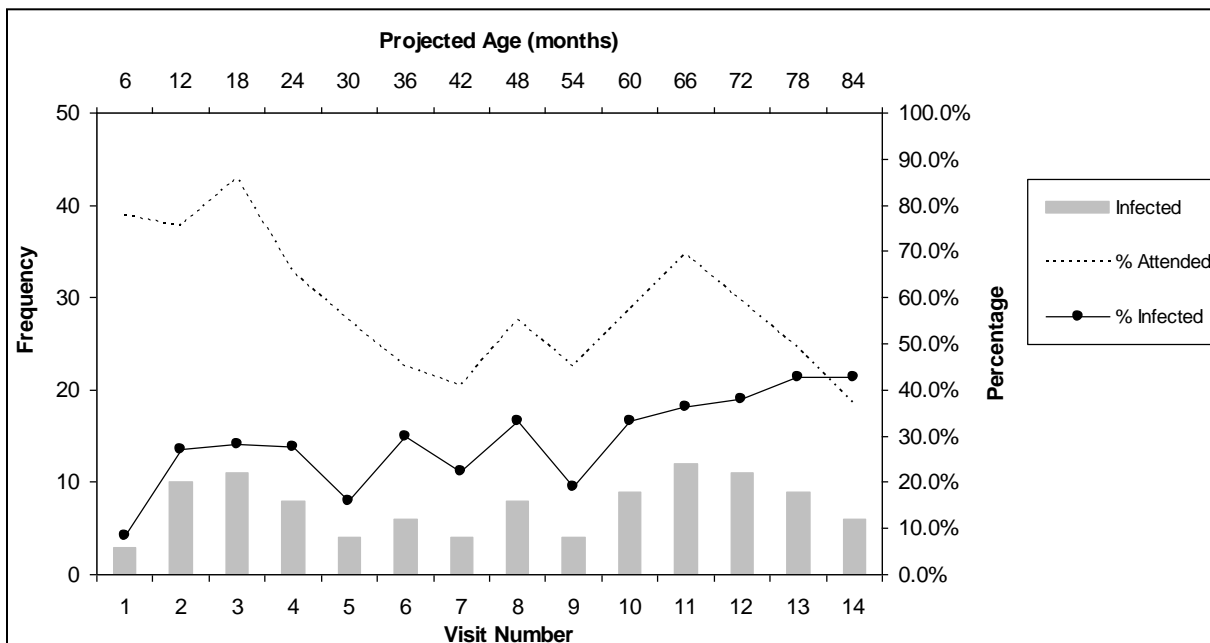
In regards to younger siblings of The Pasitos Cohort, visit attendance and *H. pylori* infection status per visit are shown in Figures 9 and 10. Figure 9 is limited to younger siblings living with an index child who did not become infected with *H. pylori* (n=52) while Figure 10 is limited to younger siblings living with an index child who did become infected with *H. pylori* during follow-up (n=88). Attendance was equivalent in younger siblings living without and with *H. pylori* infected index children ranging from 2%-67% and 1-64%, respectively. Prevalence peaked at 67% for younger siblings whose older index sibling did not become infected with *H. pylori* and 50% for children whose older index sibling did become infected with *H. pylori* was noted although overall higher prevalence per visit was observed in younger siblings whose older sibling was also infected with *H. pylori*.

When restricted to younger siblings whose older index sibling did not become infected with *H. pylori* and whose mother was seronegative at baseline (n=28), 32% of younger siblings became infected and none achieved a long-term infection. In younger siblings whose older index sibling became infected and whose mother was seronegative at baseline (n=29), 28% became infected and 13% of these children progressed to a long-term infection.

FIGURE 9. VISIT ATTENDANCE AND *HELICOBACTER PYLORI* INFECTION STATUS BY VISIT NUMBER FOR YOUNGER SIBLINGS- INDEX CHILD NOT *HELICOBACTER PYLORI* INFECTED
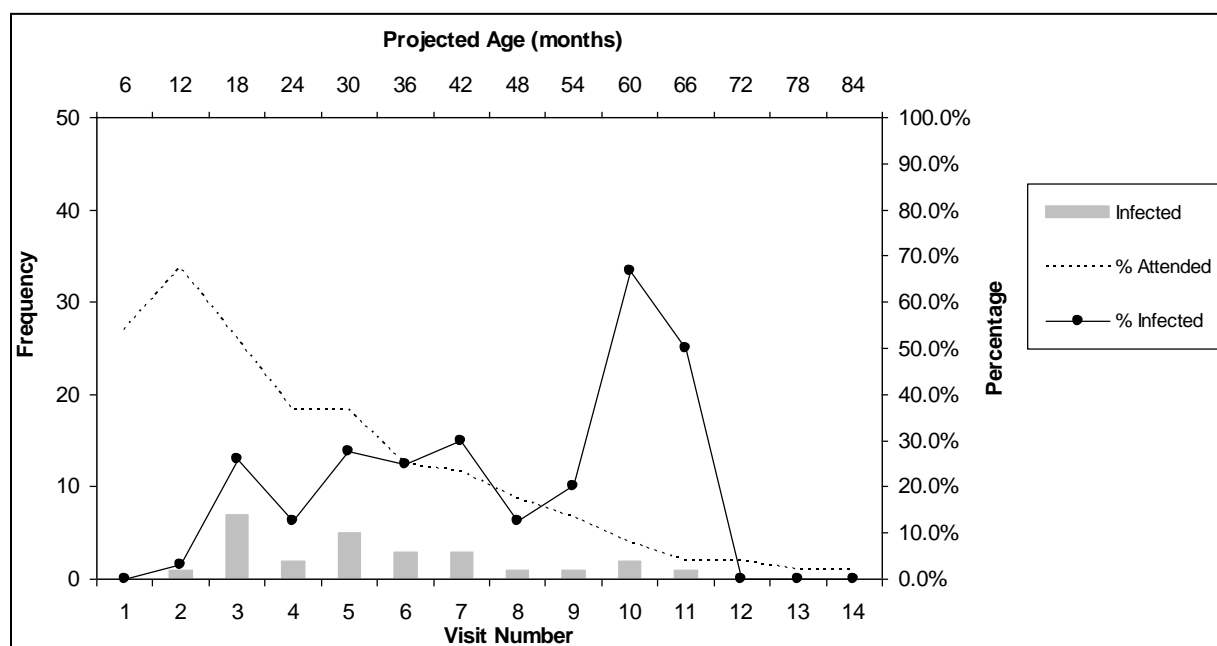
FIGURE 10. VISIT ATTENDANCE AND *HELICOBACTER PYLORI* INFECTION STATUS BY VISIT NUMBER FOR YOUNGER SIBLINGS- INDEX CHILD *HELICOBACTER PYLORI* INFECTED
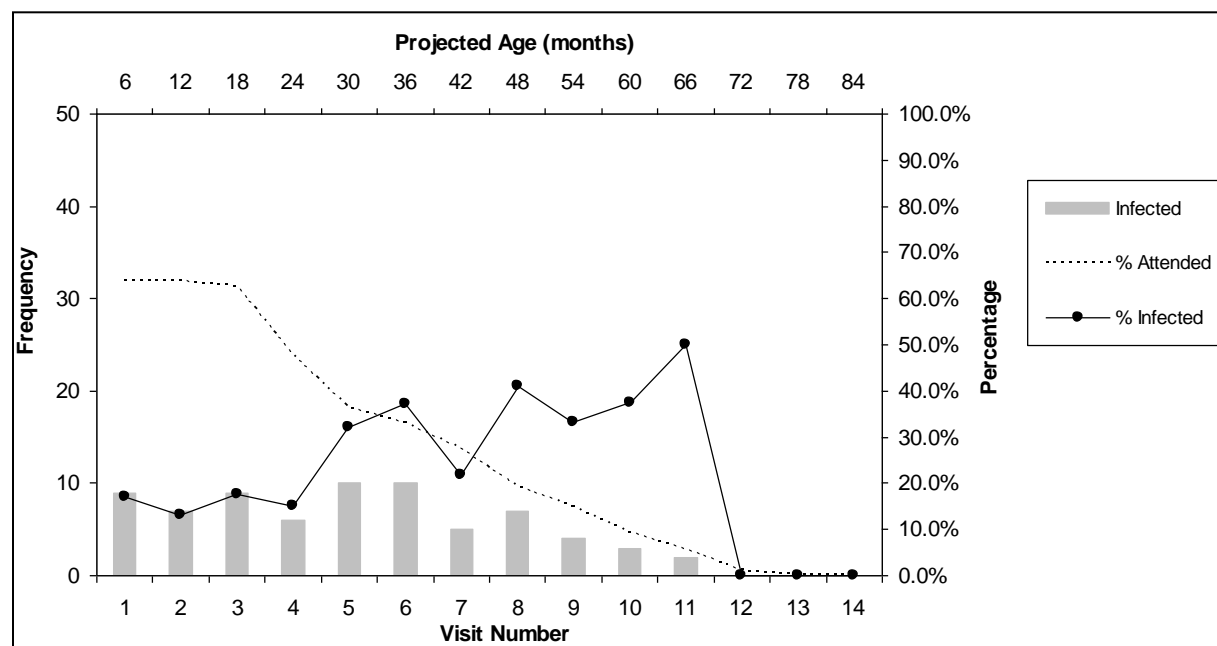
Regression Analysis

Of 122 index subject with one or more younger Pasitos siblings, there was no instance in which the younger sibling's *H. pylori* acquisition preceded that of the index subject. In all instances except four, the first incident infection in the older index sibling was acquired prior to the first incident infection in a younger sibling; in the other four occasions, the first incident infection was acquired simultaneously. In all 17 index subjects who developed a long-term *H. pylori* infection, acquisition of the index subject's long-term infection preceded the acquisition of a long-term infection in the younger sibling. Therefore, only the results of analyses examining the effect of incident and long-term infections of *H. pylori* in the direction of the older index sibling's status affecting incident and long-term infections of *H. pylori* in the younger sibling are presented.

Prior to performing Cox proportional hazards regression analysis, the proportionality of hazards assumption pertaining to *H. pylori* infection acquisition rate of the younger sibling based on the older, index child's *H. pylori* infection status was again verified by three methods previously detailed. The log minus log plot of time refers to months to *H. pylori* infection acquisition of the younger sibling based on the index child's infection status (Figure11). Visual inspection of the curves indicates that the proportional hazards assumption was likely violated: the rate for *H. pylori* infection acquisition was not proportional across follow-up for children based on whether or not the index sibling had acquired an *H. pylori* infection. The time to interaction test also found that there was significant interaction between an index child's *H. pylori* status and time to infection of his or her younger sibling (β coefficient=-0.077, p-value=0.021). This result was confirmed via a partial residual plot (Appendix T).

FIGURE 11. LONG MINUS LOG PLOT: TESTING OF PROPORTIONALITY ASSUMPTION OF *HELICOBACTER PYLORI* ACQUISITION BASED ON *HELICOBACTER PYLORI* STATUS OF THE INDEX CHILD
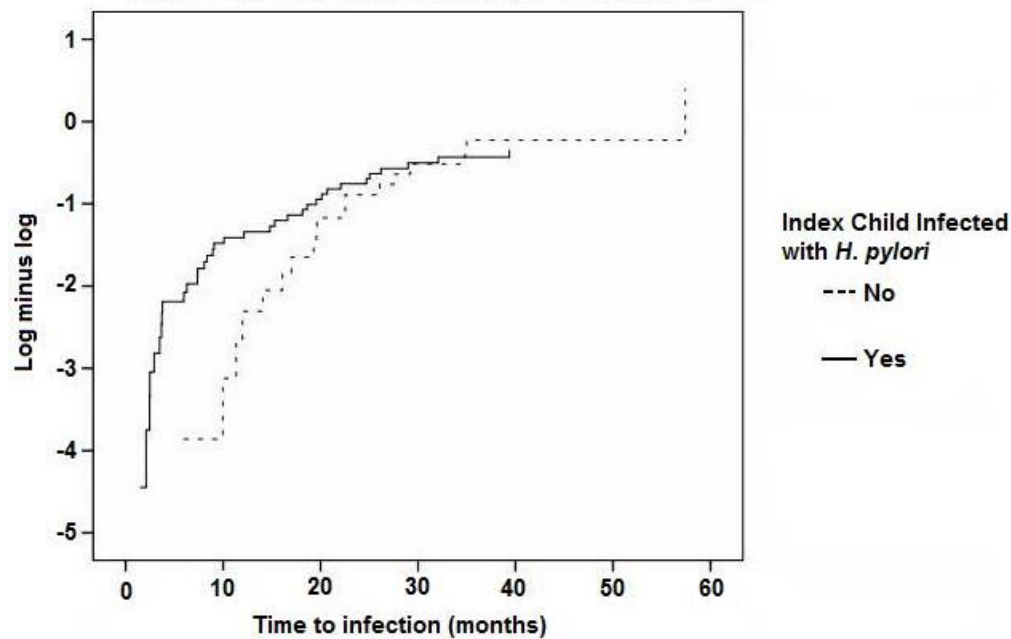
As the assumption of proportional hazards was not met, Cox regression with time dependent covariates analysis was performed to obtain hazards ratios for the association between *H. pylori* infection acquisition of the younger sibling and that of his or her older, index sibling. Results of regression analysis are shown in Appendix U. Based on crude analysis, the rate of *H. pylori* incident infection in children whose older sibling was infected with *H. pylori* at least once during follow-up was more than four times greater compared to children whose older sibling did not become infected with *H. pylori* during follow-up (crude HR= 4.6, 95% CI: 1.3, 16.3). After adjusting for crowding, mother's *H. pylori* serostatus at baseline and number of *H. pylori* active courses taken, the rate of *H. pylori* incident infection in children whose older sibling was infected with *H. pylori* at least once during follow-up was almost six times greater compared to children whose older sibling did not become infected with *H. pylori* during follow-up (adjusted

HR= 6.0, 95% CI: 1.5, 24.5). Both unadjusted and adjusted measures although uncertain in the magnitude of effect are very certain in the strong, positive direction of the estimated effect.

Upon stratifying, it was found that in Mexico the rate of *H. pylori* infection acquisition in children exposed to an older, index sibling who acquired an *H. pylori* infection was much greater relative to children not exposed to an older, index sibling who acquired an *H. pylori* infection (adjusted HR= 404.1, 95% CI: 0.3, 618067.0). In the United States, the rate of *H. pylori* infection acquisition in children exposed to an older, index sibling who acquired an *H. pylori* infection was also greater relative to children not exposed to an older, index sibling who acquired an *H. pylori* infection (adjusted HR= 3.7, 95% CI: 0.8, 16.9). The estimates within each country strata, especially the point estimate pertaining to Mexico residence, are bound by very wide confidence intervals and are highly uncertain in the magnitude and direction of effect with values of strong associations on both sides of the confidence intervals. Again due to highly imprecise estimates, effect modification may have occurred but cannot be determined in the current study due to low numbers within strata.

Due to model instability when performing time-dependent Cox proportional hazards analysis, point estimates and confidence intervals could not be calculated to estimate the effect of an index's *H. pylori* incident infection on the incident infection of the younger sibling when stratifying by age difference (≤3 years versus >3 years).

As with incident *H. pylori* infection of the older, index sibling, prior to performing Cox proportional hazards regression analysis the proportionality of hazards assumption pertaining to *H. pylori* infection acquisition rate of the younger sibling based on the

older, index child's *H. pylori* long-term infection status was verified by three methods

previously detailed. The log minus log plot of time refers to months to *H. pylori* infection

acquisition of the younger sibling based on the index child's long-term infection status

(Figure12). Visual inspection of the curves indicates that the proportional hazards

assumption was not likely violated: the rate for *H. pylori* infection acquisition was

proportional across follow-up for children based on whether or not the index sibling had

developed a long-term *H. pylori* infection. The time to interaction test also found that

there was no significant interaction between an index child's *H. pylori* status and time to

long-term infection establishment of his or her younger sibling (β coefficient=0.001, p-

value=0.986). This result was confirmed via a partial residual plot (Appendix V).

FIGURE 12. LONG MINUS LOG PLOT: TESTING OF PROPORTIONALITY ASSUMPTION OF *HELICOBACTER PYLORI* ACQUISITION BASED ON LONG-TERM *HELICOBACTER PYLORI* STATUS OF THE INDEX CHILD
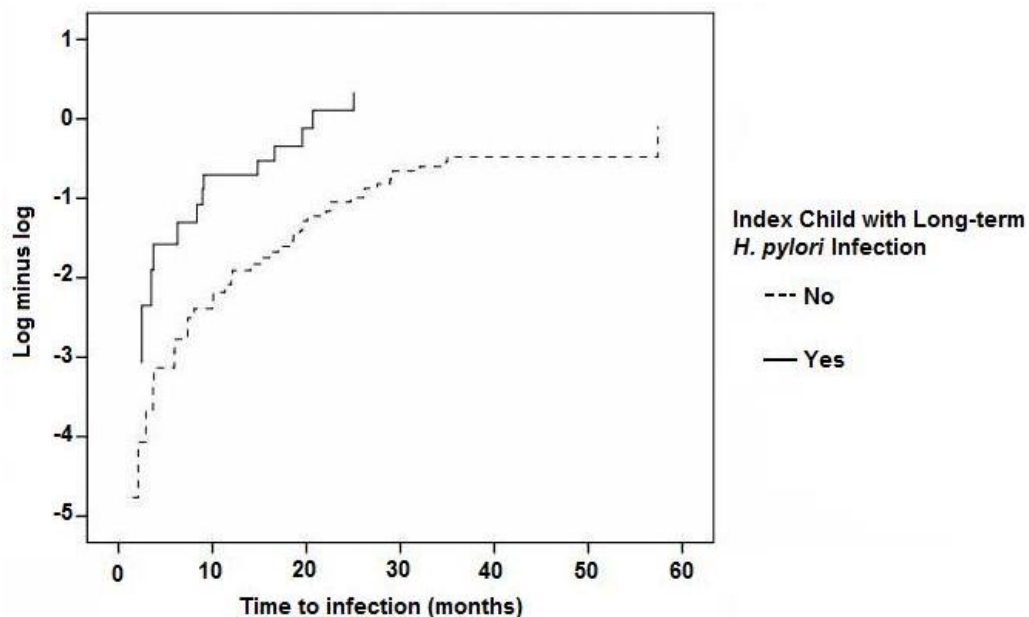
As the assumption of proportional hazards was met, Cox regression without time dependent covariates analysis was performed to obtain hazards ratios for the association between *H. pylori* infection acquisition of the younger sibling and long-term infection of his or her older, index sibling. Results of regression analysis are shown in Appendix U. Based on crude analysis, the rate of *H. pylori* incident infection in children whose older sibling developed a long-term *H. pylori* was more than three times greater compared to children whose older sibling did not develop a long-term *H. pylori* infection (crude HR=3.4, 95% CI: 1.8, 6.4). After adjusting for crowding, mother's *H. pylori* serostatus at baseline and number of *H. pylori* active courses taken, the rate of *H. pylori* incident infection in children whose older sibling developed a long-term *H. pylori* infection was still approximately three times greater compared to children whose older sibling did not develop a long-term *H. pylori* infection (adjusted HR=3.1, 95% CI: 1.6, 6.2). Both unadjusted and adjusted measures are uncertain in the magnitude of estimated effect but are highly certain in the strong, positive direction of the estimated effect.

Stratification by age difference between the index child and younger sibling (≤3 years versus > 3 years) as well as country of origin (Mexico versus the United States) was performed and the effect of exposure to an older, index child's long-term *H. pylori* infection on the acquisition of an *H. pylori* infection in a younger sibling was determined. In both stratified analyses, effect modification was noted.

It was found when the age difference between siblings was three years or less, the rate of *H. pylori* infection acquisition in children whose older, index sibling had a developed a long-term *H. pylori* infection was much greater relative to children whose

older, index sibling did not develop a long-term *H. pylori* infection (adjusted HR= 4.3, 95% CI: 1.7, 10.7). In siblings with more than three years age difference, the rate of infection acquisition in children whose older, index sibling developed a long-term infection was greater relative to children whose older, index sibling did not develop a long-term infection (adjusted HR=2.0, 95% CI: 0.7, 5.4). This magnitude of the estimated effect is less when compared to the estimate obtained for children with less than three years difference in age. But unlike the measure of association obtained for children with less than three years difference in age, is highly uncertain in the direction of effect.

In regards to country of residence, in Mexico the rate of *H. pylori* incident infection in children whose older, index sibling had developed a long-term *H. pylori* infection was much greater relative to children whose older, index sibling did not develop a long-term infection (adjusted HR=3.7, 95% CI: 1.2, 11.0). In the United States, the rate of *H. pylori* incident infection in children whose older, index sibling had developed a long-term *H. pylori* infection was also much greater relative to children whose older, index sibling did not develop a long-term (adjusted HR= 2.8, 95% CI: 1.1, 7.1). The estimates in country of residence strata although very uncertain in the magnitude of effect, are certain in the direction of effect.

The effect of an index child's long-term *H. pylori* infection on the establishment of a younger sibling's *H. pylori* long-term infection was also estimated. Prior to this analysis, the proportionality of hazards assumption for the rate of establishment of long-term *H. pylori* infection of the younger sibling based on the long-term infection of the older, index child was verified. The log minus log plots refers to months to *H. pylori* long-term

infection establishment of the younger sibling based on the index child's long-term infection status as defined in three different time-to-event scenarios previously described (Figures13-15). For the establishment of long-term *H. pylori* infection in a child, visual inspection of the three curves for all time to long-term infection scenarios indicate that the proportional hazards assumption was likely met: the rate for *H. pylori* long-term infection was proportional across follow-up whether or not their older, index sibling had a long-term *H. pylori* infection. The time to interaction test also found that there was no significant interaction between an index child's long-term *H. pylori* infection status and time to long-term infection establishment of his or her younger sibling (scenario one: β coefficient=0.028, p-value=0.600, scenario two: β coefficient=-0.019, p-value=0.709, scenario three: β coefficient=0.027, p-value=0.608). Partial residual plots (Appendices W-Y) revealed that the average value of residuals did deviate from zero for all time-to-event scenarios. But as two of three tests did indicate meeting the proportional hazards assumption, a violation of the assumption was determined not to have occurred for all three time-to-event scenarios.

As the proportional hazards assumption was met, Cox regression without time dependent covariates analysis was performed to obtain hazards ratios for all three time-to-event scenarios. Results of regression analysis are shown in Appendices Z-BB.

FIGURE 13. LONG MINUS LOG PLOT: TESTING OF PROPORTIONALITY ASSUMPTION OF *HELICOBACTER PYLORI* INFECTION ESTABLISHMENT BASED ON LONG-TERM *HELICOBACTER PYLORI* STATUS OF THE INDEX CHILD, SCENARIO 1
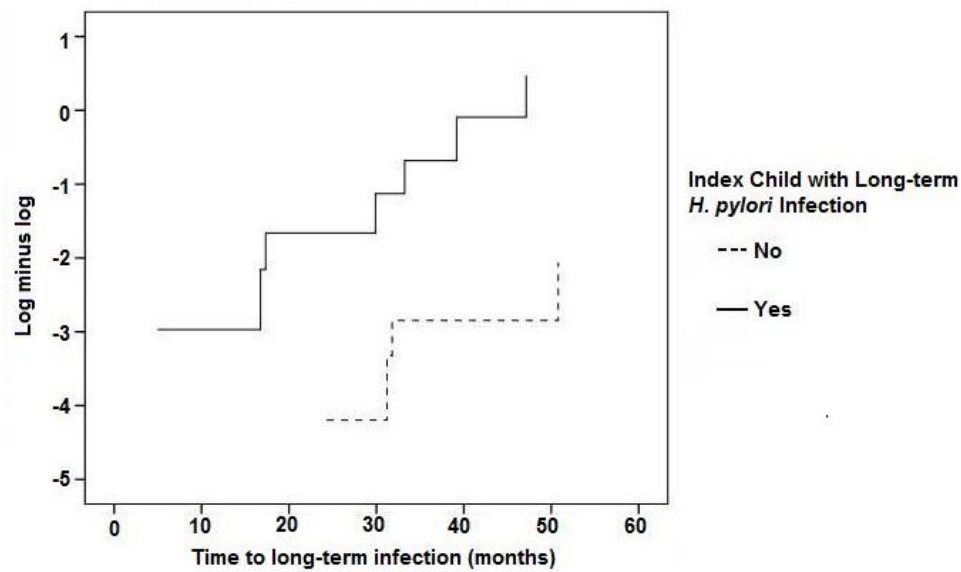
FIGURE14. LONG MINUS LOG PLOT: TESTING OF PROPORTIONALITY ASSUMPTION OF *HELICOBACTER PYLORI* INFECTION ESTABLISHMENT BASED ON LONG-TERM *HELICOBACTER PYLORI* STATUS OF THE INDEX CHILD, SCENARIO 2
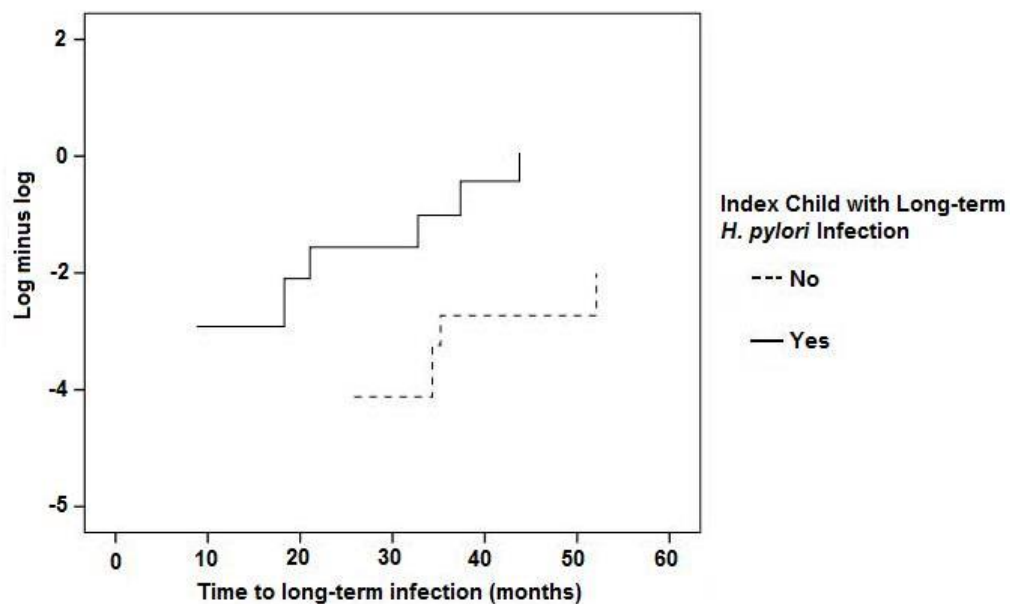
FIGURE 15. LONG MINUS LOG PLOT: TESTING OF PROPORTIONALITY ASSUMPTION OF *HELICOBACTER PYLORI* INFECTION ESTABLISHMENT BASED ON LONG-TERM *HELICOBACTER PYLORI* STATUS OF THE INDEX CHILD, SCENARIO 3
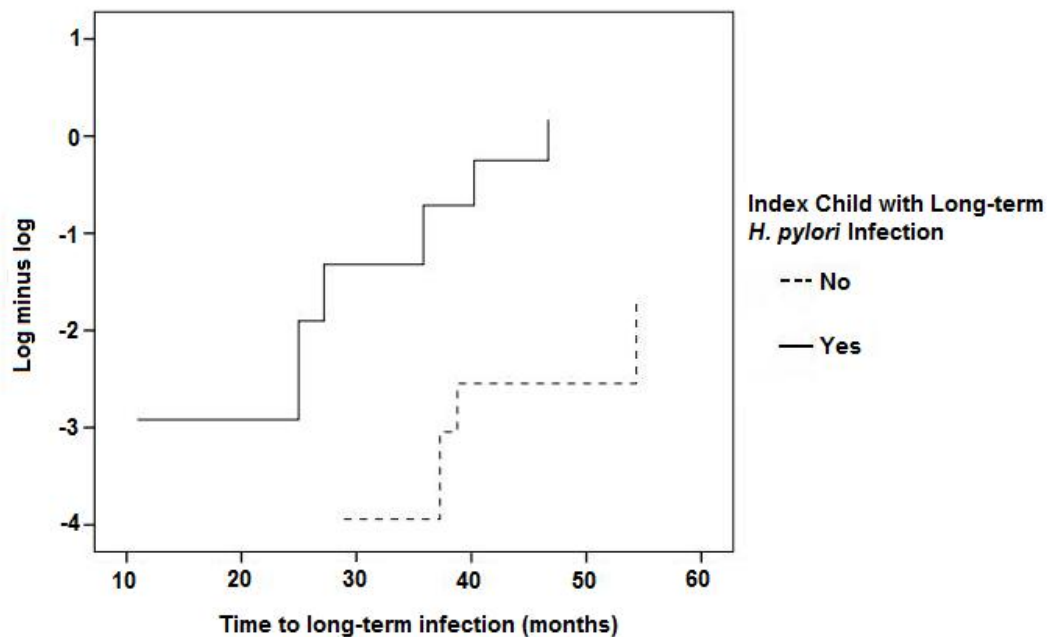
In time-to-event scenario one, crude analysis revealed that the rate of long-term *H. pylori* infection establishment in children whose older, index sibling developed a long-term *H. pylori* infection was more than thirteen times greater compared to children whose older, index sibling did not develop a long-term *H. pylori* infection (crude HR= 13.4, 95% CI: 3.9, 46.6). After adjusting for crowding, mother's *H. pylori* serostatus at baseline and the number of *H. pylori* active antibiotic courses taken, the rate of long-term *H. pylori* infection establishment in children whose older sibling developed a long-term *H. pylori* infection was nine times greater compared to children whose older sibling did not develop a long-term infection (adjusted HR= 9.1, 95% CI: 2.2, 36.6). Both unadjusted and adjusted measures are very certain in the strong, positive direction of effect but very uncertainty in the magnitude of effect.

Crude analyses for time-to-event scenarios two and three obtained similar result to scenario one: the rate of long-term *H. pylori* infection establishment in children whose older sibling developed a long-term infection was much greater relative to children whose older sibling did not developed a long-term infection (scenario two crude HR= 13.3, 95% CI: 3.8, 45.8; scenario three crude HR= 12.2, 95% CI: 3.8, 45.8). After adjusting for crowding, mother's *H. pylori* serostatus at baseline and the number of *H. pylori* active antibiotic courses taken, the rate of long-term *H. pylori* infection establishment in children whose older sibling developed a long-term *H. pylori* infection was still much greater relative to children whose older sibling did not develop a long-term infection (scenario two adjusted HR= 8.5, 95% CI: 2.2, 32.1; scenario three adjusted HR= 11.9, 95% CI: 2.7, 53.7). Again, there is a strong positive association with a high degree of certainty in the direction of the effect but a great deal of uncertainty in the magnitude of effect in these measures.

In all three scenarios stratified analyses revealed that in children from Mexico, the rate of a long-term infection in children whose older, index sibling developed a long-term infection was greater relative to children whose older, index sibling did not develop a long-term infection (scenario one adjusted HR= 2.6, 95% CI: 0.3, 21.6; scenario two adjusted HR= 4.8, 95% CI: 0.4, 52.7; scenario three adjusted HR= 4.8, 95% CI: 0.4, 59.0). In the United States, the rate of a long-term *H. pylori* infection in children with an older, index sibling who developed a long-term *H. pylori* infection was much greater relative to children who were not exposed to an older, index sibling who developed a long-term *H. pylori* infection (scenario one adjusted HR= 58.5, 95% CI: 2.7, 1251.6; scenario two adjusted HR= 44.9, 95% CI: 2.1, 964.0; scenario three adjusted HR=

113.8, 95% CI: 1.5, 8468.5). In the United States, there is uncertainty in the magnitude of effect but a great deal of certainty in the direction of effect while measures pertaining to Mexico display a great deal of uncertainty in the magnitude and direction of effect, with values of strong associations in both directions in the confidence intervals. Effect modification by country of residence likely occurred and the effects appear stronger in the United States than in Mexico but as stratified measures display a high degree of imprecision, results are inconclusive.

Due to limited number of observations upon stratifying, hazard ratios with confidence intervals could not be obtained to estimate the effect of exposure to an older sibling with a long-term *H. pylori* infection on the establishment of a long-term infection of his or her younger sibling based on age difference.

In considering the other covariates in the model for the effect of exposure to an older infected sibling on the incident infection of a younger sibling shown in Appendix CC (Model A), only the mother's *H. pylori*'s seropositivity at baseline appeared to have an independent effect on the incident infection of a younger sibling (adjusted HR=1.5, 95% CI: 0.8, 2.8); the rate of *H. pylori* acquisition in younger children whose mother was seropositive for *H. pylori* at the beginning of the study was 50% greater compared to younger children whose mother was seronegative for *H. pylori* at the beginning of the study. The magnitude of the effect of having a seropositive mother on *H. pylori* incident infection of her child is less than the magnitude of effect of having an older sibling with an incident infection on *H. pylori* incident infection in a sibling. Likewise, the magnitude of effect of an older sibling with a long-term infection on the incident infection of a

younger sibling was greater than the magnitude of effect of having a seropositive mother on the incident infection of a younger sibling (Appendix CC, Model B).

For the effect of exposure to an older sibling who developed a long-term *H. pylori* infection on the establishment of a long-term *H. pylori* infection of a younger sibling (Appendix CC), other covariates for all time-to-event scenarios indicate that crowding (scenario one adjusted HR=1.3, 95% CI: 0.7, 2.6; scenario two adjusted HR=1.5, 95% CI: 0.7, 2.9; scenario three adjusted HR= 3.2, 95% CI: 1.2, 8.5) and mother's seropositivity at baseline (scenario one adjusted HR=4.1, 95% CI: 0.5, 36.4; scenario two adjusted HR=3.8, 95% CI: 0.4, 32.4; scenario three adjusted HR=4.1, 95% CI: 0.5, 34.0) appeared to contribute to the long-term infection establishment of the younger child (Appendix CC). In both cases, the magnitude of the estimated effect of the older, index child's long-term *H. pylori* infection on the establishment of his or her younger sibling's long-term *H. pylori* infection is greater. The number of *H. pylori* active antibiotic courses taken did not appear to contribute to the rate of establishment of a long-term infection in the younger child for all time-to-event scenarios (Appendix CC).

Aim C- Breastfeeding and *Helicobacter pylori* Infection

Descriptive Analysis

In The Pasitos Cohort, there were 468 children who had one or more classifiable[13]C-UBT results and for whom breastfeeding data was available for analysis. The majority of these children were breastfed (n=335, 72%). One-hundred and twenty-three (26%) of children were breastfed for at least 6 months while 63 (13%) of children where breastfed at one year of age or older. Cohort characteristics and *H. pylori* infection status stratified by breastfeeding status are shown in Table 16. In regards to gender, among breastfed children, gender was evenly distributed. In both breastfed females and males, 47% acquired an incident infection. Among children who were not breastfed, a slightly higher percentage of children were female (53%) and a slightly higher percentage of these female (44%) children acquired an infection. More than 50% of children resided in the United States, and accordingly more than half of breastfed children (54%) were from the United States; 46% were from Mexico. A slightly greater percentage of breastfed children originating from Mexico (50%) were infected with *H. pylori* compared to breastfed children from the United States (45%). Among children who were not breastfed, the majority were from the United States (86%) although a higher percentage (50%) of non-breastfed children from Mexico became infected with H. *pylori* compared to non-breastfed children from the United States (40%).

In regards to educational status of the mother, 45% of mothers had 7-11 years of education and 47% of mothers who breastfed had 7-11 years of education. In both children who were breastfed and those who were not breastfed, the proportion of children who became infected with *H. pylori*

TABLE 16. COHORT CHARACTERISITICS BY BREASTFED STATUS AND INFECTION STATUS, THE PASITOS COHORT

Characteristics	Breastfed Children					Children not Breastfed				
	N	*N*	%[a]	Infected[b]	%[c]	*N*	*n*	%[a]	Infected[b]	%[c]
Sex	335					133				
F		169	50	79	47		71	53	31	44
M		166	50	78	47		62	47	24	39
Country	335					133				
United States		182	54	81	45		115	86	46	40
Mexico		153	46	76	50		18	14	9	50
Mother's education (years)	335					133				
0-6		60	18	38	63		15	11	8	53
7-11		156	47	72	46		55	41	23	42
12-17		119	36	47	39		63	47	24	38
Mother's *H. pylori* status at baseline	320					126				
Positive		210	66	63	30		70	56	19	27
Negative		110	34	31	28		56	44	19	34
Crowding[d]	335					133				
≤1		221	66	97	44		103	77	40	39
>1-2		84	25	44	52		28	21	14	50
>2		30	9	16	53		2	2	1	50
***H. pylori* active antibiotic courses taken**	324					131				
Yes		184	57	104	57		72	55	31	43
No		140	43	53	38		59	45	24	41
***H. pylori* infected sibling in household**	166					71				
Yes		51	31	16	31		16	23	7	44
No		115	69	36	31		55	77	17	31

[a] Percentage within group (N)
[b] Denotes children with at least one positive ^{13}C-UBT result
[c] Percentage within subgroup (n)
[d] Crowding is defined as the ratio of people living within the household to number of rooms in the household.

was greatest among children whose mother had less than seven years of education.

The greatest percentage of breastfed (66%) and non-breastfed children (77%) resided in a home in which there was at least one room per person. In breastfed and non-breastfed children, children with the lowest crowding ratio also had the lowest percentage of children infected (44% and 39%, respectively). For *H. pylori* active antibiotic courses taken, greater than half of breastfed (57%) and non-breastfed children (55%) took at least one course of *H. pylori* active antibiotics during follow-up. Among breastfed children who received at least one course of antibiotics, 57% became infected with *H. pylori* compared to 38% of children who did not receive *H. pylori* active medications. In children who were not breastfed, regardless of *H. pylori* active antibiotic status, approximately four in ten children became infected with *H. pylori*.

Concerning *H. pylori* serostatus of the mother, 66% of mothers who breastfed were seropositive at baseline; a higher percentage of mother's who did not breastfeed were also seropositive for *H. pylori* at baseline (56%). Thirty percent of breastfed children whose mother was seropositive and slightly less than 30% of non-breastfed children whose mother was seropositive for *H. pylori* at baseline acquired *H. pylori*. This differs from living in a household with one or more siblings with an *H. pylori* infection. The majority of both breastfed (69%) and non-breastfed children (77%) with siblings did not live in a household in which one or more siblings was infected or seropositive for *H. pylori*. For breastfed children, an equal percentage of children living with and without *H. pylori* infected or seropositive siblings became infected (31%). For non-breastfed children, a greater percentage of children living with an older sibling who was either infected or seropositive for *H. pylori* (44%) acquired an *H. pylori* infection. Overall, when

comparing *H. pylori* incidence between breastfed and non-breastfed children, in almost all cohort characteristics, children who were breastfed had higher proportions of *H. pylori* infection than children who were not breastfed.

Minimum, maximum, median and mean breastfeeding duration by *H. pylori* infection status is shown in Table 17. Almost half of children acquired an incident *H. pylori* infection (n=212, 45%). A short-term *H. pylori* infection occurred in 139 children (30%) while a long-term infection occurred in only 7% of children (n=34). Overall, the average duration of breastfeeding was four months with the longest duration being two years. Children who were infected were breastfed for a longer, average duration compared to children who were not infected; this is noted in both short and long-term infections. Children who developed a long-term *H. pylori* infection were breastfed, on average, a month longer than children who did not develop a long-term infection. The median breastfeeding duration only differed for children who did and did not develop an *H. pylori* infection; the median number of months of breastfeeding was longer for children who became infected with *H. pylori*.

TABLE 17. BREASTFEEDING DURATION BY *HELICOBACTER PYLORI* INFECTION STATUS, THE PASITOS COHORT

Incident Infection (n, %)	Breastfeeding Duration (months)			
	Minimum	Maximum	Median	Mean
TOTAL (N=468)	**0.0**	**24.0**	**2.0**	**4.0**
Ever infected[a]				
Yes (212, 45.3)	0.0	24.0	2.0	4.6
No (256, 54.7)	0.0	24.0	1.0	3.4
Short-term Infection[b]				
Yes (139, 29.7)	0.0	24.0	2.0	4.5
No (329, 70.3)	0.0	24.0	2.0	3.7
Long-term Infection[c]				
Yes (34, 7.3)	0.0	24.0	2.0	5.1
No (434, 92.7)	0.0	24.0	2.0	3.9

[a] Indicates at least one positive ^{13}C-UBT throughout follow-up.

[b] Indicates at least three, consecutive positive ^{13}C-UBT with no subsequent negative tests throughout follow-up.

[c] Indicates the first event in which a positive ^{13}C-UBT was followed by a negative ^{13}C-UBT.

H. pylori infection status by breastfeeding duration is shown in Table 18. Overall, the incident proportion of all infection types increased as duration of breastfeeding increased. An *H. pylori* infection was detected at least once in 47-54% of children who were breastfed. Short-term and long-term infections were detected in 29-35% and 8-11% of breastfed children, respectively. Again, overall the incidence proportion of *H. pylori* infection was 45% with short-term infections detected in 30% of children and long-term infections detected in 7% of children.

TABLE18. *HELICOBACTER PYLORI* INFECTION STATUS BY BREASTFEEDING STATUS, THE PASITOS COHORT

	N	%
Breastfed, any duration (N=335)		
Ever infected[a]	157	46.9%
Short-term Infection[b]	98	29.3%
Long-term Infection[c]	27	8.1%
Breastfed 6 months or longer (N=123)		
Ever infected[a]	66	53.7%
Short-term Infection[b]	41	33.3%
Long-term Infection[c]	14	11.4%
Breastfed at 12 months of age or longer (N=63)		
Ever infected[a]	34	54.0%
Short-term Infection[b]	22	34.9%
Long-term Infection[c]	6	9.5%

[a] Indicates at least one positive ^{13}C-UBT throughout follow-up.

[b] Indicates at least three, consecutive positive ^{13}C-UBT with no subsequent negative tests throughout follow-up.

[c] Indicates the first event in which a positive ^{13}C-UBT was followed by a negative ^{13}C-UBT.

In regards to time-to-event by *H. pylori* infection and breastfeeding status, minimum, maximum and median follow-up times are shown in Table 19 for incident *H. pylori* infections. Follow-up time did not differ greatly based on breastfeeding status. For children who did not acquire an incident *H. pylori* infection, the shortest follow-up time was about five months and the longest follow-up time was roughly 85 months. The first *H. pylori* infection was detected in slightly over two and a half months after birth overall. The median time to incident infection was shorter for children who were breastfed (17.9 months) compared to children who were not breastfed (18.7 months).

TABLE 19. TIME TO *HELICOBACTER PYLORI* INCIDENT INFECTION BY *HELICOBACTER PYLORI* AND BREASTFEEDING STATUS, THE PASITOS COHORT

Incident Infection (n, %)	Time-to-Event (Months)		
	Minimum	Maximum	Median
Breastfed[a]			
Yes (157, 46.9)	2.6	81.1	17.9
No (178, 53.1)	5.3	84.6	23.4
Not Breastfed[b]			
Yes (55, 41.4)	2.7	74.3	18.7
No (78, 58.6)	5.2	85.4	21.7

[a] Percentage of breastfed children with one or more classifiable ^{13}C-UBT results (n=335).

[b] Percentage of non-breastfed children with one or more classifiable ^{13}C-UBT results (n=133).

Table 20 shows time-to-event by *H. pylori* short-term infection and breastfeeding status. As previously stated, for children who only acquired a long-term infection, time to risk for a short-term infection was defined as the time to long-term infection. As time to a long-term infection was defined by three different scenarios as previously described, three time-to short-term infection scenarios are also presented and based respectively on long-term infection scenarios 1-3. Again, as seen in infection acquisition, time of follow-up did not vary greatly based on breastfeeding status and acquisition of a short-term *H. pylori* infection. In breastfed and non-breastfed children who did not acquire a short-term infection, the shortest follow-up time was roughly 5 months and the longest follow-up was roughly 85 months. The median follow-up time for children who did not develop a short-term infection was longer for children who had been breastfed (24 to 28 months) compared to children who had not been breastfed (18 to 21 months). In breastfed and non-breast fed children that developed a short-term infection, the earliest short-term infection occurred roughly eight and a half months after birth and the longest time to develop a short-term infection was about 80 months. The median time from birth

to development of a short-term infection was somewhat shorter for children who were

breastfed (24 to 25 months) compared to children who were not breastfed (32 months).

TABLE 20. TIME TO *HELICOBACTER PYLORI* SHORT-TERM INFECTION BY
HELICOBACTER PYLORI AND BREASTFEEDING STATUS, THE PASITOS COHORT

Short-Term Infection (n, %)	Time-to-Event or Follow-up (Months)		
	Minimum	Maximum	Median
Breastfed[a]			
Yes (98, 29.3)			
Scenario 1	8.6	79.9	25.2
Scenario 2	8.6	79.9	23.9
Scenario 3	8.6	79.9	23.9
No (237, 70.7)			
Scenario 1	5.3	84.6	24.3
Scenario 2	5.3	84.6	25.0
Scenario 3	5.3	84.6	28.0
Not Breastfed[b]			
Yes (41, 30.8)			
Scenario 1	8.5	80.3	31.9
Scenario 2	8.5	80.3	31.9
Scenario 3	8.5	80.3	31.9
No (92, 69.2)			
Scenario 1	5.2	85.4	18.4
Scenario 2	5.2	85.4	20.4
Scenario 3	5.2	85.4	20.7

[a] Percentage of breastfedchildren with one or more classifiable[13]C-UBT results (n=335).

[b] Percentage of non-breastfed children with one or more classifiable[13]C-UBT results (n=133).

In regards to long-term *H. pylori* infection, time-to-event by long-term infection and

breastfed status is shown in Table 21. Again, three time-to-long-term infection scenarios

are displayed and were previously defined. In both breastfed and non-breast fed

children who did not develop a long-term infection, the shortest length of follow-up was

roughly five and a half months and the longest follow-up was about 85 months. For

children who developed a long-term infection, among those who were breastfed, the

earliest long-term infection was detected approximately 8 months to 23 months after birth dependent on time-to-event scenario; for children who were not breastfed, this time varied from 12 months to almost 18 months after birth. The median time to a long-term infection was longer for children who were breastfed (36 to 49 months) compared to children who were not breastfed (37 to 43 months).

TABLE 21. TIME TO *HELICOBACTER PYLORI* LONG-TERM INFECTION BY *HELICOBACTER PYLORI* AND BREASTFEEDING STATUS, THE PASITOS COHORT

Long-Term Infection (n, %)	Time-to-Event or Follow-up (Months)		
	Minimum	Maximum	Median
Breastfed[a]			
Yes (27, 8.1)			
Scenario 1	7.6	65.2	36.1
Scenario 2	9.7	68.1	44.1
Scenario 3	22.8	71.1	48.6
No (308, 91.9)	5.3	84.6	42.1
Not Breastfed[b]			
Yes (7, 5.3)			
Scenario 1	12.2	65.5	36.7
Scenario 2	16.1	68.1	39.6
Scenario 3	17.7	71.3	43.1
No (126, 94.7)	5.2	85.4	30.0

[a] Percentage of breastfed children with one or more classifiable ^{13}C-UBT results (n=335).

[b] Percentage of non-breastfed children with one or more classifiable ^{13}C-UBT results (n=133).

Pertaining to the number of visits attended, Table 22 shows this information by breastfeeding status. Again, no children attended all 14 possible visits but 16% of breastfed children attended at least one visit while 23% of children who were not breastfed attended at least one visit.

TABLE 22. NUMBER AND PERCENTAGE OF COMPLETED FOLLOW-UP VISITS BY
BREASTFEEDIGN STATUS, THE PASITOS COHORT

Visits Completed	Breastfed Number of Children	%	Not Breastfed Number of Children	%
1	54	16.1	30	22.6
2	40	11.9	15	11.3
3	23	6.9	14	10.5
4	25	7.5	12	9.0
5	23	6.9	4	3.0
6	24	7.2	14	10.5
7	30	9.0	7	5.3
8	32	9.6	3	2.3
9	27	8.1	9	6.8
10	19	5.7	10	7.5
11	17	5.1	8	6.0
12	18	5.4	5	3.8
13	3	0.9	2	1.5
14	0	0.0	0	0.0
Total	**335**	**100.0**	**133**	**100.0**

When evaluating the number of classifiable[13]C-UBT (Table 23) *H. pylori* infection
status could be determined for 99% of children regardless of breastfeeding status. Two
or more classifiable[13]C-UBT results were needed to determine short-term infection
status while three of more classifiable test results needed to determine long-term
infection. Short-term infection could be determined for 279 (83%) and 100 (75%) of
breastfed and non-breastfed children, respectively. For long-term infection, this could be
determined for 239 (71%) of children who were breastfed and for 84 (63%) of children
who were not breastfed.

