SPATIAL DISTRIBUTION AND DISEASE ECOLOGY OF GASTRIC CANCER IN WESTERN HONDURAS

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ABSTRACT

Jill Marie Furgurson: Spatial Distribution and Disease Ecology of Gastric Cancer in Western Honduras
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Gastric cancer, etiologically linked to infection with *Helicobacter pylori*, is the leading infectious-related cancer and the second most common cause of cancer mortality worldwide. Previous research has shown that gastric cancer rates are higher at high altitudes; however causal factors remain poorly understood. This research examines the relationship between altitude and gastric cancer risk, and explores potential explanatory covariates related to human behavior that may help explain the spatial patterns of gastric cancer incidence. Using a case control study of gastric cancer cases in western Honduras from 2002-2012, clusters of high-incidence areas are identified. Binomial multilevel likelihood models are constructed to better understand how altitude affects gastric cancer risk and to explore how individual-level behaviors drive disease incidence patterns. While simple models often assume all individuals are identical, multilevel models incorporate individual and group-level heterogeneity in characteristics that may be related to disease dynamics. Results indicate that age-standardized rates (n=594) are twice as high for males than females (15.07 for males and 6.59 for females), and that high rates are significantly clustered at the *municipio* (local administrative unit) level. Altitude was an insignificant predictor of gastric cancer when measured both as a continuous (p=0.197) and categorical variable (high/low; p=0.192). The results of the multilevel modeling of individual-level behaviors reveal that use of refrigeration as an adult is associated with a decrease in gastric cancer risk (β = -0.9883, p=6.51e-08). The finding that altitude does not affect gastric cancer risk within the study area suggests the possibility that the study area does not contain enough altitudinal heterogeneity to accurately characterize the relationship between altitude and gastric cancer rates. The finding that use of refrigeration as an adult is protective against gastric cancer suggests that access to refrigeration may decrease dependency on salted and preserved meats and increase access to fruits and vegetables, two established factors related to

gastric cancer risk. During the past two decades it has been well-established that infection with *H. pylori* is linked to increased gastric cancer risk. However, the finding that individual-level behavior impacts disease risk supports the theory that to understand disease dynamics, host-pathogen interactions must be considered within the context of their disease ecology.

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CHAPTER 1

INTRODUCTION

Nearly two million new cancer cases were attributed to infectious agents in 2008, and 80 percent of cases occurred in less developed regions (de Martel et al., 2012). Gastric cancer, etiologically linked to infection with *Helicobacter pylori*, is the leading infectious-related cancer and the second most common cause of cancer mortality worldwide. *H. pylori* are bacteria found in the stomach and gastrointestinal tract of half the world's population and an identified risk factor for the development of gastric cancer. Genetic polymorphisms in the human host population and virulent strains of the pathogen have been linked to increased susceptibility to the disease (El-Omar et al., 2001; de Sablet et al., 2011). However, these factors alone do not explain the distinctly heterogeneous geographical distribution of incidence and mortality. Many of the lowest rates occur in North America and Western Europe, while the highest rates occur in East Asia, Latin America, and Eastern Europe. This pattern is pronounced with an up to 10-fold variation in incidence between the lowest and highest at-risk populations, but the pattern is not always straight forward (Forman and Burley, 2006). Within high risk regions, some countries have relatively low rates, and within low-risk populations, there are high-risk groups, such as Japanese and Korean ethnic groups living in Los Angeles (Parkin et al., 2002). Furthermore, although over 70 percent of gastric cancer cases occur in less developed countries, the 'developed' vs. 'less developed' dichotomy does not always hold true. Japan has one of the highest rates of gastric cancer incidence and mortality, while some poorer countries have notably low rates (Bray et al., 2013).

Countries in Latin America have some of the highest age-standardized rates (ASR) of incidence and mortality. Honduras and Guatemala are ranked highest in the region with an ASR of 26.6 per 100,000 people, and fifth worldwide. Ecuador (23.7), Costa Rica (21.8), Peru (21.8), and Chile (17.9) are all among the highest ranking 20 countries worldwide, while Mexico (9.4) and Argentina (10.4) have notably low rates (Ferlay et al., 2010).In the high altitude region of western Honduras a larger than expected proportion of gastric cancer cases have been documented, a pattern observed in regions throughout Latin America (Torres et al., 2013). Among those infected with *H. pylori*, gastric cancer rates have been observed to be higher within populations living at high altitudes than in coastal or other low altitude populations (Palmeiro et al, 1988; de Sablet et al., 2011), although altitude is thought to be a proxy for other factors. The mountainous region of western Honduras is characterized as an area of endemic gastric cancer susceptibility due to a combination of genetic pre-disposition within the host population and prolific *H. pylori* infection rates (Morgan et al., 2006). Within populations with a genetic-predisposition, marked heterogeneity in disease incidence is indicative of additional risk factors (Amieva and El-Omar, 2008). This research aims to describe the spatial distribution of gastric cancer across the high-incidence region of western Honduras and identify behavioral and environmental covariates associated with the geographical variation of gastric cancer rates.

The majority of the research investigating gastric cancer comes from the field of epidemiology, where the emphasis tends to be on individual-level risk factors and the biological underpinnings of disease development. However, a gap in the literature has resulted from the lack of studies incorporating the role of space in disease dynamics. Behavioral and environmental risk factors vary across geographical space. Increasingly, public health research is revealing the importance of considering space and area-level characteristics when analyzing health and disease (Diez-Roux, 2000; Kawachi and Berkman, 2003; Macintyre et al., 2002). Previous gastric cancer studies have identified risk factors that are associated with disease risk, including diet, infection with *H. pylori*, tobacco use, and socio-economic status (Graham and Yamaoka, 2000; Forman and Burley, 2006; Ladeiras-Lopes et al. 2008; Moayyedi et al. 2002). Less understood are how behavioral and environmental factors that vary in space affect gastric cancer risk.

This study focuses on the use of refrigeration and the use of indoor woodstoves because they might help explain the spatial patterns of gastric cancer. The use of refrigeration increases access to fruits and vegetables, and is associated with a decreased dependency on salting, smoking, and adding nitrates/nitrites to preserve meat, factors thought to be associated with increased gastric cancer risk (Tsugane, 2005; Crew and Neugut). The evidence is scant, especially in Latin America, but some studies have shown that increased refrigerator use corresponds to lower gastric cancer risk (La Vecchia et al., 1990,1995; Larsson et al., 2006). Woodstoves are common in western Honduras, and a recent study in Honduras found evidence of an association between exposure to wood smoke from the use of an indoor wood stove and increased risk of invasive cervical cancer in women with human papilloma virus (HPV) (Velema et al., 2002).

The field of medical geography emphasizes the roles of human behavior and environment in understanding spatial variation in disease incidence patterns (Audy, 1958; Meade, 1977). The disease ecology framework from the field of medical geography, which posits that a variety of behavioral, environmental, and population variables are relevant to understanding disease etiology, in combination with spatial statistical methods from spatial epidemiology and the neighborhoods and health literature are used to investigate the following research questions:

How do behavioral factors help explain the altitudinal spatial patterns in western Honduras? Specifically, how do the behavioral factors of refrigerator and woodstove use affect gastric cancer incidence patterns in western Honduras?

Using a combination of disease mapping, cluster analysis, and multi-level modeling techniques applied to a case-control study of gastric cancer incidence in western Honduras, the geographical patterns of disease prevalence are explored. Behavioral, environmental, and population covariates are examined through the lens of disease ecology, a holistic approach to a multi-factorial disease. The findings contribute to the disease ecology and medical geography bodies of literature by demonstrating the role of spatially-variable behavioral covariates in gastric cancer etiology. As the etiologies of the majority of gastric cancer cases remain largely unknown, understanding the roles of geographically-variable and behavioral covariates relevant to disease dynamics provide valuable etiological insight.

THEORETICAL FRAMEWORKS

This research is informed by the disease ecology research framework within the human-environment tradition of geography and the neighborhoods and health framework from the public health literature. The theory of disease ecology applies a holistic perspective – one that includes the relevant behavioral, environmental, and population variables – in order to understand the etiology of multi-factorial diseases like gastric cancer (Meade and Emch, 2010). This frameworks enables research that considers the numerous and diverse factors, as well as the interactions between factors, which lead to specific disease patterns in specific places and times. The theory guiding research in neighborhoods and health can be applied to disease ecology studies in order to appropriately define the specific places or 'neighborhoods' to serve as units of analysis (Diez Roux, 2001). A compliment of statistical methods and spatial techniques has been developed to support and build the theory (Subramanian et al., 2003).

Disease Ecology

The subdiscipline of medical geography falls within the human-environment tradition of the discipline of geography and emphasizes the roles of human behavior and environment in understanding spatial variation in disease incidence patterns. Behavioral variables include factors related to culture (diet, smoking, recreation, beliefs), social organization (occupation, family structure) and technology (refrigeration). Behavior impacts the disease ecology framework in several ways. (1) Cultural behaviors expose individuals and populations to certain hazards while protecting them from others; (2) cultural behavior creates built human habitats; (3) health is impacted by marriage customs, food customs, and access to/use of health technologies, such as vaccines and antibiotics (Meade and Emch, 2010).

Within the field of medical geography, disease ecology provides a framework to understand disease etiology by examining the ways human behavior interacts with population and environment

(Meade and Emch, 2010). Modern applications of disease ecology trace their roots back to concepts posited by May (1958) and Dubos (1959), namely that disease is a product of environment and sociocultural factors and results from imbalances in the human-environment equilibrium. The various aspects of the disease ecology approach can be understood as the vertices of a triangle that encompass human health and disease. Used in combination with techniques and methods of spatial analysis, the disease ecology framework facilitates the investigation of where disease is occurring and how potential contextual factors are driving the observed spatial patterns. This framework, with an underlying tenet that it is important to understand not only the clinical manifestations of disease, but also how and where the disease process begins and ends, is especially useful for an investigation of the complex etiology of gastric cancer.

Neighborhoods and Health

Citing the pronounced differences between and within populations, many studies suggest that a combination of genetic, dietary, behavioral and environmental risk factors is influencing both gastric cancer incidence and *H. pylori* infection (Tkachencko et al., 2007; Graham and Yamaoka, 2000). In order to address a complex disease etiology, this study investigates the roles of space and place. The idea that where you live impacts your health, i.e. place matters, is not a novel concept (Kawachi and Berkman, 2003). However, in recent years the development of sophisticated spatial and multilevel modeling techniques has increasingly been used to elucidate distinctions between compositional effects (differences in the people constituting a place) and physical/social contextual effects (differences in between places). These so-called neighborhood effects are represented in analyses by incorporating multiple levels of organization within the models. Lower level (e.g. individual) effects are nested within higher level (e.g. neighborhoods) effects to understand how risk factors conceptualized at multiple levels impact health outcomes (Diez Roux, 2003). A one level regression model would be inappropriate for modeling gastric cancer outcomes at the individual level because such a model ignores space-based correlations, i.e. people who live in the same spatially-defined neighborhood tend to be more similar than a random sample of the entire population, thus violating the assumption that observations are independent of one another.

Most of the gastric cancer literature to date has focused on individual level risk factors. However, human populations are organized by a number of area level factors, such as urbanization, ethnic and class residential segregation, and geographical barriers. These organizational patterns can correspond to diverse built and natural environments that in turn have diverse effects on human health and disease. These "neighborhood" effects can be protective or harmful, leading to reduced or excess risk respectively (Kawichi and Berkman, 2003). Incorporating multilevel modeling methods from the neighborhoods and health literature allows the units of analysis and the constructs relevant to explaining variability in the outcome to be defined at distinct levels, thereby providing a framework for recognizing that there is a lack of independence between observations that are clustered within groups. Such analyses attempt to differentiate between compositional differences (differences caused by differences in the kinds of people who inhabit a place) and contextual explanations (differences between the places) (Macintyre and Ellaway, 2003).