TABLE 23. NUMBER AND PERCENTAGE OF CLASSIFIABLE UREA BREATH TEST
RESULTS BY BREASTFEEDING STATUS, THE PASITOS COHORT

Classifiable ^{13}C-UBT Result	Breastfed-		Not Breastfed-	
	Frequency	%	Frequency	%
0	3	0.9	1	0.7
1	56	16.6	33	24.6
2	40	11.8	16	11.9
3	22	6.5	11	8.2
4	33	9.8	13	9.7
5	25	7.4	8	6.0
6	28	8.3	9	6.7
7	31	9.2	7	5.2
8	27	8.0	9	6.7
9	26	7.7	6	4.5
10	18	5.3	9	6.7
11	19	5.6	6	4.5
12	9	2.7	5	3.7
13	1	0.3	1	0.7
14	0	0.0	0	0.0
Total	**338**	**100.0**	**134**	**100.0**

Participation at each scheduled visit as well as information pertaining to ^{13}C-UBT results and *H. pylori* prevalence at each scheduled visit is shown in Table 24 for breastfed children and in Table 25 for non-breastfed children. Attendance was somewhat greater among children who were breastfed compared to children who were not breastfed. Attendance for children who were breastfed ranged from 20% to 83% and 15% to 78% for non-breastfed children. For both breastfed and non-breastfed children, visit one was the most attended and visit nine was the least attended visit. In regards to ^{13}C-UBT, a majority of children were tested, ranging from 94% to 100% of visit attendees for breastfed children and 90% to 100% of visit attendees for non-breastfed children. Classifiable results were obtained for 93% to 100% of children who were tested, regardless of breastfeeding status.

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Prevalence at each visit number ranged from 8% to 38% for children who were breastfed and 3% to 35% for children who were not breastfed. At approximately six months of age, 8% of breastfed children who attended visit one had acquired an *H. pylori* infection while 3% of non-breastfed children who attended visit one had acquired an *H. pylori* infection.

TABLE 24. VISIT ATTENDANCE, ^{13}C-UREA BREATH TESTING AND *HELICOBACTER PYLORI* INFECTION BY TARGET AGE FOR BREASTFED CHILDREN, THE PASITOS COHORT

Target Age (mos)[a]	Visit Number	Attended[b]	% Attended[c]	^{13}C-UBT[b,d]	% ^{13}C-UBT[e]	Classifiable[13]C-UBT[b]	% Classifiable[13]C-UBT[f]	Infected	% Infected
6	1	278	83.0	267	96.0	262	98.1	22	8.4
12	2	248	74.0	243	98.0	239	98.4	40	16.7
18	3	203	60.6	199	98.0	196	98.5	30	15.3
24	4	169	50.4	164	97.0	159	97.0	34	21.4
30	5	132	39.4	129	97.7	126	97.7	22	17.5
36	6	110	32.8	110	100.0	104	94.5	18	17.3
42	7	84	25.1	82	97.6	76	92.7	16	21.1
48	8	78	23.3	73	93.6	72	98.6	16	22.2
54	9	68	20.3	67	98.5	67	100.0	12	17.9
60	10	99	29.6	97	98.0	96	99.0	27	28.1
66	11	124	37.0	121	97.6	120	99.2	28	23.3
72	12	131	39.1	130	99.2	121	93.1	34	28.1
78	13	100	29.9	100	100.0	97	97.0	28	28.9
84	14	79	23.6	77	97.5	69	89.6	26	37.7

[a] Actual age of participant was +/- 3 months of target age.
[b] Limited to index children with one or more classifiable[13]C-UBT living who were breastfed (n=335).
[c] Proportion of participants who attended scheduled visit of those with one or more classifiable[13]C-UBT results.
[d] Number of ^{13}C-urea breath tests performed for scheduled visit number.
[e] Proportion of ^{13}C-UBT performed of those who attended the scheduled visit number.
[f] Proportion of classifiable[13]C-UBT results obtained of total number of ^{13}C-UBT performed.

TABLE 25. VISIT ATTENDANCE, ^{13}C-UREA BREATH TESTING AND *HELICOBACTER PYLORI* INFECTION BY TARGET AGE FOR NON-BREASTFED CHILDREN, THE PASITOS COHORT

Target Age (mos)[a]	Visit Number	Attended[b]	% Attended[c]	^{13}C-UBT[b,d]	% ^{13}C-UBT[e]	Classifiable ^{13}C-UBT[b]	% Classifiable ^{13}C-UBT[f]	Infected	% Infected
6	1	104	78.2	94	90.4	92	97.9%	3	3.3
12	2	96	72.2	95	99.0	95	100.0%	15	15.8
18	3	76	57.1	76	100.0	73	96.1%	13	17.8
24	4	62	46.6	61	98.4	60	98.4%	8	13.3
30	5	56	42.1	56	100.0	52	92.9%	10	19.2
36	6	45	33.8	45	100.0	43	95.6%	10	23.3
42	7	33	24.8	33	100.0	33	100.0%	6	18.2
48	8	29	21.8	29	100.0	27	93.1%	8	29.6
54	9	20	15.0	20	100.0	20	100.0%	4	20.0
60	10	34	25.6	34	100.0	34	100.0%	6	17.6
66	11	37	27.8	37	100.0	36	97.3%	8	22.2
72	12	35	26.3	35	100.0	33	94.3%	7	21.2
78	13	32	24.1	31	96.9	30	96.8%	8	26.7
84	14	23	17.3	22	95.7	20	90.9%	7	35.0

[a] Actual age of participant was +/- 3 months of target age.
[b] Limited to index children with one or more classifiable ^{13}C-UBT living who were not breastfed (n=133).
[c] Proportion of participants who attended scheduled visit of those with one or more classifiable ^{13}C-UBT results.
[d] Number of ^{13}C-urea breath tests performed for scheduled visit number.
[e] Proportion of ^{13}C-UBT performed of those who attended the scheduled visit number.
[f] Proportion of classifiable ^{13}C-UBT results obtained of total number of ^{13}C-UBT performed.

Figure 16 and Figure 17 illustrate decreasing attendance at scheduled visits as follow-up progressed with breastfed children attending at a slightly greater percentage compared to non-breastfed children. Also, the overall number of children infected with *H. pylori* at each visit as well as the percentage of children infected was higher in breastfed children as follow-up progressed.

FIGURE 16. VISIT ATTENDANCE AND *HELICOBACTER PYLORI* INFECTION STATUS BY VISIT NUMBER FOR BREASTFED CHILDREN
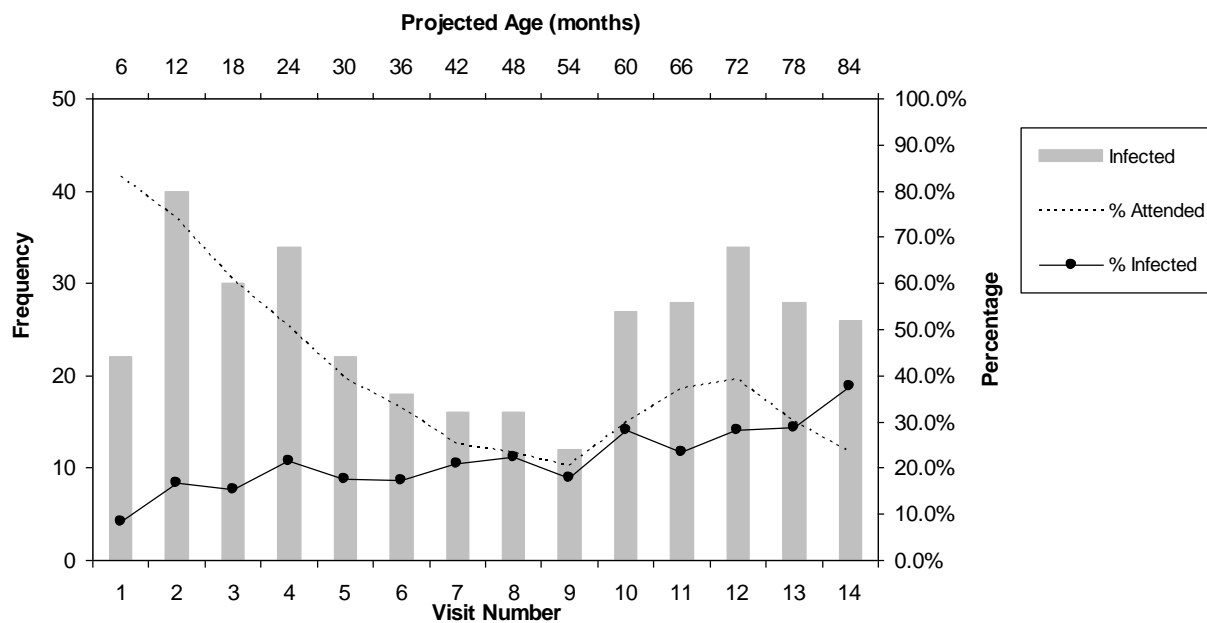
FIGURE 17. VISIT ATTENDANCE AND *HELICOBACTER PYLORI* INFECTION STATUS BY VISIT NUMBER FOR CHILDREN NOT BREASTFED
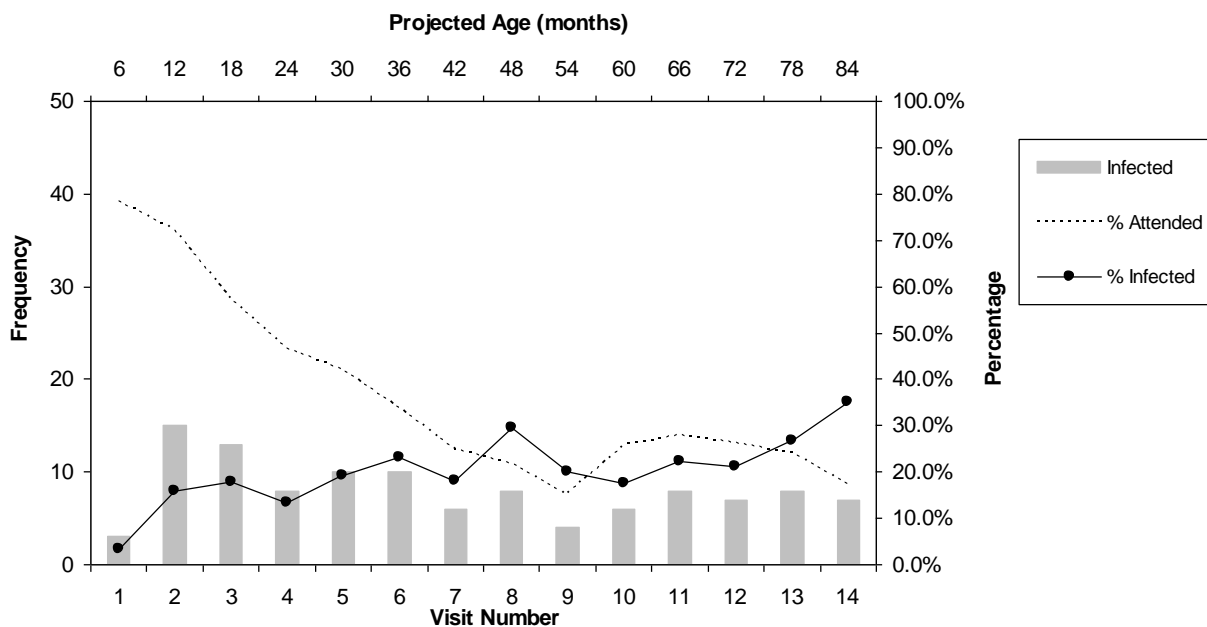
When stratifying *H. pylori* infection by serostatus of the mother, breastfed children whose mother was seropositive at baseline (N=210) had a higher *H. pylori* incidence than non-breastfed children whose mother was seropositive at baseline (N=70). Over half (51%, n=107) of breastfed children and 39% (n=27) of non-breastfed children acquired an *H. pylori* infection if their mother was seropositive for *H. pylori* at baseline. A higher percentage of *H. pylori* incident infection among breastfed versus non-breastfed children living with seropositive mothers is also seen with short-term and long-term *H. pylori* infections. Thirty percent (n=63) and 27% (n=19) of breastfed and non-breastfed children with seropositive mothers developed a short-term *H. pylori* infection at least once during follow-up. Eleven percent of breastfed (n=24) and 7% (n=5) of non-breastfed children whose mother was seropositive for *H. pylori* developed long-term infections.

When restricted to breastfed and non-breastfed children who had at least one sibling who was either infected with *H. pylori* or seropositive for *H. pylori*, breastfed children acquired *H. pylori* less often than non-breastfed children. Sixty-one percent of breastfed children (n=31) and 63% of non-breastfed children (n=10) living with an *H. pylori* positive sibling acquired *H. pylori* at least once during follow-up. For a short-term infection, again a lower percentage of breastfed children (n=16, 31%) versus non-breastfed children (n=7, 44%) living with an *H. pylori* positive sibling acquired a short-term infection. Pertaining to long-term *H. pylori* infection development, a lower percentage of breastfed children (n=9, 18%) compared to non-breastfed children (n=4, 25%) who lived with an *H. pylori* positive sibling developed a long-term *H. pylori* infection.

As differing types of *H. pylori* infections (overall acquisition, short-term and long-term) are the outcomes of interest, Table 26 shows *H. pylori* infection status by breastfed duration and the number and percentage of infections that occurred prior to defined breastfeeding length (6 months and 12 months). In regards to breastfeed duration of six months or longer, 38% (n=25) of children who acquired an *H. pylori* infection at least once during follow-up, acquired this infection prior to receiving at least 6 months of breastfeeding. For short-term and long-term *H. pylori* infections, 15% to 17% of short-term infections occurred prior to receiving 6 months of breastfeeding while 7% to 36% of long-term infections occurred prior to receiving 6 months of breastfeeding.

For children who were being breastfeed at 12 months of age, more than half (56%) acquired an *H. pylori* infection prior to being breastfed at 12 months of age. Twenty-three percent of children acquired a short-term *H. pylori* infection before 12 months of age and breastfeeding and 20% to 60% of children developed a long-term *H. pylori* infection before 12 months of age and breastfeeding.

TABLE 26. *HELICOBACTER PYLORI* INFECTION STATUS AND ORDER BY BREASTFEEDING STATUS, THE PASITOS COHORT

Breastfeeding Status	n	%[a]	Infection prior to breastfeeding	
			N	%[b]
Breastfed 6 months or longer (N=123)				
Ever infected[c]	66	53.7%		
			25	37.9%
Short-term Infection[d]	41	33.3%		
Scenario 1			7	17.1%
Scenario 2			6	14.6%
Scenario 3			6	14.6%
Long-term Infection[e]	14	11.4%		
Scenario 1			5	35.7%
Scenario 2			1	7.1%
Scenario 3			1	7.1%
Breastfed at 12 months of age or longer (N=63)				
Ever infected[c]	34	54.0%		
			19	55.9%
Short-term Infection[d]	22	34.9%		
Scenario 1			5	22.7%
Scenario 2			5	22.7%
Scenario 3			5	22.7%
Long-term Infection[e]	5	7.9%		
Scenario 1			3	60.0%
Scenario 2			1	20.0%
Scenario 3			1	20.0%

[a] Percentage within group (N)

[b] Percentage within subgroup (n)

[c] Indicates at least one positive ^{13}C-UBT throughout follow-up.

[d] Indicates at least three, consecutive positive ^{13}C-UBT with no subsequent negative tests throughout follow-up.

[e] Indicates the first event in which a positive ^{13}C-UBT was followed by a negative ^{13}C-UBT.

Regression Analysis

Descriptive analysis (Table 26) revealed that *H. pylori* infection acquisition occurred prior to being breastfed for 6 months and at 12 months of age for a large percentage of children, 38% and 56% respectively. So, by the time children had been breastfed for 6 months or at 12 months of age, they were already infected. In addition, for children who were breastfed for 6 months, short-term and long-term infections occurred prior to the exposure for up to 17% and 36% of children, respectively. For children who were breastfed at 12 months of age, 23% and up to 60% of short-term and long-term infections occurred prior to the exposure respectively. The impact of breastfeeding on developing *H. pylori* infection acquisition and establishment (short-term and long-term) is complicated. The hazards during and after breastfeeding compared with children without breastfeeding need more understanding to establish an adequate model, which may not be handled by standard survival analysis techniques. Another issue encountered is how to separate six months breastfeeding and breastfeeding at twelve months of age in the model, including whether the difference will diminish in the long run. Therefore, in this dissertation, I take an exploratory approach and only regression analysis pertaining to exposure to any duration of breastfeeding was performed and is presented. More sophisticated statistical model could be a future research topic.

The proportionality of hazards assumption concerning *H. pylori* infection acquisition rate based on breastfed status was again verified by three methods previously detailed. The log minus log plot of time refers to months to *H. pylori* infection acquisition based on being breastfed for any duration (Figure18). Visual inspection of the curves indicates that the proportional hazards assumption was likely violated: the rate for *H. pylori*

infection acquisition was not proportional across follow-up for children based on whether or not they had been breastfed. But the time to interaction test found that there was no significant interaction between a child's breastfed status and his or her time of infection (β coefficient=-0.001, p-value=0.847). This result was confirmed via a partial residual plot (Appendix DD).

FIGURE 18. LOG MINUS LOG PLOT: TESTING OF PROPORTIONALITY ASSUMPTION OF *HELICOBACTER PYLORI* ACQUISITION BASED ON BREASTFED STATUS
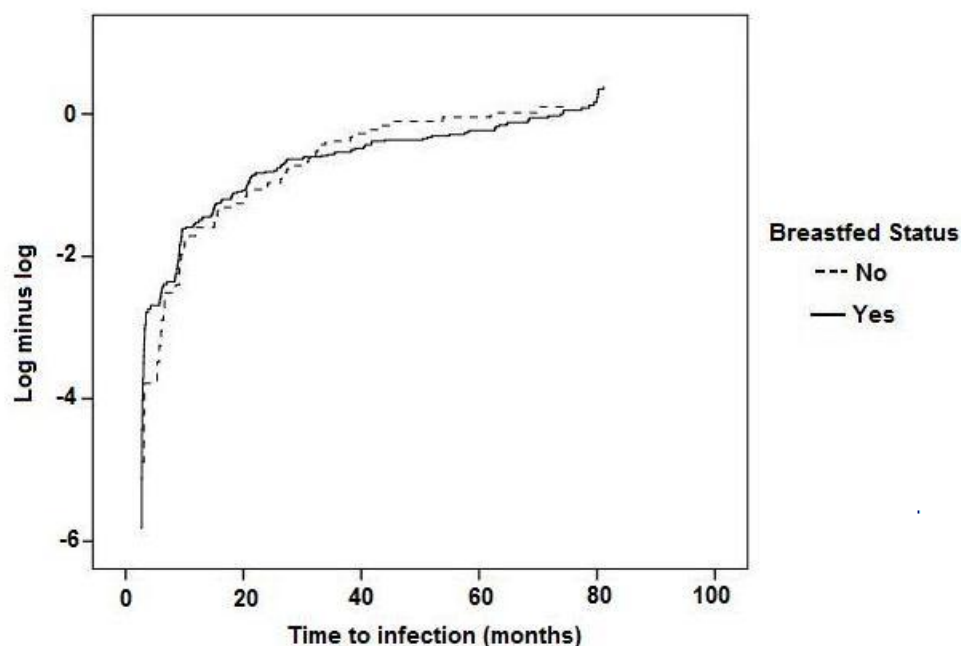
As the assumption of proportional hazards was met, Cox regression without time dependent covariates analysis was performed to obtain hazards ratios for the association between *H. pylori* infection acquisition of the child and breastfed status. Results of regression analysis are shown in Appendix EE. The rate of *H. pylori* incident infection in children who were breastfed for any duration was neither greater nor lower compared to children who were not breastfed (crude HR= 1.0, 95% CI: 0.7, 1.4). This

measure is somewhat uncertain in the magnitude and direction of effect and strong protective and positive associations can be excluded.

Stratification by *H. pylori* serostatus of the mother, *H. pylori* status of a sibling, *H. pylori* active antibiotic use and country of origin (Mexico versus the United States) was performed to determine the effect of exposure of being breastfed on the acquisition of *H. pylori* (Appendix EE). In all stratified analyses, no effect modification was indicated; estimates were weak in magnitude and imprecise.

In children whose mother was seropositive, the rate of *H. pylori* incident infection in children who were breastfed for any duration was just slightly greater relative to children who were not breastfed (crude HR= 1.1, 95% CI: 0.7, 1.7). In children whose mother was seronegative, the rate of *H. pylori* incident infection in children who were breastfed for any duration was just slightly lower relative to children who were not breastfed (crude HR=0.9, 95% CI: 0.5, 1.4).

When stratifying by *H. pylori* status of a sibling living within the same household, in children who lived with a sibling who were neither infected with *H. pylori* nor seropositive for *H. pylori* the rate of *H. pylori* incident infection in children who were breastfed was just slightly lower relative to children who were not breastfed (crude HR=0.9, 95% CI: 0.6, 1.5). In children who lived with a sibling who was either infected or seropositive for *H. pylori* the rate of *H. pylori* incident infection in children who were breastfed was just slightly greater relative to children who were not breastfed (crude HR= 1.1, 95% CI: 0.5, 2.2).

In children who did not take any *H. pylori* active antibiotic courses during follow-up, the rate of *H. pylori* infection acquisition in breastfed children was slightly lower relative

to children who were not breastfed (crude HR=0.8, 95% CI: 0.5, 1.2). For children who took at least one *H. pylori* active antibiotic course, the rate of *H. pylori* acquisition in breastfed children was slightly greater relative to children who were not breastfed (crude HR= 1.1, 95% CI: 0.5, 2.2).

Stratification by country of residence found that in Mexico, the rate of *H. pylori* incident infection in breastfed children was just slightly greater compared to non-breastfed children (crude HR= 1.1, 95% CI: 0.6, 2.2). In the United States, the rate of incident *H. pylori* infection in breastfed children was neither lower nor greater relative to children who were not breastfed (crude HR= 1.0, 95% CI: 0.7, 1.5).

In addition to determining the effect of breastfeeding on *H. pylori* infection acquisition, the effect between breastfeeding and the rate of a short-term *H. pylori* infection was also estimated. Again, three different time-to-short-term infection periods were determined as previously described. Prior to performing regression analysis, the proportionality of hazards assumption was determined concerning *H. pylori* infection acquisition rate based on breastfed status; again this was verified by three methods previously detailed. The log minus log plot of time refers to months to *H. pylori* short-term infection development based on being breastfed for any duration (Figures19-21). Visual inspection of the curves indicates that the proportional hazards assumption was likely violated for all time-to-event scenarios: the rate for *H. pylori* short-term infection development was not proportional across follow-up for children based on whether or not they had been breastfed. Both the time to interaction test (scenario one: β coefficient= -0.021, p-value=0.026; scenario two: β coefficient=-0.022, p-value=0.020; scenario

three: β coefficient= -0.022, p-value=0.020) and partial residual plots (Appendices FF-

HH) verified this violation of proportional hazards.

Again, as the proportional hazards assumption was not met, Cox regression with

time dependent covariates analysis was performed to obtain hazards ratios for the

association between *H. pylori* short-term infection development of the child and

breastfed status. Results of regression analysis are shown in Appendices II-KK.

FIGURE 19. LOG MINUS LOG PLOT: TESTING OF PROPORTIONALITY ASSUMPTION OF SHORT-TERM *HELICOBACTER PYLORI*/INFECTION ACQUISITION BASED ON BREASTFED STATUS, SHORT-TERM INFECTION SCENARIO 1
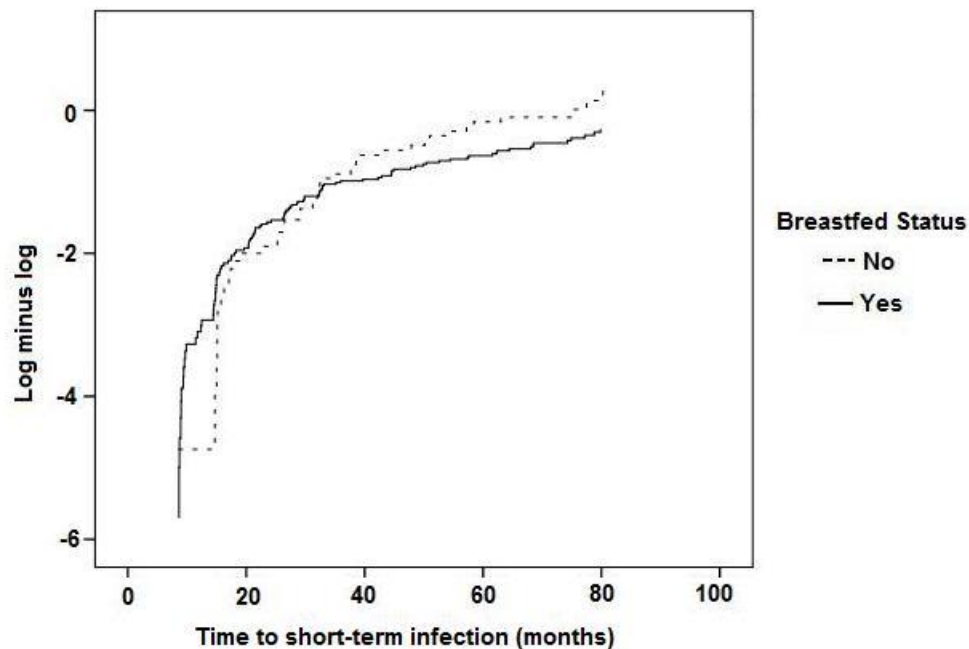
FIGURE 20. LOG MINUS LOG PLOT: TESTING OF PROPORTIONALITY ASSUMPTION OF SHORT-TERM *HELICOBACTER PYLORI* INFECTION ACQUISITION BASED ON BREASTFED STATUS, SHORT-TERM INFECTION SCENARIO 2
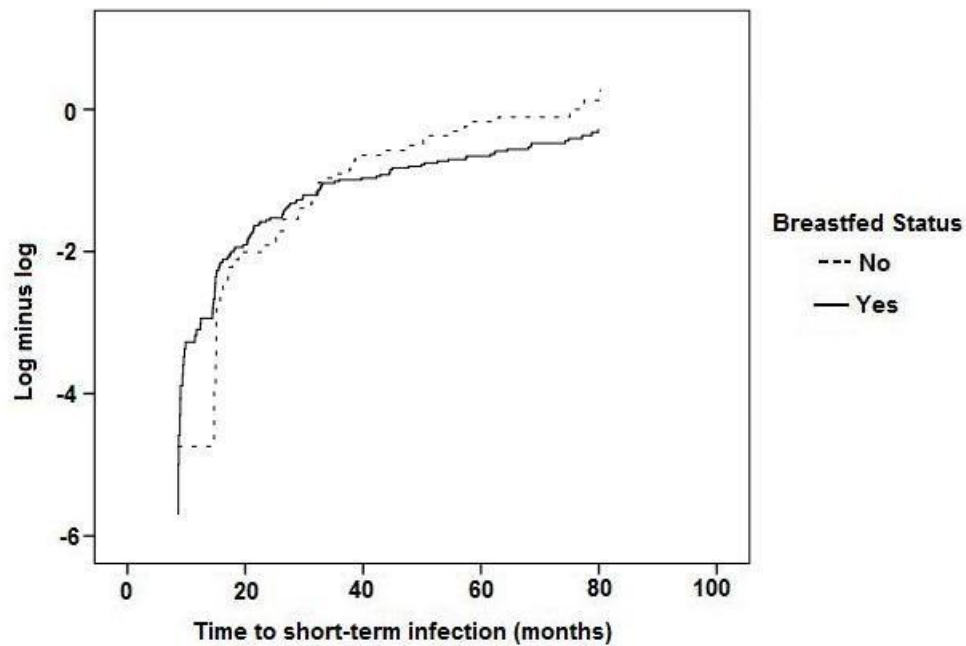
FIGURE 21. LOG MINUS LOG PLOT: TESTING OF PROPORTIONALITY ASSUMPTION OF SHORT-TERM *HELICOBACTER PYLORI* INFECTION ACQUISITION BASED ON BREASTFED STATUS, SHORT-TERM INFECTION SCENARIO 3
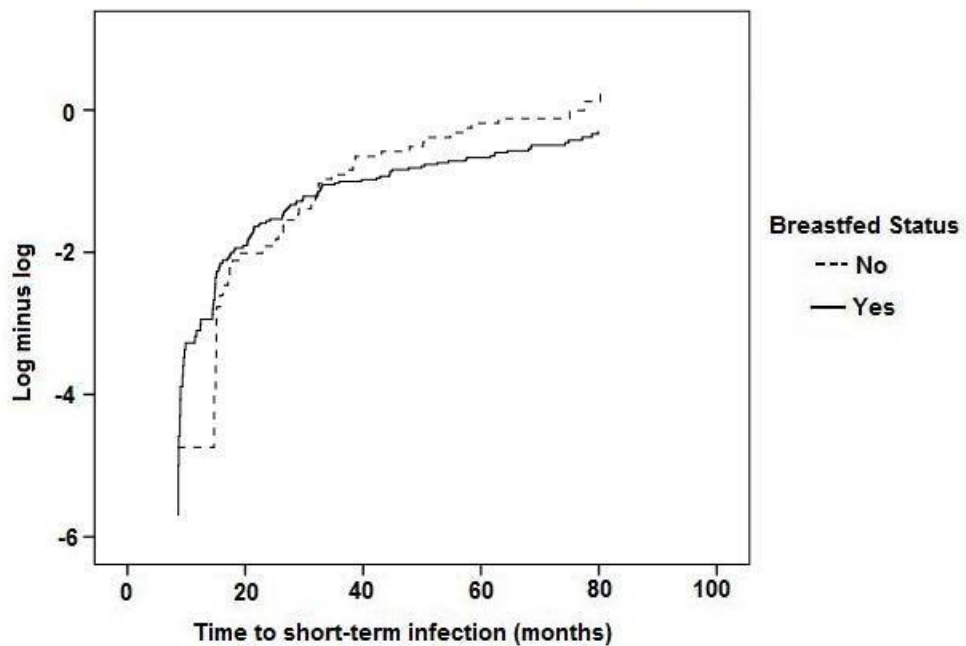
In all three time-to-event scenarios, there was a very high level of uncertainty in both magnitude and direction of estimates. The rate of short-term *H. pylori* infection development in children who were breastfed for any duration was more than one and a half times greater compared to children who were not breastfed in all time-to-short-term infection scenarios (scenario one crude HR= 1.6, 95% CI: 0.8, 3.2; scenario two HR= 1.6, 95% CI: 0.8, 3.3; scenario three crude HR= 1.6, 95% CI: 0.8, 3.2).

Stratified analysis was again performed to estimate the effect of breastfeeding on the *H. pylori* short-term infection development by *H. pylori* serostatus of the mother, *H. pylori* status of a sibling, *H. pylori* effective antibiotic use and country of residence. Overall, the estimated effect measures obtained were imprecise due to low numbers within strata and although effect modification may have occurred by country of residence and sibling's *H. pylori* status, it cannot be determined in this study.

In children whose mother was seronegative for *H. pylori*, the rate of short-term infection development in breastfed children was slightly greater relative to children who were not breastfed (scenario one crude HR= 1.4, 95% CI: 0.4, 4.8; scenario two HR= 1.4, 95% CI: 0.4, 4.8; scenario three crude HR= 1.4, 95% CI: 0.4, 4.8). In children whose mother was seropositive for *H. pylori*, the rate of short-term infection development in breastfed children was also slightly greater relative to children who were not breastfed (scenario one crude HR= 1.5, 95% CI: 0.6, 3.9; scenario two HR= 1.6, 95% CI: 0.6, 4.1; scenario three crude HR= 1.6, 95% CI: 0.6, 4.0).

It was also found in all time-to-short-term infection scenarios that in children who lived with a sibling who was either seropositive for *H. pylori* or infected with *H. pylori* the rate of a short-term *H. pylori* infection in breastfed children was 2.4 times greater compared

to non-breastfed children (scenario one crude HR= 2.4, 95% CI: 0.4, 16.4; scenario two HR= 2.4, 95% CI: 0.4, 16.5; scenario three crude HR= 2.4, 95% CI: 0.4, 16.0). In children who did not live with a sibling who was either seropositive for *H. pylori* or infected with *H. pylori* the rate of short-term infection was neither lower or greater by breastfed status of the child (scenario one crude HR= 1.0, 95% CI: 0.3, 3.3; scenario two HR= 1.1, 95% CI: 0.3, 3.3; scenario three crude HR= 1.0, 95% CI: 0.3, 3.3). In Mexican children, the rate of short-term infection development in breastfed children was much greater relative to children who were not breastfed (scenario one crude HR= 2.5, 95% CI: 0.4, 17.3; scenario two crude HR= 2.5, 95% CI: 0.4, 17.5; scenario three crude HR= 2.5, 95% CI: 0.4, 17.3). In children from the United States, the rate of short-term infection development was also much greater in breastfed children relative to children who were not breastfed (scenario one crude HR= 2.0, 95% CI: 0.8, 4.9; scenario two crude HR= 2.5, 95% CI: 0.4, 17.5; scenario three crude HR= 2.5, 95% CI: 0.4, 17.3).

Due to model instability, hazard ratios with confidence intervals could not be obtained to estimate the effect of exposure to breastfeeding on the development of a short-term infection based on *H. pylori* active antibiotic use.

Lastly, the effect of breastfeeding on the establishment of a long-term *H. pylori* infection was estimated. Prior to performing regression analysis, the proportionality of hazards assumption was determined concerning *H. pylori* long-term infection establishment rate based on breastfed status again as previously detailed. The log minus log plot of time refers to months to *H. pylori* long-term infection establishment based on being breastfed for any duration (Figures 22-24). Visual inspection of the curves indicates that the proportional hazards assumption may have been violated for

all time-to-event scenarios: the rate for *H. pylori* long-term infection establishment may

have not been proportional across follow-up for children based on whether or not they

had been breastfed. But, both the time to interaction test (scenario one: β coefficient= -

0.006, p-value=0.780; scenario two: β coefficient= -0.006, p-value=0.820; scenario

three: β coefficient= 0.000, p-value=0.984) and partial residual plots (Appendices LL-

NN) indicate that a violation of proportional hazards did not occur. As the proportional

hazards assumption was met in two of three methods, Cox regression without time

dependent covariates analysis was performed to obtain hazards ratios for the

association between *H. pylori* long-term infection establishment of the child and

breastfed status. Results of regression analysis are shown in Appendices OO-QQ.

FIGURE 22. LOG MINUS LOG PLOT: TESTING OF PROPORTIONALITY ASSUMPTION OF LONG-TERM *HELICOBACTER PYLORI* INFECTION ACQUISITION BASED ON BREASTFED STATUS, LONG-TERM INFECTION SCENARIO 1
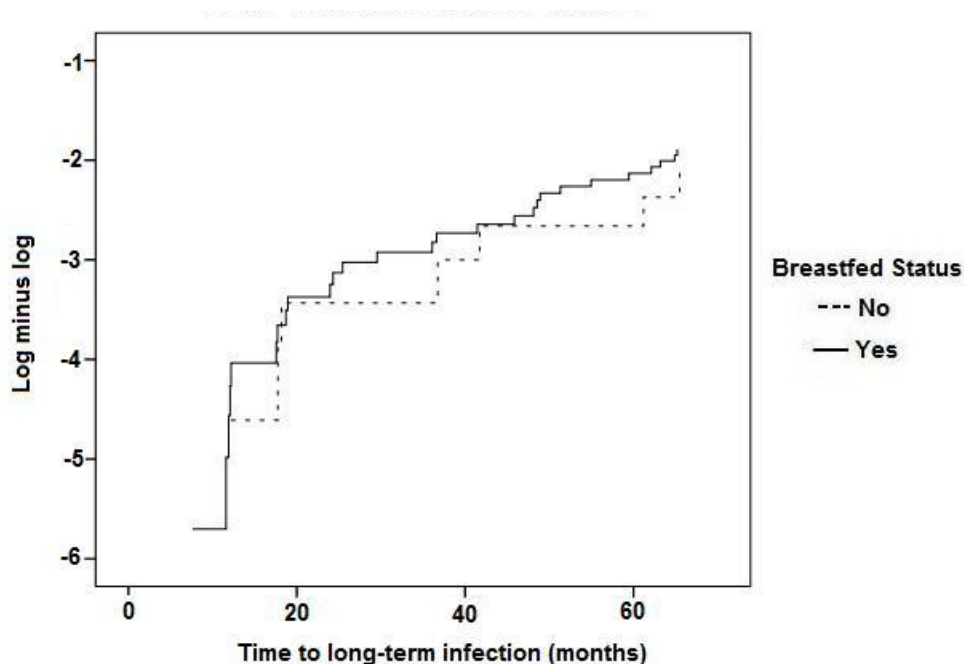
FIGURE 23. LOG MINUS LOG PLOT: TESTING OF PROPORTIONALITY ASSUMPTION OF LONG-TERM *HELICOBACTER PYLORI* INFECTION ACQUISITION BASED ON BREASTFED STATUS, LONG-TERM INFECTION SCENARIO 2
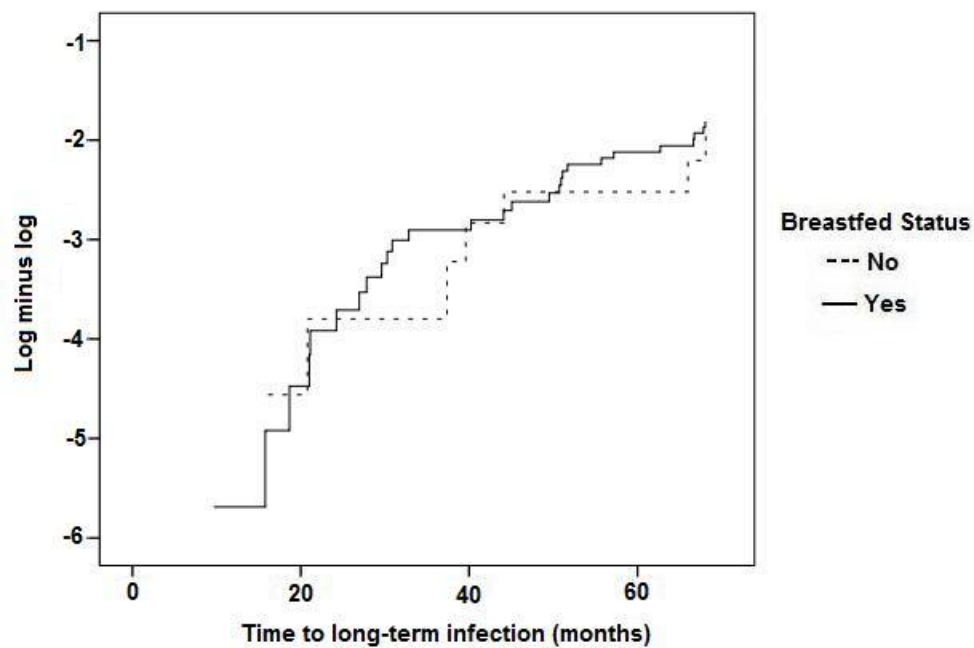
FIGURE 24. LOG MINUS LOG PLOT: TESTING OF PROPORTIONALITY ASSUMPTION OF LONG-TERM *HELICOBACTER PYLORI* INFECTION ACQUISITION BASED ON BREASTFED STATUS, LONG-TERM INFECTION SCENARIO 3

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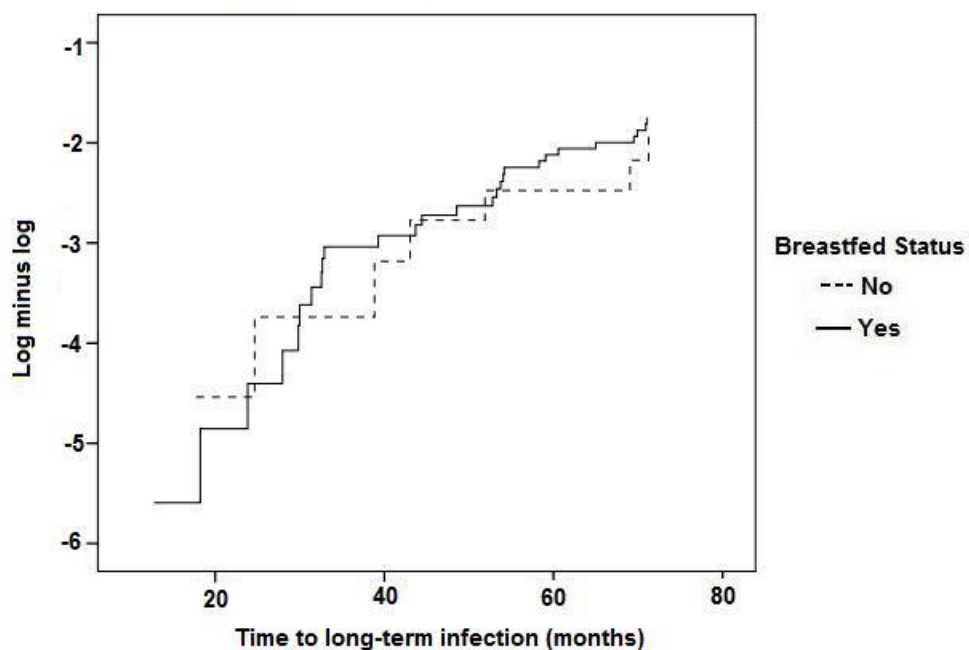
The rate of long-term *H. pylori* infection establishment in children who were breastfed for any duration was slightly greater relative to children who were not breastfed in all time-to long-term infection scenario (scenario one crude HR: 1.3, 95% CI: 0.6, 3.0; scenario two HR: 1.2, 95% CI: 0.5, 2.9; scenario three crude HR: 1.2, 95% CI: 0.5, 2.8). In these analyses, the estimates are very uncertain in the direction of effect and magnitude of effect; a strong inverse as well as a strong positive effect can be ruled out.

Stratified analysis was again performed to estimate the effect of breastfeeding on the *H. pylori* long-term infection development by *H. pylori* serostatus of the mother, *H. pylori* status of a sibling, *H. pylori* effective antibiotic use and country of residence. Effect modification was not noted by *H. pylori* status of the mother but may have occurred for the other stratified factors. But again, in all of these estimates display a very high degree of uncertainty in magnitude and direction of effect with values of strong association in both directions in the confidence intervals.

When stratifying by *H. pylori* serostatus of the mother, in children whose mother was seropositive for *H. pylori* the rate of long-term infection establishment in breastfed children was slightly greater compared to children who were not breastfed (scenario one crude HR: 1.3, 95% CI: 0.5, 3.4; scenario two HR: 1.2, 95% CI: 0.5, 3.2; scenario three crude HR: 1.2, 95% CI: 0.5, 3.2). These estimates display a very high degree of uncertainty in magnitude and direction of effect. In children whose mother was seronegative for *H. pylori*, the rate of long-term *H. pylori* infection in breastfed children was also slightly greater relative to children who were not breastfed (scenario one

crude HR: 1.4, 95% CI: 0.1, 13.3; scenario two HR: 1.3, 95% CI: 0.1, 12.2; scenario three crude HR: 1.3, 95% CI: 0.1, 12.3).

Upon stratifying by sibling's *H. pylori* status, it was found in all time-to-long-term infection scenarios that in children whose sibling was either seropositive or infected with *H. pylori* the rate of long-term *H. pylori* infection was slightly lower in breastfed children compared to non-breastfed children *(*scenario one crude HR: 0.8, 95% CI: 0.2, 2.4; scenario two HR: 0.8, 95% CI: 0.2, 2.6; scenario three crude HR: 0.9, 95% CI: 0.3, 2.8). In children who did not live with a sibling in which *H. pylori* was detected, the rate of long-term infection in breastfed children was somewhat greater compared to non-breastfed children (scenario one crude HR: 1.5, 95% CI: 0.3, 7.2; scenario two HR: 1.5, 95% CI: 0.3, 7.1; scenario three crude HR: 1.4, 95% CI: 0.3, 6.8). Effect modification may be present but as these measures are highly uncertain in regards to the magnitude and direction of effect with values of strong association in both directions in confidence intervals, this cannot be determined in the current study.

Analysis by country of origin found that in children from the United States, the rate of long-term infection in breastfed children was slightly lower compared to children who were not breastfed (scenario one crude HR=0.8, 95% CI: 0.2, 2.4; scenario two crude HR=0.8, 95% CI: 0.2, 2.4; scenario three crude HR=0.8, 95% CI: 0.2, 2.4). In children from Mexico, the rate of establishment of a long-term *H. pylori* infection in breastfed children was slightly greater compared to non-breastfed children was found for all time-to-long-term infection scenarios (one crude HR=1.3, 95% CI: 0.3, 5.6; scenario two crude HR=1.3, 95% CI: 0.3, 5.5; scenario three crude HR=1.2, 95% CI: 0.3, 5.4).

For children who took at least one course of *H. pylori* effective antibiotics, the rate of long-term infection establishment was just slightly lower in breastfed children compared to children who not breastfed for all time-to-event scenarios (scenario one crude HR=0.9, 95% CI: 0.3, 2.8; scenario two crude HR= 0.9, 95% CI: 0.3, 2.8; scenario three crude HR= 0.9, 95% CI: 0.3, 2.7). For children that did not take any *H. pylori* active antibiotics, the rate of long-term infection establishment was somewhat greater in children who were breastfed compared to children who were not breastfed (one crude HR=1.9, 95% CI: 0.6, 6.6; scenario two crude HR=1.8, 95% CI: 0.5, 6.3; scenario three crude HR=1.8, 95% CI: 0.5, 6.2).

Sensitivity Analyses

Directed Acyclic Graphs (DAGs): Alternate Role of Hygiene and Maternal Education

In order to assess the potential impact misspecification of causal pathways had on estimates of effect, alternative DAGs for overall effects were constructed and analyzed as previously described in the Methodology section. The alternate DAGs incorporate pathways connecting maternal education through unmeasured hygienic behaviors to *H. pylori* infection status of her child (Appendices RR-VV). Statistical models were constructed based on alternate DAGs and Cox proportional hazards regression analysis was utilized to calculate hazard ratios as previously described in the Methodology section.

Hazard ratios estimating the effect of exposure to an *H. pylori* infected mother on the *H. pylori* acquisition of her child are shown in Table 27. Hazard ratios calculated based on alternative DAG one (Appendix RR) reflect a 50% decrease in the relative effect from the original model presented. Hazard ratios calculated based on alternative DAG two (Appendix SS) also reflects a 50% decrease in the relative effect from the original model presented.

TABLE 27. SUFFICIENT ADJUSTMENT SETS INCLUDING HYGIENE AND MATERNAL EDUCATION: *H. PYLORI* INFECTION ACQUISITION AND MATERNAL INFECTION STATUS

Adjustment Sets	HR[a], 95% CI[b]
Crude	1.2 (0.9, 1.6)
Crowding[c]	1.2 (0.9, 1.6)
Maternal Education[d]	1.1 (0.9, 1.5)
Crowding and Maternal Education[e]	1.1 (0.9, 1.5)
[a] Hazards ratio [b] 95% confidence interval [c] Original model defined [d] Alternate model one [e] Alternate model two	

In regards to the effect of exposure to an *H. pylori* infected mother on the *H. pylori* long-term infection establishment of her child, hazard ratios estimating the effect are shown in Table 28. Hazard ratios calculated based on alternative DAG one (Appendix RR) reflect a 27% decrease in the relative effect from the original model presented. Hazard ratios calculated based on alternative DAG two (Appendix SS) also reflects a 27% decrease in the relative effect from the original model presented.

TABLE 28.SUFFICIENT ADJUSTMENT SETS INCLUDING HYGIENE AND MATERNAL EDUCATION:*H. PYLORI* INFECTION LONG-TERM INFECTION ESTABLISHMENT AND MATERNAL INFECTION STATUS

Adjustment Sets	HR[a], 95% CI[b]
Crude	2.3 (0.3, 16.6)
Crowding[c]	2.1 (0.3, 15.0)
Maternal Education[d]	1.8 (0.3, 13.2)
Crowding and Maternal Education[e]	1.8 (0.2, 12.9)
[a] Hazards ratio	
[b] 95% confidence interval	
[c] Original model defined	
[d] Alternate model one	
[e] Alternate model two	

Hazard ratios estimating the effect of exposure to breastfeeding of any duration on the *H. pylori* acquisition of a child are shown in Table 29. Hazard ratios calculated based on alternative DAG one through three (Appendices TT-VV) do not reflect an increase or decrease in the relative effect from the original model presented. No difference in relative effect from the original model presented is also noted in hazard ratios estimating the effect of breastfeeding for any duration on the *H. pylori* short-term infection establishment in a child (Table 30).

TABLE 29. SUFFICIENT ADJUSTMENT SETS INCLUDING HYGIENE AND MATERNAL EDUCATION, *H. PYLORI* INFECTION ACQUISITION AND BREASTFEEDING STATUS

Adjustment Sets	HR[a], 95% CI[b]
Crude[c]	1.0 (0.7, 1.4)
Crowding[d]	1.0 (0.7, 1.3)
Maternal Education[e]	1.0 (0.7, 1.3)
Crowding and Maternal Education[f]	1.0 (0.7, 1.3)
[a] Hazards ratio	
[b] 95% confidence interval	
[c] Original model defined	
[d] Alternate model one	
[e] Alternate model two	
[f] Alternate model three	

TABLE 30. SUFFICIENT ADJUSTMENT SETS INCLUDING HYGIENE AND MATERNAL EDUCATION, *H. PYLORI* SHORT-TERM INFECTION AND BREASTFEEDING STATUS

Adjustment Sets	HR[a], 95% CI[b]
Crude[c]	1.6 (0.8, 3.2)
Crowding[d]	1.6 (0.8, 3.2)
Maternal Education[e]	1.6 (0.8, 3.2)
Crowding and Maternal Education[f]	1.6 (0.8, 3.3)
[a] Hazards ratio	
[b] 95% confidence interval	
[c] Original model defined	
[d] Alternate model one	
[e] Alternate model two	
[f] Alternate model three	

In regards to the effect of exposure to breastfeeding of any duration on the *H. pylori* long-term infection establishment of a child, hazard ratios estimating the effect are shown in Table 31. Hazard ratios calculated based on alternative DAG one and two (Appendices TT and UU) reflect a 67% decrease in the relative effect from the original model presented. Hazard ratios calculated based on alternative DAG three (Appendix

VV) reflect a change from a 30% increase in the relative effect from the original model presented to a null relative effect in alternate model Three.

TABLE 31.SUFFICIENT ADJUSTMENT SETS INCLUDING HYGIENE AND MATERNAL EDUCATION, *H. PYLORI* LONG-TERM INFECTION AND BREASTFEEDING STATUS

Adjustment Sets	HR[a], 95% CI[b]
Crude[c]	1.3 (0.6, 3.0)
Crowding[d]	1.1 (0.5, 2.6)
Maternal Education[e]	1.1 (0.5, 2.6)
Crowding and Maternal Education[f]	1.0 (0.5, 2.4)
[a] Hazards ratio	
[b] 95% confidence interval	
[c] Original model defined	
[d] Alternate model one	
[e] Alternate model two	
[f] Alternate model three	

Timing of Infection Status

As this dissertation is the first attempt to define time to a long-term *H. pylori* infection, three distinct time-to-event scenarios were presented for Aims A-C as previously described. Hazard ratios estimating the overall effect of a mother's *H. pylori* infection on the long-term infection establishment of her child in scenarios one through three are shown in Table 32. Between scenarios one and two, there is a 93% difference in estimated effects. Between scenarios one and three there is a 43% difference in estimated effects and between scenarios two and three there is a 55% difference in estimated effects. For all time-to-event scenarios pertaining to the effect of maternal *H. pylori* infection on long-term infection establishment of her child, all confidence intervals indicate a great deal uncertainty in both the magnitude and direction of effect.

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TABLE 32. TIME TO LONG-TERM *H. PYLORI* INFECTION, *H. PYLORI* TRANSMISSION AND MATERNAL INFECTION STATUS

	HR[a]	95% CI[b]
Scenario 1	2.1	0.3, 15.0
Scenario 2	4.0	0.3, 46.5
Scenario 3	2.7	0.2, 39.7
[a] Hazards ratio		
[b] 95% confidence interval		

Hazard ratios estimating the overall effect of an older sibling's long-term *H. pylori* infection on the long-term infection establishment of his or her younger sibling in scenarios one through three are shown in Table 33. Between scenarios one and two, there is an 8% difference in estimated effects. Between scenarios one and three there is a 30% difference in estimated effects and between scenarios two and three there is a 37% difference in estimated effects. For all time-to-event scenarios pertaining to the effect of an older sibling's long-term *H. pylori* infection on long-term infection establishment of his or her younger sibling, all confidence intervals indicate a strong positive association but highly uncertainty in the magnitude of effect.

TABLE 33. TIME TO LONG-TERM *H. PYLORI* INFECTION, *H. PYLORI* TRANSMISSION AND SIBLING LONG-TERM INFECTION STATUS

	HR[a]	95% CI[b]
Scenario 1	9.1	2.3, 36.6
Scenario 2	8.5	2.2, 32.1
Scenario 3	11.9	2.7, 53.7
[a] Hazards ratio		
[b] 95% confidence interval		

As time to a short-term infection development for children who achieved a long-term infection was defined by the three scenarios, hazard ratios estimating the effect of being

breastfed for any duration on the short-term *H. pylori* infection development in children

in scenarios one through three are shown in Table 34. For all time-to-event scenarios

there were no differences in estimated effects between scenarios and confidence

intervals are very uncertain in both magnitude and direction.

TABLE 34. TIME TO SHORT-TERM *H. PYLORI* INFECTION, BREASTFEEDING AND *H. PYLORI* INFECTION

	HR[a]	95% CI[b]
Scenario 1	1.6	0.8, 3.2
Scenario 2	1.6	0.8, 3.3
Scenario 3	1.6	0.8, 3.3
[a] Hazards ratio		
[b] 95% confidence interval		

   Hazard ratios estimating the effect of being breastfed on the long-term *H. pylori*

infection establishment of a child in scenarios one through three are shown in Table 35.

As with time to a short term infection development, there are no differences in estimated

effects between scenarios one and two. There is a 40% difference in estimated effects

between scenarios one and three and two and three. Again, confidence intervals

indicate a great deal of uncertainty in both magnitude and direction.

TABLE 35. TIME TO LONG-TERM *H. PYLORI* INFECTION, BREASTFEEDING AND *H. PYLORI* INFECTION

	HR[a]	95% CI[b]
Scenario 1	1.3	0.6, 3.0
Scenario 2	1.3	0.5, 2.9
Scenario 3	1.2	0.5, 2.8
[a] Hazards ratio		
[b] 95% confidence interval		

CHAPTER 5

DISCUSSION

Aim A- *Helicobacter pylori* Transmission and Maternal Infection Status

This study aimed to determine the effect of a mother's *H. pylori* infection on the

acquisition and the long-term establishment of *H. pylori* infection of her child. Overall,

this analysis suggests that the *H. pylori* seropositive status of the mother was not

consistent with a strong inverse association but also not a strong positive association,

increasing the rate of infection acquisition in children of seropositive mothers by

approximately 20% compared to children of seronegative mothers (95% CI: 0.9, 1.6)

after adjusting for crowding.

But as detailed in the literature review, these results are in overall agreement with

current studies which indicate that the mother is positively associated with *H. pylori*

infection of her child (Appendices D-F). However, the magnitude of the estimated effect

reported in this study is lower than reported by other studies that determined active *H.

pylori* status infection of the child (Appendix E). This study differs from other studies in

that *H. pylori* incidence rate of the child was the outcome versus prevalence and hazard

ratios are the measure of effect whereas in other studies the odds ratio (OR) was used

to approximate the relative risk. By using the OR, it would be expected that the results

would be biased away from the null as the odds ratio only approximates the relative risk

when the disease is rare (~10% prevalence). The prevalence of *H. pylori* in this study

(62%) and others (up to 87%) (Ford & Axon, 2010) (Appendix D) is clearly not rare. And

therefore larger estimates of effect in other studies may be explained by the

inappropriate use of an odds ratio instead of a hazards ratio. It is also possible that

differences in covariates selected for adjustment may have contributed to the estimates

of effect being lower in this study than estimates in the literature. No other study found in the current literature utilized DAGs to identify covariates for adjustment and this could also contribute to differences in the magnitude of the point estimates. Lastly, the longitudinal nature of The Pasitos Study allowed assessment of person-time which is a more accurate determination of the denominator.

In terms of long-term *H. pylori* infection establishment, infection establishment in children of seropositive mothers was approximately twice as much compared to children whose mother was seronegative for *H. pylori* after adjusting for crowding but there is a very high degree of uncertainty in both the magnitude and direction of effect (HR= 2.1, 95% CI: 0.3, 15.0). Yet despite the high degree of uncertainty of these results, the increased positive association between *H. pylori* long-term infection establishment is in concordance with other studies evaluating maternal *H. pylori* status and infection status of her child (Appendix D-F). However, this analysis is unique in the context of the published literature in evaluating the relationship of the mother's infection status to the establishment of a long-term infection versus infection acquisition alone. In this analysis, a child was determined to have an established *H. pylori* infection if he or she had at least three consecutive positives test results with no subsequent negatives. The literature search conducted for this dissertation did not reveal other studies which defined long-term *H. pylori* infection.

This study also aimed to reveal what factors may modify the effect that a mother's *H. pylori* status has on her child's *H. pylori* infection. In regards to infection acquisition, although effect modification was noted differences in stratum-specific estimates were fairly modest and well within what would be expected from random variation. The

magnitude of the estimated effect of maternal *H. pylori* seropositivity on *H. pylori* acquisition of her child was slightly greater in Mexican children than United States children. Although these results display a moderate degree of uncertainty they may be due to behavioral and societal difference between Mexico versus the United States. For example, mothers from Mexico may spend more time as the primary caretaker of her children versus mothers from the United States (DiGirolamo and Snyder, 2008).

In regards to stratification by antibiotic use, the analyses generated estimates of low magnitude and are imprecise. In children who took at least one course of *H. pylori*-effective antibiotics the rate of infection acquisition was slightly greater in children whose mother was seropositive for *H. pylori* relative to children whose mother was seronegative while in children who did not take any *H. pylori*-effective antibiotics, there was no association in the rate of infection acquisition based on maternal serostatus. These results may be explained by the following: 1) temporality of antibiotic use was not differentiated from timing of *H. pylori* acquisition or 2) some children may have experienced symptoms of infection leading to antibiotic use.

The association between a mother's *H. pylori* seropositive status and the *H. pylori* infection acquisition of her child was slightly greater in children who lived with a sibling in which *H. pylori* was also detected than in children who lived with a sibling in which *H. pylori* was not detected. The estimated effect of a mother's *H. pylori* seropositive status on the infection acquisition of her child was just slightly greater in children whose older, index sibling was no more than three years older compared to the children whose older, index sibling was more than three years older. A high degree of uncertainty in both the direction and magnitude of effect in these analyses was noted. Again, effect

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modification was noted in both household sibling *H. pylori* status and age difference

stratified analyses and what can be gleaned is the need to consider a possible

interacting, biological role of siblings when estimating the effect of a mother's *H. pylori*

infection on that of her child. Lastly, the association between the mother's *H. pylori*

seropositive status and the *H. pylori* infection acquisition in her child was slightly greater

among children who were breastfed and differed in the direction of effect. These results

are uncertain and weak. These weak effects may be explained by an intimate contact

between mother and child to facilitate *H. pylori* transmission.

Regarding factors that modify the effect of long-term *H. pylori* infection establishment

of the child, the estimated effect of a mother's *H. pylori* seropositive status on the *H.*

pylori long-term infection establishment of her child was much greater in children from

the United States versus Mexico and suggestive of a positive association in the former

while an inverse association was noted in the latter. The magnitude of the estimated

effect of a mother's *H. pylori* seropositive status on long-term *H. pylori* infection

establishment in her child was much greater in children living with siblings who were

either seronegative for *H. pylori* or for whom *H. pylori* was not detected versus children

living with siblings who were either seropositive or for who *H. pylori* was detected.

Other studies in the current literature have not defined long-term infection

establishment nor have they have assessed effect modification by country of residence

but one study did describe effect modification by sibling *H. pylori* status (Yang et al,

2005) (Appendix D). In the study conducted by Yang, the effect of maternal *H. pylori*

seropositivity on *H. pylori* infection of her child was greater in households in which *H.*

pylori was detected in siblings which is in agreement with analyses for infection

acquisition but differs from the results presented for long-term infection establishment. But as previously described one possibility to explain this difference is the role of *H. pylori* transmission efficient strains in combination with the developing, robust immunity of the host which can create a situation of competing risks. Adults may harbor a lower percentage of *H. pylori* transmission efficient strains compared to children (Talarico et al., 2009; Gonzalez-Valencia et al., 2000). Therefore, if both the child's sibling and the mother are infected, then the child's infection may be reflective of less efficient transmission from mother to child and a child may become infected from a sibling and not the mother. But, if a child's sibling is not infected with *H. pylori*, this may be reflective of a combination of increased probability of transmission of *H. pylori* from mother to child even if the mother harbors a low proportion of *H. pylori* transmission efficient strains as well as the ability to mount a robust immune response in the child.

In summary, a positive association between maternal infection and *H. pylori* infection acquisition and long-term infection establishment of her child was observed and effect modification may have occurred but additional studies with a greater number of participants are need make more concrete conclusion of this modification.

Aim B- *Helicobacter pylori* Transmission and Sibling Infection Status

This study aimed to estimate the effect of exposure to *H. pylori* infection and a long-term infection in children on the acquisition and establishment of a long-term *H. pylori* infection in his or her siblings. This study found that the rate of incident *H. pylori* infection in younger siblings with an infected older sibling was much greater relative to children whose older sibling was not infected with *H. pylori* after controlling for crowding, mother's *H. pylori* status and the number of *H. pylori*-effective antibiotics taken. This positive association is consistent with other studies which estimated the effect of *H. pylori* status of children on the infection of their siblings but again these studies determined prevalent infection (Appendix I).

If a younger child's older, index sibling had an established, long-term *H. pylori* infection, their rate in acquiring an *H. pylori* infection was three times greater compared to children whose older sibling did not have a long-term infection after controlling for crowding, mother's *H. pylori* status and the number of *H. pylori*-effective antibiotics taken. The magnitude of the estimated effect of the older, index sibling's long-term infection on the infection acquisition on the younger sibling was much greater when the siblings had an age difference of three years or less versus when children were more than three years apart in age. This study differs from previous studies identified through systematic review of the literature as previous studies did not define and distinguish differing *H. pylori* outcomes (i.e. acquisition versus long-term *H. pylori* infection). Other studies only assessed the prevalence of *H. pylori* infection in children with varying strengths of estimated effects. In addition, although other studies estimated the effect of country of residence and age difference in *H. pylori* infection (Appendix G), no studies in

the reviewed literature assessed effect modification by these factors. Therefore the results of the present study cannot be compared to those reported from other studies.

It was found in all-time to-event scenarios that the rate of long-term infection establishment in a younger sibling's whose older sibling had an established, long-term *H. pylori* infection was much greater relative to younger siblings whose older sibling did not have a long-term infection after controlling for crowding, mother's *H. pylori* status and the number of *H. pylori*-effective antibiotics. Again, it is important to note that this is the first study in the accessible literature which defines and distinguishes long-term *H. pylori* infection and hence there are no comparable studies to which to relate these results; prior studies have only found positive cross-sectional associations of varying magnitudes between *H. pylori* status of an older sibling and that of his or her younger siblings (Appendix I).

Furthermore, as opposed to *H. pylori* acquisition in the younger sibling, the magnitude of the estimated effect of establishment of long-term *H. pylori* infections in younger siblings whose older sibling had a long-term infection was much greater in the United States than in Mexico in all time-to-event scenarios; again all of these effects were observed after controlling for the mother's *H. pylori* infection status and other potential confounders previously noted. It is possible this apparent difference in country-specific effects may be reflective of more complete follow-up (i.e. fewer missed visits) in children from the United States than in Mexico as described by Goodman and colleagues (Goodman et al., 2005). This would be more likely the case when assessing long-term infection establishment versus infection acquisition due to the manner in which long-term infection was defined. Long-term *H. pylori* infection establishment was

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define as three consecutive positive results while only one positive result was necessary to determine infection acquisition.

Previous cross-sectional studies have also suggested that family composition, specifically number of siblings, may influence *H. pylori* transmission (Appendices H-K). In the study by Goodman and Correa, the odds of a prevalent *H. pylori* infection in Aldana, Colombia increased as the number of two to nine year-old siblings in the household increased, and *H. pylori* infection was especially likely when siblings were less than four years apart in age (Goodman and Correa, 2000). In the study by Kivi et al, shared strains of *H. pylori* were most commonly observed among siblings, followed by mother-child relationships (Kivi et al., 2003). These studies were limited by their cross-sectional design, and therefore were not able to evaluate the time sequence for *H. pylori* transmission or long-term infection. They were also limited to estimating odds ratios, which may bias the estimated effect away from the null. Odds ratios can only approximate risk if an outcome is rare and as previously stated prevalence is high in this population.

In contrast, the present study estimates rate ratios for the effect of an older sibling's infection on acquiring an infection and establishing a long-term infection in a younger sibling. It also provides evidence to suggest that transmission occurs from the older to the younger siblings, given the time sequence of *H. pylori* infection in siblings. It was found there were no instances in which the younger sibling became infected with *H. pylori* prior to infection acquisition of the older sibling. In a limited number of instances, a younger sibling became infected with *H. pylori* and even progressed to a long-term infection and *H. pylori* was not detected in the older index child; in these instances, a

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large percentage of mothers were also seropositive for *H. pylori*. When long-term infection occurred in both the index and younger sibling, establishment of an *H. pylori* infection occurred first in the older sibling and later in the younger sibling, but never vice versa. Again, there were only two instances in which both older and younger child had acquired an *H. pylori* infection but only the infection of the younger child progressed to a long-term infection; in these two instances, the mother was seropositive for *H. pylori.*

In conclusion, although in the current study the seropositivity status of the mother was positively associated with acquisition of *H. pylori* in her child compared to children of seronegative mothers (HR= 1.2, 95% CI: 0.9, 1.6) (Aim A), the magnitude of the effect estimate was not as large or certain as observed for either exposure to an infected older sibling (adjusted HR= 6.0, 95% CI: 1.5, 24.5) or an older sibling with a long-term infection (adjusted HR= 3.1, 95% CI: 1.6, 6.2). Similarly, the seropositivity status of the mother was positively associated with the long-term *H. pylori* establishment of her child compared to children of seronegative mothers (adjusted HR= 2.1, 95% CI: 0.3, 15.0 for scenario one), but again the magnitude of the estimated effect was much greater and there was less uncertainty when the exposure was an older sibling with a long-term infection (adjusted HR 9.1, 95% CI 2.2, 36.6).

Aim C- Breastfeeding and *Helicobacter pylori* Infection

This study aimed to estimate the effect of being breastfed on the *H. pylori* acquisition as well as the short-term infection development, and the long-term *H. pylori* infection establishment in young children. Overall, there was no observed positive or inverse association between being breastfed and *H. pylori* acquisition of the child. Confidence interval estimation does not suggest a strong positive or inverse effect of breastfeeding

on *H. pylori* acquisition. A paucity of previous studies were conducted to estimate the association between breastfeeding and *H. pylori* status in children (Appendix K). A meta-analysis reported a weak inverse association between breastfeeding and *H. pylori* infection status in developed countries but a strong inverse association in developing countries and studies which assessed current infection via ^{13}C-UBT (Chak et al., 2009). The overall results presented here differ from this meta-analysis as this analysis is not consistent with an inverse effect. Also no modification by country of residence (United States versus Mexico) was observed. But as in this study, a high degree of uncertainty was observed in most studies assessing breastfeeding and *H. pylori* status (Chak et al., 2009). For example, the only longitudinal study conducted in children, reported by Naficy et al (2000), and included in the meta-analysis reported a positive association between breastfeeding and *H. pylori* status of a child but the confidence interval was also quite wide, including large effect sizes in both directions (OR= 1.4, 95% CI: 0.4, 5.8). The analysis by Naficy and colleagues (2000) included 196 children < 26 months of age. Hence the meta-analysis only provided sufficient power for estimating a precise effect by combining this longitudinal study with several cross-sectional studies. Despite imprecise measures obtained, this study is the only study found in the current literature to date that utilized follow-up time in children to estimate the association between breastfeeding and active *H. pylori* infection in childhood.

In regards to stratified analysis to identify effect modification estimates derived were weak and imprecise, lying just on one side of the null or the other. The association between breastfeeding and the child's acquisition of *H. pylori* did not differ greatly if the mother was seropositive for *H. pylori* or if the mother was seronegative for *H. pylori*.

Similarly, the magnitude of the estimated effect of breastfeeding on the child's acquisition of *H. pylori* did not differ greatly based on *H. pylori* infection or serostatus of the sibling or *H. pylori* effective antibiotic courses taken. Again, as estimates of stratified analyses were small in magnitude and just lie on either side of the null, stratified analyses do not suggest effect modification by these factors.

In regards to short-term *H. pylori* infection development, it was found that breastfeeding increased the rate of short-term infection development by 60% in children who were breastfed compared to children who were not breastfed in all time-to-event scenarios. One possible explanation for a positive association between short-term infection and breastfeeding may be that breastfeeding causes short-term infection as it induces the passive immune response as opposed to the active immune response. An active immune response is an important factor in establishing a long-term infection (J. Wang et al., 2001) so therefore without it, short-term infection may be a more likely outcome.

Upon stratification by *H. pylori* status of the sibling, there was a large positive association between being breastfed and short-term infection development if *H. pylori* was detected in a sibling while in children in which *H. pylori* was not detected in a sibling, no effect to very little effect was estimated dependent of the time-to-event. Again, the results pertaining to stratification by sibling serostatus are highly uncertain, with strong values on both sides of confidence intervals. Although a high degree of uncertainty exists, it is possible that breastfeeding by inducing passive immunity pathways may contribute to *H. pylori* short-term infection and in particular in combination with siblings within a household who may mainly harbor *H. pylori* strains

that are efficient in transmission (Talarico et al., 2009) hence children become infected, lose their infection and quickly become infected again.

The effect of breastfeeding on the long-term *H. pylori* infection establishment was also estimated. Overall, the rate of long-term infection establishment was slightly greater in children who were breastfed compared to children who were not breastfed in all time-to-event scenarios. These estimated effects are highly uncertain in both the magnitude and direction of effect with values of strong association on both sides of the confidence interval.

In children whose sibling was either seropositive or infected with *H. pylori,* the measure of the estimated effect of breastfeeding on *H. pylori* long-term infection establishment was inverse yet positively associated in breastfed children whose sibling was seronegative or not infected with *H. pylori*. Again, as with short-term *H. pylori* infection, these estimates are very uncertain with strong values of association on both sides of confidence intervals. Despite a great deal of uncertainty, these results may be due to *H. pylori* transmission efficient strains in siblings. In this situation, it is possible that in households in which one or more siblings are infected with *H. pylori,* breastfeeding confers passive immunity that prevents long-term infection establishment (J. Wang et al., 2001) in a household environment in which transmission efficient strains are predominate in siblings.

The magnitude of the estimated effect of being breastfed on long-term infection establishment was smaller in children from the United States versus in children from Mexico and differed in the direction of effect with an inverse effect in the United States. Results of stratification by country are highly uncertain with strong values of association

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on both sides of confidence intervals. But as previously stated differences in stratum estimates by country may also be due to more complete follow-up in the United States.

The magnitude of the estimated effect of being breastfed on long-term infection establishment was much smaller and inverse in children who took at least one course of *H. pylori*-effective antibiotics versus children who did not take *H. pylori* effective antibiotics. Effect modification by antibiotic use is again difficult to elucidate due to temporality issues: the order of antibiotic use and *H. pylori* long-term infection establishment was not determined. But it may be possible that *H. pylori* effective antibiotic use in conjunction with breastfeeding prevents long-term infection establishment.

The results of this study must be interpreted as exploratory due to a lack of statistical precision. This study like others in the literature has a high degree of uncertainty due to low statistical precision which limits the ability to make inferences with a high degree of confidence. Low statistical precision may result from small sample size or uneven distribution of variables across comparison groups (Rothman, Greenland, & Lash, 2008). A larger sample size is required to have greater certainty in the results. Also, in cohort studies larger samples are typically required as the incidence rate is utilized (Lengerich and Lengerich, 2013). Low statistical precision issues are only amplified upon stratification to assess effect modification.

But this study did incorporate parameters that are unique compared to other studies reported in the literature. This study is different from other studies which did not have follow-up of active *H. pylori* infection in children and hence were unable to estimate the risk or incidence rate. Also, no other studies identified in systematic literature review

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have differentiated short-term and long-term infections in children, or examined the association of these outcomes with being breastfed. Again, a DAG was utilized to establish that adjustment by age was sufficient to control for confounding. If the DAG is not correct, confounding may have occurred. It is important to note that it was not possible to estimate the effect of breastfeeding durations (6 months or at least at 12 months of age) on the *H. pylori* acquisition, or short-term infection development or long-term infection development as it was found that many *H. pylori* outcomes occurred during the exposure interval. Hence, semi-parametric time-to-event analysis such as Cox regression may not be the best analysis option in these situations.

Sensitivity Analyses

Directed Acyclic Graphs (DAGs): Alternate Role of Hygiene and Maternal Education

In constructing DAGs, the potential always exists for exclusion and misspecification of pathways. This may result in the differing identification of sufficient adjustment sets and hence estimated measures of effect. Regression models based on alternate DAGs for maternal infection status on infection establishment of her child (Appendices RR-SS) resulted in effect estimates that shifted towards the null upon inclusion of maternal education. In comparison to maternal education alone, crowding alone does not seem to accurately reflect the pathways from which the effect of maternal education passes. The effect of crowding and maternal education in combination highlights the minor effect of crowding in the causal pathways. Therefore, adjustment for crowding alone in estimating the effect of a mother's infection on that of her child's may not appropriately account for other factors such as unmeasured hygienic behaviors compared to maternal education. Although there is a need to define the most accurate relationships and

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pathways among variables via DAGs for this and future studies, it is important to note that the overall interpretation of the overall estimated effect of maternal infection on *H. pylori* acquisition and long-term infection establishment rate of her child is not altered.

In regards to the association of breastfeeding of any duration on *H. pylori* infection establishment in children, estimated effects calculated from regression models based on three alternate pathways (Appendices TT-VV) indicate little to no change in *H. pylori* acquisition or short-term infection establishment from the original estimates calculated. Therefore, adjustment for age alone in estimating the effect of breastfeeding of any duration on infection acquisition and short-term infection establishment may accurately define relationships and pathways among variables via DAGs for this and future studies.

For the association of breastfeeding for any duration on long-term infection establishment, estimated effects calculated from regression models based on three alternate pathways (Appendices TT-VV) indicate a shift towards the null when including crowding alone or maternal education alone in causal pathways. Crowding and maternal education in combination in causal pathways resulted in a null effect of breastfeeding on *H. pylori* long-term infection establishment. Therefore, age alone may not accurately define relationships and pathways among variables via DAGs for this and future studies. Again it is important to note that the overall interpretation of the overall estimated effects of breastfeeding for any duration on *H. pylori* long-term infection establishment in children is not altered.

Timing of Infection Status

Three time-to long-term *H. pylori* infection establishment scenarios were presented in order to assess the impact of timing definitions. For all time-to-event scenarios, long-term infection was defined as three consecutive positive ^{13}C-UBT results without a subsequent negative result. For each child who achieved a long-term infection, time to long-term infection establishment increased as time-to-event scenario increased from scenario one to scenario three.

In regards to the hazard ratios estimating the effect of *H. pylori* infection of the mother on the long-term infection establishment of her child, no patterns in percentage differences were noted as time-to-even scenarios increased. In all scenarios, there was a great deal of uncertain in both the direction and magnitude of effect with strong values of association on both sides of confidence intervals. Therefore, regardless of time-to-event scenario, there was no change in interpretation of results. For hazard ratios estimating the effect of an older sibling's *H. pylori* long-term infection on a younger sibling's infection acquisition, again no patterns in percentage differences were noted as time-to-even scenarios increased. Again, in all scenarios, there was a strong positive association but a great deal of uncertainty in magnitude of effects. Therefore, regardless of time-to-event scenario, there was no change in interpretation of results.

For all scenarios pertaining to hazard ratios estimating the effect of being breastfed on *H. pylori* short-term infection development, different time-to-event scenarios did not result in changes to hazard ratios. Only very minor changes in the upper confidence interval of scenario one were noted but all scenarios exclude a strong inverse association as well as a strong positive association between the exposure of

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breastfeeding and the short-term *H. pylori* infection development. Hence, distinct time-to-event scenarios did not change interpretations. Hazard ratios estimating the effect of being breastfed on *H. pylori* long-term infection establishment for different time-to-event scenarios resulted in a change in the hazard ratio pertaining only to scenario three. Again, in all scenarios there are strong values of association in both directions of the confidence intervals. Hence, distinct time-to-event scenarios did not change interpretations.

It is unknown what combination of host and bacterial factors result in the establishment of a long-term infection and therefore the timing to such an event has not been defined. In this dissertation, three different time-to-event scenarios were presented resulting in no change in the interpretation of overall results. But, although no patterns were noted as scenarios increased (from one to two and two to three) an increase in the magnitude of estimated effects from scenario one to scenario three was consistently noted. Therefore it is possible that increased timing biased results away from the null and this must be considered in future studies. Scenario one is also most in alignment with our current knowledge of long-term infection and immune response. It is know that long-term infection establishment relies on robust immune response of the host (J. Wang et al., 2001) and this immune response may not change throughout the course of infection but instead may be established upon infection.

CHAPTER 6

SUMMARY

Limitations and Strengths

The current studies include study design and analysis elements which were not identified in other studies reported in the literature. These include longitudinal assessment of active *H. pylori* infection in childhood and estimated measures of effect for the initial acquisition and establishment of short-term and long-term *H. pylori* infections. These studies also utilized DAGs to illustrate hypothesized causal mechanisms behind *H. pylori* transmission. As in all research, limitations must be considered. First, targeted follow-up of the Pasitos Cohort occurred by testing for *H. pylori* status at six-month intervals. It is possible that during the time interval between visits, some infection and spontaneous elimination events may have been missed leading to misclassification of the outcome for all analyses as well of the exposure for the sibling analysis. Therefore, the resulting estimates of association may be biased towards or even away from the null dependent on if there was misclassification of the outcome or exposure. The direction and degree of misclassification depend on factors which may influence adherence to a follow-up schedule such as the mother's education.

Non-differential misclassification of the exposures of breastfeeding practices and maternal serostatus may have occurred but was minimized due to prospective nature of information collection. Due to the longitudinal nature of the Pasitos Cohort study, breastfeeding practices were collected during infancy while the child was currently being either breastfed or formula fed leading to presumed higher accuracy of information and would likely not be influenced by *H. pylori* status of the child. Serostatus of the mother

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was assessed utilizing a commercially available enzyme immunoassay kit for the detection of immunoglobulin G antibodies against *H. pylori* with high sensitivity and specificity (HM-CAP Enteric Products, Westbury, NY). This assay was estimated to have a sensitivity of 95% (95% CI: 86.1%, 98.2%) and specificity of 98% (95% CI: 87.4%, 99.4%) (Meijer, Thijs, Kleibeuker, van Zwet, &Berrelkamp, 1997). Although Meijer and colleagues did not describe the population in which the assay characteristics were determined, variations may be seen by geographic location. Differential misclassification of exposures may have occurred if factors associated with *H. pylori* infection of a child such as maternal education influenced the reporting of exposures such as breastfeeding status. But attempts to minimize maternal education differences were taken as the questionnaire used to assess exposures utilized simple language, was provided in Spanish and English, and study personnel were available for assistance/explanation if needed during the home visit when the tool was administered.

In regards to household size, breastfeeding status, country of residence and age difference, non-differential misclassification may have occurred although likely minimal. Again, every effort including collection of data at the participant home was made to assess these variables at baseline and at every follow-up visit resulting in less possibility for error. For the possible confounding/effect measure modifying variable maternal years of education, errors in reporting may have occurred leading to non-differential misclassification but it is likely that this was again minimal. The mother was directly questioned regarding years of education and as previously stated, the questionnaire used was written in simple language. For the confounder/effect measure modifier antibiotic use, non-differential misclassification may have occurred. Under

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reporting of antibiotic use may have occurred as follow-up visits occurred at most every six months.

Differential misclassification of *H. pylori* infection did not likely occur in the Pasitos Cohort Study. Assessment of outcomes, *H. pylori* infection acquisition, short-term and long-term infection establishment was determined in a systematic manner regardless of potential exposures. It is possible that visit attendance in which *H. pylori* infection was assessed was lower due to exposures such as *H. pylori* status of the mother. Therefore infections may have been missed due to skipped visits resulting in differential misclassification of the outcome biasing results towards the null.

DAGs created for this study may also not accurately represent the pathways relevant to *H. pylori* transmission. DAGs which do not correctly specify relationships between variables may lead to confounded estimates (Rothman, Greenland, & Lash, 2008). Sensitivity analysis revealed that the possibility of ill-defined DAGs may have occurred in regards to maternal transmission studies (Aim A). The need to consider the role of maternal education as a proxy for hygienic factors which are difficult to measure is needed in future *H. pylori* familial transmission studies.

As the Pasitos Cohort is limited to a defined population on the U.S.-Mexico border, the results may not be generalizable to populations which differ greatly in external environment and social customs. Studies conducted in developed countries of middle to high socioeconomic status have generated results which differ from associations observed in developing countries. For example, in a meta-analysis of studies related to breastfeeding and *H. pylori* infection, it was found that breastfeeding showed an inverse effect of greater magnitude on *H. pylori* infection in developing nations than in

developed nations (Chak, et al., 2009). In the current study, different study settings and populations were considered and noted when deriving conclusions.

Another possible limitation of the proposed sibling and maternal transmission studies is the inability to adjust for *H. pylori* status of other family members within the household (e.g. the father's *H pylori* status). Some studies have found positive associations between the *H. pylori* status of the father or other members within the household and the *H. pylori* status of the child, although the majority of studies have found little or no such associations (Weyermann et al., 2009). If the infection status of the father or other family members did influence the *H. pylori* status of the Pasitos Cohort index child or younger siblings, then lack of adjustment for *H. pylori* status of other household members in the analysis may have resulted in confounding.

Also, the studies did not utilize analysis methods to account for censored time and within household dependent observations; this was also previously not done in other studies pertaining to *H. pylori* infection in childhood. In these studies, this analysis was not done due to lack of availability of analysis software necessary to perform multi-level modeling. In order to adjust for a multi-level dependency due to inclusion of multiple children within one household, modified modeling should be performed. This modeling uses a working independent general estimation equation approach to ensure corrected standard error and variance therefore providing more accurate confidence interval estimation (Lee, Wei, & Amato, 1992).

Lastly, for the current studies, no bacterial genotyping information was obtained to examine the specific strains from household members. Although not necessary to make causal inferences, genotyping based evidence could support study hypotheses and

results. If *H. pylori* bacterial strains isolated from the index child and younger siblings were identical or similar, this would support the conclusion of the epidemiological studies that have shown evidence that younger siblings had a relatively high frequency of strain concordance with an older sibling (Han et al., 2000; Herrera et al., 2008; Kivi et al., 2003; Raymond et al., 2008; J. T. Wang, Sheu, Lin, Wang, & Wu, 1993),. As the Pasitos Cohort Study aimed to identify risk factors for *H. pylori* infection in children, invasive procedures to collect biopsies and isolate *H. pylori* were not warranted and possibly detrimental to the participant. *H. pylori* infection in children typically does not result in symptoms and as previously mentioned, such infections often spontaneously resolve (Broussard et al., 2009). A more general limitation of *H. pylori* transmission studies, such as this one, is that the infection status of family members is an imperfect proxy for person-to-person transmission in that concordant status of family members is also consistent with shared exposure to another environmental infection source. The more exposure to environmental sources of infection that are shared within families, the greater the evidence of sibling-sibling or parent-child transmission will come from showing varied degrees of *H. pylori* status concordance in distinct types of family relationships.

These studies, as with all studies, have limitations that may result in biased estimates of association pertaining to initial *H. pylori* infection, short-term and long term infection establishment, yet these analyses contribute valuable evidence due to strengths previously summarized. Previous studies have aimed to examine the association between the mother and her child's *H. pylori* infection as well as association between *H. pylori* infection and infection in siblings. But the studies in the current

literature have at least one of the following potentially limiting issues: 1) cross-sectional observation of study participants; 2) lack of consideration of causal mechanisms in *H. pylori* transmission; 3) assessment of combined active and past *H. pylori* infection via antibody development in children <10 years of age; and 4) inability to detect and differentiate between *H. pylori* outcomes (infection acquisition versus short-term or long-term infection establishment). Aim B (sibling transmission study) addressed all of these issues, determining associations of sibling in *H. pylori* transmission and active infection during what is thought to be the most critical period of transmission, childhood. For Aim A pertaining to maternal-child transmission, effects on the risk of distinct active *H. pylori* infection outcomes in children were estimated and causal pathways were considered in selecting covariates for inclusion in regression models. But, unlike results obtained in Aim B, estimates of effect obtained exhibited greater uncertainty regarding the magnitude and direction of these effects. Time-to-event analysis relies on the number of observed events and sparse data led to suboptimal precision for estimating effects, particularly for Aims A and C, and particularly for the short- and long-term infection outcomes. Hence, results from Aims A and C (estimating the association of breastfeeding in *H. pylori* infection outcomes) must be considered inconclusive, particular pertaining to effects on short- and long-term infection. For the analyses of effects on overall incidence of *H. pylori* infection in children, without regard to duration, this study provides evidence that the association between the mother's and child's infection status is at most modest in strength and considerably weaker than the association between siblings' status.

Cross-sectional observations, the study design utilized by the majority of studies addressing *H. pylori* transmission, are limited in their ability to identify directionality of transmission. When attempting to determine the direction of transmission, it is critical to identify the temporal order of *H. pylori* incidence within a family. It is also important to note that as the possibility for short-term infections in childhood is high, cross-sectional observations will not identify past *H. pylori* infections and therefore may led to misclassification of the outcome in maternal transmission studies as well as the exposure in sibling studies. Furthermore, advances in *H. pylori* diagnostic methods in children have found that serological methods utilized to detect *H. pylori* infection in many studies may have diminished sensitivity in pediatric populations < 10 years of age (Rowland et al., 2006); this again may lead to misclassification of the outcome and/or exposure.

Relatively few studies have been conducted to examine the causal relationship between breastfeeding and *H. pylori* infection (Chak et al., 2009). Again, as with mother-child (Aim A) and child-child transmission (Aim B) studies, no studies in the accessible literature pertaining to breastfeeding and *H. pylori* infection have presented DAGs to illustrate hypothesized causal mechanisms in the acquisition of *H. pylori* infection. Also, to date no *H. pylori* breastfeeding studies in the accessible literature have examined the effect of breastfeeding on *H. pylori* acquisition, short and long-term *H. pylori* infection.

As with Aim A, Aim C (estimating the associations between breastfeeding and *H. pylori* infection outcomes) must be considered inconclusive due to a high degree of uncertainty. As the Pasitos Cohort Study was longitudinal in nature, it was possible to

attempt to address issues relating to the direction of *H. pylori* transmission; in addition, it was possible to define the outcome and/or exposure, *H. pylori* infection, as differing infection states. As most studies conducted to date were not longitudinal, the assessment of initial, short and long-term infection has not been examined in previous studies. Both the maternal transmission study and sibling transmission study defined the exposure and outcome in terms of initial infection and long-term infection. The breastfeeding study not only defined the outcome as initial and long-term infection, but also short-term infection. In all studies, differing time-to-event scenarios were presented to explore differing outcome definitions. Overall, the results of analyses presented in this dissertation indicate that interpretations remain unchanged from one time-to-event scenario over another especially in defining time to a short-term infection. But in defining time to long-term infection establishment on the outcome of long-term infection in a child, increasing time to event did increase the magnitude of the estimated effect increasing from scenario one to three. This increase may be an artifact of time-to-event definition which future studies will need to consider.

Public Health Significance

Research that sheds light on *H. pylori* transmission and the mechanisms that modulate the establishment of a long-term infection are critical for the goal of reducing the extensive global burden of morbidity and mortality due to *H. pylori*. Prevention of *H. pylori* infection is imperative as secondary and tertiary intervention strategies require expensive medical treatments and procedures which are often inaccessible in areas of highest *H. pylori* prevalence such as developing countries (Goodman et al., 1996). Even

when antibiotic treatment is available, eradication therapy is often not effective at reducing *H. pylori* prevalence, due to high rates of *H. pylori* re-infection (Leal-Herrera et al., 2003) or antibiotic resistance.

Helicobacter pylori infection is widespread, with approximately half of the world infected (Khalifa et al., 2010). The greatest burden of infection occurs in the developing world, with a reported prevalence of 90% or greater in some areas (Khalifa et al., 2010). Although peptic ulceration occurs in approximately 10% and stomach cancer in only roughly 1% of those with long-term infections (Mourad-Baars et al., 2010), due to high *H. pylori* prevalence, this translates to over 500,000 peptic ulcers and over 600,000 case of stomach cancer per year. This dissertation contributes to the current body of research as it addresses factors which may be integral to understanding *H. pylori* transmission and establishment of infection. The results of Aim B (sibling-to-sibling infection associations) show evidence consistent with primarily unidirectional transmission from older to younger sibling, unlike other infectious agents commonly associated with gastrointestinal involvement such as salmonella or shigella (Khalifa et al., 2010). In addition to contributing to the understanding of *H. pylori* transmission and long-term infection establishment, results from Aim C (breastfeeding association in *H. pylori* infection outcomes) although imprecise, provide some epidemiological basis to support past and future laboratory-based, experimental studies investigating the interplay of the immune response to *H. pylori* infection. Additional and supporting data may reveal factors for targeted research and intervention during childhood as this represents a unique as well as critical time period in which immune response is being developed (Szczawinska-Poplonyk, 2012). In regards to Aim A, the results pertaining to

the estimated effect of the mother's *H. pylori* infection status on the child's rate of acquiring the infection provide evidence against a strong effect on overall acquisition but inconclusive with respect to long-term infection due to highly imprecise results. Hence results from Aim A may lead to additional longitudinal studies or meta-analyses with sufficient infection events which may contribute to hypotheses.

The study pertaining to breastfeeding and *H. pylori* outcomes (Aim C) also must be considered exploratory due to sparse data. In this study, it was found that breastfed children with *H. pylori* infected siblings exhibited a lower rate of long-term infection with *H. pylori* but a greater rate of short-term infection. This may be due to an interaction of 1) *H. pylori* transmission efficient strains in older, index siblings in combination with 2) immune development of the host. Hence, this may lead to future studies focusing on the identification of *H. pylori* bacterial factors which may illicit immune stimulation for the selection of *H. pylori* strains which may then lead to a shift in *H. pylori* strains: from a predominance of strains which transmit efficiently to strains which are less efficient in transmission and possibly more adapt to the host's active and robust immune response leading to a long-term infection. It is important to note that the role of duration of infection and stain characteristics is itself an area of new research exploration and potentials but these factors may then one day contribute to the development of a vaccine. It has been estimated that an effective vaccine to protect against *H. pylori* colonization may lead to a 42% reduction in the incidence of gastric cancer (Ford & Axon, 2010). Again, research leading to possible vaccine development addresses a primary public health goal, the prevention of infection in populations most susceptible to disease and death burden.

Conclusions

The studies conducted found that although both the mother and siblings may be positively associated with *H. pylori* in childhood, the estimated effect of and older sibling's infection on that of a younger sibling's was of greater magnitude and in a unilateral fashion. In regards to maternal associations and breastfeeding studies, additional studies with sufficient data should allow for more definitive conclusions. Larger sample sizes are needed to obtain results with more certainty if time-to-event analysis is to be used. Future analysis pertaining to breastfeeding and *H. pylori* infection will include 140 additional observations of the younger siblings which were not available during the current study. Lastly, multilevel analysis should be conducted to obtain more accurate confidence interval estimations.

APPENDICIES

APPENDIX A

DIRECTED ACYCLIC GRAPH FOR MOTHER-CHILD *HELICOBACTER PYLORI* INFECTION ASSOCIATIONS
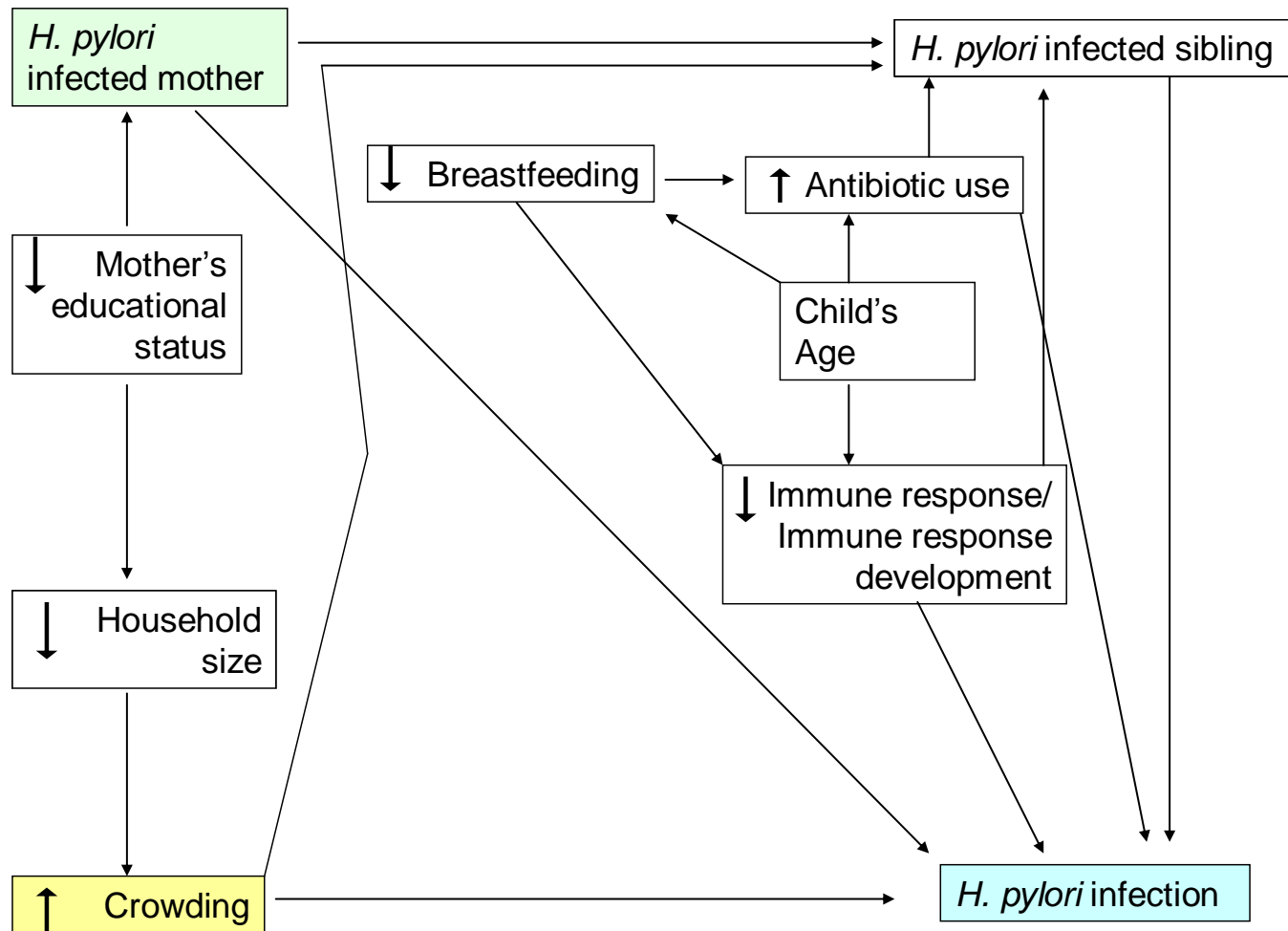
Exposure highlighted in green, outcome highlighted in blue, covariates for adjustment highlighted in yellow.

APPENDIX B

DIRECTED ACYCLIC GRAPH FOR SIBLING-CHILD *HELICOBACTER PYLORI* INFECTION ASSOCIATIONS
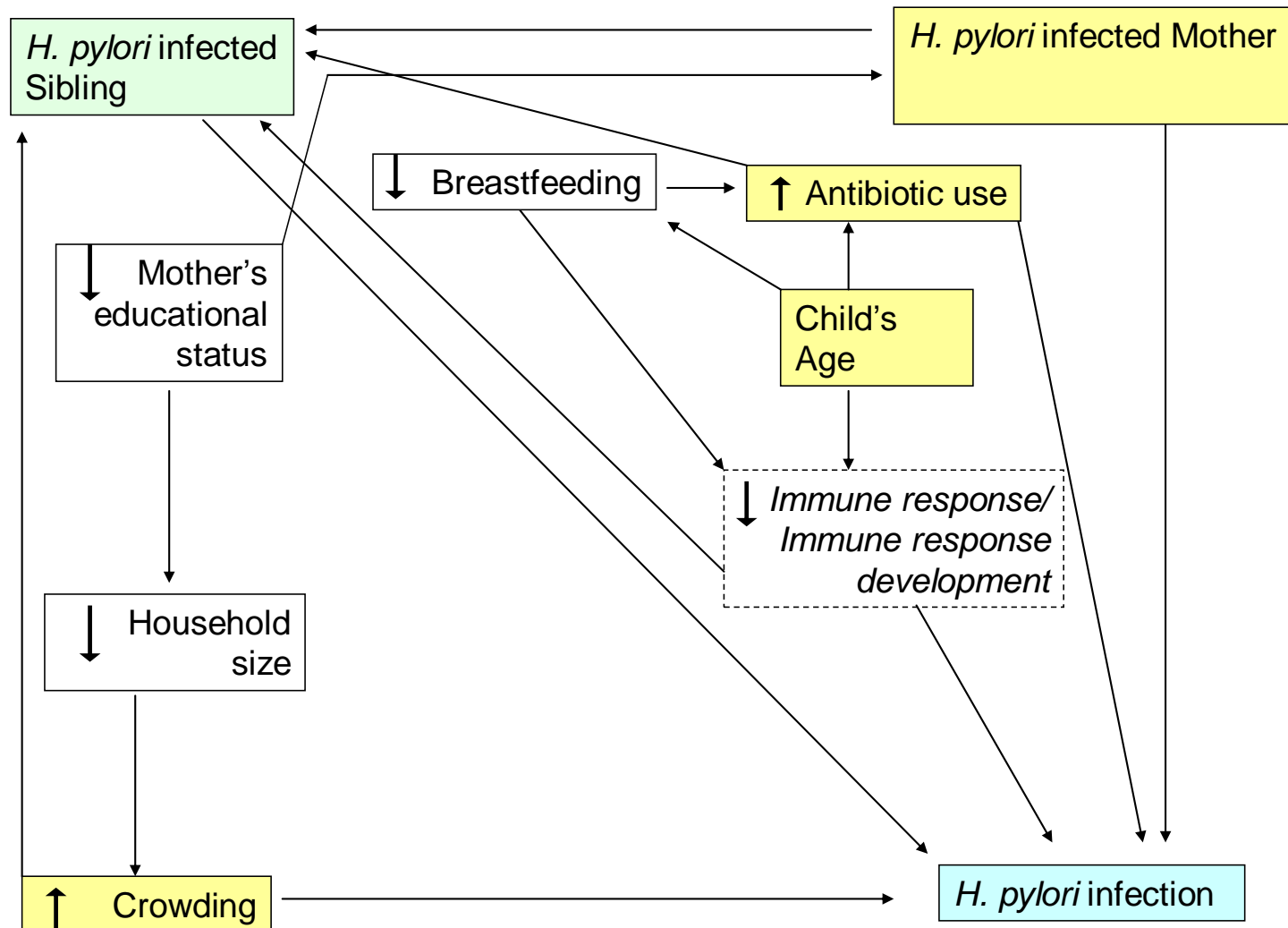
Exposure highlighted in green, outcome highlighted in blue, covariates for adjustment highlighted in yellow.