The two frameworks and their associated concepts are used to both structure and answer the research questions. Rather than using a simple causal model where disease is a result of infection with the parasite, the multilevel models created incorporate numerous environmental and behavioral components related to gastric cancer risk. This includes spatial variables such as altitude and *municipios* of residence, and behavioral variables related to use of refrigeration and a woodstove. Moving beyond a host-pathogen relationship, the disease ecology and neighborhoods and health frameworks enhance understanding of gastric cancer disease dynamics.

BACKGROUND

Helicobacter pylori

H. pylori are common bacteria found in the stomach and gastrointestinal tract of half the world's population. If left untreated, infection is linked to chronic gastritis, gastric ulcers and gastric cancer. Over the past 25 years, infection with *H. pylori* has been etiologically implicated in gastric cancer pathogenesis in numerous ecological, cohort, and case-control studies (Forman and Burley, 2006). Neither the exact sources of infection nor transmission pathways have been identified, although evidence supports person to person transmission via an oral-oral or fecal-oral routes (Frenck and Clemens, 2003). The majority of infections occur in childhood when the bacteria colonize the environment of the human stomach, where they persist for decades. Over time, infection can cause chronic inflammation of the gastric mucosa. However, the majority of people infected do not have clinical consequences to infection (Covacci et al., 1999). Genetic evidence suggests ancestral forms of *H. pylori* have coevolved with human populations for over 100,000 years, and until recently the bacterium was believed to be present in nearly all adults (Blaser, 2006). Disease symptoms appear in only a small fraction of those infected, suggesting the bacteria is most often a commensal rather than a pathogenic organism. Among those who develop disease symptoms, significant spatial heterogeneity exists on both a global and local scale.

H. pylori strains can be divided into populations and subpopulations that reveal human migratory patterns on a global scale and influence gastric cancer risk. Each distinct *H. pylori* strain is associated with a distinct geographical distribution (Falush et al., 2003). In a study of two human host populations in Venezuela, one representing an Amerindian population and one representing a mestizo - European and mixed-ancestors population, there was evidence that the Amerindian population had a prominence of East Asian *H. pylori* genotypes and thus that these genotypes are indigenous in Amerindian populations (Ghose et al. 2002). In Colombia, a 25-fold increase in gastric cancer rate is observed in a high-altitude population in the Andean mountains compared to a coastal population (de Sablet et al., 2011). The two human populations have a similar prevalence of *H. pylori* infection (~90 percent); however the study concluded that all of the strains from the high-risk mountain region were of European origin, while the low-risk region had strains of either European (34 percent) or African (66 percent) origin. The *H. pylori* strains of European origin were strongly associated with increased premalignant histological lesions and gastric epithelial DNA damage regardless of whether they were from the high-risk or low-risk human population.

Recent evidence has demonstrated that certain strains of *H. pylori*, are particularly virulent (de Sablet et al., 2011; Wroblewski et al., 2010). Blocks of DNA present in pathogenic bacteria but absent from their nonpathogenic ancestors are known as pathogenicity islands. Strains of *H. pylori* isolated from patients diagnosed with diseases associated with severe gastritis, such as peptic ulcer and gastric cancer, contain a pathogenicity island known as cagA, which is absent from strains isolated from asymptomatic infected individuals (Covacci et al., 1999). Studies have identified Cag-A positive strains of *H. pylori* that lead to an increased risk of developing gastric cancer compared to infection with a Cag-A negative strain (Peek et al., 1995, Blaser et al., 1995; Parsonnet et al., 1997). Studies report varied odds-ratios, mostly in the 2-3 range and a few studies have failed to find evidence of the association (Amieva and El-Omar, 2008). One study claims that none of the virulence factors have proven disease-specificity, although there is evidence that Cag-A strains of *H. pylori* result in increased inflammation. Rather, the study concludes that the variation in patterns of gastritis in response to *H. pylori* infection can be primarily explained through environmental factors such as diet, and secondarily by the particular strain acting synergistically with the host-environment interactions (Graham and Yamaoka, 2000). It is clear that there is some disagreement in the literature on the role pathogenic virulence factors play in disease etiology.

Gastric Cancer and H. pylori Incidence Patterns

Each year, nearly two million new cancer cases are attributed to infectious agents. Gastric cancer is the most common cancer that is related to an infectious agent, and is the second most common cause of cancer mortality overall (de Martel et al., 2012). In 2008, there were nearly 989,000 new diagnoses and over 700,000 deaths due to gastric cancer worldwide (Ferlay et al.,2010).

Countries in Eastern Asia, Eastern Europe and Latin America have some of the highest age-standardized rates (ASR) of incidence. Eastern Asia has age-standardized incidence rates of 42.4 for males and 18.3 for females (Jemal et al., 2011). Honduras and Guatemala are ranked highest in the region with an ASR of 26.6 per 100,000 population, and fifth worldwide. Ecuador (23.7), Costa Rica (21.8), Peru (21.8), and Chile (17.9) are all among the 20 highest incidence countries worldwide, while Mexico (9.4) and Argentina (10.4) have notably lower rates (Ferlay et al., 2010).

Some of the regional variation in gastric cancer incidence is attributed to differences in *H. pylori* prevalence. Current prevalence rates of *H. pylori* infection vary by geographic location and ethnicity (Brown et al., 2000). Japan and Eastern Europe have particularly high prevalence rates of *H. pylori* (71 and 82 percent respectively), but prevalence is notably lower in Central America (62 percent) (Parkin, 2006).

Wide variation in prevalence exists both within and between populations of children, suggesting that unknown environmental factors are influencing the rate of acquisition and/or transmission pathways (Tkachenko et al., 2007). Due to an unknown combination of factors related to socioeconomic status, sanitation, diet, housing conditions, environment, and genetic predisposition, *H. pylori* prevalence and acquisition rates are higher in children in developing countries than in developed countries.

Host Genetics

Although the stomachs of half the world's population are colonized by *H. pylori*, only around one percent of infected people develop gastric cancer (Amiera and El-Omar, 2008). There is extensive evidence that genetic polymorphisms in the host population influence the pro- and anti-inflammatory response to damage to the gastric mucosa (Morgan et al., 2006). Proinflammatory IL-1 gene cluster polymorphisms are associated with an increased gastric cancer risk in those infected with *H. pylori* (El-Omar et al., 2001), and evidence of the association has been found in White, Asian, and Hispanic populations (Machado et al., 2001; Furuta et al. 2002; Garza-Gonzalez et al., 2005). Those individuals with both bacterial and host high-risk genotypes had the greatest odds of developing gastric cancer (Rad et al., 2003; Figueiredo et al., 2002).

An analysis of high risk alleles within the western Honduras population reveals that 89 percent of the population carried at least one risk allele, and the overall prevalence of risk alleles in the population is among the highest reported worldwide (Morgan et al., 2006). Although the role of these risk alleles within the Hispanic population is not well understood, evidence suggests concurrent *H. pylori* infection and carriage of risk alleles increases gastric cancer risk (Yang el al., 2004; El-Omar et al., 2003). Morgan et al. (2006) found significantly increased gastric cancer risk associated with persons with multiple risk alleles in western Honduras. That study concluded that the polymorphism profile of this Hispanic population mirrored those of Asian populations, supporting the hypothesis that early human migrations from Asia populated regions of Mesoamerica and in line with the distinctly high incidence rates.

Behavior and Environmental Risk Factors

Within the literature investigating causal and preventative interactions between dietary factors and gastric cancer, several distinct relationships have emerged. In some studies, intake of fruits and vegetables is linked to a reduction in the risk of developing gastric cancer (Riboli and Norat, 2003; Block et al., 1992). Potential mechanisms for this protective effect include the antioxidant activity of fruits and vegetables, a property related to phytochemicals, beta-carotene, alpha-tocopherol and Vitamin C (Liu, R.H., 2003; Forman and Burley, 2006). Vegetables of the Allium family (e.g. garlic, onions, leeks) have been the focus of numerous studies, and consumption of garlic is associated with a reduced risk of gastric cancer, suggesting a protective effect (Fleischauer et al., 2000). The evidence for the protective effect of fruit and vegetables is stronger in case-control studies than in prospective studies (Riboli and Norat, 2003), and it is worth noting, that many studies report ambiguous results. Additional dietary factors related to higher gastric cancer risk considered in the literature are salt intake (Tsugane, 2005; van den

Brandt et al., 2003), and the consumption of pickled vegetables (Ren et al., 2012) and processed meats (Larsson et al. 2006).

In a recent systematic review of studies investigating the role of smoking and tobacco use in gastric cancer, the relative risks for current smokers compared to never-smokers were 1.62 for males and 1.20 for females (Smyth et al., 2012). After controlling for education level, diet, alcohol consumption, and body mass index (BMI), smoking tobacco was associated with an increased gastric cancer risk. The hazard ratios were 1.73 and 1.87 in currently smoking males and females respectively, and 1.45 for ever-smokers (González et al., 2003).

The availability of refrigeration is thought to contribute to the overall decrease in gastric cancer rates in recent decades. The use of refrigeration has increased access to fruits and vegetables, a factor associated with decreased gastric cancer risk, and decreased dependency on salting, smoking, and adding nitrates/nitrites to preserve meat, factors thought to be associated with increased risk (Crew and Neugut, 2006; Larsson et al., 2006). The evidence is scant, especially in Latin America, but some studies have shown that increases in the number of years of refrigerator use correspond to decreases in gastric cancer risk (La Vecchia et al., 1990; La Vecchia et al., 1995).

A recent study in Honduras investigated the effects of wood smoke associated with the use of an indoor wood stove on the risk of invasive cervical cancer in women with human papilloma virus (HPV) (Velema et al., 2002). The study found an elevated risk of cervical cancer for the use of wood as fuel in the kitchen in women infected with HPV. Wood stoves are common in western Honduras, and this study contains detailed information about the type and duration of stove use.

OVERVIEW

A recent meta-analysis of gastric cancer in Latin America examined risk factors for gastric cancer. Like other studies worldwide, the authors determined smoking, alcohol use, increased dietary salt and processed meat, and a specific genetic polymorphism were associated with increased risk. However, the meta-analysis excluded analysis of the behavioral factors the present study proposes to address, namely use of a refrigerator and use of a woodstove, as well as the environmental variable of altitude due to a limited number of studies (Bonequi et al., 2013).

The present work will contribute to this identified gap in the literature by taking a disease ecology approach to the study of gastric cancer. The key to understanding and preventing infectious-related diseases such as gastric cancer is to explore the underlying causes and interactions that facilitate the transmission of pathogens and the development of disease. Those interactions most relevant to disease dynamics and patterns include the biological, hormonal, and immunological relationships between the pathogen and host, but also include the interactions born of an individual's personal environment. Individual personal environments are in turn shaped by human behavior and external macroenvironmental forces, including political, socioeconomic and technological factors (Wasserheit, 1994).

It may appear more obvious how human behavior impacts infectious disease patterns and prevalence by considering sexually-transmitted diseases (STDs). STD patterns are shaped by a variety of human behaviors, including drug use, condom use, early health-care utilization, and compliance with therapy (Amirkhanian et al, 2013; Wasserheit, 1994). All of these behaviors act to expose or protect individuals from harmful pathogens (Meade and Emch, 2010), and similar processes are at work with all infectious-related diseases.

The present work applies the disease ecology approach in constructing multilevel models characterizing and quantifying gastric cancer risk as the outcome to a number of environmental and individual-level behaviors. The existing literature suggests that important risk factors include sex, geography, socioeconomic status and smoking status (Smyth et al., 2012; Bray et al., 2013;Moayyedi, 2002). The multilevel models control for known individual-level risk factors as well as the group-level variable of *municipio*. Most studies of regional variation in gastric cancer patterns are based on large-area studies (Bray et al., 2013, Parkin, 2006). This study is unique in its focus on the small-area units of *municipios*.

The following chapters describe the spatial patterns of gastric cancer in western Honduras and further develop the disease ecology of gastric cancer with an emphasis on exploring the relationships between altitude, individual behavior, and gastric cancer risk. In Chapter 2, the spatial patterns of gastric cancer in western Honduras are described and the relationship between altitude and gastric cancer is explored. Chapter 3 explores the disease ecology of gastric cancer in western Honduras and specifically looks at the roles of refrigeration and woodstove use. In the concluding chapter, the results are discussed in light of the existing literature and further research directions are proposed.

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CHAPTER 2: SPATIAL PATTERNS OF GASTRIC CANCER IN WESTERN HONDURAS: EXPLORING THE ALTITUDE ENIGMA

ABSTRACT: Gastric cancer is the most prevalent cancer linked to an infectious agent, and is the second most common cause of cancer mortality worldwide. In the mountains of western Honduras a larger than expected proportion of cases have been documented, a pattern observed in regions throughout Latin America where higher rates of mortality and incidence are associated with higher altitudes. Using a study of gastric cancer cases in western Honduras from 2002-2012, this study describes the spatial patterns of gastric cancer within western Honduras by calculating crude incidence rates for each gender per *municipio*(local administrative unit) (n=605) and age-standardized incidence rates for the study area as a whole (n=594). Disease clusters are identified based on the local indicators of spatial association (LISA) statistic and multilevel models are constructed from case-control data (n=1030) to explore the relationship between gastric cancer cases and altitude. The age-standardized incidence rates for the entire study site are 15.07 for men and 6.59 for women. Crude *municipio*-level rates ranged from 3.87 to 58.54 (per 100,000). Results identify significant spatial clustering for both sexes, and demonstrate that the majority of high-incidence *municipios* are located in the state of Copán. Although several studies have demonstrated altitude is identified with high rates of gastric cancer, this analysis did not find altitude to be a significant predictor of increased risk. This finding may reflect the fact that the study area overall is characterized as mountainous, with mean *municipio* elevations for the case-control study area ranging from 447 to 1406 meters. The lack of a significant relationship between altitude and gastric cancer cases may suggest that the study area as a whole encompasses a high risk population. Future research in Latin America exploring the altitude relationship should include comparisons of coastal or low-elevation *municipios* with mountainous populations.

INTRODUCTION

Worldwide, nearly one million gastric cancer cases are diagnosed each year. However, significant geographical variation in disease incidence exists both within and between countries. The disease etiology of gastric cancer is linked to infection with *Helicobacter pylori,* a common bacterium that colonizes the human stomach of half the world's population. The high-altitude region of western Honduras is characterized as an area of endemic gastric cancer susceptibility due to a combination of genetic pre-disposition within the host population and prolific *H. pylori* infection (Morgan et al., 2006). Estimated gastric cancer standardized incidence rates in western Honduras are 39 for men and 21 for women (per 100,000 population), nearly double the global estimates for males and females of 22 and 10.3 respectively (Morgan et al., 2006; Parkin et al., 2005). The observed high incidence rates represent a larger pattern within Latin America, a region where countries have some of the highest morality rates from gastric cancer worldwide (Bonequi et al., 2013).

Among those infected with *H. pylori,* a small number of studies document that gastric cancer rates have been observed to be higher within populations living at high altitudes than in coastal or other low altitude populations (Torres et al., 2013; Palmeiro et al, 1988; de Sablet et al., 2011). A recent analysis focusing on altitude in the mountainous regions of Latin America suggests altitude in itself is not thought to directly influence incidence. Rather, altitude is hypothesized to be a proxy for other spatially-clustering factors related to diet, host and pathogen genetics, and environment (Torres et al., 2013). This is a case/control study design for a 2002-2012 study period to describe the spatial patterns of gastric cancer incidence within an area of western Honduras by quantifying the relationship between altitude and disease incidence.

BACKGROUND

Each year, nearly two million new cancer cases are attributed to infectious agents. Gastric cancer is the leading infectious-related cancer, and is the second most common cause of cancer mortality (de Martel et al., 2012). The highest rates of gastric cancer are found in East Asia, Eastern Europe and Latin America, while many of the lowest rates occur in North America, parts of Africa and Western Europe (Jemal et al., 2011; Parkin et al., 2002) Although over 70 percent of gastric cancer cases occur in less developed counties (Bray et al., 2013), high gastric cancer incidence rates are not simply a reflection of development status. Many parts of Africa experience some of the lowest documented rates (Jemal et al., 2013), although this may in part reflect limited resources for detection. Japan has one of the highest rates of gastric cancer incidence and mortality, although survival rates are considered moderately good due to mass screening efforts in place since the 1960's (Parkin et al., 2002). Countries in Latin America have some of the highest age-standardized rates (ASR) of incidence and mortality. Honduras and Guatemala are ranked highest in the region with an ASR of 26.6 per 100,000 population, and fifth worldwide. Ecuador (23.7), Costa Rica (21.8), Peru (21.8), and Chile (17.9) are all among the 20 highest incidence countries worldwide, while Mexico (9.4) and Argentina (10.4) have notably lower rates (Ferlay et al., 2010). Within Central and South America, high gastric cancer rates are observed to be higher in the mountains than in coastal areas with low elevations. This association has been observed in both the high-altitude Andes and at more moderate altitudes (Torres et al., 2013; Recavarren-Arce et al., 2005; de Sablet et al., 2011). Additional factors effecting the distribution include prevalence of *H. pylori* infection and diet (Parkin et al., 2002; Parkin, 2006).

The study area includes four states in western Honduras. The high altitude region of western Honduras is an area with endemic gastric cancer due to a combination of genetic pre-disposition within the host population and prolific *H. pylori* infection rates (Morgan et al., 2006). The region has a range of elevations, making it a suitable location to investigate the relationship between gastric cancer and altitude, a relationship which is poorly represented within the extensive literature on gastric cancer. A recent meta-analysis of gastric cancer in Latin America examined risk factors for gastric cancer. Like other studies worldwide, the study found that smoking, alcohol use, increased dietary salt and processed meat, and a specific genetic polymorphism were associated with increased risk. However, the meta-analysis excluded analysis of the environmental variable of altitude due to a limited number of studies (Bonequi et al., 2013).

The present work contributes to this identified gap in the literature by looking specifically at the relationship between gastric cancer and altitude in western Honduras. The analyses presented control for known individual-level risk factors and effects to demonstrate the role of spatially-variable area effects such as altitude in gastric cancer etiology. Establishing a relationship between gastric cancer risk and altitude is the first step in understanding the underlying factors that cluster in space along altitudinal gradients. Because altitude is hypothesized to be a proxy for other factors that cluster in space, exploring altitude as both a threshold variable by using altitude classes and as a continuous variable may shed further light on the mechanisms by which altitude affects risk.

Developing effective local public health responses and prevention efforts demands a detailed understanding of the geographical patterns of disease. In addition to the investigation of the role of altitude in gastric cancer risk, a spatial analysis of *municipio*-level variation in gastric cancer in western Honduras is presented. The resulting disease maps, which are used to understand the geographical distribution of disease incidence, demonstrate where incidence rates are highest and identify significant spatial clustering.

STUDY AREA AND DATA

This study is a case-control study of 605 gastric cancer cases and 635 controls in western Honduras for the period from 2002 – 2012. The study area includes four states – Copán, Ocotepeque, Lempira, and Santa Barbara – and 40 municipalities, administrative units with an average extent of approximately 160 square kilometers (Figure 2.1). Within this mountainous region, 95% of the population is Hispanic Mestizo. The remaining 5% of the population consist of two small indigenous groups, the Lenca and the Chorti (Morgan et al., 2006). The case data were collected from the Western Regional Hospital of the Ministry of Health, the single district hospital located in the state of Copán. The hospital has an established referral pattern, well-defined catchment area, and provides the only endoscopy services in the region. Details on the validation of the catchment area and the determination of the definitive at-risk population can be found in Dominguez et al. (2012). The area is bounded by geopolitical borders and has modest geographical isolation. In addition, the region has some altitudinal variation but does not represent the entire country. Notably, the study area lacks a coast low elevation area.

The cases used in the study were drawn from the database of all individual cases presenting to Western Regional Hospital. Controls were selected in a systematic, population-based sampling design and were age and sex matched from a subset of 20 *municipios*. Villages within a 50-mile radius of the hospital with populations ranging from 3,000 to 50,000 were included (Morgan et al., 2006). Every fifth household was sampled. One limitation of the study is that sampling of controls is geography-based at the level of *municipio*, making it difficult to identify altitudinal effects. Each individual case and control has associated survey data that contains information on age, sex, *municipios* of residence, and occupation. These variables are used to control for established individual risk factors in multilevel models of gastric cancer risk.

Ancillary data on elevation are available as 90 m digital elevation data, available from the NASA Shuttle Radar Topographic Mission (SRTM). The background population statistics are obtained from the Honduras National Statistics Institute (*Instituto Nacional de Estadistica* or INE), which provides official census and population data from the most recent census in 2001 and population projections released in 2009.