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APPENDIX C

DIRECTED ACYCLIC GRAPH FOR BREASTFEEDING AND *HELICOBACTER PYLORI* INFECTION ASSOCIATIONS
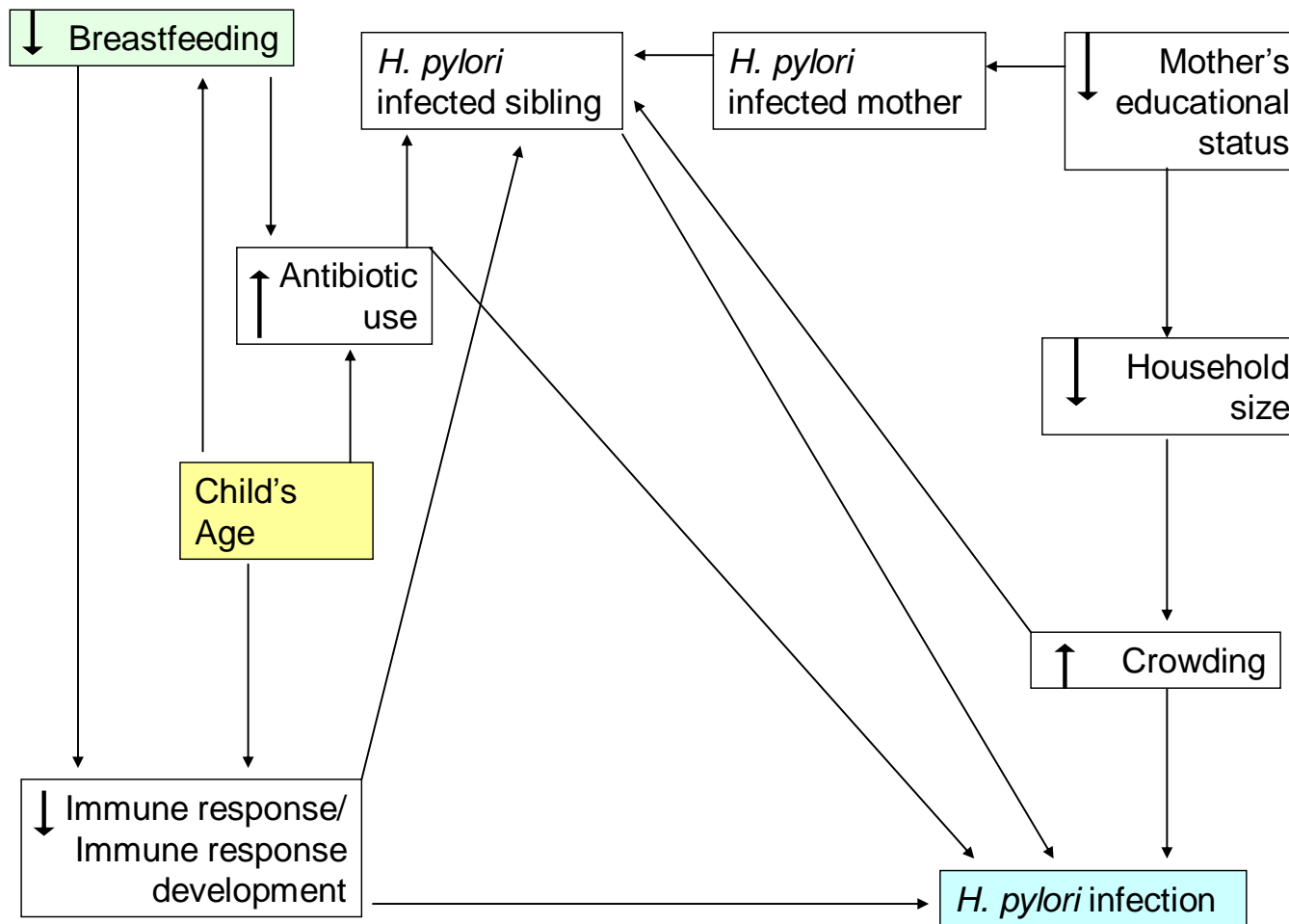
Exposure highlighted in green, outcome highlighted in blue, covariates for adjustment highlighted in yellow.

Appendix D

MOTHER-CHILD *HELICOBACTER PYLORI* INFECTION ASSOCIATIONS LITERATURE REVIEW ARTICLES

First Author	Publication Year	Study Title	Study Design	Study Population	Study Location	Inclusion & Exclusionary Criteria	Sample Size	Helicobacter pylori Detection Methodology	Reported Diagnostic Sensitivity/ Specificity	Follow-up	Statistical Analysis Methods	P-value Based Results	Measure of Association	Covariate Adjustments	Effect Measures Modifiers	Point Estimates (95% Confidence Interval) Crude	Point Estimates (95% Confidence Interval) Adjusted
Drumm, B	1990	Interfamilial clustering of *Helicobacter pylori* infection	Cross-sectional	Children (age not specified)	Toronto (Canada)	Exclusion: Children not attending The Hospital for Sick Children for endoscopy due to upper GI symptoms	93	Patients: Histology & Culture Mothers: IgG Immunoassay	Not stated	NONE	Hypothesis testing	Mother Hp seropositive in Hp infected child (36/51 or 83%) vs. Mother Hp seropositive in non-Hp infected child (2/17 or 12%) (P<0.001)	NA	NA	NONE	NA	NA
Malaty, HM	1991	Transmission of *Helicobacter pylori* Infection: Studies in families of healthy individuals	Cross-sectional	U.S. families (41 families, 151 people; 69 children); parents age range: 19-40 yrs of age; children's age range: 1- 18 yrs of age	Houston, Texas (United States)	Exclusion: Those not members of a family (two parents and at least one biological child), those not born in the United States, those not in self described good health; any family in which a member had a history of peptic ulcer or upper GI tract symptoms occurring more than once a month	69	IgG immuno assay &^{13}C-UBT (either test positive)	Not stated	NONE	Hypothesis testing	42% of Hp positive children had a mother who was also positive compared to 6% of Hp positive children whose mother was Hp negative (p<0.01)	NA	NA	NONE	NA	NA
Bamford, KB	1993	Helicobacter pylori: comparison of DNA fingerprints provides evidence for intrafamilial infection	Laboratory analysis	Children (ages not provided)	Not stated	Inclusion: patients of pediatric clinics; Exclusion: Asymptomatic children, children without abdominal pain	8 children, 4 parents	Biopsy/ Culture; Ribotyping/ RE assay	Not stated	NONE	Descriptive Analysis	7 of 8 parents Hp pos, all children symptomatic and Hp pos. It was found that in one family mother, father and child had the same Hp strain.	NA	NA	NONE	NA	NA
Gasbarrini, G	1995	A population based study of *Helicobacter pylori* infection in a European country: the San Mario study. Relations with gastrointestinal diseases	Cross-sectional	2,237 (>18 yrs of age)	San Marino (Italy)	NONE	2,237	IgG immunoassay	Not stated	NONE	Unconditional logistic regression	NA	Odds Ratios	NONE	NONE	**Dyspepsia of mother (Y/N; no referent):** 0.76 (0.53, 1.08) **Gastric cancer history of mother (Y/N; N referent):** 1.38 (0.79, 2.38)	NA

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														Point Estimates (95% Confidence Interval)	
First Author	Publication Year	Study Title	Study Design	Study Population	Study Location	Inclusion & Exclusionary Criteria	Sample Size	*Helicobacter pylori* Detection Methodology	Reported Diagnostic Sensitivity/ Specificity	Follow-up	Statistical Analysis Methods	P-value Based Results	Measure of Association	Covariate Adjustments	Effect Measures Modifiers	Crude	Adjusted
Ma, J	1998	*Helicobacter pylori* infection and mode of transmission in a population at high risk of stomach cancer	Cross-sectional	Children (3-12 yrs of age)	Linqu County (China)	NONE	49	^{13}C-UBT (children)/ IgG immunoassay (parents)	Not stated	NONE	Unconditional logistic regression	NA	Odds Ratios	Sex, age	NONE	Not provided	**Mother's Hp immuno status status (yes vs. no; no referent):** 30.1 (2.8, 325.2)
Brenner, H	1998	Parental History of gastric or duodenal ulcer and prevalence of *Helicobacter pylori* infection in preschool children: population based study	Cross-sectional	Children (6 yrs of age)	Germany	Exclusion: Children who received antibiotics 4 weeks prior to Hp testing	945	^{13}C-UBT	Not stated	NONE	Unconditional logistic regression	NA	Odds Ratios	Nationality, mother's education, father's education, housing density, birth order, history of breast feeding, attendance at nursery, antibiotic treatment, history of ulcer in father	NONE	**Mother's history of peptic ulcer (Y/N; no referent):** 2.4 (0.9, 6.4)	**Mother's history of peptic ulcer (Y/N; no referent):** 11.7 (3.8, 36.2)
Kikuchi, S	1998	*Helicobacter pylori* risk associated with sibship size and family history of gastric diseases in Japanese adults	Cross-sectional	Adults (19-69 yrs of age)	Japan	Inclusion: Restricted to public service workers	4,361	IgG Immunoassay	Not stated	NONE	Unconditional Logistic Regression	NA	Odds Ratios	Age and sex	NONE	Not Provided	**Mother's history of gastric disease (negative referent): positive:** 1.25 (0.91, 1.72); **unknown:** 1.10 (0.84, 1.44)
Dowsett, SA	1999	Helicobacter pylori infection in indigenous families of Central America: serostatus and oran and fingernail carriage	Cross-sectional	Children ≥12 yrs old	Guatemala	NONE	242	IgG Immunoassay	Not stated	NONE	Hypothesis testing	Mother Hp seropositive in Hp seropositve child vs. seronegative child (P=0.02)	NA	NA	NONE	NA	NA
Rothenbacher, D	1999	*Helicobacter pylori* among preschool children and their parents: Evidence of parent-child transmission	Cross-sectional	Children (5-8 years of age)	Ulm (Germany)	Exclusion: Children who had received antibiotics within the prior four weeks to Hp infection assessment	1,143	^{13}C-UBT	Not stated	NONE	Unconditional logistic regression	NA	Odds Ratios	**Partial Adjustment:** nationality **Full Adjustment:** nationality, age, sex, place of birth, birhtweight, education of father, education of mother, history of breast feeding, history of antibiotic use, housing density	NONE	**Mother's Hp infection status (yes vs. no; no referent):** 10.0 (5.1, 19.4) **Full Adjustment:** 16.5 (8.8, 30.8)	Partial Adjustment: Mother's Hp infection status (yes vs. no; no referent): 10.0 (5.1, 19.4) **Full Adjustment:** Mother's Hp infection status (yes vs. no; no referent): 7.9 (4.0, 15.7)

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													Point Estimates (95% Confidence Interval)	
First Author	Publication Year	Study Title	Study Design	Study Population	Study Location	Inclusion & Exclusionary Criteria	Sample Size	*Helicobacter pylori* Detection Methodology	Reported Diagnostic Sensitivity/ Specificity	Follow-up	Statistical Analysis Methods	P-value Based Results	Measure of Association	Covariate Adjustments	Effect Measures Modifiers	Crude	Adjusted
Han, SR	2000	*Helicobacter pylori*: Clonal population structure and restricted transmission within families revealed by molecular typing	Laboratory analysis	9 index children and 27 family members: 23 children 2-18 yrs of age and 4 adults 29-44 yrs of age	Mainz (Germany)	Inclusion: Patients of Dept of Pediatrics, Gutenberg University	59	Gastric biopsy/ Culture; RFLP & PFGE	Not stated	NONE	Descriptive Analysis	it was found that of nine families, mothers and sibs shared the same Hp strain in five families	NA	NA	NA	NA	NA
Zhou, H	2000	Intrafamilial spread of *Helicobacter pylori*: A prospective study using urea breath test	Case-Control	Children (age not specified);	Hong Kong (China)	Exclusion: Children who were not dyspeptic	32	Gastric biopsy/histology (^{13}C-UBT family members)	Not stated	NONE	Hypothesis testing	Hp infected mother in Hp infected children (14/16 or 88%) vs. non-infected children (5/15 or 33%) P<0.01	NA	NA	NA	NA	NA
Malaty, HM	2000	Evidence from a nine-year birth cohort study in Japan of transmission of pathways of *Helicobacter pylori* infection	Cross-sectional	46 Japanese families (Adults and children); ages not specified	Nagano Prefecture, (Japan)	NONE	116	IgG Immunoassay	Sensitivity: 93% Specificity: 97%	Annually for nine years	Unconditional logistic regression	NA	Odds Ratios	NONE	NONE	**Hp serostatus of mother (pos vs. neg; neg referent):** 5.3 (0.6, 42.8)	NA
Krumbiegel, P	2000	*Helicobacter pylori* prevalence in Leipzig's 1998 school entries: methodology and first results	Cross-sectional	Children (6 yrs old)	Leipzig, Germany	NONE	1,890	^{13}C-UBT (Childenable to provide breath sample)/ 15N urea Urine test (Children not able to) provide breathe test)	Not stated	NONE	Descriptive Analysis	of 161 pos children, 65% of mother's Hp infected	NA	NONE	NONE	NA	NA
Miyaji, H	2000	*Helicobacter pylori* infection occurs via close contact with infected individuals in early childhood	Cross-sectional	Children (10-16 yrs of age)	Japan	NONE	80	IgG Immunoassay	Not stated	NONE	Unconditional logistic regression	NA	Odds Ratios	NONE	NONE	**Hp serostatus of mother (pos vs. neg; neg referent):** 7.36 (1.57, 34.59)	NA
Miyaji, H	2000	*Helicobacter pylori* infection occurs via close contact with infected individuals in early childhood	Cross-sectional	Children (10-16 yrs of age)	Japan	NONE	86	IgG Immunoassay	Not stated	NONE	Unconditional logistic regression	NA	Odds Ratios	NONE	Younger vs. Older Sibling	**Hp serostatus of mother (pos vs. neg; neg referent):** 13.30 (1.71, 103.3)	NA

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First Author	Publication Year	Study Title	Study Design	Study Population	Study Location	Inclusion & Exclusionary Criteria	Sample Size	*Helicobacter pylori* Detection Methodology	Reported Diagnostic Sensitivity/ Specificity	Follow-up	Statistical Analysis Methods	P-value Based Results	Measure of Association	Covariate Adjustments	Effect Measures Modifiers	Point Estimates (95% Confidence Interval) Crude	Point Estimates (95% Confidence Interval) Adjusted
Brenner, H	2000	*Helicobacter pylori* infection among offspring of patients with stomach cancer	Cross-sectional	Adults (30-74 yrs of age)	Germany	Exclusion: adults < 30 and > 74	1,351	IgG Immunoassay	Sensitivity: 94% Specificity: 79%	NONE	Unconditional logistic regression	NA	Odds Ratios	Age, sex, education, alcohol consumption	NONE	**Mother's stomach cancer status (Y/N; No cancer referent): 4.8 (1.6, 14.5)**	**Mother's stomach cancer status (Y/N; No cancer referent): 3.2 (1.0, 10.2)**
Al Knawy, BA	2000	Intrafamilial clustering of *Helicobacter pylori* infection in Saudi Arabia	Cross-sectional	Children; ages not provided	Saudia Arabia	NONE	79	IgG immunoassay	Not stated	NONE	Descriptive Analysis	it was found as the number of seropositive children increases so does the % of seropositve mothers and fathers. In 79 children, If a mother is seropos, 22.8% of children were seropos while if only father was seropos, 16.4% were pos. If both mother and father seropos, 54.4% of children seropos.	NA	NA	NONE	NA	NA
Kurosawa, M	2000	*Helicobacter pylori* infection, gastritis and gastric cancer: Helicobacter pylori infection among Japanese children	Cross-sectional	Children, 6 & 14 yrs old	Japan	Exclusion: Subjects with inconsistent test results (not pos both in 1995 and 1996)	504	IgG Immunoassay	Not stated	NONE	Unconditional logistic regression	NA	Odds Ratios	sex and age	NONE	Not provided	**Maternal history of stomach disease (Y/N; N referent): 2.771 (1.083, 7.090)**
Taneike, I	2001	*Helicobacter pylori* intrafamilial infections: change in source of infection of a child from father to mother after eradication therapy	Laboratory analysis	1 family, 4 members (ages not specified); Juntendo Hospital	Tokyo, Japan	NONE	2 parents 2 children	Biopsy/ Culture; RFLP, PFGE & Ribotyping	Not stated	NONE	Descriptive Analysis	After a symptomatic child was successfully treated, it was found he harbored the identical strain of his father. All infected family members but mother (hp pos) underwent eradication therapy. 36 weeks later, Child was reinfected with identical strain of mother.	NA	NA	NONE	NA	NA

First Author	Publication Year	Study Title	Study Design	Study Population	Study Location	Inclusion & Exclusionary Criteria	Sample Size	*Helicobacter pylori* Detection Methodology	Reported Diagnostic Sensitivity/ Specificity	Follow-up	Statistical Analysis Methods	P-value Based Results	Measure of Association	Covariate Adjustments	Effect Measures Modifiers	Crude	Adjusted
Yilmaz, E	2002	Seroprevalence of *Helicobacter pylori* infection among children and their parents in eastern Turkey	Cross-sectional	Children, (6 mos-16 yrs); Firat Universtiy Medical Center	Turkey	Exclusion: Those presenting with gastrointestinal bleeding and abdominal pain, children taking antimicrobial drugs 2 months prior to study, those taking immunosuppressive drugs or chemotherapeutic drugs, children with primary and secondary immunodeficiencies	346	IgG Immunoassay	Not stated	NONE	Hypothesis testing	Child Hp seropositive with Hp seropositive mother vs. mother Hp seronegative (104 or 49% vs. 7 or 22.6%) P<0.002	NA	NA	NA	NA	NA
Miyazaki, M	2002	Intrafamilial transmission of *Helicobacter pylori*: the association between a parent and an offspring with respect to the presence of anti-CagA antibodies	Cross-sectional	Adults (22-79 yrs old)	Fukuoka (Japan)	NONE	1,910	IgG Immunoassay	Sensitivity: 94.9% Specificity: 91.3%	NONE	Unconditional logistic regression	NA	Odds Ratios	Age of offspring	NONE	Not provided	**Mother's Hp serostatus (pos vs. neg; neg referent): 8.08 (1.39, 40.04)**
Rothenbacher, D	2002	Role of infected parents in transmission of *Helicobacter pylori* to their children	Cross-sectional	Children (5-7 yrs of age)	Ulm (Germany)	Exclusion: Children who had received antibiotics within the prior four weeks to Hp infection assessment	305	¹³C-UBT (children)/ IgG saliva immunoassay (parents)	Not stated	NONE	Unconditional logistic regression	NA	Odds Ratios	Hp status of father, nationality, age, sex, place of birth, birhtweight, education of father, education of mother, history of breast feeding, history of antibiotic use, housing density	NONE	Not provided	**Mother's Hp immunostatus status (yes vs. no; no referent): 3.9 (1.4, 10.6)**
Chang, YW	2002	Role of *Helicobacter pylori* infection among offspring or siblings of gastric cancer patients	Case-Control	Korean adults (19-74 yrs old, mean 45 yr)	Korea	Exclusion: if younger than 18 and older than 75; if had any serious medical conditions; cases were first degree relatives Gca patients	726: 300 cases; 426 controls	Biopsy with rapid urease and Giemsa stain	Not stated	NONE	Unconditional logistic regression	NA	Odds Ratios	Age, sex, years of school education, socioeconomic status, place of residence, number of siblings,	Age (19-40) and (41-74)	**Mother with history of gastric cancer (compared to controls): 1.2 (0.7, 2.0)**	**Mother with history of gastric cancer (compared to controls): 1.3 (0.8, 2.3)**
Rocha, GA	2003	Transmission of *Helicobacter pylori* infection in families of preschool-aged children from Minas Gerais, Brazil	Cross-sectional	Children (2 mos- 8 yrs of age)	Minas Gerais (Brazil)	NONE	66	¹³C-UBT (Children ≤ 12 yrs of age)/ IgG immunoassay (Children > 12 years of age)	¹³C-UBT: sensitivity: 95.4-100%, specificity 93.1-100% IgG immunoassay: sensitivity: 99.5% specificity: 99.0%	NONE	Unconditional logistic regression	NA	Odds Ratios	age, gender, number of children in household, Hp status of father, Hp status of siblings	NONE	**Mother's Hp serostatus (pos vs. neg; neg referent): 21.4 (2.5, 471.7)**	**Mother's Hp serostatus (pos vs. neg; neg referent): 22.7 (2.31, 223.21)**

Point Estimates (95% Confidence Interval)

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First Author	Publication Year	Study Title	Study Design	Study Population	Study Location	Inclusion & Exclusionary Criteria	Sample Size	*Helicobacter pylori* Detection Methodology	Reported Diagnostic Sensitivity/ Specificity	Follow-up	Statistical Analysis Methods	P-value Based Results	Measure of Association	Covariate Adjustments	Effect Measures Modifiers	Crude	Adjusted
O'Rourke, K	2003	Determinants of geographic variation in *Helicobacter pylori* infection among children on the US-Mexico border	Cross-sectional	Children > 6 yrs of age	Texas (United States) & Juarez (Mexico)	Exclusion: Children > 6 yrs of age; non-sibling of index child of birth cohort	264	IgG immunoassay	Sensitivity: 97% Specificity: 94%	NONE	Unconditional logistic regression	NA	Odds Ratios	Location (El Paso/Juarez), age, maternal education, household crowding, number of indoor bathrooms	Seropositivity cut off (≥1.8=pos, >2.2=pos and ≥2.0)	Not provided	**Seropositivity cut off- ≥1.8=pos: maternal seropositivity (pos vs. neg/indeterminate; neg/ind referent): 2.63 (0.99, 7.00) >2.2=pos maternal seropositivity (pos vs. neg/indeterminate; neg/ind referent): 2.54 (0.79, 8.18) and ≥2.0 maternal seropositivity (pos vs. neg/indeterminate; neg/ind referent): 2.84 (1.0, 8.10)**
Kivi, M	2003	Concordance of *Helicobacter pylori* strains within families	Laboratory analysis	Children (10-12 yrs old)	Sweden	NONE	104	Culture; RFLP	Not stated	NONE	Descriptive Analysis	56% (10/18) mother-child strain concordance	Descriptive stats	NA	NA	NA	NA
Chang, PS	2003	Household *Helicobacter pylori* antibody survey in children with upper gastrointestinal symptoms	Cross-sectional	Children (0.9-15.3 yrs old)	Taiwan	Inclusion: Restricted to children with upper GI symptoms	112	Biopsy & culture (index); IgG immunoassay (family)	Not stated	NONE	Hypothesis testing	In Hp infected children, 84.7% of mothers were Hp seropositive versus only 70.1% of mothers of non-Hp infected children (P=0.093)	NA	NA	NA	NA	NA
Escobar, ML	2004	Evidence of mother-child transmission of *Helicobacter pylori* infection	Cross-sectional	Children (2-14 years of age)	Brazil? (not stated)	Exclusion: Those not family members of 38 dyspeptic patients (2-20 yrs of age) who were Hp positive by histology and urease rapid test	112	IgG immunoassay	Not stated	NONE	Hypothesis testing	Mother Hp seropositive in Hp infected patient (22/27 81%) vs. Mother Hp seropositive in non-Hp infected patient (2/11 18%): P=0.001	NA	NA	NA	NA	NA
Sinha, SK	2004	The incidence of *Helicobacter pylori* acquisition in children of a Canadian First Nations Community and the potential for parent-to-child transmission	Cohort	Children (1-13 yrs of age)	Wasagamack (Canada)	Exclusion: Those not belonging to First Nations American aboriginal population	50	SAT	Not stated	1 year	Hypothesis testing	50 children who tested negative in 1999 were tested via SAT; 8 were positive and it was found that all of their mothers were Hp pos and only Five of 8 had no infected siblings and 3 had infected siblings.	NA	NA	NA	NA	NA

| | | | | | | | | | | | | | | | | Point Estimates (95% Confidence Interval) | |
First Author	Publication Year	Study Title	Study Design	Study Population	Study Location	Inclusion & Exclusionary Criteria	Sample Size	*Helicobacter pylori* Detection Methodology	Reported Diagnostic Sensitivity/ Specificity	Follow-up	Statistical Analysis Methods	P-value Based Results	Measure of Association	Covariate Adjustments	Effect Measures Modifiers	Crude	Adjusted
Fujimura, S	2004	Detection of *Helicobacter pylori* in the stools of newborn infants	Longitudinal	Infants	Japan	NONE	50	SAT & PCR (children); IgG immunoassay (urine-mothers)	Not stated	3 days after birth & 24 months of age	Hypothesis testing	Detection rate of Hp DNA in newborns was higher for mother's with Hp positive IgG (60%) than those for mother's with negative Hp IgG (17%) P<0.01	NA	NA	NONE	NA	NA
Aguemon, BD	2005	Prevalence and risk-factors for *Helicobacter pylori* infection in urban and rural Beninese populations	Cross-sectional	Children (2-15 yrs of age)	Cotonou, Benin (Africa)	Exclusion: Persons with a history of gastroduodenal ulcer, with current complaints of the upper digestive tract for >2 mos (nausea, vomiting, heartburn, pyrosis or indigestion), those currently using anti-ulcer medications	446	IgG Immunoassay	Adults: sensitivity: 95.6% specificity: 94.3% Children: sensitivity: 90.1% specificity: 90.3%	NONE	Unconditional logistic regression	NA	Odds Ratios	Sleeping accommodation density	NONE	**Mother's Hp serostatus (pos vs. neg; neg referent):** 5.3 (1.52, 18.45)	**Mother's Hp serostatus (pos vs. neg; neg referent):** 3.85 (1.53, 9.67)
Kivi, M	2005	*Helicobacter pylori* status in family members as risk factors for infection in children	Cross-sectional	Children (11-13 yrs of age)	Stockholm, Sweden	Exclusion: Any children who at least one family member did not provide blood for study	162	IgG immunoassay & Western blot	IgG immunoassay: sensitivity: 98% specificity: 96% Western blot: sensitivity: 94% specificity: 98%	NONE	Unconditional logistic regression	NA	Odds Ratios	infection status of siblings, country of birth of the index child, household SES, antibiotic consumption	NONE	Not provided	**Mother's Hp serostatus (pos vs. neg; neg referent):** 11.6 (2.0, 67.9)
Yang, YJ	2005	Children of *Helicobacter pylori*-infected dyspeptic mothers are predisposed to H. pylori acquisition with subsequent iron deficiency and growth retardation	Cross-sectional	Children (1-16 yrs of age);	Taiwan	Inclusion: Dyspeptic Hp infected mothers; Exclusion: Children who had taken antibiotics or acid secretion inhibitors within the past 4 weeks	163	^{13}C-UBT & SAT	Not stated	NONE	Unconditional logistic regression	NA	Odds Ratios	NONE	Number of Hp infected Siblings (1 and >1 sibling in HH	**Hp infected mother (pos vs. neg; neg referent): living with one Hp infected sibling:** 10.8 (2.6, 45.9); **living with > 1 Hp infected sibling:** 21.7 (2.0, 231)	NA

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First Author	Publication Year	Study Title	Study Design	Study Population	Study Location	Inclusion & Exclusionary Criteria	Sample Size	*Helicobacter pylori* Detection Methodology	Reported Diagnostic Sensitivity/ Specificity	Follow-up	Statistical Analysis Methods	P-value Based Results	Measure of Association	Covariate Adjustments	Effect Measures Modifiers	Point Estimates (95% Confidence Interval) Crude	Adjusted
Konno, M	2005	Five-year follow-up study of mother-to-child transmission of *Helicobacter pylori* infection detected by a random amplified polymorphic DNA fingerprinting method	Laboratory analysis	Children, infants	Japan	Exclusion: Children whose mothers were not Hp infected (serology)	44	SAT & Culture (string tests); Lab analysis, RAPD	Not stated	5 years (4-6 mos intervals after 1st 6 mos)	Descriptive Analysis	From year 2-5, 5/44 (11%) became seropos and all of these children had the same or similar strain as the mother's hp strain	NA	NA	NA	NA	NA
Farrell, S	2005	Risk factors for *Helicobacter pylori* infection in children: an examination of the role played by intrafamilial bed sharing	Cross-sectional	Children	Ireland	NONE	178	Biopsy & urease (index)/ ^{13}C-UBT	Not stated	NONE	Unconditional logistic regression	NA	Odds Ratios	Not stated? Assume crude measure	NONE	**Hp infected mother (Y/N; No referent):** 2.52 (1.03, 6.13)	NA
Rodrigues, MN	2006	History of breastfeeding and *Helicobacter pylori* in children: results of a community based study from northeast Brazil	Cross-sectional	Children (≤ 14 years of age)	Fortaleza, Ceará, (Brazil)	NONE	353	^{13}C-UBT	Not stated	NONE	Unconditional logistic regression	NA	Odds Ratios	Age, nutritional status, education of mother, history of antibiotic use, smoking of mother, number of persons per room, number of children per household, breast feeding	NONE	**Mother Hp seropositive (Y/N, No referent):** OR=3.11 (1.57, 6.19)	**Mother Hp seropositive (Y/N, No referent):** OR=2.54 (1.12, 5.15)
Nguyen, BV	2006	Prevalence of and factors associated with *Helicobacter pylori* infection in children in the north of Vietnam	Cross-sectional	Children (6 mos-15 yrs of age)	North Vietnam	Inclusion: Inpatient of University Hospital; Exclusion: outpatient not presenting Wednesday at pediatric hospital; any children with acute diarrhea, ulcer disease, repeated abdominal pain, immunocompromised	824	IgG immunoassay	Sensitivity: 99.6%, Specificity: 97.8%	NONE	Unconditional logistic regression	NA	Odds Ratios	NA	NONE	**Mother's History of gastro-duodenal disease (Y/N, No referent)** OR=1.3 (0.8, 2.0)	NA
Weyermann, M	2006	The mother as the source of *Helicobacter pylori* infection	Cross-sectional	Children, (3 yrs of age)- Birth Cohort	Ulm (Germany)	Exclusion: Mother's who received antibiotics 4 weeks prior to UBT	834	SAT	Not stated	NONE	Unconditional logistic regression	NA	Odds Ratios	Hp status of father and nationality of parents	NONE	**Mother Hp infected (pos/neg; neg referent):** 12.4 (4.2, 36.4)	**Mother Hp infected (pos/neg; neg referent):** 12.9 (3.2, 52.5)

																Point Estimates (95% Confidence Interval)	
First Author	Publication Year	Study Title	Study Design	Study Population	Study Location	Inclusion & Exclusionary Criteria	Sample Size	*Helicobacter pylori* Detection Methodology	Reported Diagnostic Sensitivity/ Specificity	Follow-up	Statistical Analysis Methods	P-value Based Results	Measure of Association	Covariate Adjustments	Effect Measures Modifiers	Crude	Adjusted
Nguyen, BV	2006	Intra-familial transmission of *Helicobacter pylori* infection in children of households with multiple generations in Vietnam	Cross-sectional	Children and Adults; Age Not Specified (< 10 yrs of age to >70 years of age)	Hanoi (Vietnam)	Exclusion: Children < 6 mos	533	IgG Immunoassay	Sensitivity: 99.6%, Specificity: 97.8%	NONE	Unconditional logistic regression	NA	Odds Ratios	Age group in ten years, sex and sibling number on household	NONE	**Mother Hp serostatus (pos/neg; neg referent):** 2.5 (1.19, 5.26)	**Mother Hp serostatus (pos/neg; neg referent):** 2.47(1.12, 5.47)
Ito, LS	2006	Community-based familial study of *Helicobacter pylori* infection among healthy Japanese Brazilians	Cross-sectional	Japanese children (0-19 yrs of age)	San Paulo (Brazil)	Exclusion: Adopted children, either or one of parents not of Japanese descent, any households in which all of members were not living in same household, children with mental deficiency, children with chronic diseases	507	IgG Immunoassay	Not stat ed	NONE	Unconditional logistic regression	NA	Odds Ratios	Sex and age	NONE	Not provided	**Parental Hp serostatus (Mother pos/father neg vs. both parents neg; both parents neg referent):** 4.93 (1.83, 13.06)
Muhsen, K	2006	Prevalence and risk factors of *Helicobacter pylori* infection among healthy 3- to 5-year-old Israeli Arab children	Cross-sectional	Children (3-5 yrs of age)	Israel	NONE	197	SAT	Not stated	NONE	Mantel-Haenszel Analysis	NA	Odds Ratios	NONE	NONE	**Maternal Hp status (neg vs. pos; neg referent):** 2.3 (0.6, 8.3)	NA
Rowland, M	2006	Age-specific incidence of *Helicobacter pylori*	Nested case-control	Children 24-48 mos of age at enrollment	Dublin (Ireland)	Exclusion: Children with no eligible sibling tested for Hp (sibling analysis)	290	13C-UBT	Not stated	Annually for four years	Unconditional logistic regression	NA	Odds Ratios	≥2 bottles/day, infected sibling, always sleeps with mother, income <£8320/yr	NONE	**Infected mother (Y/N; No referent):** 5.40 (2.45, 11.88)	**Infected mother (Y/N; No referent):** 7.20 (1.97, 26.37)
Braga, A	2007	*Helicobacter pylori* colonization among children up to 6 years: results of a community-based study from Northeastern Brazil	Cross-sectional	Children (3 mos-6 yrs of age)	Brazil	Exclusion: Children with major congenital abnormalities, severe chronic illness	217	13C-UBT	Not stated	NONE	Unconditional logistic regression	NA	Odds Ratios	NONE	NONE	**Mother Hp seropositive (Y/N; No referent):** OR= 2.98 (1.11, 8.30)	Not provided
Ceylan, A	2007	Prevalence of *Helicobacter pylori* in children and their family members in a district in Turkey	Cross-sectional	Children (1-15 yrs of age);	Turkey	Inclusion: Yil University Pediatric Unit Exclusion: Asymptomatic children, children <1 yr of age, children >15 yrs of age, any other chronic diseases, used medicines which affect the GI system	275	IgG Immunoassay & SAT	Not stated	NONE	Hypothesis testing	45 or 69% of infected children had infected mother while 37 or 1.7% of non-infected children had infected mothers (P<0.0001)	NA	NA	NA	NA	NA

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First Author	Publication Year	Study Title	Study Design	Study Population	Study Location	Inclusion & Exclusionary Criteria	Sample Size	Helicobacter pylori Detection Methodology	Reported Diagnostic Sensitivity/ Specificity	Follow-up	Statistical Analysis Methods	P-value Based Results	Measure of Association	Covariate Adjustments	Effect Measures Modifiers	Point Estimates (95% Confidence Interval) Crude	Point Estimates (95% Confidence Interval) Adjusted
Fujimoto, Y	2007	Intrafamilial transmission of *Helicobacter pylori* among the population of endemic areas in Japan	Cross-sectional	Children (> 6 yrs of age)	Japan	NONE	197	IgG Immunoassay	Not stated	NONE	Hypothesis testing	Hp prevalence higher in children with Hp seropos mothers (21.6%, 22 of 102) than in children whose mother was seronegative (3.2%, 3 of 95, p<0.001)	NA	NA	NA	NA	NA
Kivi, M	2007	Helicobacter pylori genome variability in a framework of familial transmission	Laboratory analysis	1 mother (39 years of age) and her three children (13, 19 and 21 years of age)	Not stated	NONE	3 Children 1 Mother	Biopsy/ Culture; RFLP & PFGE	Not stated	NONE	Descriptive Analysis	NA	NA	NA	NONE	NA	NA
Herrera, PM	2008	DNA-level diversity and relatedness of *Helicobacter pylori* strains in shantytown families in Peru and transmission in a developing-country setting	Laboratory analysis	Family members	Lima, Peru	NONE	133	String Test (culture or DNA)/RAPD/ PCR/ Sequencing	Not stated	NONE	Descriptive Analysis	It was found that 30% of mother-child, 18% of child-father, 25% mother-father and 32% sib-sib strains matched at >99.5%	NA	NA	NA	NA	NA
Konno, M	2008	Predominance of mother-to-child transmission of *Helicobacter pylori* infection detected by random amplified polymorphic DNA fingerprinting analysis in Japanese families	Laboratory analysis	Japanese families, Children 4-19 yrs	Japan	NONE	66 total, 42 Children	Biopsy/ Culture OR Gastric aspiration	Not stated	NONE	RAPD	29 (69%) of Hp infected children had identical DNA patterns to mother, significantly higher than father's pattern match(P=0.01)	NA	NA	NONE	NA	NA
Weyermann, M	2009	Acquisition of *Helicobacter pylori* infection in early childhood: Independent contributions of infected mothers, fathers, and siblings	Cross-sectional	Children (4 yrs of age)	Ulm (Germany)	Exclusion: Mother's who received antibiotics4 weeks prior to UBT, families in which mothers, fathers and siblings were not all tested	493	SAT	Not stated	NONE	Unconditional logistic regression	NA	Odds Ratios	**Partial Adjustment:** nationality of parents, single child status **Full Adjustment:** nationality of parents, single child status	NONE	**Mother Hp serostatus (Pos/Neg; neg referent):** 11.7 (3.9, 35.1)	**Partial Adjustment: Mother Hp serostatus (Pos/Neg; neg referent):** 16.1 (4.0, 65.9) **Full Adjustment: Mother Hp serostatus (Pos/Neg; neg referent):** 13.0(3.0, 55.2)

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First Author	Publication Year	Study Title	Study Design	Study Population	Study Location	Inclusion & Exclusionary Criteria	Sample Size	*Helicobacter pylori* Detection Methodology	Reported Diagnostic Sensitivity/ Specificity	Follow-up	Statistical Analysis Methods	P-value Based Results	Measure of Association	Covariate Adjustments	Effect Measures Modifiers	Point Estimates (95% Confidence Interval) Crude	Point Estimates (95% Confidence Interval) Adjusted
Nahar, S	2009	Evidence of intra-familial transmission of *Helicobacter pylori* by PCR-based RAPD fingerprinting in Bangladesh	Laboratory analysis	Family members	Nandipara, Bangladesh (India)	Exclusion: A household with less than 3 children, concomitant disease, regular NSAID or corticosteriod use by one of the family members, treatment with antibiotics or proton pump inhibitors, pregnancy	138	SAT & Biopsy/gastric aspirate (culture); RAPD DNA	Not stated	NONE	Descriptive Analysis	46% of Hp isolated from mothers shared related genotypes with strains from children; 29% of Hp isolates of mother were related to the youngest child. Only 6% of parents shared similar strains.	NA	NA	NA	NA	NA
Yucel, O	2009	The factors associated with asymptomatic carriage of *Helicobacter pylori* in children and their mothers living in three socio-economic settings	Cross-sectional	Children (2-12 yrs of age)	Turkey	Exclusion: Symptomatic children	165	SAT	Not stated	NONE	Hypothesis testing	Mother Hp seropositive in Hp infected child (15/18 or71%) (P<0.001)	NA	NA	NONE	NA	NA
Roma, E	2009	Intrafamilial spread of *Helicobacter pylori* infection in Greece	Cross-sectional	Children (age not provided)	Greece	Exclusion: Asymptomatic children (restricted to children with upper GI symptoms including epigastric or abdominal pain, vomiting and upper GI bleeding); children with history of antibiotic use within 3 mos of study	100	^{13}C-UBT & SAT (children less than 2 yrs of age); for index children two of the following: culture, histology, rapid urease test, ^{13}C-UBT	Not stated	NONE	Unconditional logistic regression	NA	Odds Ratios	NONE	Current and previous Hp status of family member	**Hp infected mother (pos vs. neg; neg referent) :** 3.2 (1.37, 7.49) **Previous Hp with eradication therapy:** 5.14 (1.96, 13.49)	NA
Fialho, AMN	2010	Younger Siblings Play a Major Role in *Helicobacter pylori* Transmission Among Children from a Low-income Community in the Northeast of Brazil	Cross-sectional	Children ﹥6 mos-14 yrs of age)	Fortaleza, Ceara (Brazil)	NONE	351	^{13}C-UBT (Children < 14 yrs of age)/ IgG immunoassay (Children ≥ 14 years of age)	^{13}C-UBT: Sensitivity: 93.8% Specificity: 99.1% IgG immunoassay: Sensitivity: 95.4% Specificity: 100%	NONE	Unconditional logistic regression	NA	Odds Ratios	Partial Adjustment: age, number of children per household, number of people per room. Full Adjustment: age, number of children per household, number of people per room and siblings Hp status	NONE	**Maternal Hp serostatus (Hp - vs. Hp +; Hp - referent):** 3.0 (1.6, 5.7)	**Partial Adjustment- Maternal Hp serostatus (Hp - vs. Hp +; Hp - referent):** 2.9 (1.5, 5.6) **Partial Adjustment- Maternal Hp serostatus (Hp - vs. Hp +; Hp - referent):**2.2 (1.0, 4.6)

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Appendix E

FOREST PLOT OF MATERNAL *HELICOBACTER PYLORI* TRANSMISSION STUDIES: COMPARISON OF ACTIVE *H. PYLORI* INFECTION AND IMMUNOASSAY DIAGNOSTICS IN DETERMINING *H. PYLORI* INFECTION ASSOCIATIONS BETWEEN MOTHER AND CHILD

	OR (95% CI)
OVERALL ESTIMATE	**3.8 (2.5, 5.8)**
Ito, S (2006)	4.9 (1.8, 13.1)
Nguyen, BV (2006)	2.5 (1.1, 5.5)
Kivi, M (2005)	11.6 (2.0, 67.9)
Aguemon, BD (2005)	3.9 (1.5, 9.7)
O'Rourke, K (2003)	2.8 (1.0, 8.1)
Miyaji, H (2000)	7.4 (1.8, 34.6)**
Malaty, HM (2000)	5.3 (0.6, 42.8)**
OVERALL ESTIMATE	**4.4 (3.3, 5.7)**
Fialho, AMN (2010)	2.2 (1.0, 4.6)
Roma, E (2009)	5.1 (2.0, 13.5)**
Weyermann, M (2009)	13.0 (3.0, 55.2)
Braga, A (2007)	3.0 (1.1, 8.3)
Rowland, M (2006)	7.2 (2.0, 26.4)
Muhsen, K (2006)	2.3 (0.6, 8.3)**
Weyermann, M (2006)	12.9 (3.2, 52.5)
Rodrigues, MN (2006)	2.5 (1.1, 5.2)
Yang, S (2005)	10.8 (2.6, 45.9)**
Farrell, S (2005)	2.5 (1.0, 6.1)**
Rocha, GA (2003)	22.7 (2.3, 223.2)
Rothenbacher, D (2002)	3.9 (1.4, 10.6)
Rothenbacher, D (1999)	7.9 (4.0, 15.7)
Ma, J (1998)	30.1 (2.8, 325.2)

Studies assessing active *H. pylori* infection of index child shown in red. Studies utilizing immunoassays for *H. pylori* diagnosis shown in blue. Mother's *H. pylori* status determined via varying immunoassay and active infection methods.

** indicates crude point estimates; all other measures adjusted for covariates as stated in appendix D.

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Appendix F

FOREST PLOT OF MATERNAL *HELICOBACTER PYLORI* TRANSMISSION STUDIES: COMPARISON OF *H. PYLORI*

STATUS OF THE MOTHER VERSUS GASTRIC DISEASE OUTCOMES IN DETERMINING *H. PYLORI* INFECTION

ASSOCIATIONS BETWEEN MOTHER AND CHILD

	OR (95% CI)
OVERALL ESTIMATE	2.8 (2.5, 5.8)
Ito, S (2006)	4.9 (1.8, 13.1)
Nguyen, BV (2006)	2.5 (1.1, 5.5)
Kivi, M (2005)	11.6 (2.0, 67.9)
Aguemon, BD (2005)	3.9 (1.5, 9.7)
O'Rourke, K (2003)	2.8 (1.0, 8.1)
Miyaji, H (2000)	7.4 (1.8, 34.6)**
Malaty, HM (2000)	5.3 (0.6, 42.8)**
OVERALL ESTIMATE	1.2 (1.0, 1.5)
Nguyen, BV (2006)	1.3 (0.8, 2.0)
Chang, YW (2002)	1.3 (0.8, 2.3)
Kurosawa, M (2000)	2.8 (1.1, 7.1)
Brenner, H (2000)	3.2 91.0, 10.2)
Kikuchi, S (1998)	1.3 (0.9, 1.7)
Brenner, H (1998)	11.7 (3.8, 36.2)
Gasbarrini, G (1995)	1.4 (0.8, 2.4)**
Gasbarrini, G (1995)	0.8 (0.5, 1.1)**
OVERALL ESTIMATE	5.6 (3.8, 8.2)
Roma, E (2009)	5.1 (2.0, 13.5)**
Rowland, M (2006)	7.2 (2.0, 26.4)
Muhsen, K (2006)	2.3 (0.6, 8.3)**
Weyermann, M (2006)	12.9 (3.2, 52.5)
Yang, S (2005)	10.8 (2.6, 45.9)**
Farrell, S (2005)	2.5 (1.0, 6.1)**
Rothenbacher, D (1999)	7.9 (4.0, 15.7)

Studies assessing active *H. pylori* infection of the mother and child shown in pink. Studies utilizing immunoassays for *H. pylori* diagnosis of the mother and her child shown in blue. Studies utilizing maternal gastric disease as a proxy for *H. pylori* infection shown in green; child's status determined by varying methods.