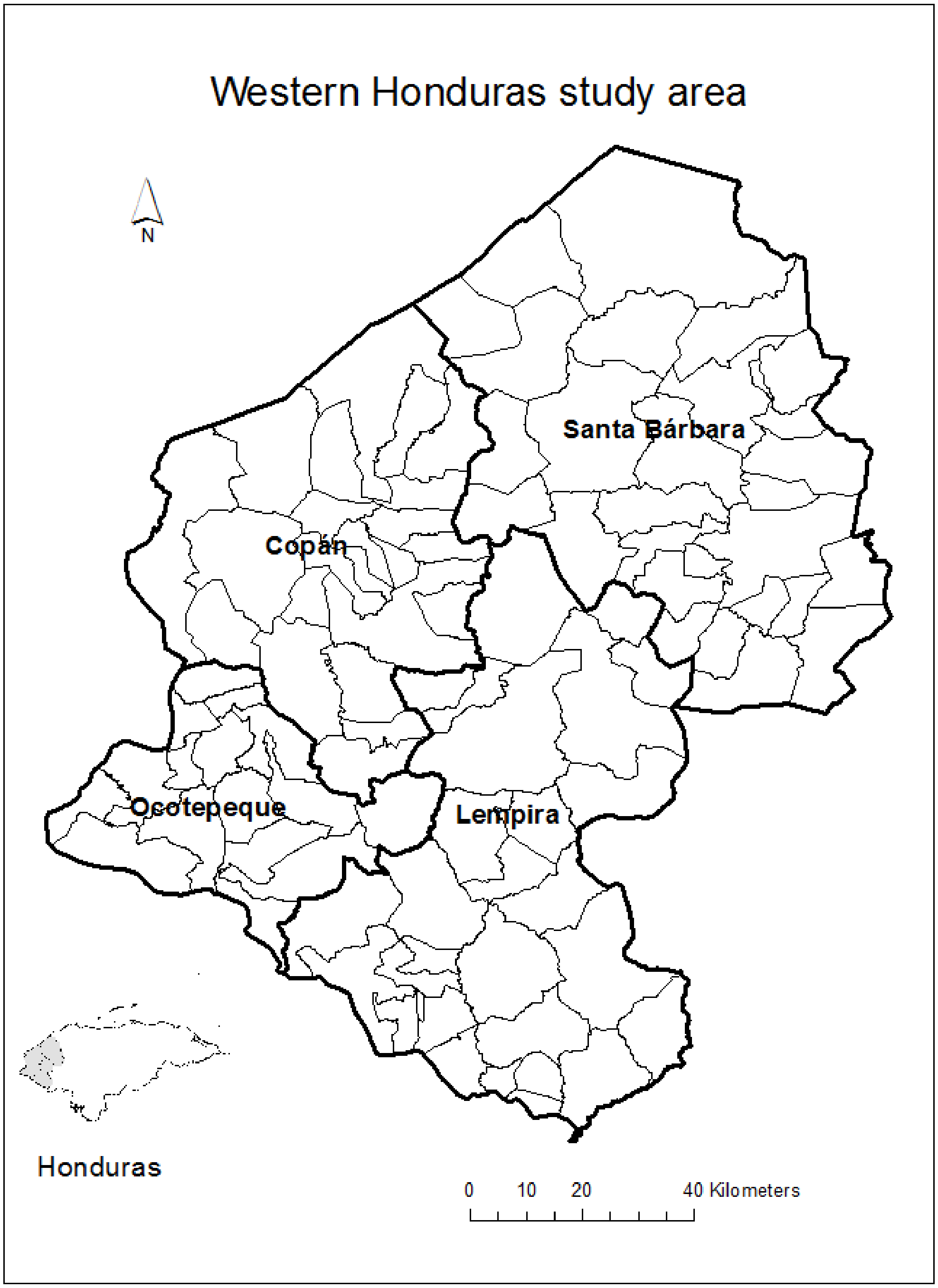

Figure 2.1 Study area states and *municipios* in western Honduras

METHODS

Multilevel models were constructed to examine how variation in the contextual factor of altitude affects gastric cancer risk within western Honduras. Altitude is thought to be a proxy for neighborhood factors directly related to gastric cancer, such as higher concentrations of genetic phenotypes and diet. Incorporating altitude as an area-level factor is important to account for the nested nature of the data, and failing to do so violates the regression assumption of independence between observations.

The role of altitude in gastric cancer risk was assessed via two pathways. As an area-level predictor, a mean altitude value was assigned to each *municipio* and used in the multilevel analyses. However, the relationship between altitude and high rates of gastric cancer was not expected to be a linear one. Therefore, in a second analysis two altitude classes were created (low = 0 – 900 m, high = 900 – 1400 m). These classes were defined based on the geography of the interior highlands of Honduras in which the valleys attend to occur at 300-900 m elevation. This distinction reflects the hypothesis that altitude differences represent differences in the characteristics of populations rather than a direct effect of altitude, such as hypoxia. Each *municipio* was then assigned to an altitude class based on their mean altitude value. In both analyses the area-level effect of altitude was examined by controlling for individual level confounders.

The basic model is a binomial multi-level likelihood model, where y_i takes the form 0 or 1 for each individual in group j (0 = control, 1 = case):

$$Y_i \sim \text{Binomial}\ (p_i)$$

$$\text{Log}[p_i/(1-p_i)] = \beta_o + \beta X_i\ \mu_j$$

Where p_i is the probability of response 1, X_i is a matrix of individual and area-level covariates associated with individual i, μ_j is the random effect term at the area level. The individual-level compositional factors of age, sex, smoking, and occupation were controlled for in the models. The area-level predictors include *municipio* and altitude, both as a linear and class factor.

In order to examine the neighborhood effects on gastric cancer rates, statistical analyses incorporate multiple levels of organization. Multilevel modeling is recognized as an asset for incorporating random effects, correctly modeling correlated error that occurs in clustered data, and to account for some spatial autocorrelation (Garson, 2012; Lawson et al., 2003). The use of hierarchal modeling will help to elucidate distinctions between contextual (characteristics of the population) and compositional factors (physical/social characteristics of place).

Using the case data of incident gastric cancer cases from 2002 - 2012 and background population data from the Honduras National Statistics Institute (*Instituto Nacional de Estadistica* or INE), age-standardized incidence rates (ASIR) were calculated for the study area as a whole and maps of *municipios* incidence rates were created. ASIR of gastric cancer for the study area as a whole was calculated by first calculating the crude incidence rate for each gender for each age class and then multiplying these values by the WHO world standard rate (Ahmad et al., 2001). Crude incidence rates were calculated for both males and females by *municipio* of residence. The median year 2007 was chosen for background population counts and sex ratios. Disease clusters were detected based on the local indicators of spatial association (LISA) statistic. LISA analysis is used to identify local patterns of spatial autocorrelation and indicates the significant clustering of similar values in space (Anselin, 1995). The term “local” is used to indicate that the relationship being evaluated is between an observation and neighboring values. The features of interest, in this case the *municipios*, are weighted based on their relationship to neighboring features. The analysis provides a z-score; a high positive z-score is indicative of a cluster of higher or lower than expected values. In this study, LISA analysis provides a measure of significant clustering of high rates of gastric cancer cases in the study area. ArcGIS was used for analysis and the creation of incident rate maps.

RESULTS

Table 2.1 displays mean altitude values and incident gastric cancer case rates per 100,000 by sex for each *municipio* within the Western Regional Hospital catchment area. Overall, the mean altitude values ranged from 447 meters above sea level in Macuelizo, Santa Barbara to 1406 meters above sea level in San Marcos, Ocotepeque (mean = 996 m). The case rates ranged for males from 3.87 (per 100,000) in Ocotepeque, Ocotepeque to 58.54 in San Agustin, Copán (mean = 15.72) (Figure 2.2). The female case rates ranged from 1.61 in Naranjito, Santa Barbara to 19.43 (mean = 7.82) in San Agustin, Copán (Figure 2.3). The age-standardized incidence rates for the entire study site are 15.07 for men and 6.59 for women. Similar to previous studies that have found rates to be higher for males than females, (Crew and Neugut, 2006; Jemal et al., 2008), this analysis found males to experience rates of gastric cancer nearly twice those of females.

Municipio	**State**	**Male Crude IR (per 100,000)**	**Female Crude IR (per 100,000)**	**Mean Altitude (m)**
Santa Rosa	Copán	17.09	8.12	855
Cabañas	Copán	4.62	3.05	1156
Concepción	Copán	15.92	8.51	1136
Corquin	Copán	16.77	6.34	1237
Cucuyagua	Copán	18.50	6.93	985
Dolores	Copán	18.51	16.79	1010
Dulce Nombre	Copán	25.64	12.58	1023
El Paraiso	Copán	15.75	5.23	790
Florida	Copán	12.36	5.93	837
La Jigua	Copán	23.24	4.06	614
La Union	Copán	5.97	6.68	1072
Nueva Arcadia	Copán	13.20	6.16	665

San Agustin	Copán	58.54	19.43	1179
San Antonio	Copán	9.94	14.70	1016
San Jeronimo	Copán	9.88	15.35	977
San Jose	Copán	17.39	12.85	746
San Juan de Opoa	Copán	43.69	13.19	733
San Nicolas	Copán	16.95	2.90	874
San Pedro	Copán	15.02	2.93	1214
Santa Rita	Copán	11.73	3.25	1019
Trinidad	Copán	17.62	17.39	933
Veracruz	Copán	35.97	11.70	987
Gracias	Lempira	5.82	2.53	1317
Guarita	Lempira	10.53	6.51	716
Las Flores	Lempira	8.15	2.07	918
Lepaera	Lempira	9.23	7.36	1052
Talgua	Lempira	15.09	19.15	1018
Ocotepeque	Ocotepeque	3.87	3.54	1637
La Encarnacion	Ocotepeque	4.16	4.47	1367
La Labor	Ocotepeque	11.63	6.87	1197
Lucerna	Ocotepeque	29.20	17.33	1351
San Fernando	Ocotepeque	18.81	6.39	1132
San Francisco del Valle	Ocotepeque	16.96	2.48	1277
San Marcos	Ocotepeque	4.00	3.80	1406
Senseti	Ocotepeque	11.20	11.74	1078
Azacualpa	Santa Barbara	6.76	4.92	590
Macuelizo	Santa Barbara	6.79	2.88	447
Naranjito	Santa Barbara	21.83	1.61	704
Proteccion	Santa Barbara	12.04	2.40	711

Nueva Frontera	Santa Barabara	8.46	2.50	869
Mean	**Total**	**15.72**	**7.82**	**996**

Table 2.1 Incidence rates of gastric cancer cases by sex and mean altitude value per *municipio*

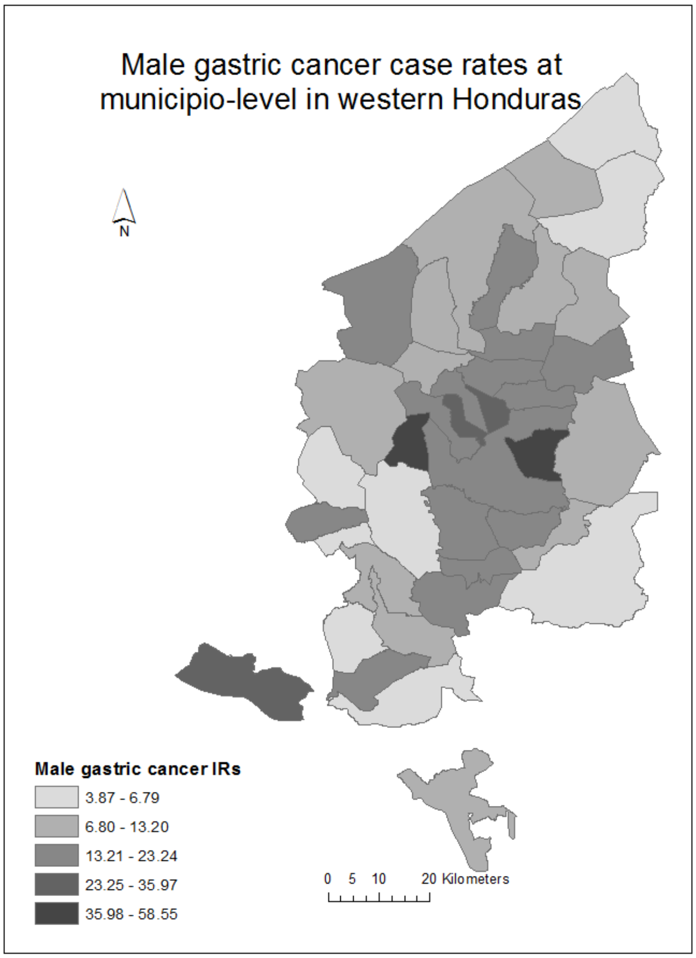

Figure 2.2 Map of male gastric cancer rates in western Honduras

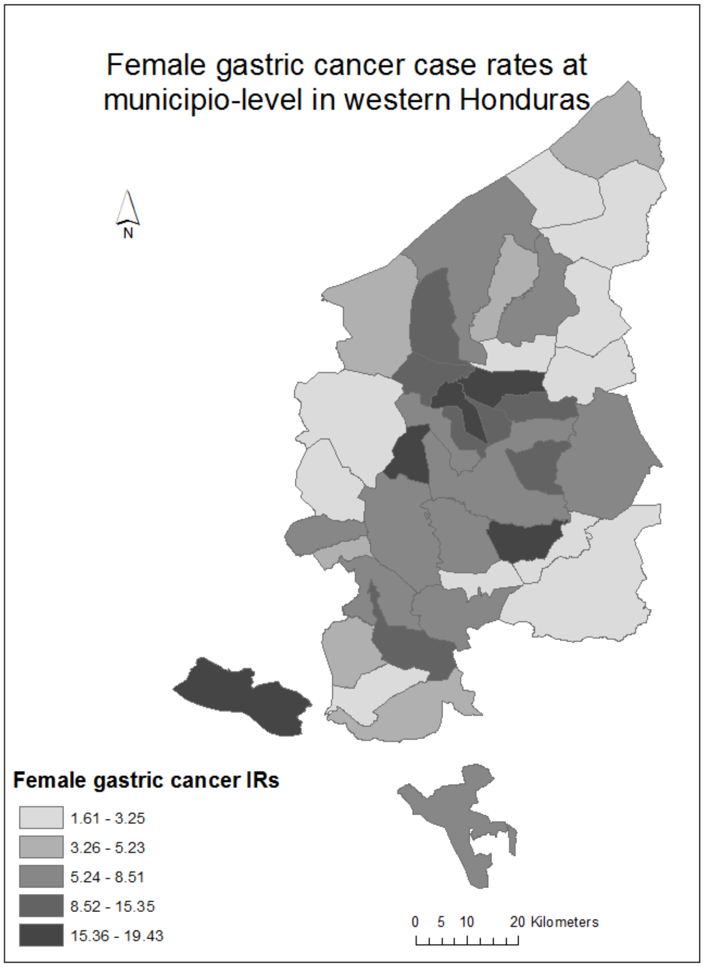

Figure 2.3 Map of female gastric cancer rates in western Honduras

The multilevel analysis of altitude and gastric cancer risk based on the case/control data found no significant effect of altitude on gastric cancer risk when measured as a mean value or as a categorical value of high/low (Table 2.2, Table 2.3). The relationship between altitude and gastric cancer incidence rates is shown graphically in Figure 2.2.

Variable	**Type**	**Parameter estimate (β)**	**SE (β)**	**p-value**

Altitude	Continuous	0.0020	0.0015	0.1883
Age	Continuous	0.0433	0.0058	1.26e-13***
Smoking	Binary	0.6596	0.2040	0.0012**
Sex	Binary	-0.3420	0.3388	0.3127
Occupation	Categorical			
Professionals		-1.9128	0.7482	0.0106*
Technicians		-0.7550	0.5705	0.1857
Trade/Craft		-1.5602	0.5701	0.0062**
Home maker		-1.5284	0.5410	0.0047**
Street Vendor		-1.2599	1.1729	0.2828
Construction		-1.1457	0.7232	0.1131
Agriculture		-0.2331	0.4705	0.6202
Other		-2.8364	0.8992	0.0016**

Table 2.2 Results from the multi-level analysis of mean altitude and gastric cancer risk.
* p <. 05, ** p < .01, *** p < .001

Variable	**Type**	**Parameter estimate (β)**	**SE (β)**	**p-value**
Altitude	Categorical	-1.0050	0.7569	0.1842
Age	Continuous	0.0434	0.0059	1.15e-13***
Smoking	Binary	0.6614	0.2042	0.0012**
Sex	Binary	-0.3298	0.3393	0.3310
Occupation	Categorical			
Professionals		-1.9066	0.7489	0.0109*
Technicians		-0.7524	0.5719	0.1883
Trade/Craft		-1.5656	0.5714	0.0062**

Home maker		-1.5167	0.5421	0.0051**
Street Vendor		-1.2578	1.1733	0.2837
Construction		-1.1509	0.7245	0.1121
Agriculture		-0.2343	0.4711	0.6190
Other		-2.8310	0.9001	0.0017**

Table 2.3 Results from the multi-level analysis of altitude class and gastric cancer risk.
* p <. 05, ** p < .01, *** p < .001

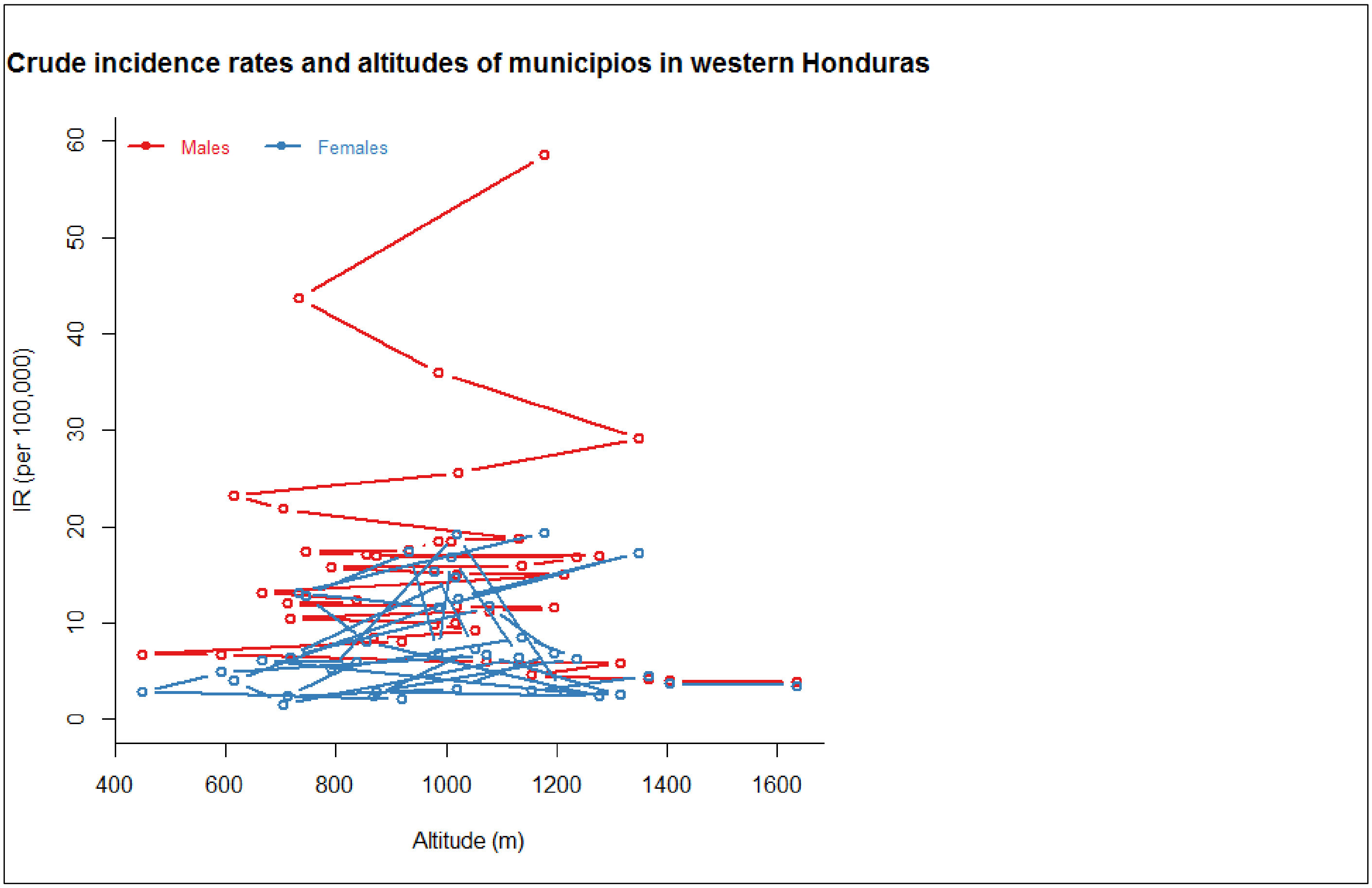

Figure 2.4 Crude incidence rates and mean altitude (m) values for 40 *municipios* in western Honduras. Each circle represents a *municipio*.

Results of the local Moran's I analysis of spatial clustering of the disease indicate that there are three *municipio*-level clusters for males and seven *municipio*-level clusters for females. The three male clusters are characterized by high positive z-scores, indicating that surrounding *municipios* have similar high case rate values. Six of the seven female clusters are also characterized by high positive z-scores, however one of the seven has a low negative z-score (<-1.96) which indicates a significant spatial outlier, in this case a high case rate *municipio* surrounded by lower case rates (ESRI, 2011). The three *municipios* with male high case rate clustering are Dulce Nombre, San Juan de Opoa, and Veracruz, all within the state of Copán. The six *municipios* with female high case rate clustering are Dolores, Dulce Nombre, San Agustin, San Jeronimo, Santa Rosa de Copán, and Trinidad, all within the state of Copán. The spatial outlier of a high case rate surrounded by low case rates is Lapaera in the state of Lempira. Significant spatial clustering at the *municipio* level for males and females is displayed in Figures 2.3and 2.4.

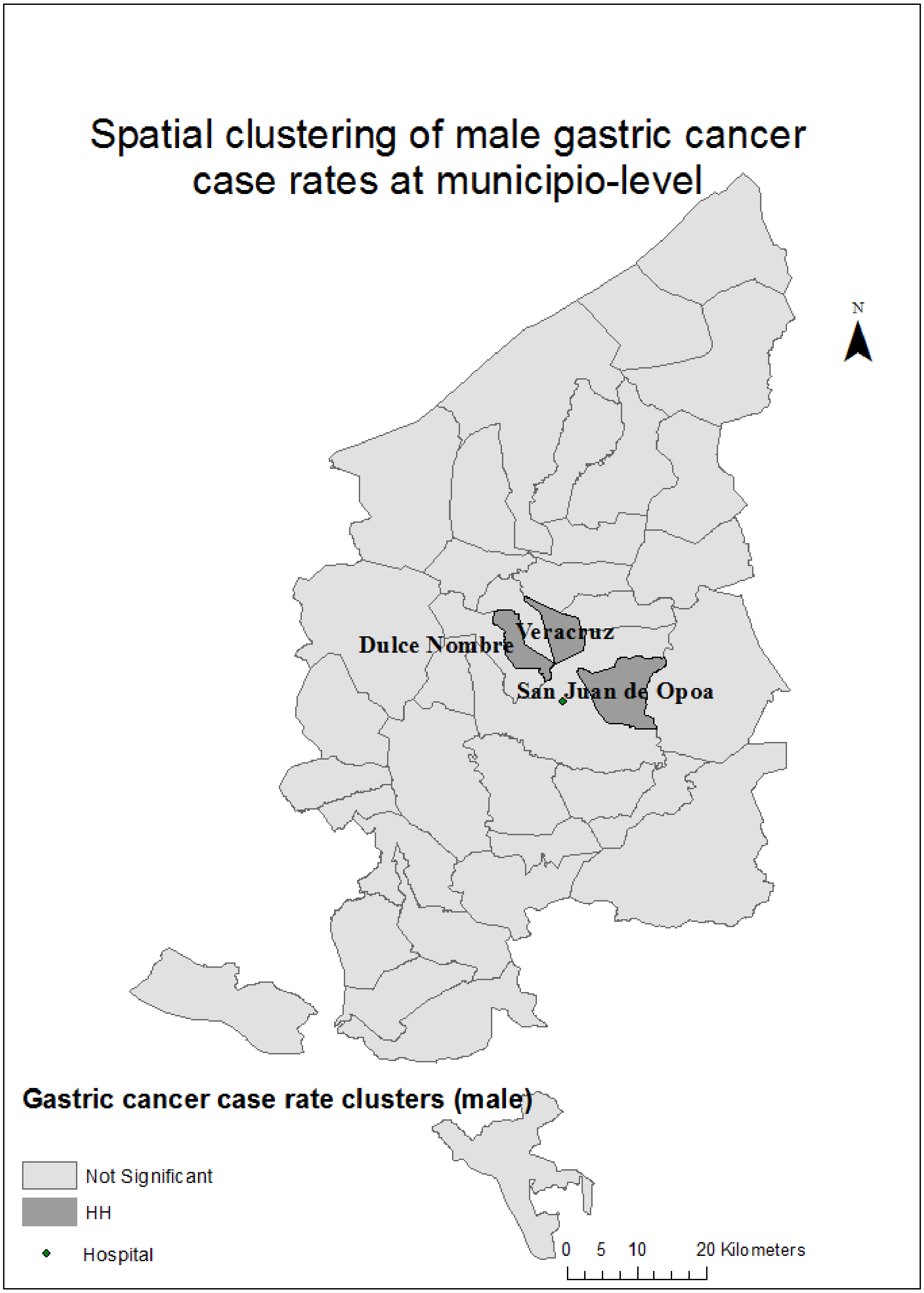

Figure 2.5 Significant spatial clustering of male gastric cancer cases. HH Case Cluster indicates clustering of higher than expected incidence rates.