** indicates crude point estimates; all other measures adjusted for covariates as stated in appendix D.

APPENDIX G

SIBLING-CHILD *HELICOBACTER PYLORI* INFECTION ASSOCIATIONS LITERATURE REVIEW ARTICLES

First Author	Publication Year	Study Title	Study Design	Study Population	Study Location	Inclusion & Exclusionary Criteria	Sample Size	*Helicobacter pylori* Detection Methodology	Reported Diagnostic Sensitivity/ Specificity	Follow-up	Statistical Analysis Methods	P-value Based Results	Measure of Association	Covariate Adjustments	Effect Measures Modifiers	Point Estimates (95% Confidence Interval) Crude	Point Estimates (95% Confidence Interval) Adjusted
Oderda, G	1991	*Helicobacter pylori* in children with peptic ulcer and their families	Cross-sectional	Children (<18 years of age)	Italy	NONE	15	Biopsy with rapid urease (index)	Not stated	NONE	Hypothesis testing	8/13 (61%) Siblings of Hp positive children with peptic ulcer Hp infected vs. 0/4 (0%) of siblings of Hp negative with peptic ulcer (P>0.05, NS)	NA	NA	NONE	NA	NA
Wang, JT	1993	Direct DNA amplification and restriction pattern analysis of *Helicobacter pylori* in patients with duodenal ulcer and their families	Laboratory analysis	14 patients their families (mean age	Taiwan	restricted to patients diagnosed with duodenal ulcer; did not received antibiotics or bismuth 6 mos prior to study; controls (post transfusion hepatitis) were age, sex matched who were negative for Hp antibodies	14 case and families; 14 controls and families	IgG immunoassay AND Biopsy/PCR; DNA diagnostics, PCR/RE DNA typing	Not stated	NONE	Descriptive Analysis	Of the 6 families in which biopsies were performed, ALL siblings within a family had the same RE pattern/type. In two families the mother had the same type as the children	NA	NA	NA	NA	NA
Mitchell, JD	1993	*Helicobacter pylori* infection within families	Cross-sectional	Children with hepatitis, 3-17 yrs of age	Australia	NONE	17	IgG Immunoassay	Not stated	NONE	Unconditional logistic regression	NA	Odds Ratios	NONE	NONE	**Sib Hp seropositive (negative referent):** 16.2 (2.1, 122.2)	NA
Webb, PM	1994	Relation between infection with *Helicobacter pylori* and living conditions in childhood: evidence for person to person transmission in early life	Cross-sectional	Males, 18-65 yrs of age	Stoke on Trent (England)	NONE	471	IgG Immunoassay	Sensitivity: 96% Specificity: 93%	NONE	Unconditional Logistic Regression	NA	Odds Ratios	Age, manual occupation, father manual occupation, crowding, shared bed	NONE	Not provided	**Siblings, per sibling at ages 5-16 yrs of age:** 1.09 (0.94, 1.27)
Tsugane, S	1994	Salty food intake and risk of *Helicobacter pylori* infection	Cross-sectional	Men (40-49 yrs of age)	Tokyo, Japan	NONE	628	IgG Immunoassay	Sensitivity: 96% Specificity: 86%	NONE	Unconditional logistic regression	NA	Odds Ratios	Area (5 different areas of Japan with varying Hp prevalence)	NONE	Not provided	**Number of siblings (0-1 referent): 2-3:** 0.83 (0.42, 1.63); **4-5:** 0.69 (0.34, 1.32); **≥6:** 0.69 (0.35, 1.39)
Patel, P	1994	*Helicobacter pylori* infection in childhood: risk factors and effect on growth	Cross-sectional	Children, 11 years of age	Edinburgh (Scotland)	NONE	554	IgG Immunoassay	Sensitivity: 98% Specificity: 94%	NONE	Hypothesis testing	Children at home at age 7: 1 to >4: Chi trend P=0.68	NA	NA	NA	NA	NA

First Author	Publication Year	Study Title	Study Design	Study Population	Study Location	Inclusion & Exclusionary Criteria	Sample Size	Helicobacter pylori Detection Methodology	Reported Diagnostic Sensitivity/ Specificity	Follow-up	Statistical Analysis Methods	P-value Based Results	Measure of Association	Covariate Adjustments	Effect Measures Modifiers	Point Estimates (95% Confidence Interval) Crude	Point Estimates (95% Confidence Interval) Adjusted
Gasbarrini, G	1995	A population based study of *Helicobacter pylori* infection in a European country: the San Mario study. Relations with gastrointestinal diseases	Cross-sectional	2,237 (>18 yrs of age)	San Marino (Italy)	NONE	2,237	IgG Immunoassay	Not stated	NONE	Unconditional logistic regression	NA	Odds Ratios	NONE	NONE	**Dyspepsia of brother/sister (Y/N; no referent):** 1.36 (1.0, 1.87) **Gastric cancer of brother. sister (Y/N; N referent):** 1.27 (0.70, 2.28)	NA
Blaser, MJ	1995	Age at establishment of *Helicobacter pylori* infection and gastric ulcer, and duodenal ulcer risk	nested case-control/ Matched C/C	Adults (Japanese men)	Hawaii	Exclusion: 5 persons who developed cancer of the gastric cardia; controls were age (same at examination) and date sera collected; Control alive at time of case hospitalization	313	IgG Immunoassay (1960's)	Sensitivity: 96.3% Specificity: 93.9%	late 1960's until 1989	Conditional and Unconditional logistic regression	NA	Odds Ratios	Age, birth order	Siblingship size	Not provided	**Gastric cancer: Sib size (1-2 referent): 3-6:** 2.48 (1.04, 5.90) **7:** 1.89 (0.75, 4.75) **Gastric ulcer: sib size (1-2 referent): 3-6:** 0.99 (0.38, 2.54) **7:** 1.02 (0.39, 2.70). **Duodenal ulcer: sib size (1-2 referent): 3-6:** 2.72 (0.70, 10.51) **7:** 1.26 (0.35, 4.60)
Blaser, MJ	1995	Age at establishment of *Helicobacter pylori* infection and gastric ulcer, and duodenal ulcer risk	nested case-control/Matched C/C	Adults (Japanese men)	Hawaii	Exclusion: 5 persons who developed cancer of the gastric cardia; controls were age (same at examination) and date sera collected; Control alive at time of case hospitalization	313	IgG Immunoassay (1960's)	Sensitivity: 96.3% Specificity: 93.9%	late 1960's until 1989	Conditional and Unconditional logistic regression	NA	Odds Ratios	Age, birth order	Siblingship size & Hp Positive status	Not provided	**IN ONLY Hp POS: Gastric cancer: Sib size (1-2 referent): 3-6:** 2.07 (1.02, 4.20) **7:** 2.05 (0.98, 4.28) **Gastric ulcer: sib size (1-2 referent): 3-6:** 1.08 (0.58, 2.0) **7:** 1.0 (0.53, 1.91). **Duodenal ulcer: sib size (1-2 referent): 3-6:** 1.63 (0.57, 4.67) **7:** 1.10 (0.38, 3.23)
McCallion, WA	1996	*Helicobacter pylori* infection in children: relation with current household living conditions	Cross-sectional	Children 3-15 years of age, day surgery (non gastro)	Belfast (Ireland)	NONE	367	IgG immunoassay	Sensitivity: 100% Specificity: 92%	NONE	Unconditional logistic regression	NA	Odds Ratios	age, social class	NONE	Not provided	**5 children or more in household (Y/N; N referent):** 3.58 (1.35, 9.49)

First Author	Publication Year	Study Title	Study Design	Study Population	Study Location	Inclusion & Exclusionary Criteria	Sample Size	*Helicobacter pylori* Detection Methodology	Reported Diagnostic Sensitivity/ Specificity	Follow-up	Statistical Analysis Methods	P-value Based Results	Measure of Association	Covariate Adjustments	Effect Measures Modifiers	Point Estimates (95% Confidence Interval) Crude	Point Estimates (95% Confidence Interval) Adjusted
Goodman, KJ	1996	*Helicobacter pylori* infection in the Colombian Andes: A population-based study of Transmission Pathways	Cross-sectional	Children (2-9 yrs old)	Colombia	NONE	684	^{13}C-UBT	Sensitivity: 96% Specificity: 93%	NONE	Max Like LR with logistic-binomial random effects model	NA	Odds Ratios	Person-to-person exposures No. of children in home No. of adults in home Presence of £50 year-olds in home Shares drinking cups Mother's hand washing habits after cleaning child's faces Location of latrine relative to hand washing facility Waterborne exposures Raw vegetable consumption Lifetime drinking water source Swims in rivers Swims in swimming pools Zoonotic exposure Cares for or plays with sheep Background covariates Age Sex Principal household	NONE	**No of children (1 referent): 2-3:** 1.8 (1.0, 3.0); **4-9: 2.8** (1.6, 5.0)	**No of children (1 referent): 2-3:** 2.4 (1.1, 5.4); **4-9: 3.5** (1.5, 8.4)
Goodman, KJ	1996	*Helicobacter pylori* infection in the Colombian Andes: A population-based study of Transmission Pathways	Cross-sectional	Children (2-9 yrs old)	Colombia	NONE	684	^{13}C-UBT	Sensitivity: 96% Specificity: 93%	NONE	Max Like LR with logistic-binomial random effects model	NA	Odds Ratios	Person-to-person exposures No. of children in home No. of adults in home Presence of £50 year-olds in home Shares drinking cups Mother's hand washing habits after cleaning child's faces Location of latrine relative to hand washing facility Waterborne exposures Raw vegetable consumption Lifetime drinking water source Swims in rivers Swims in swimming pools Zoonotic exposure Cares for or plays with sheep Background covariates Age Sex Principal household	NONE	**Birth order (1 referent):** 2: 1.5 (1.0, 2.3); **3-9:** 1.8 (1.2, 2.7)	**Birth order (1 referent):** 2: 1.5 (0.7, 2.9); **3-9:** 1.6 (0.7, 3.6)
Fall, CHD	1997	Growth in infancy, infant feeding, childhood living conditions and *Helicobacter pylori* infection at age 70	Cross-sectional	Adults (65-75 years of age)	Hertfordshire (England)	Exclusion: Those not born in Hertfordshire between 1920-1930 and not residents of Hertfordshire	838	IgG immunoassay	Sensitivity: 93-94%, Specificity: 88-93%	NONE	Unconditional logistic regression	NA	Odds Ratios	Sex, birth order, birth weight, weight at 1 yr of age, social class at birth, current social class	NONE	Not provided	**No. of siblings (0 referent): 1 to 2:** OR= 1.8 (1.1, 3.0); **3 to 4:** OR= 2.6 (1.6, 4.3); **≥5:** OR= 3.1 (1.9, 5.1)
Fall, CHD	1997	Growth in infancy, infant feeding, childhood living conditions and *Helicobacter pylori* infection at age 70	Cross-sectional	Adults (65-75 years of age)	Hertfordshire (England)	Exclusion: Those not born in Hertfordshire between 1920-1930 and not residents of Hertfordshire	838	IgG immunoassay	Sensitivity: 93-94%, Specificity: 88-93%	NONE	Unconditional logistic regression	NA	Odds Ratios	Sex, No of siblings, birth weight, weight at 1 yr of age, social class at birth, current social class	NONE	Not provided	**Birth Order (1st referent): 2-3:** OR= 1.1 (0.8 1.4); **4-5:** OR= 1.6 (1.1, 2.4); **>5:** OR= 1.8 (1.2, 2.7)

First Author	Publication Year	Study Title	Study Design	Study Population	Study Location	Inclusion & Exclusionary Criteria	Sample Size	*Helicobacter pylori* Detection Methodology	Reported Diagnostic Sensitivity/ Specificity	Follow-up	Statistical Analysis Methods	P-value Based Results	Measure of Association	Covariate Adjustments	Effect Measures Modifiers	Point Estimates (95% Confidence Interval) Crude	Point Estimates (95% Confidence Interval) Adjusted
Rothenbacher, D	1997	*Helicobacter pylori* in out-patients of a general practitioner: prevalence and determinants of current infection	Cross-sectional	Adults (15-79 yrs old); out patients of general practitioner	Ulm, Germany	Exclusion: those with previous Hp infection and successful eradication	501	^{13}C-UBT	Not stated	NONE	Unconditional logistic regression	NA	Odds Ratios	Nationality, age	NONE	Not Provided	**Number of siblings (0 referent); 1:** 1.2 (0.5, 2.7); **2 or 3:** 1.5 (0.7, 3.4); **≥4:** 2.7 (1.02 6.1)
Rothenbacher, D	1998	Prevalence and determinants of *Helicobacter pylori* infection in preschool children: a population-based study from Germany	Cross-sectional	Children (5-8 years of age)	Ulm (Germany)	NONE	945	^{13}C-UBT	Not stated	NONE	Cox w/Breslow extension	NA	Prevalence Rate Ratios	Nationality, sex, age, persons living in household, education of mother, education of father	NONE	Not provided	**Birth order (1st referent): 2nd:** 0.9 (0.6, 1.4); **3rd:** 0.9 (0.5, 1.5); **>3rd:** 1.2 (0.5, 2.8)
Kikuchi, S	1998	*Helicobacter pylori* risk associated with sibship size and family history of gastric diseases in Japanese adults	Cross-sectional	Adults (19-69 yrs of age)	Japan	Exclusion: Restricted to public service workers	4,361	IgG Immunoassay	Not stated	NONE	Unconditional Logistic Regression	NA	Odds Ratios	Age, sex, smoking habit, father's history of gastric disease	NONE	Not Provided	**Sibship size (1 referent): 2-3:** 1.17 (0.85, 1.61); **4+:** 1.48 (1.02, 2.13); **unknown:** 0.62 (0.31, 1.23)
Kikuchi, S	1998	*Helicobacter pylori* risk associated with sibship size and family history of gastric diseases in Japanese adults	Cross-sectional	Adults (19-69 yrs of age)	Japan	Exclusion: Restricted to public service workers	4,361	IgG Immunoassay	Not stated	NONE	Unconditional Logistic Regression	NA	Odds Ratios	Age and sex	NONE	Not Provided	**Sibling's history of gastric disease (negative referent): positive:** 1.71 (1.12, 2.60); **unknown:** 1.07 (0.83, 1.37)
Dowsett, SA	1999	*Helicobacter pylori* infection in indigenous families of Central America: serostatus and oran and fingernail carriage	Cross-sectional	Children ≥12 yrs old	Guatemala	NONE	242	IgG Immunoassay	Not stated	NONE	Correlation Coefficient	Sibling seropositivity correlation 0.63	NA	NA	NONE	NA	NA
Lin, DB	1999	Seroepidemiology of *Helicobacter pylori* infection among preschool children in Taiwan	Cross-sectional	Children (3-6 yrs old)	Taiwan	NONE	2,551	IgG Immunoassay	Not stated	NONE	Unconditional logistic regression	NA	Odds Ratios	Age, sex	NONE	Not provided	**Number of siblings (0 referent); 1:** 1.7 (0.7, 4.0); **2:** 1.6 (0.7, 3.8); **≥3:** 2.4 (1.0, 5.8)

																Point Estimates (95% Confidence Interval)	
First Author	Publication Year	Study Title	Study Design	Study Population	Study Location	Inclusion & Exclusionary Criteria	Sample Size	*Helicobacter pylori* Detection Methodology	Reported Diagnostic Sensitivity/ Specificity	Follow-up	Statistical Analysis Methods	P-value Based Results	Measure of Association	Covariate Adjustments	Effect Measures Modifiers	Crude	Adjusted
Rowland, M	1999	Low rates of *Helicobacter pylori* reinfection in children	Cohort	Children (1-17 yrs of age)	Dublin (Ireland)	Exclusion: Restricted to children treated and eradicated of Hp	52	Hp culture OR Histology and urease (infection and eradication); ^{13}C-UBT (eradication) and siblings	^{13}C-UBT: Sensitivity: 100%, specificity 97%	6 mos after eradication (6-62 mos)	Cox Proportional Hazards/ Survival analysis	NA	Risk Ratio	NONE	NONE	**>2 older siblings Hp positive (y/n; n referent):** 7.1 (1.4, 35.1)	NA
Han, SR	2000	*Helicobacter pylori*: Clonal population structure and restricted transmission within families revealed by molecular typing	Laboratory analysis	9 index children and 27 family members: 23 children 2-18 yrs of age and 4 adults 29-44 yrs of age	Mainz (Germany)	Inclusion: Patients of Dept of Pediatrics, Gutenberg University	59	Gastric biopsy/Culture; RFLP & PFGE	Not stated	NONE	Descriptive Analysis	NA	NA	NA	NA	NA	NA
Zhou, H	2000	Intrafamilial spread of *Helicobacter pylori*: A prospective study using urea breath test	Case-Control	Children (age not specified);	Hong Kong, China	Exclusion: Children who were not dyspeptic	32	Gastric biopsy/ histology (^{13}C-UBT family members)	Not stated	NONE	Hypothesis testing	Hp infected sibling in Hp infected children (12/17 or 71%) vs. non-infected children (2/20 or 10%) P<0.01	NA	NA	NA	NA	NA
Malaty, HM	2000	Evidence from a nine-year birth cohort study in Japan of transmission of pathways of *Helicobacter pylori* infection	Cross-sectional	46 Japanese families (Adults and children); ages not specified	Nagano Prefecture, (Japan)	NONE	116	IgG Immunoassay	Sensitivity: 93% Specificity: 97%	Annually for nine years	Descriptive Analysis	100% of children who seroconverted had Hp seropositive mothers (4 of 48) none had seropositive sibs	NA	NONE	NONE	**NA**	NA
Krumbiegel, P	2000	*Helicobacter pylori* prevalence in Leipzig's 1998 school entries: methodology and first results	Cross-sectional	Children (6 yrs old)	Leipzig, Germany	NONE	1,890	^{13}C-UBT (Child enable to provide breath sample)/ 15N urea Urine test (Children not able to) provide breathe test)	Not stated	NONE	Descriptive Analysis	of 161 pos children, 31% of sisters and 45% brothers Hp infected	NA	NONE	NONE	NA	NA
Miyaji, H	2000	*Helicobacter pylori* infection occurs via close contact with infected individuals in early childhood	Cross-sectional	Children (10-16 yrs of age)	Japan	NONE	125	IgG Immunoassay	Not stated	NONE	Unconditional logistic regression	NA	Odds Ratios	NONE	NONE	**Hp serostatus of older siblings (pos vs. neg; neg referent):** 3.97 (1.79, 8.84)	NA

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First Author	Publication Year	Study Title	Study Design	Study Population	Study Location	Inclusion & Exclusionary Criteria	Sample Size	*Helicobacter pylori* Detection Methodology	Reported Diagnostic Sensitivity/ Specificity	Follow-up	Statistical Analysis Methods	P-value Based Results	Measure of Association	Covariate Adjustments	Effect Measures Modifiers	Point Estimates (95% Confidence Interval)	
																Crude	Adjusted
Miyaji, H	2000	*Helicobacter pylori* infection occurs via close contact with infected individuals in early childhood	Cross-sectional	Children (10-16 yrs of age)	Japan	NONE	86	IgG Immunoassay	Not stated	NONE	Unconditional logistic regression	NA	Odds Ratios	Maternal Hp serostatus	NONE	**Hp serostatus of older siblings (pos vs. neg; neg referent):** 3.60 (1.25, 10.39)	NA
Goodman, KJ	2000	Transmission of *Helicobacter pylori* among siblings	Cross-sectional	Children (2-9 yrs old)	Colombia	NONE	684	13C-UBT	Not stated	NONE	Max Like Logistic Regression	NA	Odds Ratios	Number of children in family	NONE	**Birth order (1st referent): 2nd:** 1.9 (1.1, 3.5); **3rd:** 2.5 (1.2, 5.1); **4-9th:** 3.0 (1.4, 6.6)	**Birth order (1st referent): 2nd:** 1.8 (1.0, 3.3); **3rd:** 2.1 (1.0, 4.4); **4-9th:** 2.2 (0.9, 5.2)
Goodman, KJ	2000	Transmission of *Helicobacter pylori* among siblings	Cross-sectional	Children (2-9 yrs old)	Colombia	NONE	684	13C-UBT	Not stated	NONE	Max Like Logistic Regression	NA	Odds Ratios	NONE	NONE	**Total number of siblings (0 referent): 1:** 1.4 (0.8, 2.6); **2:** 2.3 (1.1, 4.8); **3:** 2.6 (0.8, 7.9); **4-5:** 4.3 (0.8, 24.7)	NA
Goodman, KJ	2000	Transmission of *Helicobacter pylori* among siblings	Cross-sectional	Children (2-9 yrs old)	Colombia	NONE	684	13C-UBT	Not stated	NONE	Max Like Logistic Regression	NA	Odds Ratios	Number of younger children	NONE	**Not provided**	**Number of older siblings (0 referent): 1:** 2.1 (1.2, 3.8); **2:** 3.2 (1.3, 8.0); **3-5:** 4.7 (1.3, 17.9)
Goodman, KJ	2000	Transmission of *Helicobacter pylori* among siblings	Cross-sectional	Children (2-9 yrs old)	Colombia	NONE	684	13C-UBT	Not stated	NONE	Max Like Logistic Regression	NA	Odds Ratios	Number of older siblings	NONE	**Not provided**	**Number of younger siblings (0 referent): 1:** 1.2 (0.7, 2.1); **2:** 1.0 (0.4, 2.4); **3-5:** 1.1 (0.3, 4.9)

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First Author	Publication Year	Study Title	Study Design	Study Population	Study Location	Inclusion & Exclusionary Criteria	Sample Size	*Helicobacter pylori* Detection Methodology	Reported Diagnostic Sensitivity/ Specificity	Follow-up	Statistical Analysis Methods	P-value Based Results	Measure of Association	Covariate Adjustments	Effect Measures Modifiers	Point Estimates (95% Confidence Interval) Crude	Point Estimates (95% Confidence Interval) Adjusted
Goodman, KJ	2000	Transmission of *Helicobacter pylori* among siblings	Cross-sectional	Children (2-9 yrs old)	Colombia	NONE	684	^{13}C-UBT	Not stated	NONE	Max Like Logistic Regression	NA	Odds Ratios	NONE	NONE	**Total number Hp Positive of siblings (0 referent): 1:** 1.5 (1, 2.3); **2:** 3.2 (1.7, 6.2); **3:** 5.6 (1.8, 17.7); **4:** 7.1 (1.4, 36.6)	**NA**
Goodman, KJ	2000	Transmission of *Helicobacter pylori* among siblings	Cross-sectional	Children (2-9 yrs old)	Colombia	NONE	684	^{13}C-UBT	Not stated	NONE	Max Like Logistic Regression	NA	Odds Ratios	Corresponding number of negative children	NONE	**Not provided**	**Number of positive Hp older siblings (0 referent): 1:** 1.9 (1.2, 3.1); **2-4:** 4.4 (2.0, 9.4)
Goodman, KJ	2000	Transmission of *Helicobacter pylori* among siblings	Cross-sectional	Children (2-9 yrs old)	Colombia	NONE	684	^{13}C-UBT	Not stated	NONE	Max Like Logistic Regression	NA	Odds Ratios	Corresponding number of negative children	NONE	**Not provided**	**Number of younger siblings (0 referent): 1:** 1.6 (1.0, 2.7) **2-4:** 2.8 (0.9, 8.7)
Luzza, F	2000	Evidence favoring the gastro-oral route in the transmission of *Helicobacter pylori* infection in children	Cross-sectional	Children (up to 16 yrs, mean 9 yrs)	Italy	NONE	100	^{13}C-UBT	Not stated	NONE	Unconditional logistic regression	NA	Odds Ratios	Age, crowding	NONE	Not provided	**History of vomiting in siblings (Y/N; N referent): 2.4** (1.3, 4.3)
Kurosawa, M	2000	*Helicobacter pylori* infection, gastritis and gastric cancer: Helicobacter pylori infection among Japanese children	Cross-sectional	Children, 6 & 14 yrs old	Japan	Exclusion: Subjects with inconsistent test results (not pos both in 1995 and 1996)	504	IgG Immunoassay	Not stated	NONE	Unconditional logistic regression	NA	Odds Ratios	sex and age	NONE	Not provided	**Siblingship size (referent 1-2): 3+:** 1.636 (0.732, 3.658)
Malaty, HM	2001	*Helicobacter pylori* infection in preschool and school-aged minority children: effect of socioeconomic indicators and breast feeding practices	Cross-sectional	Children (2-16 yrs of age); centers	Houston, Texas (United States)	Inclusion: Black and Hispanic, attending daycare Exclusion: White, non-daycare attending children; acute or chronic stomach problems or gastric surgery; current use of antibiotics (within 1 mo)	356	^{13}C-UBT	Not stated	NONE	Unconditional logistic regression	NA	Odds Ratios	Age	NONE	**No. of children living at home (0 or 1 referent): 2 or 3** OR=1.2 (0.6, 2.1); **≥4** OR=3.2 (1.7, 7.3)	**No. of children living at home (0 or 1 referent): 2 or 3** OR=1.4 (0.5, 1.8); **≥4** OR=2.4 (1.1, 5.1)

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First Author	Publication Year	Study Title	Study Design	Study Population	Study Location	Inclusion & Exclusionary Criteria	Sample Size	*Helicobacter pylori* Detection Methodology	Reported Diagnostic Sensitivity/ Specificity	Follow-up	Statistical Analysis Methods	P-value Based Results	Measure of Association	Covariate Adjustments	Effect Measures Modifiers	Point Estimates (95% Confidence Interval)	
																Crude	Adjusted
Herbarth, O	2001	*Helicobacter pylori* prevalence and risk factors among school beginners in a German urban center and its rural county	Cross-sectional	Children entering 1st grade (5-7 yrs of age)	Leipzig, Germany	NONE	2,888	^{13}C-UBT	Not stated	NONE	Unconditional logistic regression	NA	Odds Ratios	NONE? Not stated	City vs. County (rural)	**>3 children in household (≤3 referent)- County:** 4.2, 1.2, 14.6; **County + City:** 2.6 (1.1, 6.6)	NA
Glynn, MK	2002	Seroincidence of *Helicobacter pylori* infection in a cohort of rural Bolivian children: acquisition and possible analysis of risk factors	Cross-sectional	Children (6 mos-> 6 yrs)	Bolivia	Exclusion: Children without reported age, seropositive at baseline, and indeterminate serostatus	188	IgG Immunoassay	Not stated	1 year (baseline and then 1 year follow-up)	GEE to account for family clustering	NA	Odds Ratios	Age, family clustering	NONE	Not Provided	**Presence of seropositive sibling (Y/N; No referent):** 2.2 (1.0, 4.7)
Chang, YW	2002	Role of *Helicobacter pylori* infection among offspring or siblings of gastric cancer patients	Case-Control	Korean adults (19-74 yrs old, mean 45 yr)	Korea	Excluded if younger than 18 and older than 75; if had any serious medical conditions; cases were first degree relatives Gca patients	726: 300 cases; 426 controls	Biopsy with rapid urease and Giemsa stain	Not stated	NONE	Unconditional logistic regression	NA	Odds Ratios	Age, sex, years of school education, socioeconomic status, place of residence, number of siblings,	Age (19-40) and (41-74)	**Sibling with history of gastric cancer (compared to controls):** 5.3 (2.1, 13.7)	**Sibling with history of gastric cancer (compared to controls):** 5.3 (2.0, 13.9)
Chang, YW	2002	Role of *Helicobacter pylori* infection among offspring or siblings of gastric cancer patients	Case-Control	Korean adults (19-74 yrs old, mean 45 yr)	Korea	Exclusion: if younger than 18 and older than 75; if had any serious medical conditions; cases were first degree relatives Gca patients	726: 300 cases; 426 controls	Biopsy with rapid urease and Giemsa stain	Not stated	NONE	Unconditional logistic regression	NA	Odds Ratios	Age, sex, years of school education, socioeconomic status, place of residence, number of siblings,	Age (19-40) and (41-74)	**Not provided**	**19-40 yrs of age- Sibling with history of gastric cancer (compared to controls):** >999.9; **41-74 yrs of age- Sibling with history of gastric cancer (compared to controls):** 4.2 (1.6, 11.3)

																Point Estimates (95% Confidence Interval)	
First Author	Publication Year	Study Title	Study Design	Study Population	Study Location	Inclusion & Exclusionary Criteria	Sample Size	*Helicobacter pylori* Detection Methodology	Reported Diagnostic Sensitivity/ Specificity	Follow-up	Statistical Analysis Methods	P-value Based Results	Measure of Association	Covariate Adjustments	Effect Measures Modifiers	Crude	Adjusted
Moayyedi, A	2002	Relation of adult lifestyle and socioeconomic factors to the prevalence of *Helicobacter pylori* infection	Cross-sectional	Adults, (40-49 yrs of age)	England	Exclusion: Excluded if taken antibiotics proton pump inhibitors or bismuth salts within previous 2 weeks; those unwilling to give up alcohol for 1 weeks, allergies to macrolides, proton pump inhibitors, 5-nitroimidazole, and those taking warfaring, digoxin, cisapride, antihistamines or theophyllines	7452	^{13}C-UBT	Sensitivity: 98% Specificity: 96%	NONE	Unconditional Logistic Regression	NA	Odds Ratios	Country of birth, ethnic origin, gender, age, house type at age 8, crowding at age 8, bathroom at age 8, shared bedroom as a child, shared bed with siblings, shared bed with parent, social class at age 8, full time education status, present housing, present central heating, present telephone ownership, present social class, smoker, drinks alcohol, drinks coffee	NONE	**No of siblings (0-1 referent): 2-4:** 1.59 (1.42, 1.79); **5-7:** 3.57 (3.00, 4.26); **>7:** 5.61 (4.19, 7.51)	**No of siblings (0-1 referent): 2-4:** 1.33 (1.17, 1.52); **5-7:** 2.14 (1.74, 2.63); **>7:** 2.97 (2.16, 4.08)
Ueda, M	2003	*Helicobacter pylori* risk associated with childhood home environment	Cross-sectional	Adults, age not specified	Japan	Inclusion: Stomach cancer examination system participants	5,854	IgG Immunoassay ? Not specified	Not stated	NONE	Unconditional logistic regression	NA	Odds Ratios	Type of toilet, residential area, sex, age, district	NONE	**Birth order (1 referent): 2-3:** OR=1.022 (0.932, 1.36) **4-5:** OR=1.188 (1.001, 1.408) **6 or more:** OR= 1.470 (1.166, 1.854)	**Birth order (1 referent): 2-3:** OR=1.015 (0.893, 1.153) **4-5:** OR=1.149 (0.969, 1.364) **6 or more:** OR= 1.389 (1.100, 1.753)
Ertem, D	2003	*Helicobacter pylori* infection in Turkish preschool and school children: role of socioeconomic factors and breast feeding	Cross-sectional	Children, (3 12 yrs old)	Turkey	Exclusion: Children who received oral or parenteral antibiotics	327	^{13}C-UBT	Not stated	NONE	Unconditional logistic regression	NA	Odds Ratios	Socioeconomic class, heating system, breast feeding, age, weight percentile, height percentile, household density, education of parents	NONE	Not provided	**Number of Siblings (None referent): 1:** OR=1.94 (1.20, 3.86); **≥2:** OR=3.30 (0.96, 3.49)
Rocha, GA	2003	Transmission of *Helicobacter pylori* infection in families of preschool-aged children from Minas Gerais, Brazil	Cross-sectional	Children (2 mos- 8 yrs of age)	Minas Gerais (Brazil)	NONE	66	^{13}C-UBT (Children ≤ 12 yrs of age)/ IgG immunoassay (Children > 12 years of age)	^{13}C-UBT: sensitivity: 95.4-100%, specificity 93.1-100% IgG immunoassay: sensitivity: 99.5% specificity: 99.0%	NONE	Unconditional logistic regression	NA	Odds Ratios	age, gender, number of children in household, Hp status of father, Hp status of mother	NONE	**Number of positive siblings (≤ 2 vs. >2; ≤ 2 referent):** 9.9 (1.1, 459.7)	**Number of positive siblings (≤ 2 vs. >2; ≤ 2 referent):** 1.81 (1.01, 3.30)

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Kivi, M	2003	Concordance of *Helicobacter pylori* strains within families	Laboratory analysis	Children (10-12 yrs old)	Sweden	NONE	104	Culture; RFLP	Not stated	NONE	Descriptive Analysis	81% (29/36) sib-sib strain concordance; 82% (14/17)sib-sib (mother different strain) concordance	Descriptive stats	NA	NA	NA	NA
Chang, PS	2003	Household *Helicobacter pylori* antibody survey in children with upper gastrointestinal symptoms	Cross-sectional	Children (0.9-15.3 yrs old)	Taiwan	Exclusion: Restricted to children with upper GI symptoms	112	Biopsy & culture (index); IgG immunoassay (family)	Not stated	NONE	Hypothesis testing	In Hp infected children, 47% of siblings were Hp seropositive versus only 23% of siblings of non-Hp infected children (P=0.259)	NA	NA	NA	NA	NA
Reshetnikov, OV	2003	*Helicobacter pylori* seropositivity among adolescents in Novosibirsk, Russia: Prevalence and associated factors	Cross-sectional	Children (11-14 yrs of age)	Siberia (Russia)	NONE	423	IgG immunoassay	Sensitivity: 96.3% Specificity: 91.5%		Unconditional Logistic Regression	NA	Odds Ratios	NONE	NONE	**Siblings (No referent): one:** 0.92 (0.59, 1.44); **more than 1:** 0.95 (0.41, 2.22)	NA
Escobar, ML	2004	Evidence of mother-child transmission of *Helicobacter pylori* infection	Cross-sectional	Children (2-14 years of age)	Brazil? (not stated)	Exclusion: Those not family members of 38 dyspeptic patients (2-20 yrs of age) who were Hp positive by histology and urease rapid test	112	IgG immunoassay	Not stated	NONE	Hypothesis testing	Siblings Hp seropositive in Hp infected patient (25/53 76%) vs. Mother Hp seropositive in non-Hp infected patient (1/5 20%): P=0.0.27	NA	NA	NA	NA	NA
Sinha, SK	2004	The incidence of *Helicobacter pylori* acquisition in children of a Canadian First Nations Community and the potential for parent-to-child transmission	Cohort	Children (1-13 yrs of age)	Wasagamack (Canada)	Exclusion: Those not belonging to First Nations American aboriginal population	50	SAT	Not stated	1 year	Hypothesis testing	50 children who tested negative in 1999 were tested via SAT; 8 were positive and it was found that all of their mothers were Hp pos and only Five of 8 had no infected siblings and three had infected siblings. No stat testing done	NA	NA	NA	NA	NA

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Kivi, M	2005	*Helicobacter pylori* status in family members as risk factors for infection in children	Cross-sectional	Children (11-13 yrs of age)	Stockholm, Sweden	Exclusion: Any children who at least one family member did not provide blood for study	162	IgG immunoassay/ Western blot	IgG immunoassay: sensitivity: 98% specificity: 96% Western blot: sensitivity: 94% specificity: 98%	NONE	Unconditional logistic regression	NA	Odds Ratios	infection status of mother, country of birth of the index child, household SES, antibiotic consumption	NONE	Not provided	**Sibling's Hp serostatus (one or more pos vs. all neg; all neg referent): 8.1 (1.8, 37.3)**
Yang, YJ	2005	Children of *Helicobacter pylori*-infected dyspeptic mothers are predisposed to H. pylori acquisition with subsequent iron deficiency and growth retardation	Cross-sectional	Children (1-16 yrs of age);	Taiwan	Inclusion: Dyspeptic Hp infected mothers; Exclusion: Children who had taken antibiotics or acid secretion inhibitors within the past 4 weeks	163	^{13}C-UBT & SAT	Not stated	NONE	Unconditional logistic regression	NA	Odds Ratios	Hp infection status of mother	NONE	**Hp infected sibling (1 vs. 0; 0 referent): 2.0 (0.8, 5.3)**	NA
Santos, IS	2005	Prevalence of *Helicobacter pylori* infection and associated factors among adults in Southwestern Brazil: a population-based cross-sectional study	Cross-sectional	Adults (20 years and older	Rio Grande do Sol (Brazil)	NONE	363	^{13}C-UBT	Not stated	NONE	Poisson regression with family cluster	NA	Prevalence Ratios	Past socioeconomic characteristics: urban vs. urban/rural living, level of formal education of the parents, number of siblings, attendance to day care center in childhood	NONE	**Number of Siblings (0-2, 3-4, 5-6, ≥7; 0-2 referent): 3-4: 1.44 (1.13, 1.83) 5-6: 1.32 (1.02, 1.71) ≥7: 1.59 (1.28, 1.98)**	**Number of Siblings (0-2, 3-4, 5-6, ≥7; 0-2 referent): 3-4: 1.44 (1.11, 1.86) 5-6: 1.27 (0.96, 1.68) ≥7: 1.55 (1.22, 1.98)**
Farrell, S	2005	Risk factors for *Helicobacter pylori* infection in children: an examination of the role played by intrafamilial bed sharing	Cross-sectional	Children	Ireland	NONE	178	Biopsy & urease (index)/ ^{13}C-UBT	Not stated	NONE	Unconditional logistic regression	NA	Odds Ratios	Not stated? Assume crude measure	NONE	**Sharing a bed with an Hp infected sibling (Y/N; No referent): 4.84 (1.54, 15.20)**	NA
Magista, AM	2005	*Helicobacter pylori* status and symptom assessment two years after eradication in pediatric patients from a high prevalence area	Cohort	Children (5-17 yrs of age)	Southern Italy	Exclusion: Restricted to children treated and eradicated of Hp	65	Histology and urease (infection); ^{13}C-UBT (eradication) and siblings	Not stated	18 mos	Descriptive Analysis	Of children reinfected after eradication, 100% (9/9) of siblings were Hp infected versus only 35% (11/31) of sibling of children not reinfected	NA	NA	NA	NA	NA

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First Author	Publication Year	Study Title	Study Design	Study Population	Study Location	Inclusion & Exclusionary Criteria	Sample Size	*Helicobacter pylori* Detection Methodology	Reported Diagnostic Sensitivity/ Specificity	Follow-up	Statistical Analysis Methods	P-value Based Results	Measure of Association	Covariate Adjustments	Effect Measures Modifiers	Point Estimates (95% Confidence Interval)	
																Crude	Adjusted
Koch, A	2005	Seroprevalence and risk factors for *Helicobacter pylori* infection in Greenland	Cross-sectional	Children (5-18 yrs of age)	Greenland	Exclusion: Inconclusive test results	193	IgG Immunoassay	Sensitivity: 99.2% Specificity: 90.1%	NONE	Unconditional Logistic Regression	NA	Odds Ratios	Age and sex	NONE	Not provided	**No of children < 18 yrs in HH (< 1 referent): 2:** 2.63 (0.78, 8.89); **3:** 1.49 (0.41, 5.41); **4+:** 7.27 (2.06, 25.6)
Koch, A	2005	Seroprevalence and risk factors for *Helicobacter pylori* infection in Greenland	Cross-sectional	Children (5-18 yrs of age)	Greenland	Exclusion: Inconclusive test results	193	IgG Immunoassay	Sensitivity: 99.2% Specificity: 90.1%	NONE	Unconditional Logistic Regression	NA	Odds Ratios	Age and sex	NONE	Not provided	**No of older siblings (0 referent): 1-2:** 0.99 (0.47, 2.1) **3+:** 2.23 (0.68, 7.32)
Koch, A	2005	Seroprevalence and risk factors for *Helicobacter pylori* infection in Greenland	Cross-sectional	Children (5-18 yrs of age)	Greenland	Exclusion: Inconclusive test results	193	IgG Immunoassay	Sensitivity: 99.2% Specificity: 90.1%	NONE	Unconditional Logistic Regression	NA	Odds Ratios	Age and sex	NONE	Not provided	**No of younger siblings (0 referent): 1-2:** 1.03 (0.5, 2.15); **3+:** 4.61 (1.44, 14.8)
Koch, A	2005	Seroprevalence and risk factors for *Helicobacter pylori* infection in Greenland	Cross-sectional	Children (5-18 yrs of age)	Greenland	Exclusion: Inconclusive test results	193	IgG Immunoassay	Sensitivity: 99.2% Specificity: 90.1%	NONE	Unconditional Logistic Regression	NA	Odds Ratios	Age and sex	NONE	Not provided	**Distance to nearest older sibling (0-1 yr referent): 2 yrs:** 0.36 (0.09, 1.49) **3-4 yrs:** 0.74 (0.23, 2.39); **5+ yrs:** 0.15 (0.04, 0.53)
Koch, A	2005	Seroprevalence and risk factors for *Helicobacter pylori* infection in Greenland	Cross-sectional	Children (5-18 yrs of age)	Greenland	Exclusion: Inconclusive test results	193	IgG Immunoassay	Sensitivity: 99.2% Specificity: 90.1%	NONE	Unconditional Logistic Regression	NA	Odds Ratios	Age and sex	NONE	Not provided	**Distance to nearest younger sibling (0-1 yr referent): 2 yrs:** 1.36 (0.42, 4.42) **3-4 yrs:** 1.22 (0.43, 3.43); **5+ yrs:** 0.43 (0.12, 1.53)

First Author	Publication Year	Study Title	Study Design	Study Population	Study Location	Inclusion & Exclusionary Criteria	Sample Size	*Helicobacter pylori* Detection Methodology	Reported Diagnostic Sensitivity/ Specificity	Follow-up	Statistical Analysis Methods	P-value Based Results	Measure of Association	Covariate Adjustments	Effect Measures Modifiers	Point Estimates (95% Confidence Interval)	
																Crude	Adjusted
Koch, A	2005	Seroprevalence and risk factors for *Helicobacter pylori* infection in Greenland	Cross-sectional	Children (5-18 yrs of age)	Greenland	Exclusion: Inconclusive test results	193	IgG Immunoassay	Sensitivity: 99.2% Specificity: 90.1%	NONE	Unconditional Logistic Regression	NA	Odds Ratios	Age, sex, no of older siblings, distance to nearest older sibling	NONE	Not provided	**No of children < 18 yrs in HH (1 referent): 2:** 2.9 (0.75, 11.2); **3:** 1.51 (0.38, 6.07); **4+:** 6.33 (1.52, 26.4)
Koch, A	2005	Seroprevalence and risk factors for *Helicobacter pylori* infection in Greenland	Cross-sectional	Children (5-18 yrs of age)	Greenland	Exclusion: Inconclusive test results	193	IgG Immunoassay	Sensitivity: 99.2% Specificity: 90.1%	NONE	Unconditional Logistic Regression	NA	Odds Ratios	Age, sex, no children < 18 yrs of age in HH, distance to nearest older sibling	NONE	Not provided	**No of older siblings (0 referent): 1-2:** 1.44 (0.37, 5.58) **3+:** 8.58 (1.45, 50.7)
Koch, A	2005	Seroprevalence and risk factors for *Helicobacter pylori* infection in Greenland	Cross-sectional	Children (5-18 yrs of age)	Greenland	Exclusion: Inconclusive test results	193	IgG Immunoassay	Sensitivity: 99.2% Specificity: 90.1%	NONE	Unconditional Logistic Regression	NA	Odds Ratios	Age, sex, no of older siblings, distance to nearest older sibling	NONE	Not provided	**Distance to nearest older sibling (0-1 yr referent): 2 yrs:** 0.57 (0.1, 3.37) **3-4 yrs:** 1.31 (0.3, 5.68) **5+ yrs:** 0.17 (0.03, 0.9)
Ito, LS	2006	Community-based familial study of *Helicobacter pylori* infection among healthy Japanese Brazilians	Cross-sectional	Japanese children (0-19 yrs of age)	San Paulo (Brazil)	Exclusion: Adopted children, either or one of parents not of Japanese descent, any households in which all of members were not living in same household, children with mental deficiency, children with chronic diseases	507	IgG Immunoassay	Not stat ed	NONE	Unconditional logistic regression	NA	Odds Ratios	Sex and age	NONE	Not provided	**No of siblings (0-2; 0 referent):1 sibling** 1.39 (0.38, 5.01) **2 siblings** 1.52 (0.41, 5.60)
Muhsen, K	2006	Prevalence and risk factors of *Helicobacter pylori* infection among healthy 3- to 5-year-old Israeli Arab children	Cross-sectional	Children (3-5 yrs of age)	Israel	NONE	197	SAT	Not stated	NONE	Unconditional logistic regression	NA	Odds Ratios	village, crowding index, siblings 0-5 yrs of age	NONE	Not provided	**Sibling's H. pylori status (Neg vs. Pos; neg referent):** 4.4 (1.3, 14.6)

First Author	Publication Year	Study Title	Study Design	Study Population	Study Location	Inclusion & Exclusionary Criteria	Sample Size	*Helicobacter pylori* Detection Methodology	Reported Diagnostic Sensitivity/ Specificity	Follow-up	Statistical Analysis Methods	P-value Based Results	Measure of Association	Covariate Adjustments	Effect Measures Modifiers	Point Estimates (95% Confidence Interval) Crude	Point Estimates (95% Confidence Interval) Adjusted
Garg, PK	2006	Concordance of *Helicobacter pylori* infection among children in extended-family homes	Cross-sectional	Children (2-18 yrs of age); mainly Hispanic	California (United States)	Inclusion: Restricted to symptomatic children (diarrhea and/or vomiting of suspected infectious etiology presenting at cooperation clinics, children > 2 years of age; excluded household with less than 2 distinct family units with at least one child 2-18 years of age	241	IgG Immunoassay	Sensitivity: 94% Specificity: 91%	NONE	Unconditional logistic regression	NA	Odds Ratios	Age; restricted to children with at least one sibling	Number of Hp infected non-siblings 0 vs. 1+	Not provided	**Within 0 infected non-siblings: Living with 1+ Hp infected sibling (living with no infected sibling or non-sibling referent):** 4.4 (1.4, 13.9); **Within 1+ infected non-siblings: Living with no infected siblings ((living with no infected sibling or non-sibling referent):** 0.68 (0.12, 3.6); Living with 1+ infected siblings: 14.3 (4.5, 45.8)
Halitim, F	2006	High rate of *Helicobacter pylori* reinfection in children and adolescents	Cohort	French children, developed co (<18 yr of age)	Lille (France)	Inclusion: Restricted to children who had undergone Hp eradication therapy	45	Baseline: Histology or culture; Hp eradication confirmed by histology or ^{13}C-UBT 4-6 wks after treatment; For study: ^{13}C-UBT	Not stated	1 year (min) post eradication of Hp	Not stated	NA	Risk Ratio	NONE	NONE	**Siblings younger than 5 yrs (younger than 5 vs. 5 and older; 5 and older referent):** 3.3 (0.9, 11.7)	NA
Halitim, F	2006	High rate of *Helicobacter pylori* reinfection in children and adolescents	Cohort	French children, developed co (<18 yr of age)	Lille (France)	Inclusion: Restricted to children who had undergone Hp eradication therapy	45	Baseline: Histology or culture; Hp eradication confirmed by histology or ^{13}C-UBT 4-6 wks after treatment; For study: ^{13}C-UBT	Not stated	1 year (min) post eradication of Hp	Not stated	NA	Risk Ratio	NONE	NONE	**Siblings with history of Hp infection (yes vs. no no referent):** 1.3 (1.1, 11.7)	NA

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First Author	Publication Year	Study Title	Study Design	Study Population	Study Location	Inclusion & Exclusionary Criteria	Sample Size	*Helicobacter pylori* Detection Methodology	Reported Diagnostic Sensitivity/ Specificity	Follow-up	Statistical Analysis Methods	P-value Based Results	Measure of Association	Covariate Adjustments	Effect Measures Modifiers	Point Estimates (95% Confidence Interval) Crude	Point Estimates (95% Confidence Interval) Adjusted
Leung, WK	2006	*Helicobacter pylori* infection in 1st degree relatives of Chinese gastric cancer patients	Case-Control	Adults (18-75 yrs of age)	Hong Kong, China	Inclusion: Restricted to 1st degree relatives (siblings or offspring) of patients with confirmed gastric adenocarcinoma; exclusion: those who had previous gastric surgery, received eradication therapy for Hp, were pregnant, or had other serious medical conditions Controls- age and gender matched dyspeptic patients who underwent endoscopy in same hospital as cases	270	One of the following: 1) Histology ID of Hp 2) Positive urease rapid test or 3) IgG immunoassay	Not stated	NONE	Unconditional logistic regression	NA	Odds Ratios	Age > 45 yrs, youngest among all siblings	NONE	**Sibling had gastric cancer (Yes/No; No referent):** 2.00 (1.22, 3.29)	**Sibling had gastric cancer (Yes/No; No referent):** 1.92 (1.13, 3.25)
Leung, WK	2006	*Helicobacter pylori* infection in 1st degree relatives of Chinese gastric cancer patients	Case-Control	Adults (18-75 yrs of age)	Hong Kong, China	Inclusion: Restricted to 1st degree relatives (siblings or offspring) of patients with confirmed gastric adenocarcinoma; exclusion: those who had previous gastric surgery, received eradication therapy for Hp, were pregnant, or had other serious medical conditions Controls- age and gender matched dyspeptic patients who underwent endoscopy in same hospital as cases	270	One of the following: 1) Histology ID of Hp 2) Positive urease rapid test or 3) IgG immunoassay	Not stated	NONE	Unconditional logistic regression	NA	Odds Ratios	Age > 45 yrs, sibling has gastric cancer	NONE	**youngest among all siblings (Yes/No; No referent):** 0.36 (0.20, 0.66)	**youngest among all siblings (Yes/No; No referent):** 0.45 (0.24, 0.84)
Rowland, M	2006	Age-specific incidence of *Helicobacter pylori*	Nested case-control	Children 24-48 mos of age at enrollment	Dublin (Ireland)	Exclusion: Children with no eligible sibling tested for Hp (sibling analysis)	290	^{13}C-UBT	Not stated	Annually for four years	Unconditional logistic regression	NA	Odds Ratios	≥2 bottles/day, infected mother, always sleeps with mother, income <£8320/yr	NONE	**Infected Sibling (Y/N; No referent):** 7.30 (3.50, 15.23)	**Infected Sibling (Y/N; No referent):** 4.90 (2.15, 11.55)
Mbulaiteye, SM	2006	*H. pylori*-infection and antibody immune response in a rural Tanzanian population	Cross-sectional	Children and adults (age not specified)	Tanzania (Africa)	NONE	788	IgG Immunoassay	Sensitivity: 89-96% Specificity: 92-97%	NONE	Unconditional Logistic Regression (GEE for intrafamilial dependency)	NA	Odds Ratios	NONE	NONE	**Birth order (1 referent): 2:** 3.3 (1.7, 6.8); **≥3:** 11.1 (3.7, 34)	NA

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First Author	Publication Year	Study Title	Study Design	Study Population	Study Location	Inclusion & Exclusionary Criteria	Sample Size	*Helicobacter pylori* Detection Methodology	Reported Diagnostic Sensitivity/ Specificity	Follow-up	Statistical Analysis Methods	P-value Based Results	Measure of Association	Covariate Adjustments	Effect Measures Modifiers	Point Estimates (95% Confidence Interval) Crude	Adjusted
Mbulaiteye, SM	2006	*H. pylori*-infection and antibody immune response in a rural Tanzanian population	Cross-sectional	Children and adults (age not specified)	Tanzania (Africa)	NONE	788	IgG Immunoassay	Sensitivity: 89-96% Specificity: 92-97%	NONE	Unconditional Logistic Regression (GEE for intrafamilial dependency)	NA	Odds Ratios	NONE	NONE	**next older sibling to case (seroneg referent): Seropositive**: 2.7 (0.9, 8.34)	NA
Ceylan, A	2007	Prevalence of *Helicobacter pylori* in children and their family members in a district in Turkey	Cross-sectional	Children (1-15 yrs of age);	Turkey	Inclusion: Yil University Pediatric Unit Exclusion: Asymptomatic children, children <1 yr of age, children >15 yrs of age, any other chronic diseases, used medicines which affect the GI system	275	IgG Immunoassay/ SAT	Not stated	NONE	Hypothesis testing	P<0.0001 (17 or 30% of infected children had infected siblings while 7 or 3% of non-infected children had infected siblings)	NA	NA	NA	NA	NA
Ford, AC	2007	Effect of sibling number in the household and birth order on prevalence of *Helicobacter pylori*: a cross-sectional study	Cross-sectional	Adults (50-59 yrs of age)	West Yorkshire (Northern England)	NONE	3,928	^{13}C-UBT	Not stated	NONE	Unconditional logistic regression	NA	Odds Ratios	gender, inside toilet, bathroom, shared bedroom, shared bed with other siblings, type of housing, household crowding as a child, father's social class, birth order	Birth Order per unit increase	**Number of siblings in household during childhood (0 to 16; 0 referent): 1:** 1.08 (0.85, 1.37); **2:** 1.27 (0.99, 1.63); **3:** 1.79 (1.36, 2.35); **4:** 2.21 (1.62, 3.01); **5:** 3.31 (2.31, 4.76); **6:** 2.78 (1.77, 4.36); **7:** 4.86 (2.68, 8.83); **8-16:** 6.69 (4.06, 11.03)	Number of siblings in household during childhood (0 to 16; 0 referent): 1: 1.02 (0.77, 1.35); 2: 1.19 (0.88, 1.62); 3: 1.51 (1.06, 2.15); 4: 1.85 (1.23, 2.77); 5: 2.72 (1.72, 4.30); 6: 2.33 (1.31, 4.13); 7: 3.47 (1.69, 7.15); 8-16: 5.70 (2.92, 11.14); with effect modifier of birth order- Number of siblings in household during childhood (0 to 16; 0 referent): 1: 0.99 (0.74, 1.32); 2: 1.01 (0.83, 1.24); 3: 1.00 (0.83, 1.24); 4: 0.87 (0.71, 1.05); 5: 1.03 (0.84, 1.25); 6: 0.97 (0.76, 1.23); 7-16: 0.97 (0.85, 1.12)

First Author	Publication Year	Study Title	Study Design	Study Population	Study Location	Inclusion & Exclusionary Criteria	Sample Size	*Helicobacter pylori* Detection Methodology	Reported Diagnostic Sensitivity/ Specificity	Follow-up	Statistical Analysis Methods	P-value Based Results	Measure of Association	Covariate Adjustments	Effect Measures Modifiers	Point Estimates (95% Confidence Interval) Crude	Point Estimates (95% Confidence Interval) Adjusted
Ford, AC	2007	Effect of sibling number in the household and birth order on prevalence of *Helicobacter pylori*: a cross-sectional study	Cross-sectional	Adults (50-59 yrs of age)	West Yorkshire (Northern England)	NONE	3,928	^{13}C-UBT	Not stated	NONE	Unconditional logistic regression	NA	Odds Ratios	gender, inside toilet, bathroom, shared bedroom, shared bed with other siblings, type of housing, household crowding as a child, father's social class, sibling number	Sibling number per unit increase	**Birth order of individual: 1st to 16th; 1st referent): 2:** 1.12 (0.95, 1.33); **3:** 1.46 (1.18, 1.82); **4:** 1.64 (1.16, 2.63); **5:** 1.75 (1.62, 3.01); **6:** 2.97 (1.69, 5.23); **7-16:** 4.16 (2.41, 7.17)	Birth order of individual: 1st to 16th; 1st referent): 2: 1.01 (0.83, 1.23); 3: 0.96 (0.73, 1.25); 4: 0.90 (0.62, 1.29); 5: 0.70 (0.43, 1.15); 6: 0.82 (0.42, 1.60); 7-16: 0.82 (0.40, 1.67); With Effect Modifier of Sibling number- Birth order of individual: 1st to 16th; 1st referent): 1: 1.15 (1.15, 1.26); 2: 1.26 (1.13, 1.41); 3: 1.20 (1.05, 1.38); 4: 1.11 (0.93, 1.33); 5: 1.12 (0.69, 1.82); 6: 1.93 (0.83, 4.49) 7-16: 1.23 (0.89, 1.69)
Muhsen, K	2006	Prevalence and risk factors of *Helicobacter pylori* infection among healthy 3- 5-year-old Israeli Arab children	Cross-sectional	Children (3-5 yrs of age)	Israel	NONE	197	SAT	Not stated	NONE	Mantel-Haenszel Analysis	NA	Odds Ratios	NONE	NONE	**Number of siblings (≤3 vs. > 3; ≤ 3 referent):** 1.5 (0.7, 2.9)	NA
Herrera, PM	2008	DNA-level diversity and relatedness of *Helicobacter pylori* strains in shantytown families in Peru and transmission in a developing-country setting	Laboratory analysis	Family members	Lima, Peru	NONE	133	String Test (culture or DNA)/RAPD/ PCR/ Sequencing	Not stated	NONE	Descriptive Analysis	It was found that 30% of mother-child, 18% of child-father, 25% mother-father and 32% sib-sib strains matched at >99.5%	NA	NA	NA	NA	NA
Raymond, J	2008	Using macro-arrays to study routes of infection of *Helicobacter pylori* in three families	Laboratory analysis	three families (French, Morocco)	Not stated	NONE	3 families	Biopsy & Culture; Micro array analysis	Not stated	NONE	Descriptive Analysis	One family sibs had similar strains, diff from parents. Was an older sib. Still can be common source for both sibs not necessarily sib-sib transmission	NA	NONE	NA	NA	NA
Muhsen, K	2006	Prevalence and risk factors of *Helicobacter pylori* infection among healthy 3- to 5-year-old Israeli Arab children	Cross-sectional	Children (3-5 yrs of age)	Israel	NONE	197	SAT	Not stated	NONE	Mantel-Haenszel Analysis	NA	Odds Ratios	NONE	NONE	**Number of siblings ages 0-5 yrs (≤1 vs. > 1; ≤ 1 referent):** 2.3 (1.1, 4.9)	NA

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First Author	Publication Year	Study Title	Study Design	Study Population	Study Location	Inclusion & Exclusionary Criteria	Sample Size	*Helicobacter pylori* Detection Methodology	Reported Diagnostic Sensitivity/ Specificity	Follow-up	Statistical Analysis Methods	P-value Based Results	Measure of Association	Covariate Adjustments	Effect Measures Modifiers	Point Estimates (95% Confidence Interval)	
																Crude	Adjusted
Weyermann, M	2009	Acquisition of *Helicobacter pylori* infection in early childhood: Independent contributions of infected mothers, fathers, and siblings	Cohort	Children (4 yrs of age)	Ulm (Germany)	Exclusion: Mother's who received antibiotics 4 weeks prior to UBT, families in which mothers, fathers and siblings were not all tested	493	SAT	Not stated	12, 24, 36 and 48 mos	Unconditional logistic regression	NA	Odds Ratios	**Partial Adjustment:** nationality of parents, single child status **Full Adjustment:** nationality of parents, single child status	NONE	**Sibling Hp Status (all neg or no sibs/at least one pos; neg or none referent):** 7.7 (2.5, 20.6)	**Partial Adjustment: Sibling Hp Status (all neg or no sibs/at least one pos; neg or none referent):** 6.1 (0.9, 41.1) **Full Adjustment: Sibling Hp Status (all neg or no sibs/at least one pos; neg or none referent):**3.7 (0.5, 26.2)
Yucel, O	2009	The factors associated with asymptomatic carriage of *Helicobacter pylori* in children and their mothers living in three socio-economic settings	Cross-sectional	Children (2-12 yrs of age)	Turkey	Exclusion: Symptomatic children	165	SAT	Not stated	NONE	Hypothesis testing	Number of siblings in Hp infected child (P<0.001)	NA	NA	NONE	NA	NA
Roma, E	2009	Intrafamilial spread of *Helicobacter pylori* infection in Greece	Cross-sectional	Children (age not provided)	Greece	Exclusion: Asymptomatic children (restricted to children with upper GI symptoms including epigastric or abdominal pain, vomiting and upper GI bleeding); children with history of antibiotic use within 3 mos of study	100	^{13}C-UBT & SAT (children less than 2 yrs of age); for index children two of the following: culture, histology, rapid urease test, ^{13}C-UBT	Not stated	NONE	Unconditional logistic regression	NA	Odds Ratios	NONE	Current and previous Hp status of family member	**at least 1 Hp infected sibling (pos vs. neg; neg referent) :** 19.06 (3.96, 91.66) **Previous Hp with eradication therapy:** 21.37 (4.44, 102.9)	NA
Sykora, J	2009	Epidemiology of *Helicobacter pylori* infection in asymptomatic children: a prospective population-based study from the Czech Republic. Application of a monoclonal-based antigen-in-stool enzyme immunoassay	Cross-sectional	Children (0-15 yrs of age)	Czech Republic	Exclusion: use of antibiotics, acid suppressants, prokinetics, bismuth compounds, or anti-inflammatory drugs 2 mos prior to stool sampling; also any children with acute or chronic stomach complaints, gastric surgery, mental illness, or history of eradication	1572	SAT	Not stated	NONE	Unconditional logistic regression	NA	Odds Ratios	NONE	NONE	**Number of children ≤18 yrs of age in HH (1 referent): 2:** 1.21 (0.68, 2.61); **3:** 2.63 (1.36, 4.21); **4 or more:** 4.90 (1.43, 7.81)	NA

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First Author	Publication Year	Study Title	Study Design	Study Population	Study Location	Inclusion & Exclusionary Criteria	Sample Size	Helicobacter pylori Detection Methodology	Reported Diagnostic Sensitivity/ Specificity	Follow-up	Statistical Analysis Methods	P-value Based Results	Measure of Association	Covariate Adjustments	Effect Measures Modifiers	Point Estimates (95% Confidence Interval) Crude	Point Estimates (95% Confidence Interval) Adjusted
Sykora, J	2009	Epidemiology of Helicobacter pylori infection in asymptomatic children: a prospective population-based study from the Czech Republic. Application of a monoclonal-based antigen-in-stool enzyme immunoassay	Cross-sectional	Children (0-15 yrs of age)	Czech Republic	Exclusion: use of antibiotics, acid suppressants, prokinetics, bismuth compounds, or anti-inflammatory drugs 2 mos prior to stool sampling; also any children with acute or chronic stomach complaints, gastric surgery, mental illness, or history of eradication	1572	SAT	Not stated	NONE	Unconditional logistic regression	NA	Odds Ratios	Lack of formal education of the father, institutionalized children	NONE	**Not provided**	**Number of children ≥2 (< 2 referent):** 4.26 (1.91, 9.80)
Sykora, J	2009	Epidemiology of Helicobacter pylori infection in asymptomatic children: a prospective population-based study from the Czech Republic. Application of a monoclonal-based antigen-in-stool enzyme immunoassay	Cross-sectional	Children (0-15 yrs of age)	Czech Republic	Exclusion: use of antibiotics, acid suppressants, prokinetics, bismuth compounds, or anti-inflammatory drugs 2 mos prior to stool sampling; also any children with acute or chronic stomach complaints, gastric surgery, mental illness, or history of eradication	1572	SAT	Not stated	NONE	Unconditional logistic regression	NA	Odds Ratios	NONE	NONE	**Number of siblings (0referent): 1:** 1.71 (0.70, 4.01); **2:** 1.92 (0.9, 4.2); **3 or more:** 2.41 (1.12, 5.8)	NA
Chi, H	2009	Prevalence of Helicobacter pylori infection in High-school students on Lanyu Island, Taiwan: risk factor analysis and effect on growth	Cross-sectional	Lanyu Island adolescents mean 14 yrs of age	Lanyu Island, Taiwan	NONE	106	^{13}C-UBT	Not stated	NONE	Unconditional logistic regression?	NA	Odds Ratios	NONE	NONE	**Sibship: ≤4 referent: >4:** 0.99 (0.45, 2.15)	NA

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First Author	Publication Year	Study Title	Study Design	Study Population	Study Location	Inclusion & Exclusionary Criteria	Sample Size	*Helicobacter pylori* Detection Methodology	Reported Diagnostic Sensitivity/ Specificity	Follow-up	Statistical Analysis Methods	P-value Based Results	Measure of Association	Covariate Adjustments	Effect Measures Modifiers	Point Estimates (95% Confidence Interval) Crude	Point Estimates (95% Confidence Interval) Adjusted
Chi, H	2009	Prevalence of *Helicobacter pylori* infection in High-school students on Lanyu Island, Taiwan: risk factor analysis and effect on growth	Cross-sectional	Lanyu Island adolescents mean 14 yrs of age	Lanyu Island, Taiwan	NONE	106	13C-UBT	Not stated	NONE	Unconditional logistic regression?	NA	Odds Ratios	NONE	NONE	**Siblings (0 or 1 referent): 2 or 3:** 0.5 (0.16, 1.58); **≥4:** 0.66 (0.21, 2.09)	NA
Fialho, AMN	2010	Younger Siblings Play a Major Role in *Helicobacter pylori* Transmission Among Children from a Low-income Community in the Northeast of Brazil	Cross-sectional	Children)6 mos-14 yrs of age)	Fortaleza, Ceara (Brazil)	NONE	351	13C-UBT (Children < 14 yrs of age)/ IgG immunoassay (Children ≥ 14 years of age)	13C-UBT: Sensitivity: 93.8% Specificity: 99.1% IgG immunoassay: Sensitivity: 95.4% Specificity: 100%	NONE	Unconditional logistic regression	NA	Odds Ratios	Partial Adjustment: age, number of children per household, number of people per room. Full Adjustment: age, number of children per household, number of people per room and Mother's Hp status	NONE	**Siblings Hp status (all Hp - vs. at least one Hp +; all Hp - referent):** 5.2 (2.9, 8.9)	**Partial Adjustment-Siblings Hp status (all Hp - vs. at least one Hp +; all Hp - referent):** 4.8 (2.7, 8.4) **Siblings Hp status (all Hp - vs. at least one Hp +; all Hp - referent):** 2.2 (1.0, 4.6)
Muhsen, K	2010	Presence of *Helicobacter pylori* in a sibling is associated with a long term increased risk of H. pylori infection in Israeli Arab children	Cross-sectional	Children (6-9 yrs of age)	Israel	NONE	192	SAT	Not stated	twice: 2004 and then again in 2007-2009	Unconditional logistic regression	NA	Prevalence Ratios	Maternal education, crowding index	Late acquisition of Hp (testing pos during second follow-up) and Early/ persistent Hp (testing pos during both first and second follow up)	Not provided	**Siblings Hp status (pos vs. neg; neg referent): Late Acquisition:** 4.62 (0.76, 28.23) **Early/ persistent:** 2.86 (0.99, 8.27)

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Appendix H

FOREST PLOT OF SIBLING *HELICOBACTER PYLORI* TRANSMISSION STUDIES: COMAPRISON OF *H. PYLORI*

DETERMINATION OF SIBLING VERSUS NUMBER OF CHILDREN IN HOUSEHOLD IN DETERMINING *H. PYLORI*

INFECTION ASSOCIATIONS BETWEEN SIBLINGS

	OR (95% CI)
OVERALL ESTIMATE	**3.6 (2.6, 4.9)**
Fialho, AMN (2010)	4.8 (2.7, 8.4)
Roma, E (2009)	19.1 (4.0, 91.6)**
Weyermann, M (2009)	6.1 (0.9, 41.1)
Rowland, M (2006)	4.9 (2.2, 11.6)
Muhsen, K (2006)	4.4 (1.3, 14.6)
Yang, YJ (2005)	2.0 (0.8, 5.3)**
Rocha, GA (2003)	1.8 (1.0, 3.3)
Goodman, KJ (2000)	7.1 (1.4, 36.6)**
OVERALL ESTIMATE	**3.6 (2.3, 5.5)**
Mbulaiteye, SM (2006)	2.7 (0.9, 8.3)**
Garg, PK (2006)	4.4 (1.4, 13.9)**
Kivi, M (2005)	8.1 (1.8, 37.3)
Glynn, MK (2002)	2.2 (1.0, 4.7)
Miyaji, H (2000)	4.0 (1.8, 8.8)**
Mitchell, JD (1993)	16.2 (2.1, 122.2)**
OVERALL ESTIMATE	**2.1 (1.8, 2.4)**
Chi, H (2009)	1.0 (0.5, 2.2)**
Ford, AC (2007)	4.9 (1.4, 7.8)**
Santos, IS (2005)	2.7 (1.7, 4.3)
Moyyedi, A (2002)	1.3 (1.0, 1.7)
Goodman (2000)	2.4 (1.1, 5.1)
Rothenbacher, D (1997)	4.3 (0.8, 24.7)
Goodman, KJ (1996)	2.7 (1.0, 6.1)
OVERALL ESTIMATE	**3.5 (1.5, 8.4)**
Koch, A (2005)	**2.1 (1.5, 3.0)**
Lin, DB (1999)	6.3 (1.5, 26.4)
Fall, CHD (1997)	2.4 (1.0, 5.8)
McCallion, WA (1996)	3.1 (1.9, 5.1)
Tsugane, S (1994)	3.6 (1.4, 9.5)
	0.7 (0.3, 1.3)

Studies assessing active *H. pylori* infection of index child and sibling shown in red. Studies utilizing immunoassays for *H. pylori* diagnosis of index child and sibling shown in pink. Studies assessing active *H. pylori* infection of the index child but utilizing four or more siblings or children in household as the exposure shown in dark blue. Studies utilizing immunoassays for *H. pylori* diagnosis of index child but utilizing four or more siblings or children in household as the exposure shown in light blue.

** indicates crude point estimates; all other measures adjusted for covariates as stated in appendix D.

APPENDIX I

FOREST PLOT OF SIBLING *HELICOBACTER PYLORI* TRANSMISSION STUDIES: COMAPRISON OF *H. PYLORI*

DETERMINATION OF SIBLING VERSUS BIRTH ORDER AND OLDER SIBLINGS *H. PYLORI* STATUS IN

DETERMINING *H. PYLORI* INFECTION ASSOCIATIONS BETWEEN SIBLINGS

	Measure of Association (95% CI)
OVERALL ESTIMATE	3.6 (2.6, 4.9)
Fialho, AMN (2010)	4.8 (2.7, 8.4)
Roma, E (2009)	19.1 (4.0, 91.6)**
Weyermann, M (2009)	6.1 (0.9, 41.1)
Rowland, M (2006)	4.9 (2.2, 11.6)
Muhsen, K (2006)	4.4 (1.3, 14.6)
Yang, YJ (2005)	2.0 (0.8, 5.3)**
Rocha, GA (2003)	1.8 (1.0, 3.3)
Goodman, KJ (2000)	7.1 (1.4, 36.6)**
OVERALL ESTIMATE	3.6 (2.3, 5.5)
Mbulaiteye, SM (2006)	2.7 (0.9, 8.3)**
Garg, PK (2006)	4.4 (1.4, 13.9)**
Kivi, M (2005)	8.1 (1.8, 37.3)
Glynn, MK (2002)	2.2 (1.0, 4.7)
Miyaji, H (2000)	4.0 (1.8, 8.8)**
Mitchell, JD (1993)	16.2 (2.1, 122.2)**
OVERALL ESTIMATE	4.0 (2.4, 6.7)
Mbulaiteye, SM (2006)	2.7 (0.9, 8.3)**
Goodman, KJ (2000)	4.4 (2.0, 9.4)
Miyaji, H (2000)	3.6 (1.3, 10.4)**
Rowland, M (1999)	7.1 (1.4, 35.1)+ **
OVERALL ESTIMATE	1.5 (1.3, 1.7)
Ford, AC (2007)	1.2 (0.9, 1.7)
Mbulaiteye, SM (2006)	11.1 (3.7, 34.0)**
Ueda, M (2003)	1.4 (1.1, 1.8)
Goodman, KJ)2000)	2.2 (0.9, 5.2)
Rothenbacher, D (1998)	1.2 (0.5, 2.8)
Fall, CHD (1997)	1.8 (1.2, 2.7)
Goodman, KJ (1996)	1.6 (0.7, 3.6)

0.1   1.0   10.0   100.0   1000.0

Studies assessing active *H. pylori* infection of index child and sibling shown in red. Studies utilizing immunoassays for *H. pylori* diagnosis of index child and sibling shown in pink. Studies assessing *H. pylori* status of an older sibling as the exposure shown in dark blue. Studies assessing birth order (highest compared to lowest) as the exposure shown in light blue.

** indicates crude point estimates; all other measures adjusted for covariates as stated in appendix D.
+ indicates risk ratio; all other measures shown are odds ratios.

APPENDIX J

FOREST PLOT OF SIBLING *HELICOBACTER PYLORI* TRANSMISSION STUDIES: COMPARISON OF *H. PYLORI*

DETERMINATION OF SIBLING VERSUS SIBLING GASTRIC DISEASE OUTCOMES IN DETERMINING *H. PYLORI*

INFECTION ASSOCIATIONS BETWEEN SIBLINGS

	OR (95% CI)
OVERALL ESTIMATE	3.6 (2.1, 4.0)
Fialho, AMN (2010)	4.8 (2.7, 8.4)
Roma, E (2009)	19.1 (4.0, 91.6)**
Weyermann, M (2009)	6.1 (0.9, 41.1)
Rowland, M (2006)	4.9 (2.2, 11.6)
Muhsen, K (2006)	4.4 (1.3, 14.6)
Yang, YJ (2005)	2.0 (0.8, 5.3)**
Rocha, GA (2003)	1.8 (1.0, 3.3)
Goodman, KJ (2000)	7.1 (1.4, 36.6)**
OVERALL ESTIMATE	3.6 (2.3, 3.5)
Mbulaiteye, SM (2006)	2.7 (0.9, 8.3)**
Garg, PK (2006)	4.4 (1.4, 13.9)**
Kivi, M (2005)	8.1 (1.8, 37.3)
Glynn, MK (2002)	2.2 (1.0, 4.7)
Miyaji, H (2000)	4.0 (1.8, 8.8)**
Mitchell, JD (1993)	16.2 (2.1, 122.2)**
OVERALL ESTIMATE	1.9 (1.5,2.4)
Leung, K (2006)	1.9 (1.1, 3.3)
Chang, YW (2002)	5.3 (2.0, 13.9)
Luzza, F (2000)	2.4 (1.3, 4.3)
Kikuchi, S (1998)	1.7 (1.1, 2.6)
Blaser, MJ (1995)	1.9 (0.8, 4.8)
Gasbarrini, G (1995)	1.3 (0.7, 2.3)
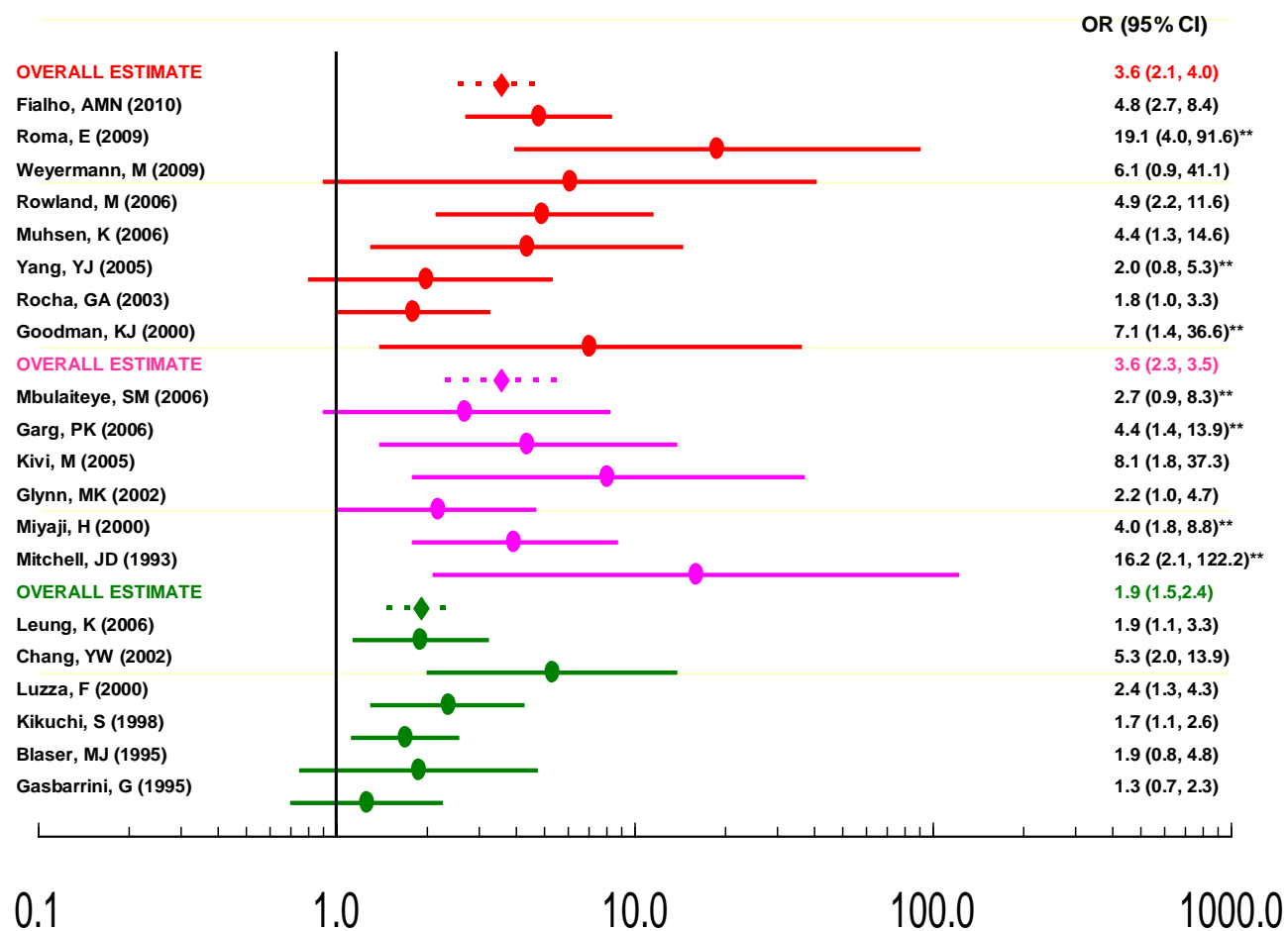
Studies assessing active *H. pylori* infection of index child and sibling shown in red. Studies utilizing immunoassays for *H. pylori* diagnosis of index child and sibling shown in pink. Studies assessing sibling gastric disease as the exposure shown in green; H. pylori status of the child (outcome) via varied methods.

** indicates crude point estimates; all other measures adjusted for covariates as stated in appendix D.

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Appendix K

BREASTFEEDING AND *HELICOBACTER PYLORI* ASSOCIATIONS LITERATURE REVIEW ARTICLES

First Author	Publication Year	Study Title	Study Design	Study Population	Study Location	Inclusion & Exclusionary Criteria	Sample Size	Helicobacter pylori Detection Methodology	Reported Diagnostic Sensitivity/ Specificity	Follow-up	Statistical Analysis Methods	P-value Based Results	Measure of Association	Covariate Adjustments	Effect Measures Modifiers	Crude	Adjusted
Weaver, LT	1995	Aspects of *Helicobacter pylori* infection in the developing and developed world: Helicobacter pylori infection, nutrition and growth of West African infants	Cohort	Infants	Gambia (Africa)	NONE	12	^{13}C-UBT	Not stated	3, 6, 9, 12 mos	Hypothesis testing	When children were divided into those who were Hp pos and neg at 9 mos of age, those who were neg, the breast milk of their mothers had 2-4 times higher IgA antibody levels between 13-39 wks post partum compared to Hp pos infants.P=0.004	NA	NA	NONE	NA	NA
Mahalanabis, D	1996	*Helicobacter pylori* infection in the young in Bangladesh: Prevalence, socioeconomic and nutritional aspects	Cross-sectional	Children (1-99 mos old)	Nandipara, Bangladesh (India)	Exclusion: Children receiving antimicrobials 6 wks prior to UBT	469	^{13}C-UBT	Not stated	NONE	Hypothesis testing	Currently breast fed children at age 2 yrs Hp pos vs. Hp Neg P=0.024; at age 5 yrs P=0.049	NA	NA	NA	NA	NA
McCallion, WA	1996	*Helicobacter pylori* infection in children: relation with current household living conditions	Cross-sectional	Children 3-15 years of age, day surgery (non gastro)	Belfast (Ireland)	NONE	367	IgG immunoassay	Sensitivity: 100% Specificity: 92%	NONE	Hypothesis testing	Breast feeding (never or <2 weeks) vs. 2 weeks or more (P=0.03)	NA	NA	NA	NA	**NA**
Gold, BD	1997	*Helicobacter pylori* acquisition in infancy after decline of maternal passive immunity	Cross-sectional	Infants	Taiwan	NONE	80	IgG immunoassay	Sensitivity: 96%, Specificity: 95%	1,2,3,6,12 and 14 mos of age	Not stated; unconditional Logistic Regression?	NA	Odds Ratios	NONE	Mother's status as primary care giver	**Breast feeding (Y/N; No referent): Mother as primary care giver:** OR= 13.2 (1.2, 347)	NA
Naficy, AB	2000	Seroepidemiology of *Helicobacter pylori* infection in a population of Egyptian children	Cohort	Children (< 24 mos of age)	Egypt	Exclusion: Children with major congenital abnormalities, severe chronic illness	196	IgG immunoassay	Sensitivity: 98.4%, Specificity: 96.4%	Not stated	Unconditional logistic regression	NA	Odds Ratios	Age	NONE	**Breast fed (Any/None; None referent):** OR=3.1 (not provided)	**Breast fed (Any/None; None referent):** OR=1.4 (0.37, 5.8)
Okuda, M	2001	Breast-feeding prevents *Helicobacter pylori infection* in early childhood	Cross-sectional	Children (1-12 yrs of age)	Japan	Inclusion: Inpatient at Wakayama Rosai Hospital; Exclusion: Gastric Symptoms	484	HpSA	Not stated	NONE	Hypothesis testing	**Increase per Breast feeding months: 1-12 yr old children:** P=0.02; **1-3 yr old children:** P=0.003	NA	NA	NA	NA	NA

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First Author	Publication Year	Study Title	Study Design	Study Population	Study Location	Inclusion & Exclusionary Criteria	Sample Size	*Helicobacter pylori* Detection Methodology	Reported Diagnostic Sensitivity/ Specificity	Follow-up	Statistical Analysis Methods	P-value Based Results	Measure of Association	Covariate Adjustments	Effect Measures Modifiers	Crude	Adjusted
Malaty, HM	2001	*Helicobacter pylori* infection in preschool and school-aged minority children: effect of socioeconomic indicators and breast feeding practices	Cross-sectional	Children (2-16 yrs of age); centers	Houston, Texas (United States)	Inclusion: Black and Hispanic, attending daycare Exclusion: White, non-daycare attending children; acute or chronic stomach problems or gastric surgery; current use of antibiotics (within 1 mo)	356	^{13}C-UBT	Not stated	NONE	Unconditional logistic regression	NA	Odds Ratios	Age	NONE	**Breast Fed (Y/N, Yes referent):** OR=2.7 (1.4, 4.2)	**Breast Fed (Y/N, Yes referent):** OR=3.0 (1.5, 5.9)
Omar, AA	2001	Prevalence and possible risk factors of *Helicobacter pylori* infection among children attending Damanhour teaching hospital	Cross-sectional	Children (1.5-16 yrs of age); outpatients of Damanhour Teaching Hospital	Egypt	NONE		IgG immunoassay	Not stated	NONE	Unconditional logistic regression	NA	Odds Ratios	increasing age (≥5 yrs), overcrowding	NONE	Not provided	**Breast Feeding duration (<1 yr vs. ≥1 yr; ≥ 1 yr referent):** 2.5 (1.1, 5.9)
Rothenbacher, D	2002	History of breastfeeding and *Helicobacter pylori* in pre-school children: results of a population based study from Germany	Cross-sectional	Children (5-8 years of age)	Ulm (Germany)	NONE	1,221	^{13}C-UBT	Sensitivity & Specificity: 100%	NONE	Unconditional logistic regression	NA	Odds Ratios	**Partial Adjustment:** *H.pylori* status of mother **Full Adjustment:** *H. pylori* status of mother, nationality, age, sex, place of birth, birhtweight, education of father, education of mother, history of antibiotic use, housing density, number of siblings, household smoking of mother, household smoking of father	NONE	**Breastfed (Y/N, No referent):** OR=1.22 (0.68, 2.22) **Duration of Breast feeding (never referent):** <3mos: OR=1.00 (0.48, 2.05) 3-6 mos: OR=1.10 (0.53, 2.26) ≥6 mos: OR=1.46 (0.77, 2.75)	Partial Adjustment: Breastfed (Y/N, No referent): OR=1.67 (0.89, 3.14) Duration of Breast feeding (never referent): <3mos: OR=1.21 (0.57, 2.59) 3-6 mos: OR=1.34 (0.62, 2.87) ≥6 mos: OR=2.38 (1.20, 4.72) Full Adjustment: Breastfed (Y/N, No referent): OR=1.56 (0.47, 2.46) Duration of Breast feeding (never referent): <3mos: OR=1.07 (0.47, 2.46) 3-6 mos: OR=1.19 (0.52, 2.75) ≥6 mos: OR=2.57 (1.19, 5.55)
Dore, MP	2002	Risk factors associated with *Helicobacter pylori* infection among children in a defined geographic area	Cross-sectional	Children (5-16 yrs of age)	Sardinia (Italy)	NONE	2,810	IgG immunoassay	Sensitivity: 98.7%, Specificity: 100.0%	NONE	Unconditional logistic regression	NA	Odds Ratios	Age, sex, occupational category for head of household, ownership of animals, daycare attendance	Rural vs. Urban areas	Not provided	**Breast fed (Y/N, No referent):** OR=1.4 (1, 2); **Rural:** OR=0.8 (0.6, 1.1) **Urban:** OR=0.6 (0.4, 1.1)

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| First Author	Publication Year	Study Title	Study Design	Study Population	Study Location	Inclusion & Exclusionary Criteria	Sample Size	*Helicobacter pylori* Detection Methodology	Reported Diagnostic Sensitivity/ Specificity	Follow-up	Statistical Analysis Methods	P-value Based Results	Measure of Association	Covariate Adjustments	Effect Measures Modifiers	Point Estimates (95% Confidence Interval) Crude	Point Estimates (95% Confidence Interval) Adjusted
Ueda, M	2003	*Helicobacter pylori* risk associated with childhood home environment	Cross-sectional	Adults, age not specified	Japan	Inclusion: Stomach cancer examination system participants	5,854	IgG Immunoassay? Not specified	Not stated	NONE	Unconditional logistic regression	NA	Odds Ratios	NA	NONE	**Breast feeding (breast fed referent): other:** OR=0.937 (0.821, 1.069)	NA
Ertem, D	2003	*Helicobacter pylori* infection in Turkish preschool and school children: role of socioeconomic factors and breast feeding	Cross-sectional	Children, (3-12 yrs old)	Turkey	Exclusion: Children who received oral or parenteral antibiotics	327	13C-UBT	Not stated	NONE	Unconditional logistic regression	NA	Odds Ratios	Socioeconomic class, number of siblings, heating system, age, weight percentile, height percentile, household density, education of parents	NONE	Not provided	**Breast fed (Y/N; Y referent):** OR=4.45 (1.04, 19.1)
Thomas, JE	2004	Specific immunoglobulin A antibodies in maternal milk and delayed *Helicobacter pylori* colonization in Gambian infants	Laboratory analysis	Children (3 mos-1 yr old)	Gambia (Africa)	NONE	65	13C-UBT	Not stated	4 wks- 1yr of age (monthly)	Hypothesis testing	There was a sig difference in Hp IgA anti urease in breast milk for children who were Hp seropos vs. seroneg (P=<0.05)	NA	NA	NONE	NA	NA
Pearce, MS	2005	Does increased duration of exclusive breastfeeding protect against *Helicobacter pylori* infection? The Newcastle Thousand Family Cohort Study at age 49-51 years	Cohort	Adults (50 years old)	New Castle (England)	NONE	407	IgG immunoassay	Sensitivity & Specificity: >95%	Breastfeeding: Up to 6 weeks in infancy and quarterly up to age five	Unconditional logistic regression	NA	Odds Ratios	Parental occupational social class, adverse housing at birth	Gender	NA	**Per 30 days exclusive breast feeding: All:** OR=0.9 (0.8, 1.1) **Males:** OR=0.8 (0.8, 0.98) **Females:** OR=0.9 (0.6, 0.9)
Rodrigues, MN	2006	History of breastfeeding and *Helicobacter pylori* in children: results of a community based study from northeast Brazil	Cross-sectional	Children (≤ 14 years of age)	Fortaleza, Ceará, (Brazil)	NONE	353	13C-UBT	Not stated	NONE	Unconditional logistic regression	NA	Odds Ratios	*H. pylori* status of mother, age, nutritional status, education of mother, history of antibiotic use, smoking of mother, number of persons per room, number of children per household	NONE	**Breastfed (Y/N):** OR=1.12 (0.45, 2.82) **Duration of Breast feeding (≤6 mos referent): > 6 mos:** OR=0.86 (0.56, 1.35)	**Breastfed (Y/N):** OR=1.12 (0.39, 3.20) **Duration of Breast feeding (≤6 mos referent): > 6 mos:** OR=0.64 (0.35, 1.18)

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First Author	Publication Year	Study Title	Study Design	Study Population	Study Location	Inclusion & Exclusionary Criteria	Sample Size	*Helicobacter pylori* Detection Methodology	Reported Diagnostic Sensitivity/ Specificity	Follow-up	Statistical Analysis Methods	P-value Based Results	Measure of Association	Covariate Adjustments	Effect Measures Modifiers	Point Estimates (95% Confidence Interval) Crude	Point Estimates (95% Confidence Interval) Adjusted
Nguyen, BV	2006	Prevalence of and factors associated with *Helicobacter pylori* infection in children in the north of Vietnam	Cross-sectional	Children (6 mos-15 yrs of age)	North Vietnam	Inclusion: Inpatient of University Hospital; Exclusion: outpatient not presenting Wednesday at pediatric hospital; any children with acute diarrhea, ulcer disease, repeated abdominal pain, immunocompromised	824	IgG Immunoassay	Sensitivity: 99.6%, Specificity: 97.8%	NONE	Unconditional logistic regression	NA	Odds Ratios	Child's age, mother's age, number of offspring, history of allergy and history of gastro-duodenal disease, breast feeding duration, sharing bed with parents, collective life initiation	NONE	**Breast feeding (≤6 mos referent): >6 mos:** OR=0.6 (0.4, 0.8)	**Breast feeding (≤6 mos referent): >6 mos:** OR=0.5 (0.3, 0.9)
Ito, LS	2006	Community-based familial study of *Helicobacter pylori* infection among healthy Japanese Brazilians	Cross-sectional	Japanese children (0-19 yrs of age)	San Paulo (Brazil)	Exclusion: Adopted children, either or one of parents not of Japanese descent, any households in which all of members were not living in same household, children with mental deficiency, children with chronic diseases	507	IgG immunoassay	Not stated	NONE	Unconditional logistic regression	NA	Odds Ratios	Sex and age	NONE	Not provided	**Breast Fed Status (<6 mos vs. ≥6 mos referent):** 1.05 (0.44, 2.51)
Rowland, M	2006	Age-specific incidence of *Helicobacter pylori*	Nested case-control	Children 24-48 mos of age at enrollment	Dublin (Ireland)	Exclusion: Children with no eligible sibling tested for Hp (sibling analysis)	290	^{13}C-UBT	Not stated	Annually for four years	Unconditional logistic regression	NA	Odds Ratios	NONE	NONE	**Breast fed (Y/N; No referent):** 0.54 (0.26, 1.09)	NA
Suoglu, OD	2007	Association of *Helicobacter pylori* infection with gastroduodenal disease, epidemiologic factors and iron-deficiency anemia in Turkish children undergoing endoscopy, and impact on growth	Case-Control	Children (<6 yrs of age); Istanbul Medical Facility	Turkey	Exclusion: Children with no symptoms, who did not undergo endoscopy for symptoms; patients with any chronic diseases that may cause anemia, upper or lower GI bleeding, history of using iron preparations or antibiotics within 1 month of study, endoscopic and/or histopathologic evidence of inflammatory bowel disease or portal hypertension, adolescent girls with excessive menstrual blood loss	70	Gastric biopsy /Histology	Not stated	NONE	Unconditional logistic regression	NA	Odds Ratios	Age, socioeconomic status	NONE	Not provided	**Exclusively breast fed (≥4 mos; < 4 mos referent):** OR=7.385 (2.049, 26.617); **Odds of being Hp negative**

First Author	Publication Year	Study Title	Study Design	Study Population	Study Location	Inclusion & Exclusionary Criteria	Sample Size	*Helicobacter pylori* Detection Methodology	Reported Diagnostic Sensitivity/ Specificity	Follow-up	Statistical Analysis Methods	P-value Based Results	Measure of Association	Covariate Adjustments	Effect Measures Modifiers	Point Estimates (95% Confidence Interval) Crude	Adjusted
Braga, A	2007	*Helicobacter pylori* colonization among children up to 6 years: results of a community-based study from Northeastern Brazil	Cross-sectional	Children (3 mos-6 yrs of age)	Brazil	Exclusion: Children with major congenital abnormalities, severe chronic illness	217	^{13}C-UBT	Not stated	NONE	Unconditional logistic regression	NA	Odds Ratios	NONE	NONE	**Breast feeding (Y/N; No referent):** OR= 1.14 (0.48, 2.74); **Breast Feeding duration (<6 mos vs. ≥6 mos; < 6 mos referent):** 1.69 (0.84, 3.41)	Not provided
Chak, E	2009	The role of breast-feeding in the prevention of *Helicobacter pylori* infection: A systematic review	Meta-Analysis	14 breast feeding studies	Various	Exclusion: Any studies which did not include RR, OR, or 95% CI, or crude data to calculate measures of association; case reports, review articles	14 breast feeding studies	Various	NA	NA	Meta Analysis	NA	Various	Various	NA	Summary OR=0.78 (0.61, 0.99); 9 of 14 studies reported OR<1.0; Summary OR with UBT: OR=0.67 (0.32, 1.39); Summary OR with Immunoassay: OR=0.91 (0.74, 1.11)	NA
Baldassarre, ME	2009	The source of *Helicobacter pylori* infection in the neonatal period	laboratory analysis	Infants (newborns)	Italy	Inclusion: children born in the University of Bari OBGYN unit	172	SAT & ^{13}C-UBT	Not stated	1,6,12, and 18 mos	Hypothesis testing	4/48 (8.3%) formula fed SAT pos while 1/124 breast fed SAT pos (P=0.02)	NA	NA	NONE	NA	NA
Yucel, O	2009	The factors associated with asymptomatic carriage of *Helicobacter pylori* in children and their mothers living in three socio-economic settings	Cross-sectional	Children (2-12 yrs of age)	Turkey	Exclusion: Symptomatic children	165	SAT	Not stated	NONE	Hypothesis testing	Breast feeding duration (months) in Hp infected child (P<0.02)	NA	NA	NONE	NA	NA
Sykora, J	2009	Epidemiology of *Helicobacter pylori* infection in asymptomatic children: a prospective population-based study from the Czech Republic. Application of a monoclonal-based antigen-in-stool enzyme immunoassay	Cross-sectional	Children (0-15 yrs of age)	Czech Republic	Exclusion: use of antibiotics, acid suppressants, prokinetics, bismuth compounds, or anti-inflammatory drugs 2 mos prior to stool sampling; also any children with acute or chronic stomach complaints, gastric surgery, mental illness, or history of eradication	1,572	SAT	Not stated	NONE	Unconditional logistic regression	NA	Odds Ratios	NONE	NONE	**Breast Feeding (Y/N; yes referent):** 2.51 (1.41, 4.90)	NA

First Author	Publication Year	Study Title	Study Design	Study Population	Study Location	Inclusion & Exclusionary Criteria	Sample Size	*Helicobacter pylori* Detection Methodology	Reported Diagnostic Sensitivity/ Specificity	Follow-up	Statistical Analysis Methods	P-value Based Results	Measure of Association	Covariate Adjustments	Effect Measures Modifiers	Point Estimates (95% Confidence Interval) Crude	Adjusted
Monajemzadeh, M	2010	Breastfeeding and *Helicobacter pylori* infection in children with digestive symptoms	Case-Control	Children (2-14 yrs of age)	Iran	Inclusion: Children's Medical Center Exclusion: Children not attending Children's Medical center, not diagnosed with digestive symptoms, no endoscopy; those with chronic debilitating disease; children with unknown or mixed breast feeding history	154 (77 case/77 controls)	Gastric biopsy	Not stated	NONE	Not stated; Unconditional logistic regression?	NA	Odds Ratios	Not stated? Assume crude measure	NONE	**Breast feeding (exclusive breast feeding versus formula feeding; bf referent):** OR= 2.92; (CI not provided)	N A

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APPENDIX L

PARTIAL RESIDUALS PLOT FOR TESTING PROPORTIONALITY HAZARDS

ASSUMPTION OF ACQUIRING AN INCIDENT *HELICOBACTER PYLORI* INFECTION

BY*HELICOBACTER PYLORI* STATUSOF THE MOTHER, THE PASITOS COHORT

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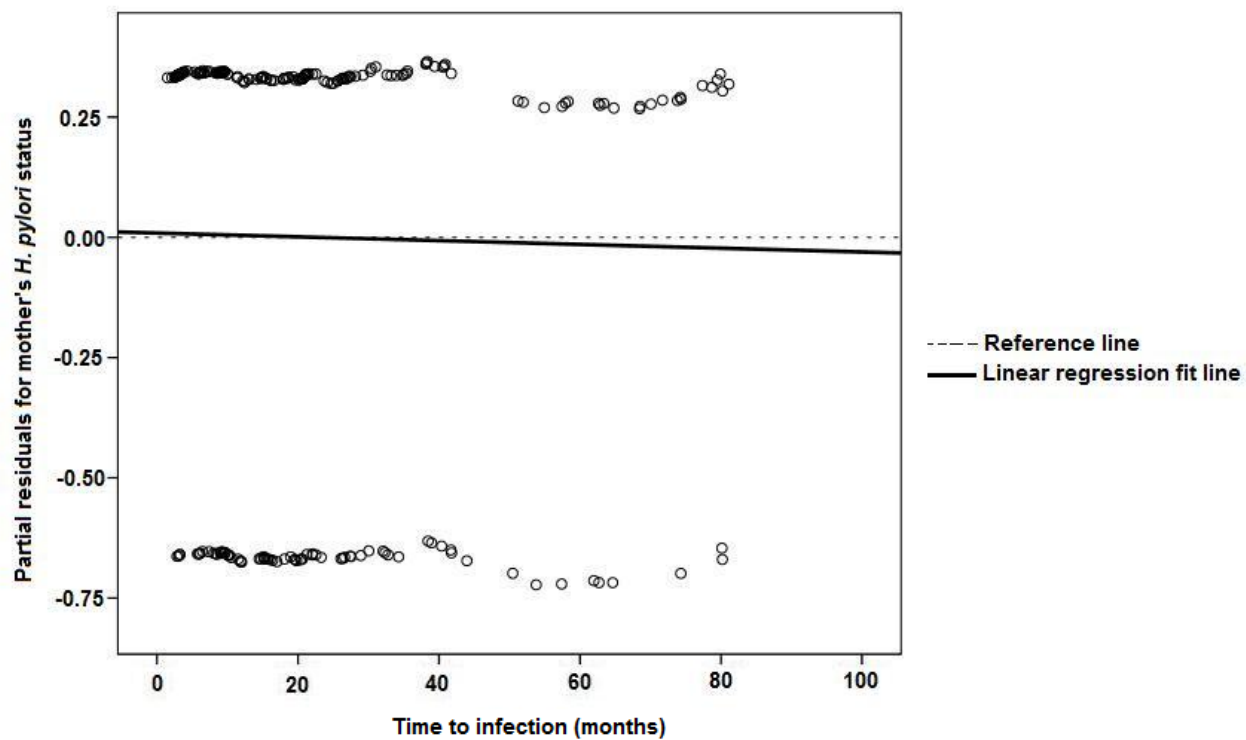
APPENDIX M

EFFECT OF EXPOSURE TO AN *HELICOBACTER PYLORI* SEROPOSITIVE

MOTHER ON HER CHILD ACQUIRING AN INCIDENT *HELICOBACTER PYLORI*

INFECTION, THE PASITOS COHORT

Exposure Variable	Incident Infections (n)[a]	Person-Time (Months)[b]	Hazard Ratio[c]	95% CI[d]
Overall	250	15,906		
Seropositive Mother[e]	167	9,978	1.2	0.9, 1.6
Seronegative Mother	83	5,928	1.0	
***H. pylori* Detected in Sibling[f]**	69	3,911		
Seropositive Mother[e]	53	2,542	1.6	0.9, 2.9
Seronegative Mother	16	1,369	1.0	
***H. pylori* Not Detected in Sibling[f]**	92	6,150		
Seropositive Mother[e]	54	3,375	1.2	0.8, 1.8
Seronegative Mother	38	2,775	1.0	
≤3 years of age between siblings	30	2,328		
Seropositive Mother[e]	19	1,238	1.4	0.7, 3.0
Seronegative Mother	11	1,090	1.0	
> 3 years of age between siblings	19	775		
Seropositive Mother[e]	14	576	1.2	0.4, 3.5
Seronegative Mother	5	199	1.0	
Mexico	96	6,426		
Seropositive Mother[e]	78	4,750	1.5	0.9, 2.5
Seronegative Mother	18	1,676	1.0	
United States	154	9,480		
Seropositive Mother[e]	89	5,227	1.1	0.8, 1.6
Seronegative Mother	65	4,253	1.0	
***H. pylori* Active Antibiotic Use**	153	10,152		
Seropositive Mother[e]	100	6,081	1.3	0.9, 1.8
Seronegative Mother	53	4,071	1.0	
No *H. pylori* Antibiotic Use	97	5,632		
Seropositive Mother[e]	67	3,816	1.0	0.7, 1.6
Seronegative Mother	30	1,816	1.0	
Breastfed, any duration	150	9,557		
Seropositive Mother[e]	107	6,417	1.2	0.9, 1.7
Seronegative Mother	43	3,140	1.0	
Not Breastfed	51	3,246		
Seropositive Mother[e]	27	1,747	0.9	0.6, 1.7
Seronegative Mother	24	1,499	1.0	

[a]Number of incident infections in the numerator of the crude rate

[b]Person months used in the denominator of the crude rate

[c]Adjusted for crowding defined as ratio of number of people within a household to number of rooms in a home.

[d] Confidence interval

[e]*H. pylori* serostatus of mother as determined at baseline.

[f]*H. pylori* serostatus for older, non-index siblings living within the same household. *H. pylori* status determined by ^{13}C-UBT for index and younger siblings enrolled in The Pasitos Cohort.