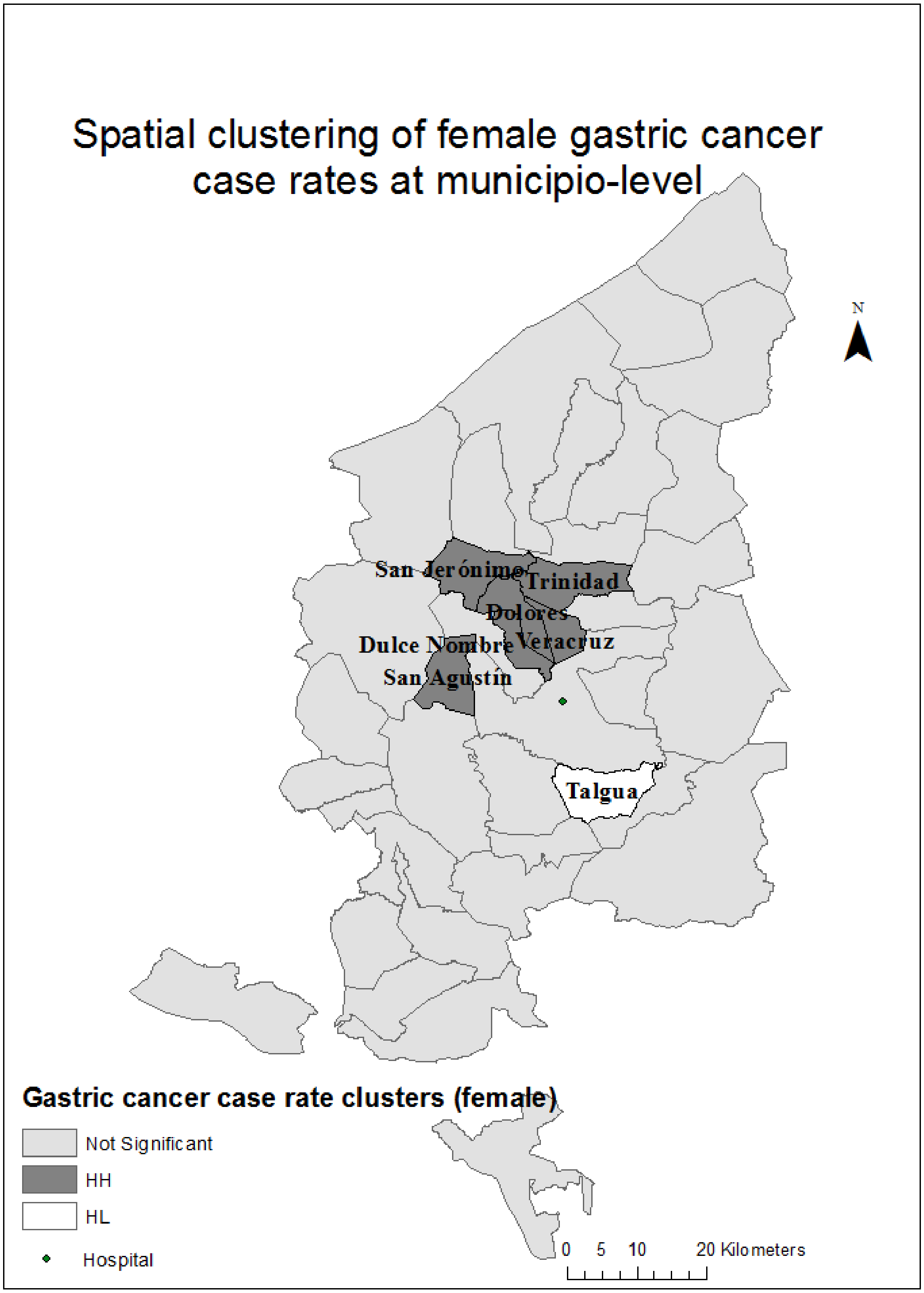

Figure 2.6 Significant spatial clustering of female gastric cancer cases. HH case cluster indicates clustering of higher than expected incidence rates. HL Case Cluster indicates spatial outlier of high incidence rate surrounded by lower rates.

Four occupational categories were found to have significant protective effects on gastric cancer risk. These include working as a home maker; working as a professional, which includes all college graduates; working at craft or trade, which includes work as a seamstress, cook, carpenter, tailor, electrical work, etc.; and work falling under the category “other”.

DISCUSSION

Gastric cancer is a major public health issue in many regions of Latin America (Torres et al., 2013; Bonequi et al., 2013; Ferlay et al., 2010). During the period 2002 – 2012, there were over 600 cases documented in western Honduras, and the highest rates were observed within the state of Copán. All three male clusters of disease and six of the seven female clusters of disease were found in the state of Copán, and the *municipio* of San Agustin contained the highest rates for both males and females. This study aims to identify the extent to which gastric cancer risk was associated with altitude in western Honduras. Previous studies of gastric cancer have documented higher rates are associated with higher altitudes (Torres et al., 2013; Recavarren-Arce et al., 2005; de Sablet et al., 2011), however this analysis did not find altitude to be a significant predictor of gastric cancer risk. Although this study did not find altitude to be significantly associated with risk, it is not believed that altitude itself is a direct risk factor for gastric cancer. Rather, it is thought that altitude is a proxy for other risk factors that may cluster in space, such as host genetics, bacterial strains, and dietary practices (Torres et al., 2013). The entire western Honduras catchment area is characterized as mountainous with elevations ranging from approximately 450 – 1400 meters. One explanation for the lack of a significant finding for the variable of altitude is that the study area may not encompass enough diversity in risk factors associated with altitude to find a significant result. The region has endemic infection with *H. pylori* and the population has a prevalence of risk alleles among the highest reported (Morgan et al., 2006). Within this region, established risk factors such as diet and socioeconomic status (Riboli and Norat, 2003; Tsugane, 2005; Moayyedi et al., 2002) may be relatively similar and therefore the study catchment area may not capture enough diversity in risk factors associated with altitude to demonstrate the effect of the variable. In addition, in this high incidence, mountainous area we do not have an included coastal region, where rates have been found to be lower than in the mounatins (Torres et al., 2012).

This study found that four occupational categories had a significant protective effect on gastric cancer risk. There are a number of epidemiological studies reporting relationships between gastric cancer risk and occupation, and evidence suggests a link between increased gastric cancer risk and occupations that result in exposure to dusty and high temperature environments (Santibanez et al., 2012; Krstev et al., 2005). It is possible that professional work and work in the home results in decreased exposures and therefore are protective. However, none of the occupational categories in this study were associated with increased risk.

The results of this analysis support previous findings that rates of gastric cancer in Latin America are among the highest worldwide (Bonequi et al., 2013), and that those rates exhibit great geographical heterogeneity (Lazcano-Ponce and Martinez, 2013). Although infection with *Helicobacter pylori* is a well-established risk factor for gastric cancer (Forman and Burley, 2006), there are clearly other risk factors operative that act within a landscape of low socio-economic status, genetic susceptibility and unknown behavioral and environmental determinants. Improving understanding of where disease occurs is critical for public health research. While improvements in diet, sanitation and treatment options will lead to decreased rates of gastric cancer in Latin America, it remains a priority to identify the unknown factors contributing to heterogeneous patterns of disease.

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CHAPTER 3: THE DISEASE ECOLOGY OF GASTRIC CANCER IN WESTERN HONDURAS: THE ROLE OF REFRIGERATION.

ABSTRACT: Gastric cancer is the second most common cause of mortality worldwide. Infection with *Helicobacter pylori* and genetic polymorphisms within the host population are linked to increased risk of gastric cancer, however behavioral risk factors receive far less attention within the literature. The population of western Honduras experiences endemic *H. pylori* infection in combination with a prevalence of identified risk alleles that is among the highest reported, making this region an ideal location to study less understood operative risk factors. This study uses a case control design (n = 1030) of gastric cancer in western Honduras to understand how the behavioral risk factors of refrigerator use and woodstove use effect gastric cancer risk. Results indicate that use of a wood stove does not affect gastric cancer risk. However, this finding may reflect the fact that 88 percent of respondents use a wood stove in the home, and therefore there may be insufficient variability in the predictor variable. Use of refrigeration as an adult significantly decreases gastric cancer risk ($p < .001$), while smoking significantly increases risk ($p < .01$). These findings suggest that human behaviors impact individual gastric cancer risk and refrigeration should be made available to at-risk populations.

INTRODUCTION

Honduras, like many Latin American countries, has notably high rates of gastric cancer incidence and mortality. Estimated gastric cancer standardized incidence rates in western Honduras are 39 for men and 21 for women (per 100,000 population), nearly double the global averages for males and females of 22 and 10.3 respectively (Morgan et al., 2006; Parkin et al., 2005). Established risk factors for gastric cancer include infection with *Helicobacter pylori*, identified host genetic polymorphisms, diet, and smoking (Graham and Yamaoka, 2000; El-Omar et al., 2001; Forman and Burley, 2006; Smyth et al., 2013). Within the literature, the causal factors behind the exceptionally high rates of gastric cancer in Latin America remain poorly understood (Bonequi et al., 2013). Gastric cancer is a complex disease and understanding the disease etiology requires that investigators move beyond host-pathogen physiology.

This study emphasizes the roles of human behavior in understanding geographical variation in disease incidence patterns. The use of refrigeration increases access to fruits and vegetables, and is associated with a decreased dependency on salting, smoking, and adding nitrates/nitrites to preserve meat, factors thought to be associated with increased gastric cancer risk. The evidence is scant, especially in Latin America, but some studies have shown that increased refrigerator use corresponds to decreases in gastric cancer risk (Crew and Neugat, 2006; Larsson et al., 2006). A recent study in Honduras found an increased risk of invasive cervical cancer among women with human papilloma virus (HPV) was associated with the use of an indoor wood stove (Velema et al., 2002).

BACKGROUND

Worldwide, nearly one million gastric cancer cases are diagnosed each year. However, significant geographical variation in disease incidence exists both within and between countries. Honduras, like many Latin American countries, has some of the highest rates of gastric cancer incidence and mortality across the globe (Torres et al., 2013; Morgan et al., 2006). Several individual risk factors have been identified, including diet, genetic polymorphisms, and infection with *Helicobacter pylori*, a common bacterium found in the human gastrointestinal tract (Graham and Yamaoka, 2000; El-Omar et al., 2001; Forman and Burley, 2006). Nevertheless, gastric cancer has a complex disease etiology and these factors alone do not explain the distinctly heterogeneous patterns of disease.