PARTIAL RESIDUALS PLOT FOR TESTING PROPORTIONALITY HAZARDS

ASSUMPTION OF ACQUIRING A LONG-TERM *HELICOBACTER PYLORI*

INFECTION BY*HELICOBACTER PYLORI* STATUSOF THE MOTHER: TIME-TO-

EVENT SCENARIO ONE, THE PASITOS COHORT

PARTIAL RESIDUALS PLOT FOR TESTING PROPORTIONALITY HAZARDS

ASSUMPTION OF ACQUIRING A LONG-TERM *HELICOBACTER PYLORI*

INFECTION BY*HELICOBACTER PYLORI* STATUSOF THE MOTHER:TIME-TO-

EVENT SCENARIO TWO, THE PASITOS COHORT

Partial residuals for mother's *H. pylori* status

Time to long-term infection (months)

- - - - Reference line
——— Linear regression fit line

APPENDIX P

PARTIAL RESIDUALS PLOT FOR TESTING PROPORTIONALITY HAZARDS

ASSUMPTION OF ACQUIRING A LONG-TERM *HELICOBACTER PYLORI*

INFECTION BY*HELICOBACTER PYLORI* STATUSOF THE MOTHER:TIME-TO-

EVENT SCENARIO THREE, THE PASITOS COHORT

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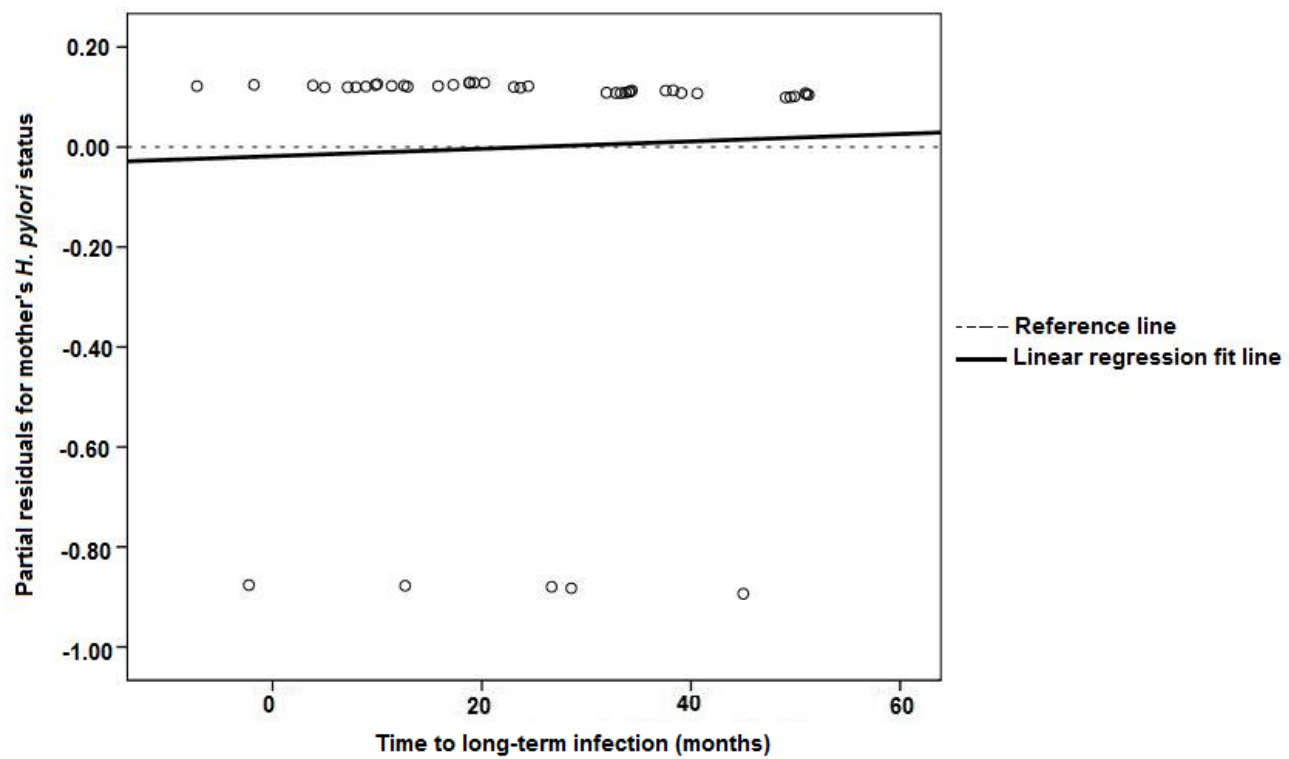
APPENDIX Q

EFFECT OF EXPOSURE TO AN *HELICOBACTER PYLORI* SEROPOSITIVE

MOTHER ON HER CHILD ACQUIRING A LONG-TERM *HELICOBACTER PYLORI*

INFECTION: TIME-TO-EVENT SCENARIO ONE, THE PASITOS COHORT

Exposure Variable	Long-Term Infections (n)[a]	Person-Time (Months)[b]	Hazard Ratio[c]	95% CI[d]
Overall	43	22,851		
Seropositive Mother[e]	38	14,514	2.1	0.3, 15.0
Seronegative Mother	5	8,337	1.0	
H. pylori Detected in Sibling[f]	20	5,559		
Seropositive Mother[e]	18	3,684	1.1	0.0, 30.9
Seronegative Mother	2	1,875	1.0	
H. pylori Not Detected in Sibling[f]	11	8,763		
Seropositive Mother[e]	9	5,088	5.5	0.2, 137.1
Seronegative Mother	2	3,675	1.0	
≤3 years of age between siblings	9	2,788		
Seropositive Mother[e]	8	1,500	59.2	0.0, 535579.2
Seronegative Mother	1	1,288	1.0	
> 3 years of age between siblings	1	975		
Seropositive Mother[e]	1	714	***	***
Seronegative Mother	0	261	***	
Mexico	27	9,067		
Seropositive Mother[e]	24	6,870	0.7	0.1, 9.2
Seronegative Mother	3	2,197	1.0	
United States	16	13,784		
Seropositive Mother[e]	14	7,644	5.4	0.2, 157.1
Seronegative Mother	2	6,140	1.0	
H. pylori Active Antibiotic Use	20	14,751		
Seropositive Mother	18	8,956	◊◊◊	◊◊◊
Seronegative Mother	2	5,795	◊◊◊	
No *H. pylori* Antibiotic Use	23	7,979		
Seropositive Mother	20	5,477	◊◊◊	◊◊◊
Seronegative Mother	3	2,502	◊◊◊	
Breastfed, any duration	27	14,244		
Seropositive Mother	24	9,626	2.7	0.2, 31.1
Seronegative Mother	3	4,618	1.0	
Not Breastfed	6	4,845		
Seropositive Mother	5	2,675	◊◊◊	◊◊◊
Seronegative Mother	1	2,170	◊◊◊	

[a]Number of long-term infections in the numerator of the crude rate

[b]Person months used in the denominator of the crude rate

[c]Adjusted for crowding defined as ratio of number of people within a household to number of rooms in a home.
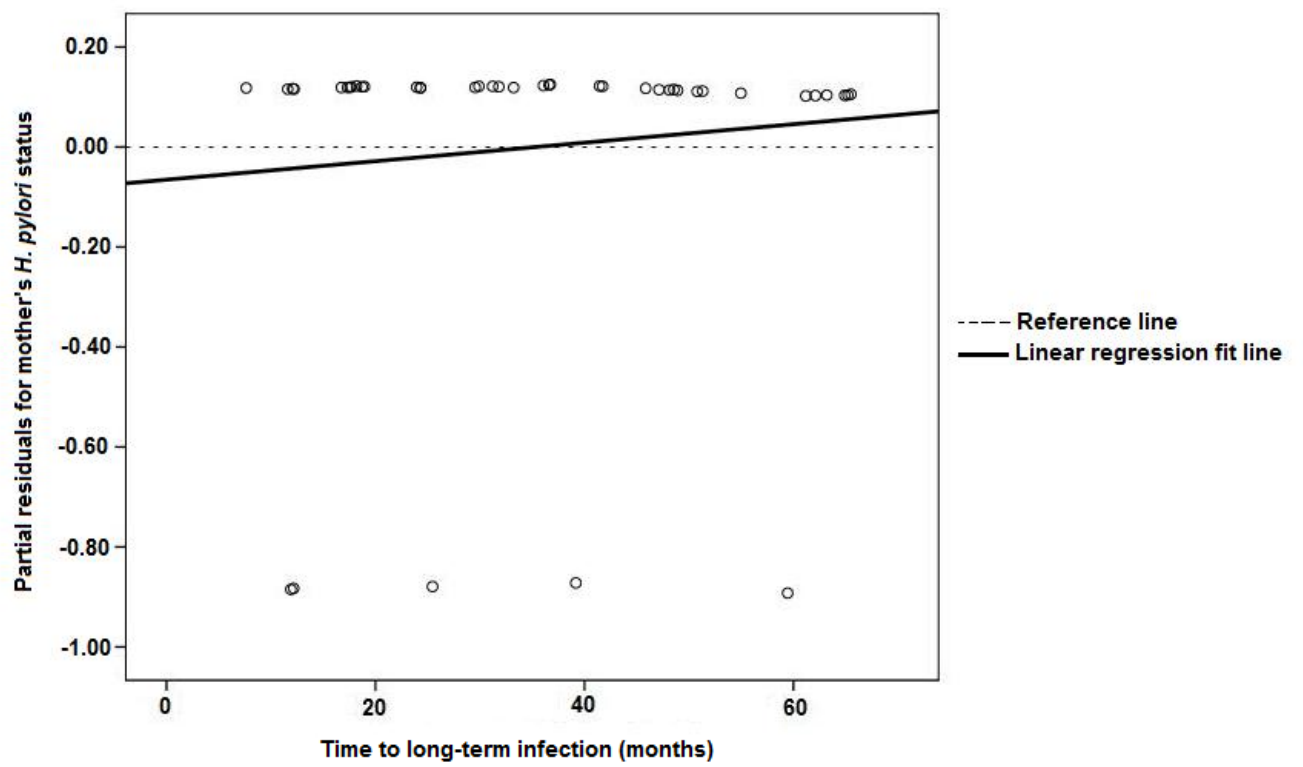
[d] Confidence interval

[e]*H. pylori* serostatus of mother as determined at baseline.

[f]*H. pylori* serostatus for older, non-index siblings living within the same household. *H. pylori* status determined by ^{13}C-UBT for index and younger siblings enrolled in The Pasitos Cohort.
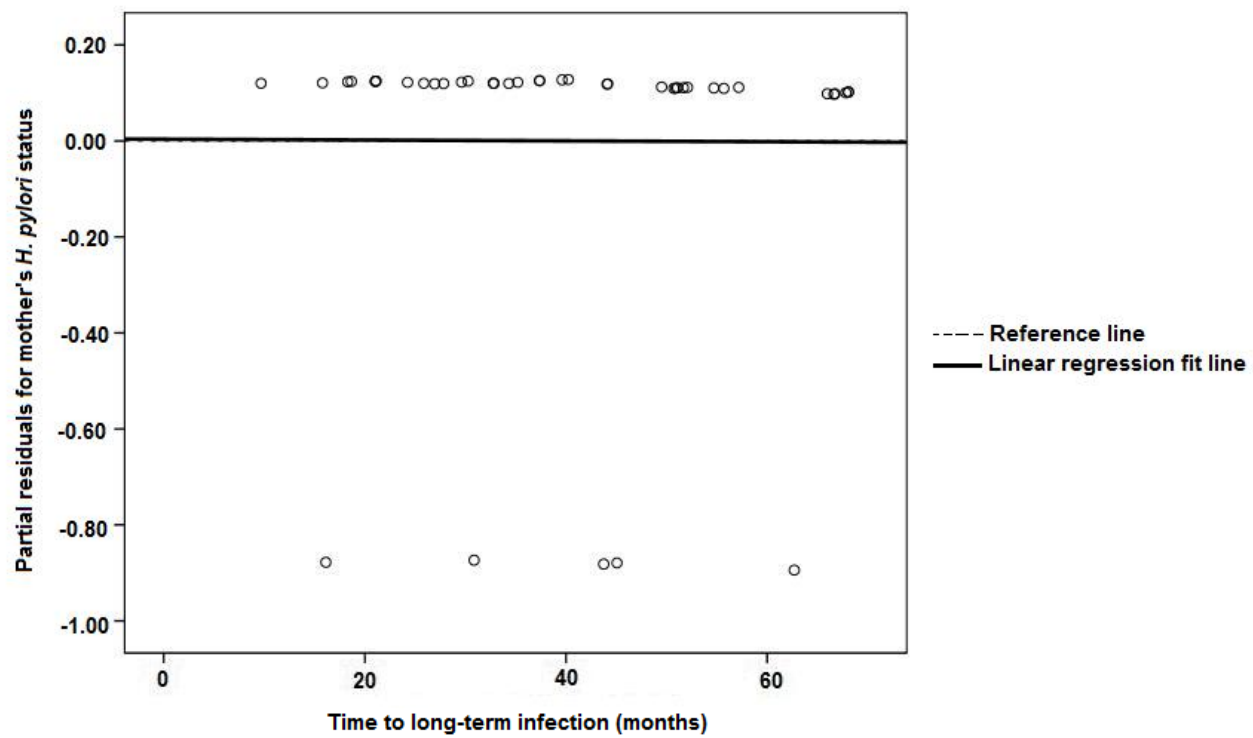
*** Hazard ratios and confidence intervals could not be calculated due to zero counts.

◊◊◊ Hazard ratios and confidence intervals could not be calculated due to model instability.

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APPENDIX R

EFFECT OF EXPOSURE TO AN *HELICOBACTER PYLORI* SEROPOSITIVE

MOTHER ON HER CHILD ACQUIRING A LONG-TERM *HELICOBACTER PYLORI*

INFECTION: TIME-TO-EVENT SCENARIO TWO, THE PASITOS COHORT

Exposure Variable	Long-Term Infections (n)[a]	Person-Time (Months)[b]	Hazard Ratio[c]	95% CI[d]
Overall	43	23,079		
Seropositive Mother[e]	38	14,692	4.0	0.3, 46.5
Seronegative Mother	5	8,387	1.0	
***H. pylori* Detected in Sibling**	20	5,668		
Seropositive Mother[e]	18	3,769	2.4	0.1, 128.8
Seronegative Mother	2	1,899	1.0	
***H. pylori* Not Detected in Sibling**	11	8,810		
Seropositive Mother[e]	9	5,128	5.9	0.2, 224.8
Seronegative Mother	2	3,682	1.0	
≤3 years of age between siblings	9	2,820		
Seropositive Mother[e]	8	1,527	98.1	0.0, 1557821.9
Seronegative Mother	1	1,293	1.0	
> 3 years of age between siblings	1	976		
Seropositive Mother[e]	1	715	***	***
Seronegative Mother	0	261	***	
Mexico	27	9,241		
Seropositive Mother[e]	24	7,000	2.4	0.8, 72.4
Seronegative Mother	3	2,241	1.0	
United States	16	13,838		
Seropositive Mother[e]	14	7,692	5.5	0.2, 199.7
Seronegative Mother	2	6,146	1.0	
***H. pylori* Active Antibiotic Use**	20	14,837		
Seropositive Mother	18	9,035	◊◊◊	◊◊◊
Seronegative Mother	2	5,802	◊◊◊	
No *H. pylori* Antibiotic Use	23	8,120	◊◊◊	◊◊◊
Seropositive Mother	20	5,575	◊◊◊	
Seronegative Mother	3	2,545		
Breastfed, any duration	27	14,402		
Seropositive Mother	24	9,742	9.2	0.3, 297.2
Seronegative Mother	3	4,660	1.0	
Not Breastfed	6	4,880		
Seropositive Mother	5	2,707	◊◊◊	◊◊◊
Seronegative Mother	1	2,173	◊◊◊	

[a]Number of long-term infections in the numerator of the crude rate

[b]Person months used in the denominator of the crude rate

[c]Adjusted for crowding defined as ratio of number of people within a household to number of rooms in a home.

[d] Confidence interval

[e]*H. pylori* serostatus of mother as determined at baseline.

[f]*H. pylori* serostatus for older, non-index siblings living within the same household. *H. pylori* status determined by ^{13}C-UBT for index and younger siblings enrolled in The Pasitos Cohort.

*** Hazard ratios and confidence intervals could not be calculated due to zero counts.

◊◊◊ Hazard ratios and confidence intervals could not be calculated due to model instability.

APPENDIX S

EFFECT OF EXPOSURE TO AN *HELICOBACTER PYLORI* SEROPOSITIVE

MOTHER ON HER CHILD ACQUIRING A LONG-TERM *HELICOBACTER PYLORI*

INFECTION: TIME-TO-EVENT SCENARIO THREE, THE PASITOS COHORT

Exposure Variable	Long-Term Infections (n)[a]	Person-Time (Months)[b]	Hazard Ratio[c]	95% CI[d]
Overall	43	23,260		
Seropositive Mother[e]	38	14,860	2.7	0.2, 39.7
Seronegative Mother	5	8,400	1.0	
H. pylori **Detected in Sibling**	20	5,756		
Seropositive Mother[e]	18	3,853	1.4	0.0, 107.7
Seronegative Mother	2	1,903	1.0	
H. pylori **Not Detected in Sibling**	11	8,849		
Seropositive Mother[e]	9	5,163	4.9	0.1, 239.8
Seronegative Mother	2	3,686	1.0	
≤3 years of age between siblings	9	2,838		
Seropositive Mother[e]	8	1,542	125.4	0.0, 6258651.7
Seronegative Mother	1	1,296	1.0	
> 3 years of age between siblings	1	985		
Seropositive Mother[e]	1	724	***	***
Seronegative Mother	0	261	***	
Mexico	27	9,346		
Seropositive Mother[e]	24	7,098	1.7	0.0, 82.6
Seronegative Mother	3	2,248	1.0	
United States	16	13,914		
Seropositive Mother[e]	14	7,763	3.4	0.8, 143.9
Seronegative Mother	2	6,151	1.0	
H. pylori **Active Antibiotic Use**	20	14,931		
Seropositive Mother	18	9,125	◊◊◊	◊◊◊
Seronegative Mother	2	5,806	◊◊◊	
No *H. pylori* **Antibiotic Use**	23	8,206		
Seropositive Mother	20	5,653	◊◊◊	◊◊◊
Seronegative Mother	3	2,553	◊◊◊	
Breastfed, any duration	27	14,525		
Seropositive Mother	24	9,858	6.7	0.2, 282.3
Seronegative Mother	3	4,667	1.0	
Not Breastfed	6	4,901		
Seropositive Mother	5	2,726	◊◊◊	◊◊◊
Seronegative Mother	1	2,175	◊◊◊	

[a]Number of long-term infections in the numerator of the crude rate

[b]Person months used in the denominator of the crude rate

[c]Adjusted for crowding defined as ratio of number of people within a household to number of rooms in a home.

[d] Confidence interval

[e]*H. pylori* serostatus of mother as determined at baseline.

[f]*H. pylori* serostatus for older, non-index siblings living within the same household. *H. pylori* status determined by ^{13}C-UBT for index and younger siblings enrolled in The Pasitos Cohort.

*** Hazard ratios and confidence intervals could not be calculated due to zero counts.

◊◊◊ Hazard ratios and confidence intervals could not be calculated due to model instability.

APPENDIX T

PARTIAL RESIDUALS PLOT FOR TESTING PROPORTIONALITY HAZARDS

ASSUMPTION OF YOUNGER SIBLING ACQUIRING AN INCIDENT *HELICOBACTER*

PYLORI INFECTION BY*HELICOBACTER PYLORI* INFECTION STATUSOF THE

INDEX SIBLING, THE PASITOS COHORT
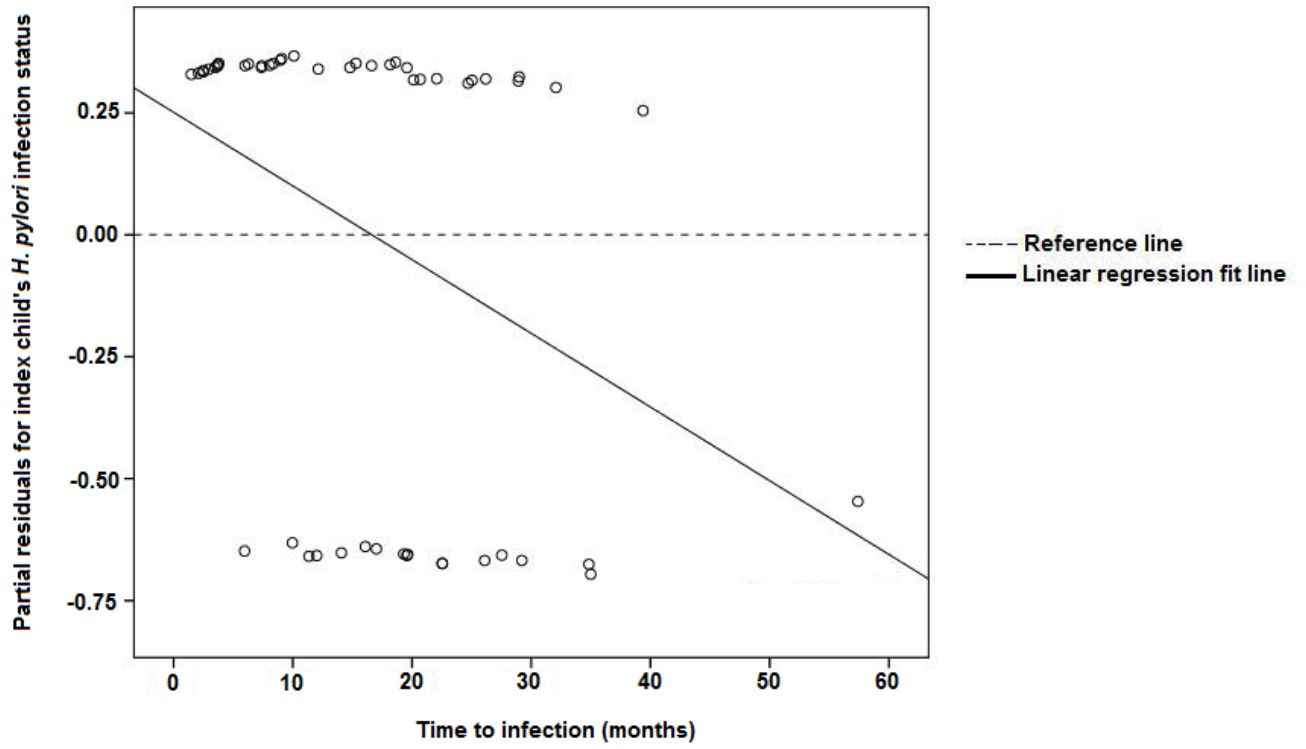
APPENDIX U

EFFECT OF EXPOSURE TO AN *HELICOBACTER PYLORI* INFECTED OLDER

INDEX SIBLING ON THE ACQUISITION OF AN INCIDENT *HELICOBACTER PYLORI*

INFECTION OF A YOUNGER SIBLING, THE PASITOS COHORT

Exposure Variable	Incident Infections (n)[a]	Person-Time (Months)[b]	Hazard Ratio[c]	95% CI[c,d]
Overall	53	3,201		
Infected Older Sibling[e]	35	1,995	6.0	1.5, 24.5
Uninfected Older Sibling	18	1,206	1.0	
Long-Term Infection in Older Sibling[f]	14	310	3.1	1.6, 6.2
No Long-Term Infection in Older Sibling	39	2,891	1.0	
≤3 years of age between siblings	32	2,401		
Infected Older Sibling[e]	19	1,397	***	***
Uninfected Older Sibling	13	1,004	***	
Long-Term Infection in Older Sibling[f]	10	208	4.3	1.7, 10.7
No Long-Term Infection in Older Sibling	22	2,193	1.0	
> 3 years of age between siblings	21	800	***	***
Infected Older Sibling[e]	16	598	***	
Uninfected Older Sibling	5	202	1.0	
Long-Term Infection in Older Sibling[f]	1	8	2.0	0.7, 5.4
No Long-Term Infection in Older Sibling	20	792	1.0	
Mexico	17	1,169		
Infected Older Sibling[e]	14	633	404.1	0.3, 618067.0
Uninfected Older Sibling	3	536	1.0	
Long-Term Infection in Older Sibling[f]	7	163	3.7	1.2, 11.0
No Long-Term Infection in Older Sibling	10	1,005	1.0	
United States	36	2,033		
Infected Older Sibling[e]	21	1,363	3.7	0.8, 16.9
Uninfected Older Sibling	15	670	1.0	
Long-Term Infection in Older Sibling[f]	7	146	2.8	1.1, 7.1
No Long-Term Infection in Older Sibling	29	1,887	1.0	

[a]Number of incident infections in the numerator of the crude rate

[b]Person months used in the denominator of the crude rate

[c]Adjusted for crowding (defined as ratio of number of people within a household to number of rooms in a home), mother's *H. pylori* serostatus at baseline, and number of *H. pylori* active antibiotic courses taken.

[d] Confidence interval

[e]One or more ^{13}C-UBT positive for *H. pylori* during follow-up.

[f]Three or more consecutive ^{13}C-UBT positive for *H. pylori* during follow-up.

*** Hazard ratios and confidence intervals could not be calculated due to model instability.

APPENDIX V

PARTIAL RESIDUALS PLOT FOR TESTING PROPORTIONALITY HAZARDS

ASSUMPTION OF YOUNGER SIBLING ACQUIRING AN INCIDENT *HELICOBACTER*

PYLORI INFECTION BY*HELICOBACTER PYLORI*LONG-TERM INFECTION

STATUSOF THE INDEX SIBLING, THE PASITOS COHORT
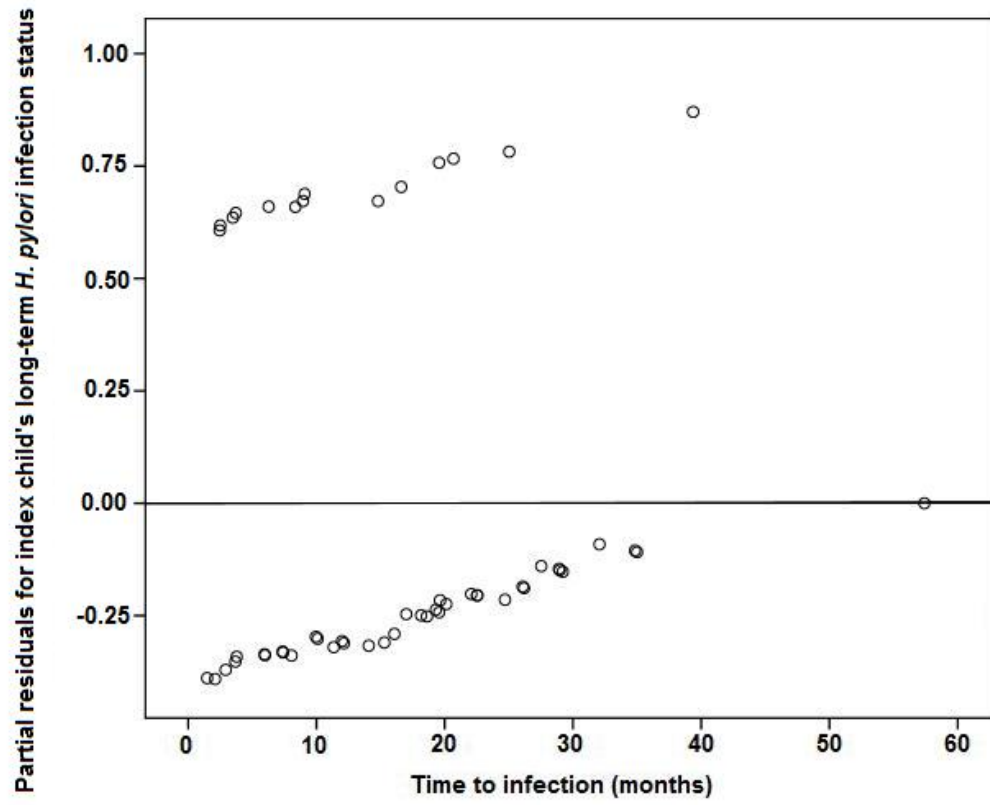
APPENDIX W

PARTIAL RESIDUALS PLOT FOR TESTING PROPORTIONALITY HAZARDS

ASSUMPTION OF YOUNGER SIBLING ACQUIRING A LONG-TERM

HELICOBACTER PYLORI INFECTION BY LONG-TERM *HELICOBACTER PYLORI*

INFECTION STATUS OF THE INDEX SIBLING: TIME-TO-EVENT SCENARIO ONE,
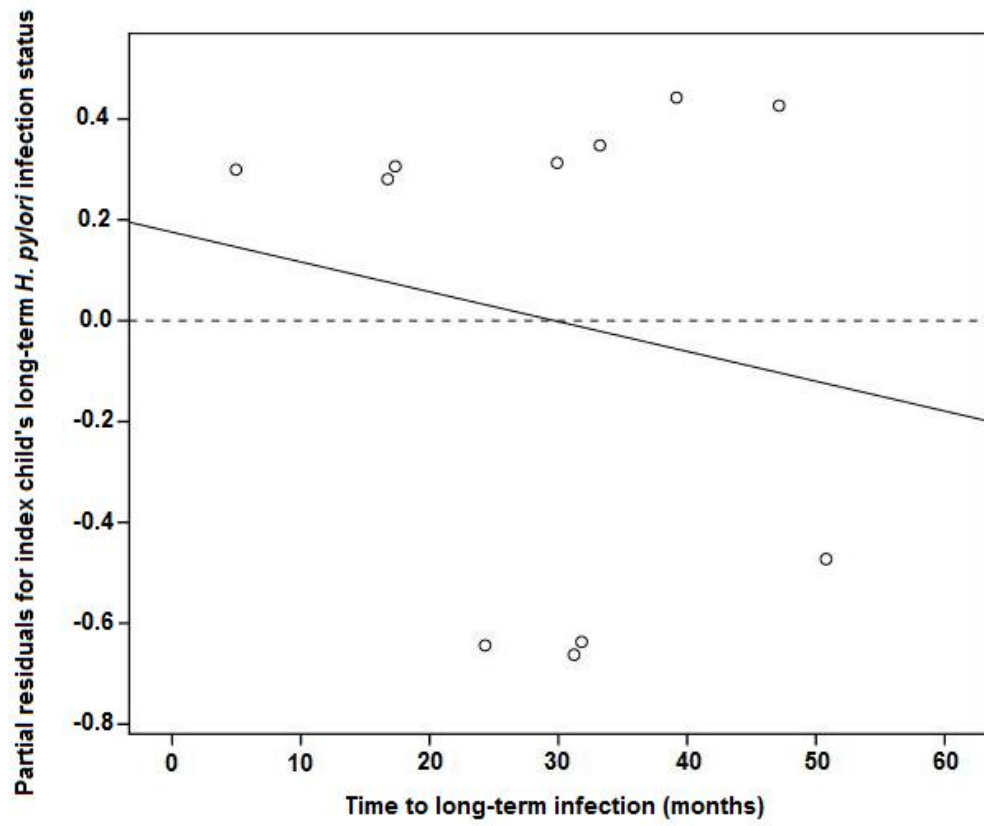
THE PASITOS COHORT

APPENDIX X

PARTIAL RESIDUALS PLOT FOR TESTING PROPORTIONALITY HAZARDS

ASSUMPTION OF YOUNGER SIBLING ACQUIRING A LONG-TERM

HELICOBACTER PYLORI INFECTION BY LONG-TERM *HELICOBACTER PYLORI*

INFECTION STATUSOF THE INDEX SIBLING: TIME-TO-EVENT SCENARIO

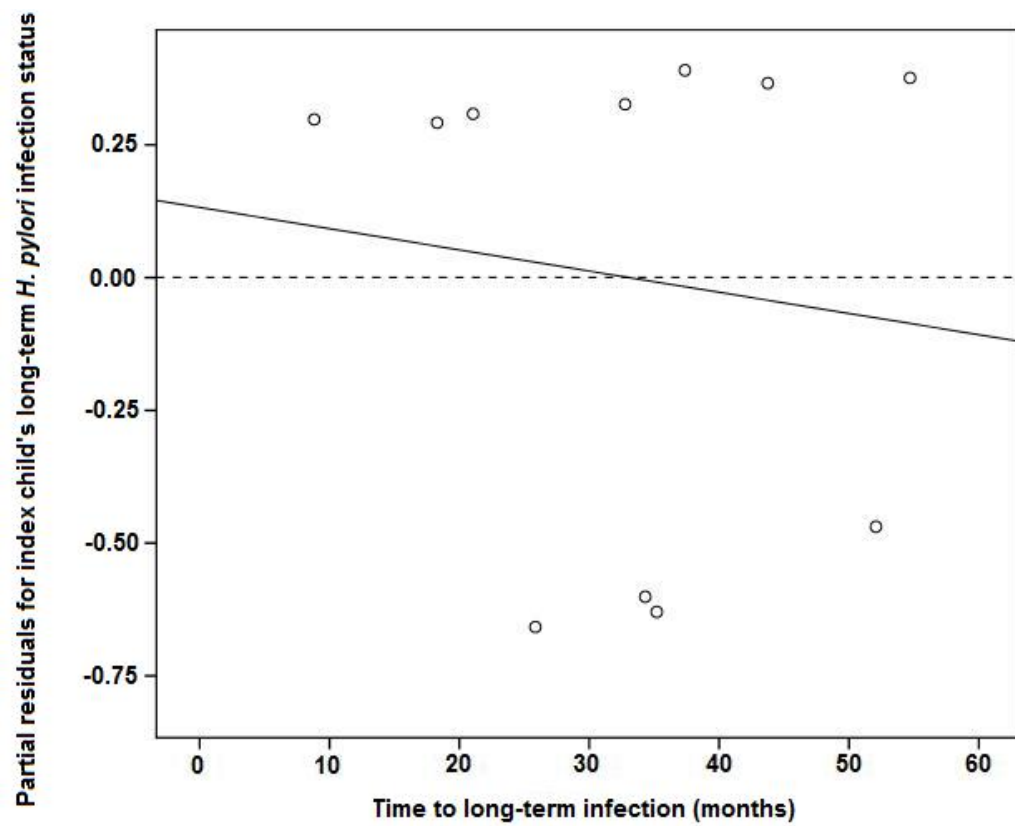TWO,THE PASITOS COHORT

APPENDIX Y

PARTIAL RESIDUALS PLOT FOR TESTING PROPORTIONALITY HAZARDS

ASSUMPTION OF YOUNGER SIBLING ACQUIRING A LONG-TERM

HELICOBACTER PYLORI INFECTION BY LONG-TERM *HELICOBACTER PYLORI*

INFECTION STATUS OF THE INDEX SIBLING: TIME-TO-EVENT SCENARIO THREE,

THE PASITOS COHORT

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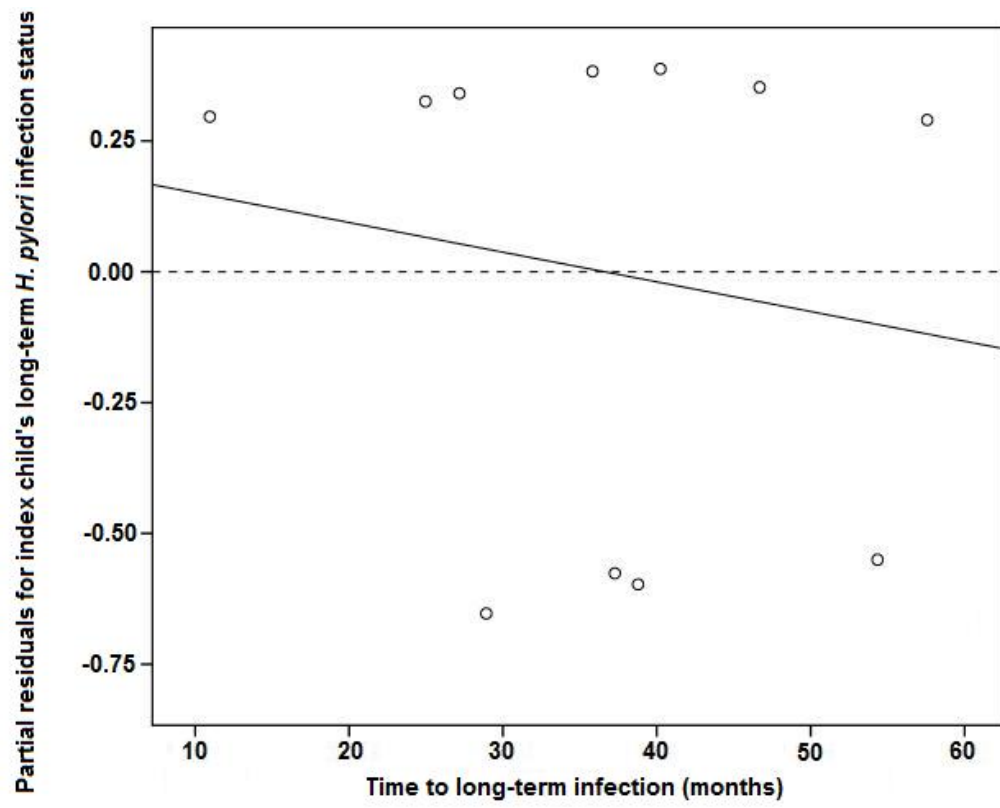APPENDIX Z

EFFECT OF EXPOSURE TO AN *HELICOBACTER PYLORI* INFECTED OLDER

INDEX SIBLING ON THE ESTABLISHMENT OF A LONG-TERM *HELICOBACTER*

PYLORI INFECTION OF A YOUNGER SIBLING: TIME-TO EVENT SCENARIO ONE,

THE PASITOS COHORT

Exposure Variable	Incident Infections (n)[a]	Person-Time (Months)[b]	Hazard Ratio[c]	95% CI[c,d]
Overall	11	3,896		
Long-Term Infection in Older Sibling[e]	7	510	9.1	2.3, 36.6
No Long-Term Infection in Older Sibling	4	3,386	1.0	
≤3 years of age between siblings	10	2,872		
Long-Term Infection in Older Sibling[e]	6	273	10.1	2.4, 42.3
No Long-Term Infection in Older Sibling	4	2,599	1.0	
> 3 years of age between siblings	1	1,024		
Long-Term Infection in Older Sibling[e]	1	238	929.0	***
No Long-Term Infection in Older Sibling	0	786	1.0	
Mexico	5	1,409		
Long-Term Infection in Older Sibling[e]	2	313	2.6	0.3, 21.6
No Long-Term Infection in Older Sibling	3	1,096	1.0	
United States	6	2,487		
Long-Term Infection in Older Sibling[e]	5	198	58.5	2.7, 1251.0
No Long-Term Infection in Older Sibling	1	2,289	1.0	

[a]Number of incident infections in the numerator of the crude rate

[b]Person months used in the denominator of the crude rate

[c]Adjusted for crowding (defined as ratio of number of people within a household to number of rooms in a home), mother's *H. pylori* serostatus at baseline, and number of *H. pylori* active antibiotic courses taken.

[d] Confidence interval

[e]Three or more consecutive ^{13}C-UBT positive for *H. pylori* during follow-up.

*** Confidence interval could not be calculated due to model instability.

APPENDIX AA

EFFECT OF EXPOSURE TO AN *HELICOBACTER PYLORI* INFECTED OLDER

INDEX SIBLING ON THE ESTABLISHMENT OF A LONG-TERM *HELICOBACTER*

PYLORI INFECTION OF A YOUNGER SIBLING: TIME-TO EVENT SCENARIO TWO,

THE PASITOS COHORT

Exposure Variable	Incident Infections (n)[a]	Person-Time (Months)[b]	Hazard Ratio[c]	95% CI[c,d]
Overall	11	3,934		
Long-Term Infection in Older Sibling[e]	7	539	8.5	2.2, 32.1
No Long-Term Infection in Older Sibling	4	3,395	1.0	
≤3 years of age between siblings	10	2,908		
Long-Term Infection in Older Sibling[e]	6	300	8.7	2.2, 34.6
No Long-Term Infection in Older Sibling	4	2,608	1.0	
> 3 years of age between siblings	1	1,026		
Long-Term Infection in Older Sibling[e]	1	239	1154.4	***
No Long-Term Infection in Older Sibling	0	787	1.0	
Mexico	5	1,423		
Long-Term Infection in Older Sibling[e]	2	321	4.8	0.4, 52.7
No Long-Term Infection in Older Sibling	3	1,102	1.0	
United States	6	2,511		
Long-Term Infection in Older Sibling[e]	5	218	44.9	2.1, 964.0
No Long-Term Infection in Older Sibling	1	2,293	1.0	

[a]Number of incident infections in the numerator of the crude rate

[b]Person months used in the denominator of the crude rate

[c]Adjusted for crowding (defined as ratio of number of people within a household to number of rooms in a home), mother's *H. pylori* serostatus at baseline, and number of *H. pylori* active antibiotic courses taken.

[d] Confidence interval

[e]Three or more consecutive ^{13}C-UBT positive for *H. pylori* during follow-up.

*** Confidence interval could not be calculated due to model instability.

APPENDIX BB

EFFECT OF EXPOSURE TO AN *HELICOBACTER PYLORI* INFECTED OLDER

INDEX SIBLING ON THE ESTABLISHMENT OF A LONG-TERM *HELICOBACTER*

PYLORI INFECTION OF A YOUNGER SIBLING: TIME-TO EVENT SCENARIO

THREE, THE PASITOS COHORT

Exposure Variable	Incident Infections (n)[a]	Person-Time (Months)[b]	Hazard Ratio[c]	95% CI[c,d]
Overall	11	3,972		
Long-Term Infection in Older Sibling[e]	7	565	11.9	2.7, 53.7
No Long-Term Infection in Older Sibling	4	3,407	1.0	
≤3 years of age between siblings	10	2,938		
Long-Term Infection in Older Sibling[e]	6	318	12.1	2.5, 58.6
No Long-Term Infection in Older Sibling	4	2,620	1.0	
> 3 years of age between siblings	1	1,034		
Long-Term Infection in Older Sibling[e]	1	247	120.0	***
No Long-Term Infection in Older Sibling	0	787	1.0	
Mexico	5	1,438		
Long-Term Infection in Older Sibling[e]	2	327	4.8	0.4, 59.0
No Long-Term Infection in Older Sibling	3	1,111	1.0	
United States	6	2,534		
Long-Term Infection in Older Sibling[e]	5	238	113.8	1.5, 8468.5
No Long-Term Infection in Older Sibling	1	2,296	1.0	

[a]Number of incident infections in the numerator of the crude rate

[b]Person months used in the denominator of the crude rate

[c]Adjusted for crowding (defined as ratio of number of people within a household to number of rooms in a home), mother's *H. pylori* serostatus at baseline, and number of *H. pylori* active antibiotic courses taken.

[d] Confidence interval

[e]Three or more consecutive ^{13}C-UBT positive for *H. pylori* during follow-up.

*** Confidence interval could not be calculated due to model instability.

APPENDIX CC

HAZARD RATIOS FOR THE EFFECT OF EXPOSURE TO A *HELICOBACTER PYLORI* INFECTED OLDER SIBLING ON A YOUNGER SIBLING ACQUIRING AN INCIDENT OR LONG-TERM *HELICOBACTER PYLORI* INFECTION AND MODEL COVARIATES, THE PASITOS COHORT

	Younger sibling acquiring incident infection - Model A		Younger sibling acquiring incident infection- Model B		Younger sibling acquiring a long-term infection: Time-to-event scenario 1		Younger sibling acquiring a long-term infection: Time-to-event scenario 2		Younger sibling acquiring a long-term infection: Time-to-event scenario 3	
	Hazard Ratio[b]	95% CI[a,b]	Hazard Ratio[b]	95% CI[a,b]	Hazard Ratio[c]	95% CI[a,c]	Hazard Ratio[d]	95% CI[a,d]	Hazard Ratio[e]	95% CI[a,e]
Infected older index sibling	6.0	1.5, 24.5								
Long-term Infection in older index sibling			3.1	1.6, 6.2	9.1	2.2, 36.6	8.5	2.24, 32.06	11.9	2.6, 53.7
Crowding	0.9	0.6, 1.4	1.0	0.7, 1.4	1.3	0.7, 2.6	1.5	0.7, 2.9	3.2	1.2, 8.5
Mother's *H. pylori* Seropositivity (baseline)	1.5	0.8, 2.8	1.2	0.6, 1.4	4.1	0.5, 36.4	3.8	0.4, 32.4	4.1	0.5, 34.0
H. pylori Active Antibiotic Courses(per course increase)	0.9	0.7,1.2	0.9	0.7, 1.2	1.2	0.7, 2.1	1.0	0.6, 1.8	0.9	0.5, 1.7

[a]Confidence Interval

[b]Overall person-time was 3,201 person months

[c]Overall person-time was 3,896 person months

[d]Overall person-time was 3,934 person months

[e]Overall person-time was 3,972 person months

APPENDIX DD

PARTIAL RESIDUALS PLOT FOR TESTING PROPORTIONALITY HAZARDS

ASSUMPTION OF ACQUIRING AN INCIDENT *HELICOBACTER PYLORI* INFECTION

BYBREASTFED STATUS, THE PASITOS COHORT
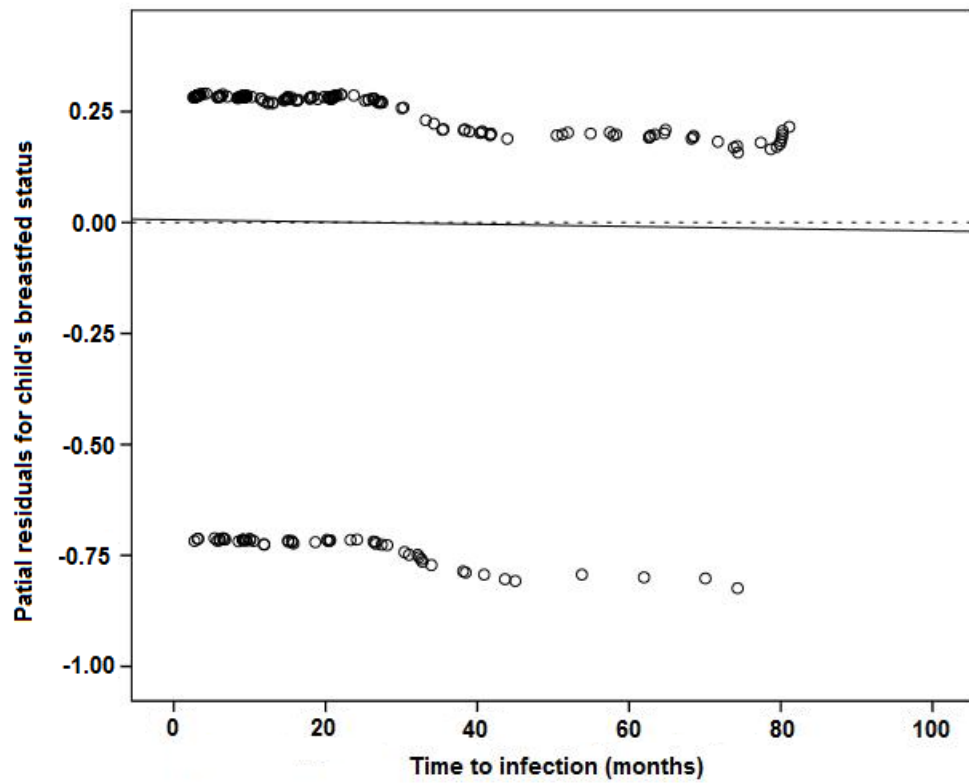
APPENDIX EE

EFFECT OF EXPOSURE TO BREASTFEEDING ON THE AQCUISITION OF A

HELICOBACTER PYLORI INFECTION, THE PASITOS COHORT

Exposure Variable	Incident Infections (n)[a]	Person-Time (Months)[b]	Hazard Ratio[c]	95% CI[c,d]
Overall	212	13,442		
Breastfed	157	9,979	1.0	0.7, 1.4
Not Breastfed	55	3,463	1.0	
Mother *H. pylori* seropositive[e]	134	8,165		
Breastfed	107	6,417	1.1	0.7, 1.7
Not Breastfed	27	1,748	1.0	
Mother *H. pylori* seronegative[e]	67	4,639		
Breastfed	43	3,140	0.9	0.5, 1.4
Not Breastfed	24	1,499	1.0	
***H. pylori* Detected in Sibling[f]**	41	1,410		
Breastfed	31	888	1.1	0.5, 2.2
Not Breastfed	10	522	1.0	
***H. pylori* Not Detected in Sibling[f]**	78	5,222		
Breastfed	54	3,707	0.9	0.6, 1.5
Not Breastfed	24	1,515	1.0	
***H. pylori* Active Antibiotic Use**	135	8,954		
Breastfed	104	6,630	1.1	0.8, 1.8
Not Breastfed	31	2,324	1.0	
No *H. pylori* Antibiotic Use	77	4,352		
Breastfed	53	3,233	0.8	0.5, 1.2
Not Breastfed	24	1,119	1.0	
Mexico	85	5,652		
Breastfed	76	5,001	1.1	0.6, 2.2
Not Breastfed	9	651	1.0	
United States	127	7,790		
Breastfed	81	4,978	1.0	0.7, 1.4
Not Breastfed	46	2,812	1.0	

[a]Number of incident infections in the numerator of the crude rate

[b]Person months used in the denominator of the crude rate

[c]Crude, unadjusted hazard ratio

[d] Confidence interval

[e]*H. pylori* serostatus of mother as determined at baseline.

[f]*H. pylori* serostatus for older, non-index siblings living within the same household. *H. pylori* status determined by ^{13}C-UBT for index and younger siblings enrolled in The Pasitos Cohort.