The majority of the research investigating gastric cancer comes from the field of epidemiology, where the emphasis tends to be on the biological underpinnings of disease development. Within the literature, environmental and behavioral variables have received less attention. Previous gastric cancer studies have identified risk factors that are associated with disease risk, including diet, infection with *H. pylori*, tobacco use, and socio-economic status (Graham and Yamaoka, 2000; Forman and Burley, 2006; Ladeiras-Lopes et al. 2008; Moayyedi et al. 2002). Less understood are how environmental and behavioral factors that vary in space, such as refrigerator and woodstove use, affect gastric cancer risk.

The availability of refrigeration is thought to contribute to the overall decrease in gastric cancer rates in recent decades. The use of refrigeration has increased access to fruits and vegetables, a factor associated with decreased gastric cancer risk (Crew and Neugut, 2006), and decreased dependency on salting, smoking, and adding nitrates/nitrites to preserve meat, factors thought to be associated with increased risk (Larsson et al., 2006). The evidence is scant, especially in Latin America, but some studies have shown that increases in the number of years of refrigerator use correspond to decreases in gastric cancer risk (La Vecchia et al., 1990; La Vecchia et al., 1995).

A recent study in Honduras investigated the effects of wood smoke associated with the use of an indoor wood stove on the risk of invasive cervical cancer in women with human papilloma virus (HPV) (Velema et al., 2002). The study found an elevated risk of cervical cancer for the use of wood as fuel in the kitchen in women infected with HPV. Wood stoves are common in western Honduras and inflammation is known to contribute to gastric cancer pathogenesis (Fox and Wang, 2007).

A recent meta-analysis of gastric cancer in Latin America examined risk factors for gastric cancer. Like other studies worldwide, the authors determined smoking, alcohol use, increased dietary salt and processed meat, and a specific genetic polymorphism were associated with increased risk. However, the meta-analysis excluded analysis of the behavioral factors the present study proposes to address, namely use of a refrigerator, use of a woodstove, and occupation, as well as the environmental variable of altitude due to a limited number of studies (Bonequi et al., 2013).

Used in combination with techniques and methods of spatial analysis, the disease ecology framework will facilitate the investigation of where disease is occurring and how potential contextual factors are driving the observed spatial patterns. This framework, with an underlying tenet that it is important to understand not only the clinical manifestations of disease, but also how and where the disease process begins and ends, is especially useful for an investigation of the complex etiology of gastric cancer. As the etiologies of the majority of gastric cancer cases remain largely unknown, understanding the roles of geographically-variable and behavioral covariates can provide valuable etiological insight.

STUDY AREA AND DATA

This study uses a case-control design (n = 1030) in western Honduras for the period from 2002 – 2012. The study area includes four states – Copán, Ocotepeque, Lempira, and Santa Barbara – and 20 municipalities, administrative units with an average extent of approximately 160 square kilometers (Figure 3.2). Within this mountainous region, 95% of the population is Hispanic Mestizo. The remaining 5% of the population consist of two small indigenous groups, the Lenca and the Chorti (Morgan et al., 2006). The case data come from the gastric cancer and pathology database from the Western Regional Hospital of the Ministry of Health, the single district hospital located in the state of Copán. The hospital has an established referral pattern, well-defined catchment area, and provides the only endoscopy services in the region. Details on the validation of the catchment area and the determination of the definitive at-risk population can be found in Dominguez et al. (2012). The area is bounded by geopolitical borders and has modest geographical isolation. In addition, the region has some altitudinal variation but does not represent the entire country. Notably, the study area lacks a coast low elevation area.

The cases used in the study were drawn from the database of all individual cases presenting to Western Regional Hospital. Controls were selected in a systematic, population-based sampling design and were age and sex matched from a subset of 20 *municipios*. Villages within a 50-mile radius of the hospital with populations ranging from 3,000 to 50,000 were included (Morgan et al., 2006). Every fifth household was sampled. One limitation of the study is that sampling of controls is geography-based at the level of *municipio*, making it difficult to identify altitudinal effects. Each individual case and control has associated survey data that contains information on age, sex, *municipios* of residence, and occupation. These variables are used to control for established individual risk factors in multilevel models of gastric cancer risk.

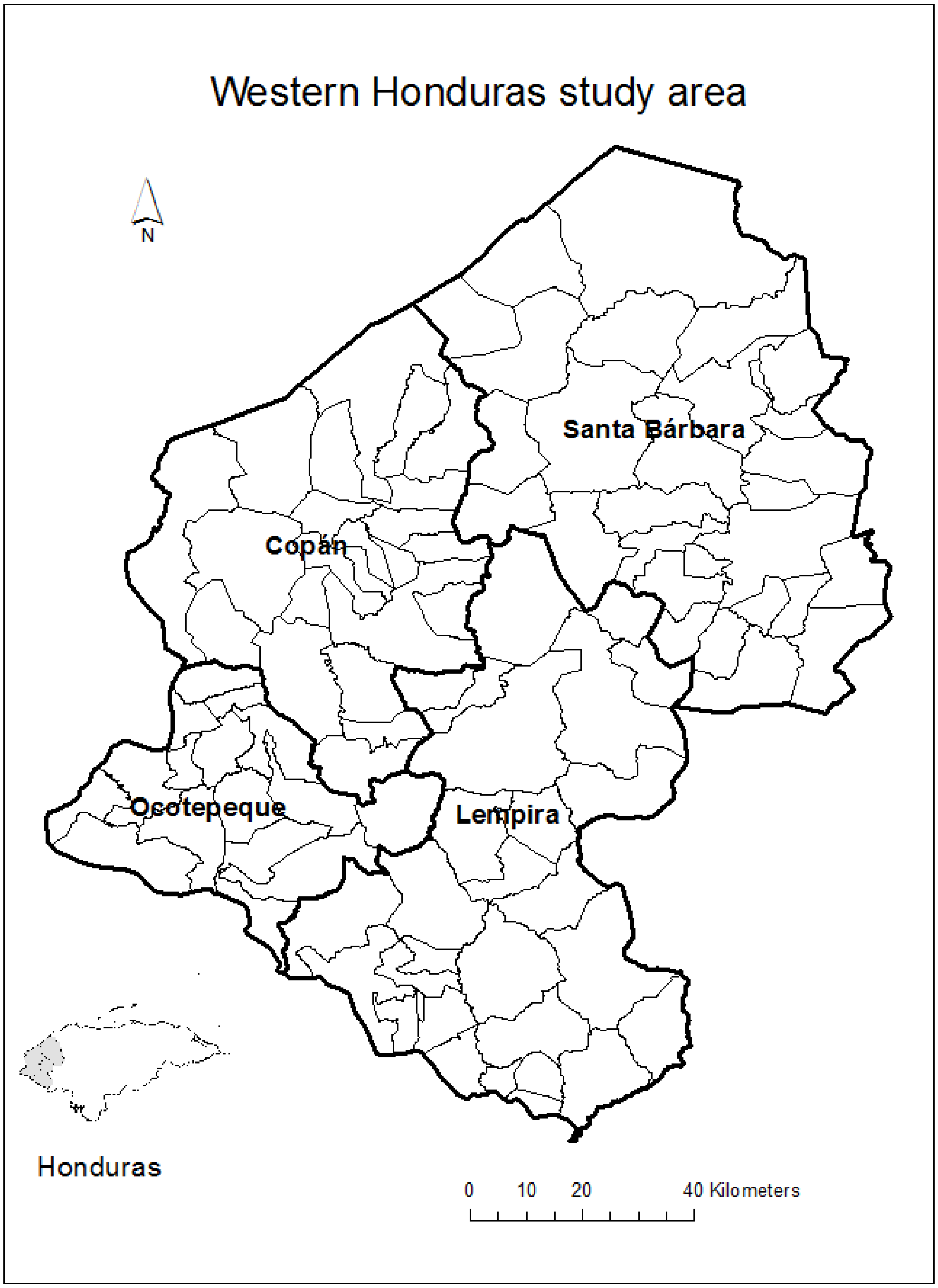

Figure 3.1 Study area states and *municipios* in western Honduras

METHODS

Multilevel analysis of public health data has emerged as a statistical approach for simultaneously analyzing individual and area, or “neighborhood”-level, variables on individual-level outcomes such as disease incidence (Diez-Roux, 2000). Increasingly, public health research is revealing the importance of considering space and area-level characteristics when analyzing health and disease data (Kawachi and Berkman, 2003). Such research aims to make the distinction between the level of “people” and the level of “place”, and subsequently maintain these levels within analyses (Subramanian et al., 2001). Binomial multilevel likelihood models were constructed to examine how the use of a refrigerator and the use of a woodstove affect gastric cancer risk within western Honduras. The 397 cases and 633 controls are nested within 20 *municipios* of residence. Using the area-level factors of *municipio* and altitude combined with individual-level behavioral covariates, the role of use of a refrigerator and use of a woodstove on gastric cancer risk are investigated. The unit of analysis is the individual, however the use of multi-level models allows incorporation of both individual-level and area-level predictors (Diez Roux, 2001). Multilevel modeling is recognized as an asset for incorporating random effects, correctly modeling correlated error that occurs in clustered data, and to account for some spatial autocorrelation (Garson, 2012; Lawson et al., 2003). The use of hierarchal modeling helps to elucidate distinctions between contextual (characteristics of the population) and compositional factors (physical/social characteristics of place).

The basic model is a binomial multi-level likelihood model, where y_i takes the form 0 or 1 for each individual in group j (0 = control, 1 = case):

$Y_i \sim \text{Binomial}\ (p_i)$

$\text{Log}[p_i/(1\text{-}p_i)] = \beta_o + \beta X_i\ \mu_j$

Where p_i is the probability of response 1, X_i is a matrix of individual and area-level covariates associated with individual i, μ_j is the random effect term at the area level.

The area-level predictors include *municipio* and altitude. The individual-level compositional factors of age, sex, and smoking are controlled for in the models. These variables have been shown to be significantly associated with gastric cancer risk in previous research. In this analysis, occupation is used as a proxy for socioeconomic status. Additional individual-level variables include refrigeration and woodstove use. The variables come from the associated survey data for each case/control (Table 3.1). Table 3.2 displays descriptive statistics for the behavioral variables of interest.

Factor	**Data**	**Source**	**Type**
Environment	90 m digital elevation	NASA Shuttle Radar Topographic Mission (SRTM)	Continuous
Behavior	Refrigerator Use (adult/childhood)	Survey data	Binary
	Smoking	Survey data	Binary
	Woodstove Use (adult/childhood)	Survey data	Binary
	Occupation	Survey data	Categorical
Population	Sex Age	Survey data Survey data	Binary Continuous

Table 3.1 Contextual and compositional covariates and data sources.

Variable	**Males**	**Females**
Smoking		
Yes	189	28
No	296	398
Refrigerator Use		
Yes	233	220
No	356	208
Woodstove Use		

Yes	489	345
No	69	45
Occupation	n=569	n=432
Agriculture	383	5
Home Maker	29	376
Commercial/Ar	49	11
tisan	26	0
Construction	15	8
Professional	29	22
Technician	6	4
Street Vendor	32	6
Other		

Table 3.2 Behavioral variables descriptive statistics

The final mixed logit model specifies that the outcome data comes from a binomial distribution and uses a logit link function. The lme4 package for the statistical program R was used for all multilevel analyses. This package allows for random effects with varying intercepts, a component of multilevel analysis. In this case, *municipio* of residence is the neighborhood-level random effect variable. This allows the slope to vary by *municipio* of residence.

RESULTS

Table 3.2 displays the results of the final multilevel model. Neither altitude nor use of a woodstove had a significant effect on gastric cancer. The effect of use of refrigeration as an adult was significant (-0.8627, p-value=<.001), indicating that using a refrigerator as an adult decreases gastric cancer risk. Similar to other studies of gastric cancer and smoking (Wu et al., 2001; Trédaniel et al., 1997), this study found that smoking was also significantly associated with risk (0.6836, p-value=<.01).

Parameter	β (log odds)	SE	p-value
Refrigerator use, adult	-0.8621 (-1.22,-0.43)	0.200	3.63e-05 ***
Woodstove use, adult	0.0130 (-0.57, 0.59)	0.2962	0.9649
Altitude	-0.0017 (-0.005 0.001	0.0016	0.2849
Smoking	0.6674 (.24, 1.09)	0.2174	0.0021**
Sex	-0.4220 (-1.13, 0.29)	0.3623	0.2441
Age	0.0460(.03, .06)	0.0062	1.25e-13***
Occupation			
Agriculture	-0.5682 (-1.54, 0.40)	0.4948	0.2508
Home Maker	-1.7085 (-2.83, -0.59)	0.5724	0.0028**
Commercial/Artisan	-1.3812 (-2.56, -0.20)	0.6011	0.0216*
Construction	-1.7169 (-3.23, -0.20)	0.7735	0.0264*
Professional	-1.5939 (-3.13, -0.06)	0.7851	0.0423*
Technician	-0.8928 (-2.05, 0.26)	0.5882	0.1290
Street Vendor	-1.3581 (-3.70, 0.98)	1.1946	0.2556
Other	-2.9584 (-4.77, -1.15)	0.9227	0.0013**

Table 3.3 Results from two-level model of gastric cancer risk in western Honduras, 2002 – 2012.
*** p-value < .001, **p-value < .01, *p-value < .05

DISCUSSION

This study found that in western Honduras the use of a refrigerator is protective against gastric cancer. Among adults that used refrigeration, gastric cancer risk was significantly protective ($\beta = -0.8627$, $p<.001$). This finding suggests that access to refrigeration is an important component to preventing gastric cancer. The protective mechanisms are hypothesized to be related to dietary changes brought about though access to fresh fruits and vegetables and decreased dependency on preserved meats. Refrigeration can keep fruits and vegetables fresh longer, maintain higher anti-oxidant levels and reduce the use of salt for preservation (Tsugane, 2005; Cai et al., 2002).

Gastric cancer is declining in most parts of the world, most likely due to a number of factors. These factors include reductions in the number of chronic *H. pylori* infections (Chen et al., 2007; Tkachenko et al., 2007), decreasing smoking rates in developed countries (Howson et al., 1996), dietary changes brought about through refrigeration, such as a reduction in the consumption of salted meats and increases in the consumption of fruits and vegetables (Crew and Neugut, 2006; Cai et al., 2002), and improving socioeconomic conditions. Despite the decline in many developed countries, gastric cancer remains an important problem for Latin America, where rates remain high.

Much of the existing literature on cancer risk and behavioral factors focuses on smoking and the use of tobacco (Coups and Ostroff, 2005). While the present study adds to the existing literature on smoking, the findings address an important gap in the literature on gastric cancer in Latin America by providing population-based estimates of a variety of behavioral risk factors, including refrigeration and the use of a woodstove. Although it was hypothesized that the use of a woodstove would lead to increased gastric cancer risk, no significant relationship was found. However, inferences are somewhat limited by the lack of variability in the predictor variable. The non-significant finding may be based on the fact that in our study area, 88 percent of responding adults use a woodstove.

Although these results shed light on the role behavioral factors play in gastric cancer risk, important questions remain. This is one of the first studies to examine the relationship between woodstove use and gastric cancer risk. Due to previous studies that suggest airborne exposures may be linked to gastric cancer risk (Sjodahl et al., 2007; Stellman et al., 1988) , further investigation into detrimental effects of wood stoves in the home is warranted.

Our findings indicate that while gastric cancer etiology is complex and not fully understood, there are opportunities for disease prevention. In high-risk areas such as western Honduras where infection with *H. pylori* is endemic, modification of behavioral risk factors brought about through increased access to refrigeration and cessation/prevention of smoking may contribute to the worldwide decline in gastric cancer rates.

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CHAPTER 4: CONCLUSIONS

The framework of disease ecology, which emphasizes a holistic perspective to disease etiology, combined with spatial statistical methods from the spatial epidemiology and neighborhoods and health literature, were used to investigate the roles of environment and human behavior in understanding spatial variation in gastric cancer incidence patterns in western Honduras. The disease ecology framework posits that to understand where disease begins, how it spreads through populations, and how to prevent incidence and mortality, a combination of environment, population, and behavioral variables must be considered. This approach is especially important for diseases with complex etiologies, such as gastric cancer, and in poorer geographic areas where socioeconomic factors may contribute to disease.

Worldwide, ten percent of cancer deaths are attributed to gastric cancer and seventy percent of cases are fatal, a figure that is significantly higher than the fatality-to-case ratio of both prostate (30 percent) and breast (33 percent) cancers (Guggenheim, 2012). However, high rates are often concentrated in developing countries where socioeconomic circumstances, lack of medical treatment, and dietary factors likely contribute to incidence and mortality. In contrast, in Japan where rates are historically high, reduced rates in recent decades are attributed to improvements in diet, prevention and treatment efforts, and childhood socioeconomic conditions (Tanaka et al., 2012).

Citing the significant heterogeneity inherent in the geographical patterns of incidence, many studies suggest that a combination of genetic, dietary, behavioral and environmental risk factors is influencing both gastric cancer incidence and *H. pylori* infection (Tkachencko et al., 2007; Graham and Yamaoka, 2000). The idea that where you live impacts your health is increasingly being incorporated into public health research (Kawachi and Berkman, 2003). In recent years the development of sophisticated spatial and multilevel modeling techniques has increasingly been used to elucidate distinctions between compositional effects (differences in the people constituting a place) and physical/social contextual effects (differences in between places). These so-called neighborhood effects are represented in analyses by incorporating multiple levels of organization within the models. That spatial approach as used in this study is supported by a disease ecology perspective. The various aspects of the disease ecology approach can be understood as the vertices of a triangle that encompass human health and disease (Meade and Emch, 2010) (Figure 4.1).

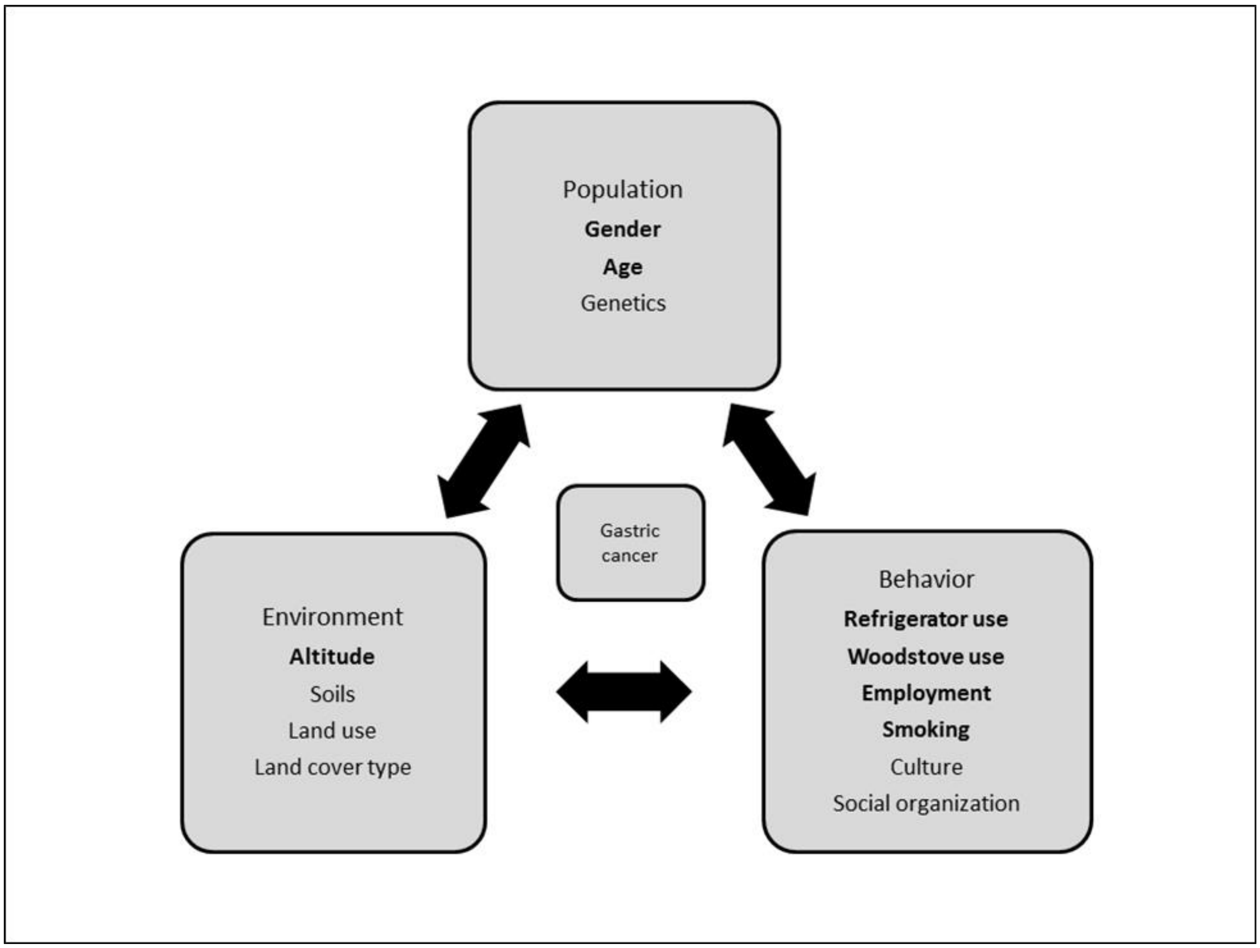

Figure 4.1 Gastric cancer triangle of disease ecology. Factors in bold were examined in the present analyses.

The results of the analyses support existing literature that provides evidence that men are twice as likely as females to experience gastric cancer (Kelley and Duggan, 2003), smoking is associated with an increased risk of gastric cancer (Smyth et al., 2012), and the use of refrigeration is inked to a decreased risk (La Vecchia et al., 1990). The lack of significant findings for the variables of altitude and the use of a woodstove may be related to issues of scale and how demographic factors such as host genetics and socioeconomic status cluster in space. The entire study area is characterized as mountainous and the regional population has one of the highest prevalence rates of risk alleles recorded (Morgan et al., 2006), and 88 percent of respondents report using a woodstove as an adult. Therefore, the study area may be too homogenous in regards to the factors of interest to be able investigate causal relationships.

Varying geographical patterns of gastric cancer incidence patterns as reported here and worldwide warrant further investigation into the complex disease etiology. disease ecology approach is important in order to fully encompass the relevant population, environment, and behavioral risk factors. This study found that age, sex, *municipio* of residence, smoking status, use of refrigeration, and occupation modify gastric cancer incidence in western Honduras and should be considered when examining disease prevention and treatment.

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