*** Hazard ratios and confidence intervals could not be calculated due to model instability.

APPENDIX FF

PARTIAL RESIDUALS PLOT FOR TESTING PROPORTIONALITY HAZARDS

ASSUMPTION OF ACQUIRING A SHORT-TERM *HELICOBACTER PYLORI*

INFECTION BY BREASTFED STATUS: TIME-TO-EVENT SCENARIO ONE, THE
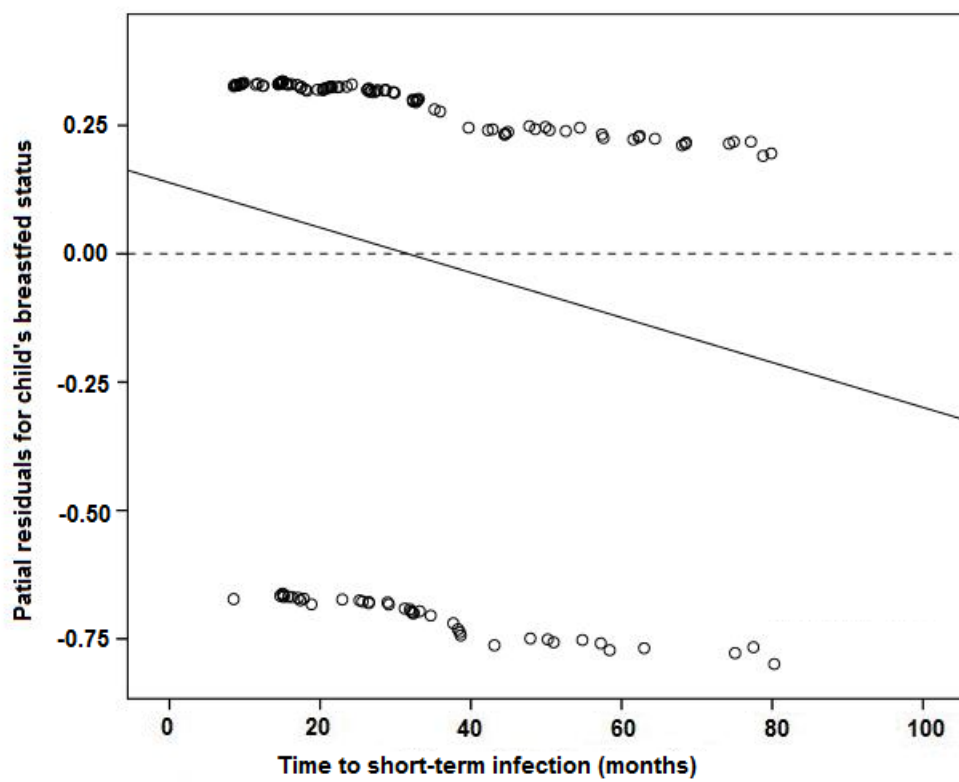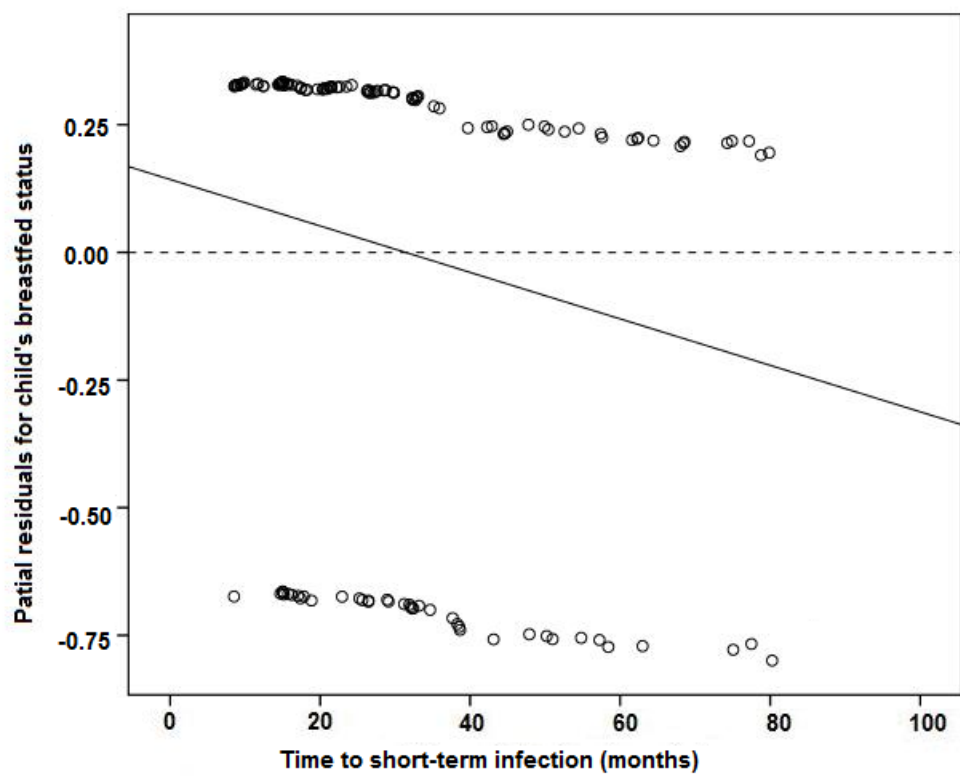
PASITOS COHORT

APPENDIX GG

PARTIAL RESIDUALS PLOT FOR TESTING PROPORTIONALITY HAZARDS

ASSUMPTION OF ACQUIRING A SHORT-TERM *HELICOBACTER PYLORI*

INFECTION BY BREASTFED STATUS: TIME-TO-EVENT SCENARIO TWO, THE
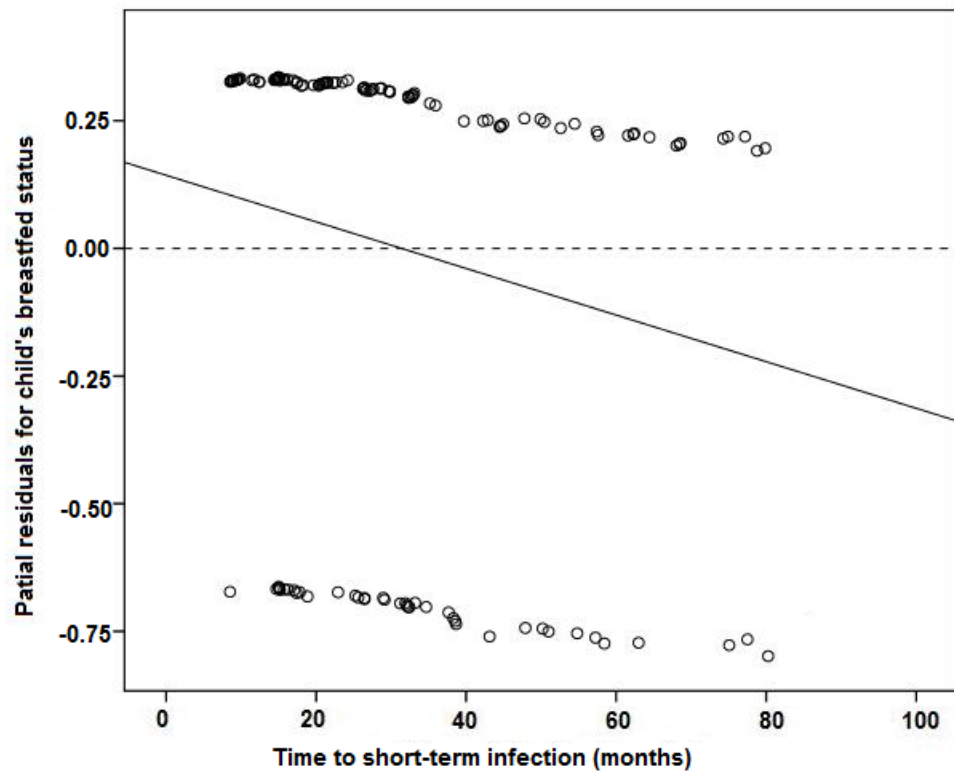
PASITOS COHORT

APPENDIX HH

PARTIAL RESIDUALS PLOT FOR TESTING PROPORTIONALITY HAZARDS

ASSUMPTION OF ACQUIRING A SHORT-TERM *HELICOBACTER PYLORI*

INFECTION BY BREASTFED STATUS: TIME-TO-EVENT SCENARIO THREE, THE

PASITOS COHORT

APPENDIX II

EFFECT OF EXPOSURE TO BREASTFEEDING ON THE ESTABLISHMENT OF A

SHORT-TERM *HELICOBACTER PYLORI* INFECTION: TIME-TO-EVENT SCENARIO

ONE, THE PASITOS COHORT

Exposure Variable	Incident Infections (n)[a]	Person-Time (Months)[b]	Hazard Ratio[c]	95% CI[c,d]
Overall	139	15,466		
Breastfed	98	11,534	1.6	0.8, 3.2
Not Breastfed	41	3,932	1.0	
Mother *H. pylori* seropositive[e]	82	9,548		
Breastfed	63	7,553	1.5	0.6, 3.9
Not Breastfed	19	1,995	1.0	
Mother *H. pylori* seronegative[e]	50	5,210		
Breastfed	31	3,519	1.4	0.4, 4.8
Not Breastfed	19	1,691	1.0	
***H. pylori* Detected in Sibling[f]**	23	2,513		
Breastfed	16	1,871	2.4	0.4, 16.4
Not Breastfed	7	642	1.0	
***H. pylori* Not Detected in Sibling[f]**	53	5,961		
Breastfed	36	4,253	1.1	0.3, 3.3
Not Breastfed	17	1,708	1.0	
***H. pylori* Active Antibiotic Use**	97	10,130		
Breastfed	71	7,561	***	***
Not Breastfed	26	2,570	***	
No *H. pylori* Antibiotic Use	42	5,200		
Breastfed	27	3,858	***	***
Not Breastfed	15	1,342	***	
Mexico	44	6,657		
Breastfed	38	5,902	2.5	0.4, 17.3
Not Breastfed	6	754	1.0	
United States	95	8,809		
Breastfed	60	5,631	2.0	0.8, 4.9
Not Breastfed	35	3,178	1.0	

[a]Number of incident infections in the numerator of the crude rate

[b]Person months used in the denominator of the crude rate

[c]Crude, unadjusted hazard ratio

[d] Confidence interval

[e]*H. pylori* serostatus of mother as determined at baseline.

[f]*H. pylori* serostatus for older, non-index siblings living within the same household. *H. pylori* status determined by ^{13}C-UBT for index and younger siblings enrolled in The Pasitos Cohort.

*** Hazard ratios and confidence intervals could not be calculated due to model instability.

APPENDIX JJ

EFFECT OF EXPOSURE TO BREASTFEEDING ON THE ESTABLISHMENT OF A

SHORT-TERM *HELICOBACTER PYLORI* INFECTION: TIME-TO-EVENT SCENARIO

TWO, THE PASITOS COHORT

Exposure Variable	Incident Infections (n)[a]	Person-Time (Months)[b]	Hazard Ratio[c]	95% CI[c,d]
Overall	139	15,596		
Breastfed	98	11,633	1.6	0.8, 3.3
Not Breastfed	41	3,963	1.0	
Mother *H. pylori* seropositive[e]	82	9,630		
Breastfed	63	7,611	1.6	0.6, 4.1
Not Breastfed	19	2,019	1.0	
Mother *H. pylori* seronegative[e]	50	5,256		
Breastfed	31	3,561	1.4	0.4, 4.8
Not Breastfed	19	1,695	1.0	
***H. pylori* Detected in Sibling[f]**	23	2,591		
Breastfed	16	1,924	2.4	0.4, 16.5
Not Breastfed	7	666	1.0	
***H. pylori* Not Detected in Sibling[f]**	53	6,001		
Breastfed	36	4,285	1.1	0.3, 3.3
Not Breastfed	17	1,715	1.0	
***H. pylori* Active Antibiotic Use**	97	10,139		
Breastfed	71	7,563	***	***
Not Breastfed	26	2,576	***	
No *H. pylori* Antibiotic Use	42	5,322		
Breastfed	27	3,955	***	***
Not Breastfed	15	1,367	***	
Mexico	44	6,795		
Breastfed	38	6,019	2.5	0.4, 17.5
Not Breastfed	6	776	1.0	
United States	95	8,801		
Breastfed	60	5,614	2.1	0.9, 5.2
Not Breastfed	35	3,187	1.0	

[a]Number of incident infections in the numerator of the crude rate

[b]Person months used in the denominator of the crude rate

[c]Crude, unadjusted hazard ratio

[d] Confidence interval

[e]*H. pylori* serostatus of mother as determined at baseline.

[f]*H. pylori* serostatus for older, non-index siblings living within the same household. *H. pylori* status determined by ^{13}C-UBT for index and younger siblings enrolled in The Pasitos Cohort.

*** Hazard ratios and confidence intervals could not be calculated due to model instability.

APPENDIX KK

EFFECT OF EXPOSURE TO BREASTFEEDING ON THE ESTABLISHMENT OF A

SHORT-TERM *HELICOBACTER PYLORI* INFECTION: TIME-TO-EVENT SCENARIO

THREE, THE PASITOS COHORT

Exposure Variable	Incident Infections (n)[a]	Person-Time (Months)[b]	Hazard Ratio[c]	95% CI[c,d]
Overall	139	15,718		
Breastfed	98	11,737	1.6	0.8, 3.2
Not Breastfed	41	3,981	1.0	
Mother *H. pylori* seropositive[e]	82	9,739		
Breastfed	63	7,707	1.6	0.6, 4.0
Not Breastfed	19	2,032	1.0	
Mother *H. pylori* seronegative[e]	50	5,265		
Breastfed	31	3,568	1.4	0.4, 4.8
Not Breastfed	19	1,697	1.0	
***H. pylori* Detected in Sibling[f]**	23	2,647		
Breastfed	16	1,967	2.4	0.3, 16.0
Not Breastfed	7	680	1.0	
***H. pylori* Not Detected in Sibling[f]**	53	6,030		
Breastfed	36	4,310	1.1	0.3, 3.3
Not Breastfed	17	1,720	1.0	
***H. pylori* Active Antibiotic Use**	97	6,387		
Breastfed	71	3,801	***	***
Not Breastfed	26	2,586	***	
No *H. pylori* Antibiotic Use	42	5,391		
Breastfed	27	4,015	***	***
Not Breastfed	15	1,376	***	
Mexico	44	6,879		
Breastfed	38	6,098	2.5	0.4, 17.3
Not Breastfed	6	781	1.0	
United States	95	8,839		
Breastfed	60	5,639	2.2	0.9, 5.2
Not Breastfed	35	3,200	1.0	

[a]Number of incident infections in the numerator of the crude rate

[b]Person months used in the denominator of the crude rate

[c]Crude, unadjusted hazard ratio

[d] Confidence interval

[e]*H. pylori* serostatus of mother as determined at baseline.

[f]*H. pylori* serostatus for older, non-index siblings living within the same household. *H. pylori* status determined by ^{13}C-UBT for index and younger siblings enrolled in The Pasitos Cohort.

*** Hazard ratios and confidence intervals could not be calculated due to model instability.
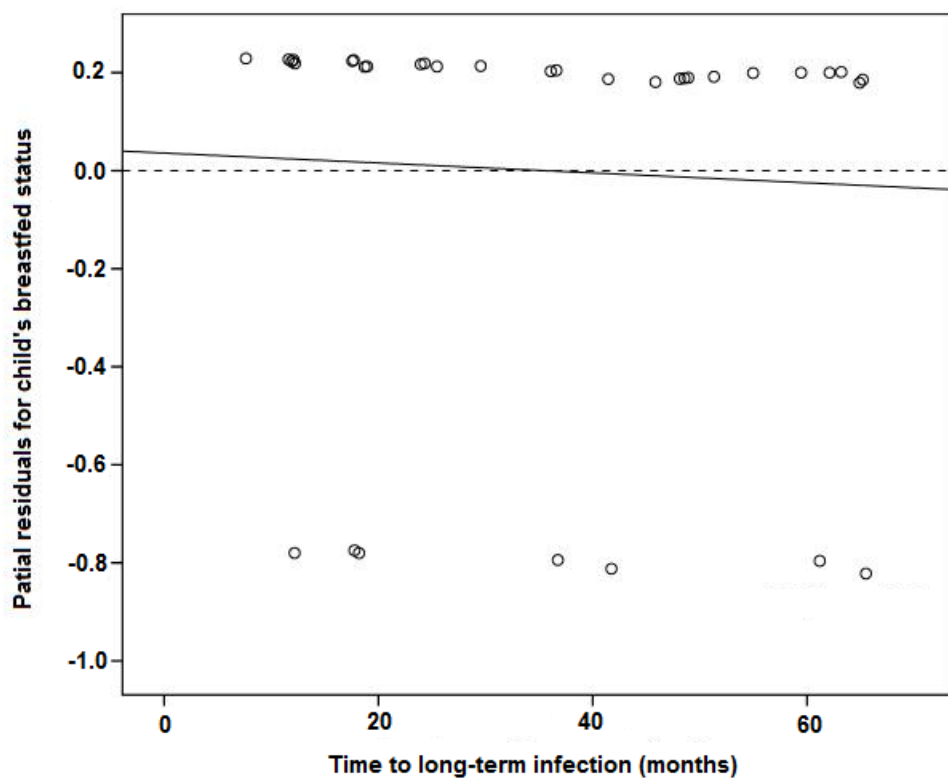
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APPENDIX LL

PARTIAL RESIDUALS PLOT FOR TESTING PROPORTIONALITY HAZARDS

ASSUMPTION OF ACQUIRING A LONG-TERM *HELICOBACTER PYLORI*

INFECTION BY BREASTFED STATUS: TIME-TO-EVENT SCENARIO ONE, THE
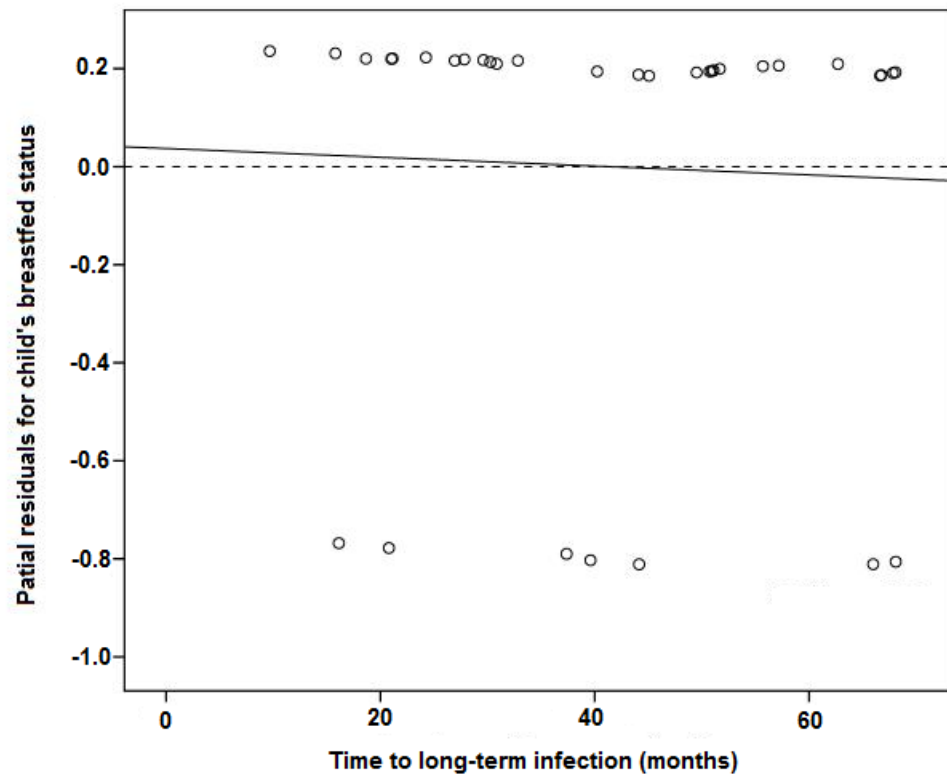
PASITOS COHORT

APPENDIX MM

PARTIAL RESIDUALS PLOT FOR TESTING PROPORTIONALITY HAZARDS

ASSUMPTION OF ACQUIRING A LONG-TERM *HELICOBACTER PYLORI*

INFECTION BY BREASTFED STATUS: TIME-TO-EVENT SCENARIO TWO, THE
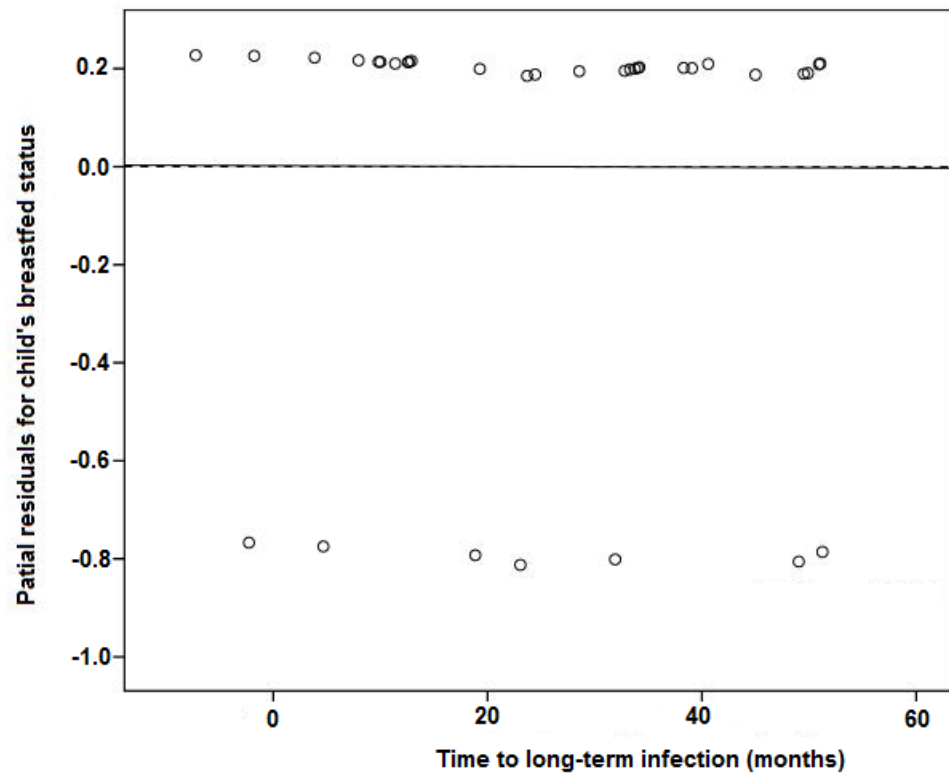
PASITOS COHORT

APPENDIX NN

PARTIAL RESIDUALS PLOT FOR TESTING PROPORTIONALITY HAZARDS

ASSUMPTION OF ACQUIRING A LONG-TERM *HELICOBACTER PYLORI*

INFECTION BY BREASTFED STATUS: TIME-TO-EVENT SCENARIO THREE, THE

PASITOS COHORT

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APPENDIX OO

EFFECT OF EXPOSURE TO BREASTFEEDING ON THE ESTABLISHMENT OF A

LONG-TERM *HELICOBACTER PYLORI* INFECTION: TIME-TO-EVENT SCENARIO

ONE, THE PASITOS COHORT

Exposure Variable	Incident Infections (n)[a]	Person-Time (Months)[b]	Hazard Ratio[c]	95% CI[c,d]
Overall	34	20,075		
Breastfed	27	14,905	1.3	0.6, 3.0
Not Breastfed	7	5,171	1.0	
Mother *H. pylori* seropositive[e]	29	12,301		
Breastfed	24	9,626	1.3	0.5, 3.4
Not Breastfed	5	2,675	1.0	
Mother *H. pylori* seronegative[e]	4	6,788		
Breastfed	3	4,618	1.4	0.1, 13.3
Not Breastfed	1	2,170	1.0	
***H. pylori* Detected in Sibling[f]**	13	3,397		
Breastfed	9	2,518	0.8	0.2, 2.4
Not Breastfed	4	879	1.0	
***H. pylori* Not Detected in Sibling[f]**	9	7,729		
Breastfed	7	5,442	1.5	0.3, 7.2
Not Breastfed	2	2,287	1.0	
***H. pylori* Active Antibiotic Use**	15	13,479		
Breastfed	11	10,099	0.9	0.3, 2.8
Not Breastfed	4	3,380	1.0	
No *H. pylori* Antibiotic Use	19	6,461		
Breastfed	16	4,691	1.9	0.6, 6.6
Not Breastfed	3	1,771	1.0	
Mexico	22	8,196		
Breastfed	20	7,266	1.3	0.3, 5.6
Not Breastfed	2	931	1.0	
United States	12	11,879		
Breastfed	7	7,639	0.8	0.2, 2.4
Not Breastfed	5	4,240	1.0	

[a]Number of incident infections in the numerator of the crude rate

[b]Person months used in the denominator of the crude rate

[c]Crude, unadjusted hazard ratio

[d] Confidence interval

[e]*H. pylori* serostatus of mother as determined at baseline.

[f]*H. pylori* serostatus for older, non-index siblings living within the same household. *H. pylori* status determined by ^{13}C-UBT for index and younger siblings enrolled in The Pasitos Cohort.

*** Hazard ratios and confidence intervals could not be calculated due to model instability.

APPENDIX PP

EFFECT OF EXPOSURE TO BREASTFEEDING ON THE ESTABLISHMENT OF A

LONG-TERM *HELICOBACTER PYLORI* INFECTION: TIME-TO-EVENT SCENARIO

TWO, THE PASITOS COHORT

Exposure Variable	Incident Infections (n)[a]	Person-Time (Months)[b]	Hazard Ratio[c]	95% CI[c,d]
Overall	34	20,273		
Breastfed	27	15,063	1.3	0.5, 2.9
Not Breastfed	7	5,209	1.0	
Mother *H. pylori* seropositive[e]	29	12,449		
Breastfed	24	9,743	1.2	0.5, 3.2
Not Breastfed	5	2,707	1.0	
Mother *H. pylori* seronegative[e]	4	6,834		
Breastfed	3	4,660	1.3	0.1, 12.2
Not Breastfed	1	2,174	1.0	
***H. pylori* Detected in Sibling[f]**	13	3,480		
Breastfed	9	2,574	0.8	0.2, 2.6
Not Breastfed	4	906	1.0	
***H. pylori* Not Detected in Sibling[f]**	9	7,772		
Breastfed	7	5,478	1.5	0.3, 7.1
Not Breastfed	2	2,294	1.0	
***H. pylori* Active Antibiotic Use**	15	13,549		
Breastfed	11	10,154	0.9	0.3, 2.8
Not Breastfed	4	3,394	1.0	
No *H. pylori* Antibiotic Use	19	6,589		
Breastfed	16	4,794	1.8	0.5, 6.3
Not Breastfed	3	1,796	1.0	
Mexico	22	8,354		
Breastfed	20	7,402	1.3	0.3, 5.5
Not Breastfed	2	953	1.0	
United States	12	11,918		
Breastfed	7	7,662	0.8	0.2, 2.4
Not Breastfed	5	4,257	1.0	

[a]Number of incident infections in the numerator of the crude rate

[b]Person months used in the denominator of the crude rate

[c]Crude, unadjusted hazard ratio

[d] Confidence interval

[e]*H. pylori* serostatus of mother as determined at baseline.

[f]*H. pylori* serostatus for older, non-index siblings living within the same household. *H. pylori* status determined by ^{13}C-UBT for index and younger siblings enrolled in The Pasitos Cohort.

*** Hazard ratios and confidence intervals could not be calculated due to model instability.

APPENDIX QQ

EFFECT OF EXPOSURE TO BREASTFEEDING ON THE ESTABLISHMENT OF A

LONG-TERM *HELICOBACTER PYLORI* INFECTION: TIME-TO-EVENT SCENARIO

THREE, THE PASITOS COHORT

Exposure Variable	Incident Infections (n)[a]	Person-Time (Months)[b]	Hazard Ratio[c]	95% CI[c,d]
Overall	34	20,421		
Breastfed	27	15,187	1.2	0.5, 2.8
Not Breastfed	7	5,234	1.0	
Mother *H. pylori* seropositive[e]	29	12,584		
Breastfed	24	9,858	1.2	0.5, 3.2
Not Breastfed	5	2,726	1.0	
Mother *H. pylori* seronegative[e]	4	6,843		
Breastfed	3	4,668	1.3	0.1, 12.3
Not Breastfed	1	2,175	1.0	
***H. pylori* Detected in Sibling[f]**	13	3,542		
Breastfed	9	2,619	0.9	0.3, 2.8
Not Breastfed	4	922	1.0	
***H. pylori* Not Detected in Sibling[f]**	9	7,805		
Breastfed	7	5,506	1.4	0.3, 6.8
Not Breastfed	2	2,299	1.0	
***H. pylori* Active Antibiotic Use**	15	13,622		
Breastfed	11	10,212	0.9	0.3, 2.7
Not Breastfed	4	3,410	1.0	
No *H. pylori* Antibiotic Use	19	6,664		
Breastfed	16	4,860	1.8	0.5, 6.2
Not Breastfed	3	1,804	1.0	
Mexico	22	8,599		
Breastfed	20	7,489	1.3	0.3, 5.4
Not Breastfed	2	1,110	1.0	
United States	12	11,974		
Breastfed	7	7,698	0.8	0.2, 2.4
Not Breastfed	5	4,276	1.0	

[a]Number of incident infections in the numerator of the crude rate

[b]Person months used in the denominator of the crude rate

[c]Crude, unadjusted hazard ratio

[d] Confidence interval

[e]*H. pylori* serostatus of mother as determined at baseline.

[f]*H. pylori* serostatus for older, non-index siblings living within the same household. *H. pylori* status determined by ^{13}C-UBT for index and younger siblings enrolled in The Pasitos Cohort.

*** Hazard ratios and confidence intervals could not be calculated due to model instability.

APPENDIX RR

DIRECTED ACYCLIC GRAPH FOR MOTHER-CHILD *HELICOBACTER PYLORI* INFECTION ASSOCIATIONS:
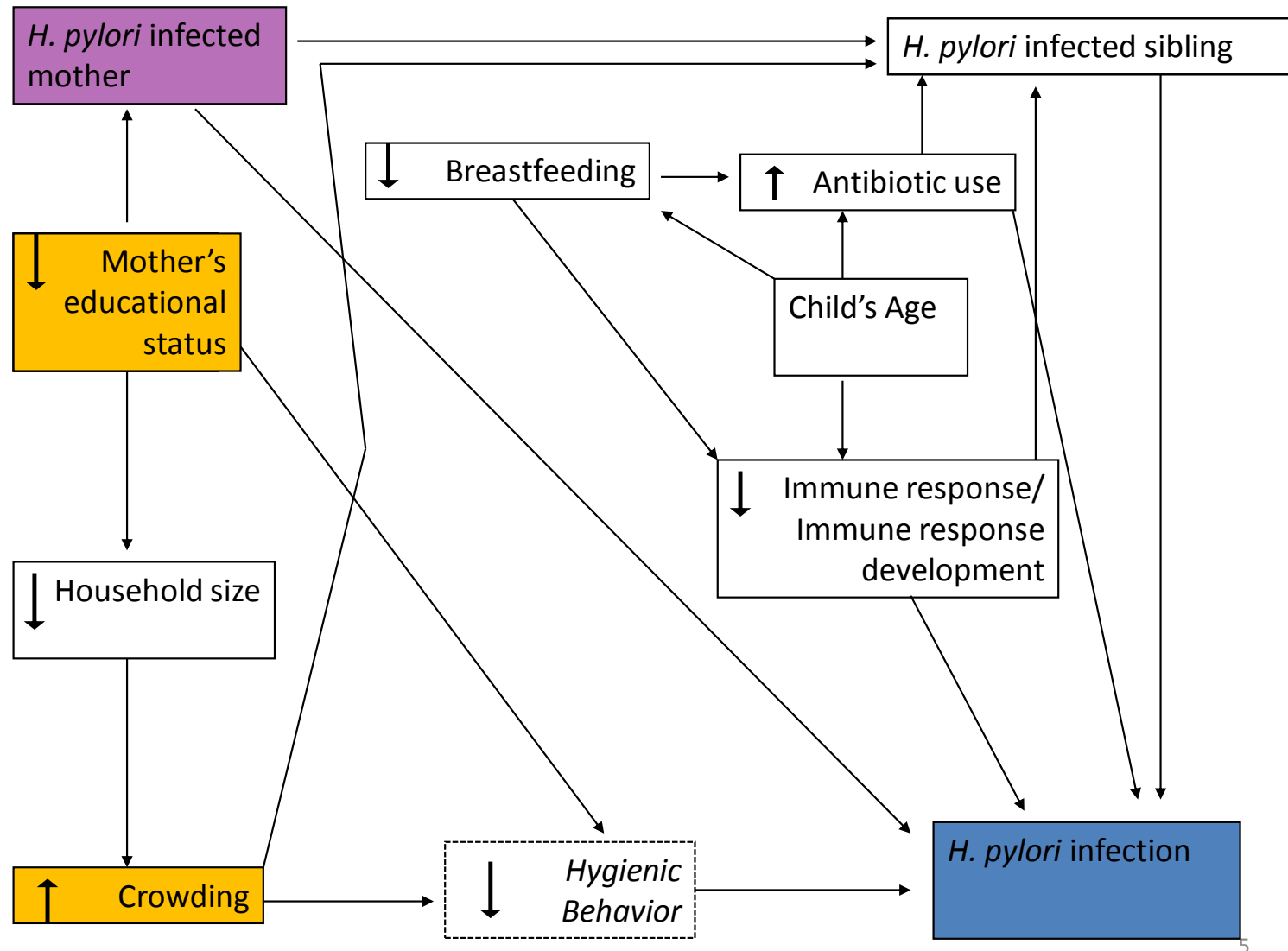
MATERNAL EDUCATION AND HYGIENE, ALTERNATIVE ONE
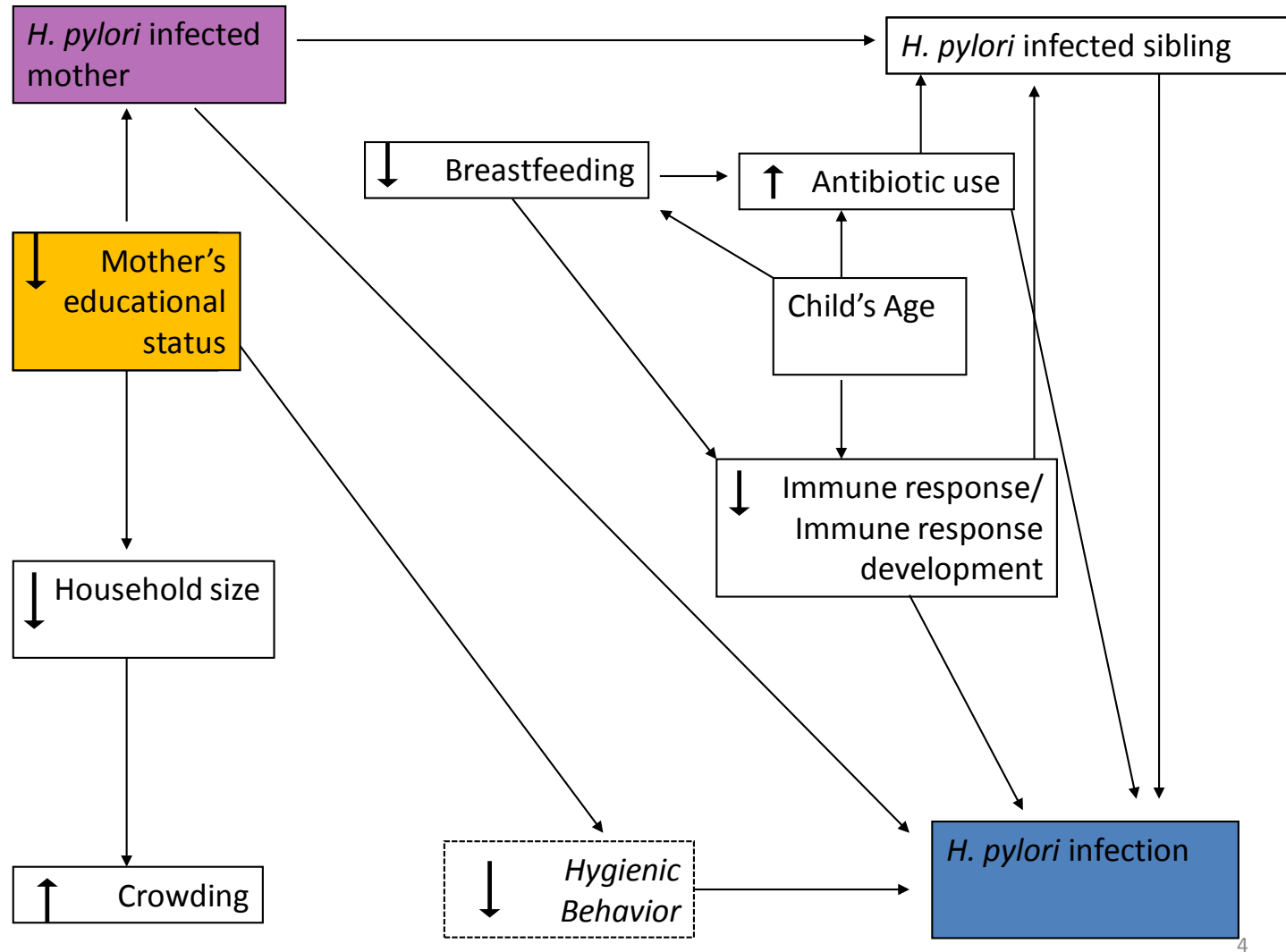
Exposure highlighted in purple, outcome highlighted in blue, covariates for adjustment highlighted in orange.
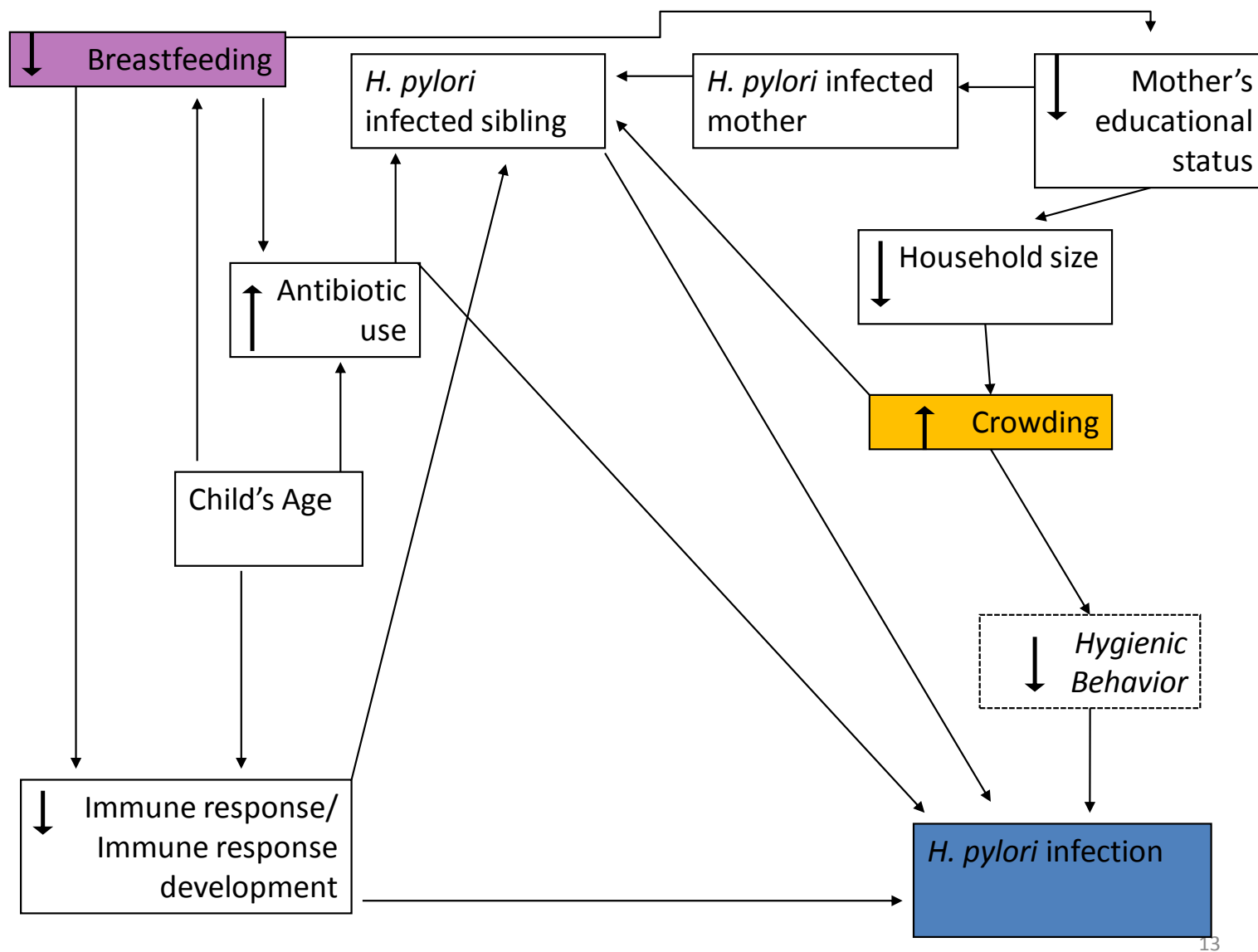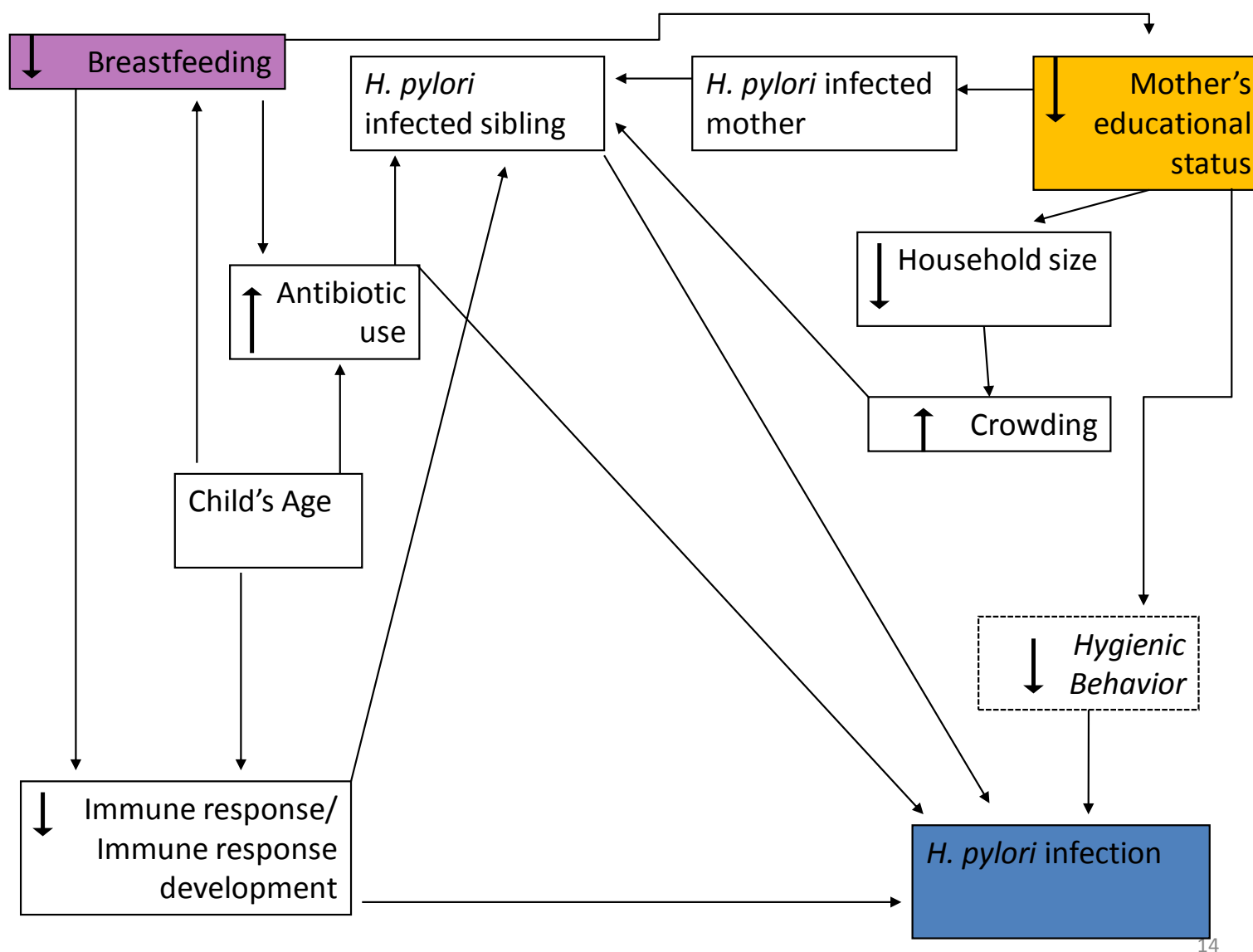
APPENDIX SS

DIRECTED ACYCLIC GRAPH FOR MOTHER-CHILD *HELICOBACTER PYLORI* INFECTION ASSOCIATIONS:

MATERNAL EDUCATION AND HYGIENE, ALTERNATIVE TWO

Exposure highlighted in purple, outcome highlighted in blue, covariates for adjustment highlighted in orange.

APPENDIX TT

DIRECTED ACYCLIC GRAPH FOR BREASTFEEDING AND *HELICOBACTER PYLORI* INFECTION ASSOCIATIONS:
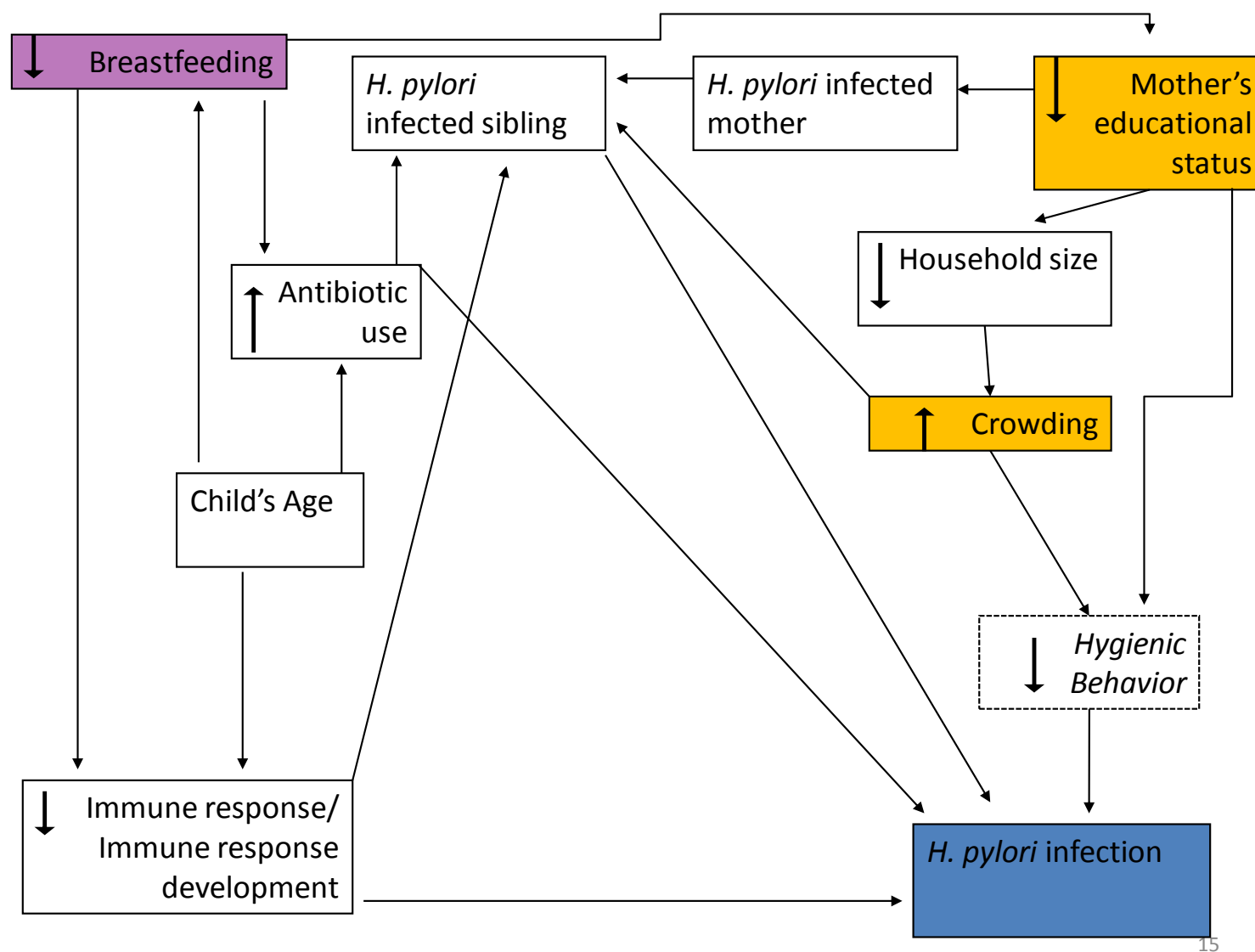
MATERNAL EDUCATION AND HYGIENE, ALTERNATIVE ONE

Exposure highlighted in purple, outcome highlighted in blue, covariates for adjustment highlighted in orange.

APPENDIX UU

DIRECTED ACYCLIC GRAPH FOR BREASTFEEDING AND *HELICOBACTER PYLORI* INFECTION ASSOCIATIONS:

MATERNAL EDUCATION AND HYGIENE, ALTERNATIVE TWO

Exposure highlighted in purple, outcome highlighted in blue, covariates for adjustment highlighted in orange.

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APPENDIX VV

DIRECTED ACYCLIC GRAPH FOR BREASTFEEDING AND *HELICOBACTER PYLORI* INFECTION ASSOCIATIONS:

MATERNAL EDUCATION AND HYGIENE, ALTERNATIVE THREE

Exposure highlighted in purple, outcome highlighted in blue, covariates for adjustment highlighted in orange.

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