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Mapping and modeling of neglected tropical diseases in Brazil and Bolivia

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MAPPING AND MODELING OF NEGLECTED TROPICAL DISEASES IN BRAZIL AND BOLIVIA

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
School of Veterinary Medicine
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

In

The Interdepartmental Program in
Veterinary Medical Sciences
Through the Department of Pathobiological Sciences

By

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Abstract

Accurately defining disease distributions and calculating disease risk is an important step in the control and prevention of diseases. This study used geographical information systems and remote sensing technologies within the MaxEnt ecological niche modeling program to create predictive risk maps for leprosy and Schistosomiasis in Brazil and Chagas disease in both Brazil and Bolivia.

New disease cases were compiled for leprosy, Schistosomiasis, and Chagas disease from the Brazilian ministry of Health for 2001 to 2009 and the data was stratified to a 10,000 population for each municipality. Bolivian Chagas prevalence rates were calculated from 2007 to 2009 survey data. Environmental data was compiled from MODIS satellite imagery, and WorldClim data for both countries. Socioeconomic data was compiled from the Brazilian IBGE and the Bolivian INE.

Leprosy results showed that areas of lower moisture and specific temperature ranges were related to areas of high leprosy case detection especially in the central western, north eastern and northern regions of the country. The states of Bahia and Minas Gerais continue to show the highest levels of new Schistosomiasis cases and also were predicted to have some of the highest risks for the disease in our study. This study confirmed the importance of sanitation and educational level in relation to Schistosomiasis, which has been previously established in other studies.

Chagas disease models identified altitude as being important, as well as lower levels of precipitation, and higher ranges of temperature which correspond to the biological requirements of the insect vectors. Information for housing materials was only found for Bolivia, but demonstrated the importance of improved housing materials. Adobe wall materials were found to be highly related to the disease while areas with hardwood floors demonstrated a direct negative correlation.

These studies demonstrated that MaxEnt can be successfully adapted to disease prevalence and incidence studies and provides governmental agencies with an easily understandable method to define disease risk area for use in resource planning, targeting, and implementation. This study emphasizes the need for more refined socioeconomic data to create better socioeconomic and smaller regional study areas to better elucidate region specific disease characteristics.

Chapter 1: Introduction

1.1 Introduction

Neglected tropical diseases (NTDs) affect approximately 1.4 billion people worldwide and in many areas the population may suffer from two or more of these diseases (Hotez 2011). In Latin America and the Caribbean (LAC), NTDs rival human immunodeficiency virus (HIV) and malaria in terms of disease burden (Hotez 2008a). NTDs disproportionately affect the poorest and marginalized communities in Latin America (Allotey 2010, Ault 2007, Conteh 2010). Approximately 78 million rural people live in poverty in LAC and 165 million in urban areas live in poverty (Ault 2007). These diseases not only occur in relation to poverty, but they induce and promote poverty through malnutrition and anemia (trichuriasis, ascariasis, schistosomiasis), disability (leishmaniasis, Chagas disease, schistosomiasis), and deformity and social stigma (leprosy) (Allotey 2010, Conteh 2010, Spiegel 2010). Increased attention and awareness is being placed on NTDs and their relationship to poverty as various organizations such as the United Nations work to achieve Millennium Development goals to end extreme poverty, hunger and disease by 2015 (Hotez 2007).

The country of Brazil provides an interesting example of the link between NTDs and poverty as most of the NTD disease burden in LAC occurs in this country (Hotez 2008b). Brazil is one of the richest countries in LAC, but also has the greatest disparity between the wealthy and poor in the world (Hotez 2008b). Brazil accounts for 93 percent of all cases of leprosy (PAHO 2007a) and 83 percent of schistosomiasis cases (Steinmann 2006) in LAC, and is also home to the highest number of leishmaniasis and leptospirosis cases (Hotez 2008b).

Bolivia, like Brazil, has a large disparity between the wealthy and the poor as well and is home to a large number of neglected tropical diseases. Unlike Brazil, Bolivia has not had the success that Brazil had with its Chagas control program. Also in contrast, Bolivia has not had a disease reporting system in place until recently.

In order to better prevent, monitor, and treat neglected tropical disease in LAC, it is essential to have information about the prevalence and geographic distribution for each disease. Geographic information systems (GIS) are useful in both understanding and explaining the spatial distribution of diseases as well as the potential relationship of diseases to geographical, climatological, socio-cultural, and health system-related factors (Tanser 2002). GIS and remote sensing technologies have successfully been utilized in control campaigns against schistosomiasis (Bavia 1999, Malone 2001, Zhou 2008), malaria (Hay 2004) and fascioliasis (Malone 1998). Through the use of this technology, relationships between environmental and socioeconomic factors and disease can be used to define at-risk target populations for intervention and allotment of resources. The goal of this research was to develop a methodology for using ecological niche models to assist in governmental decision making with regard to resource allocation for control and prevention programs. The objective of this study was to use multiple regression and ecological niche modeling to predict the occurrence of leprosy and schistosomiasis in Brazil and Chagas disease in Bolivia and Brazil.

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Chapter 2: Introduction to Leprosy in Brazil

2.1 History and Geographic Distribution

Leprosy, now known as Hansen's disease, is a chronic infectious disease caused by the intracellular bacterium *Mycobacterium leprae*. The causative agent of leprosy was first discovered in 1873 in Norway by G.H. Armauer Hansen (Araujo 2003), but the disease has been recorded as far back as 600 B.C. in China, India and Egypt, and is believed to have originated in the Indian subcontinent (Monot 2005). Globally the prevalence is estimated at 0.4 million people, with India and then Brazil as the top two countries most affected by the disease Lindoso 2009 (Figure 1).

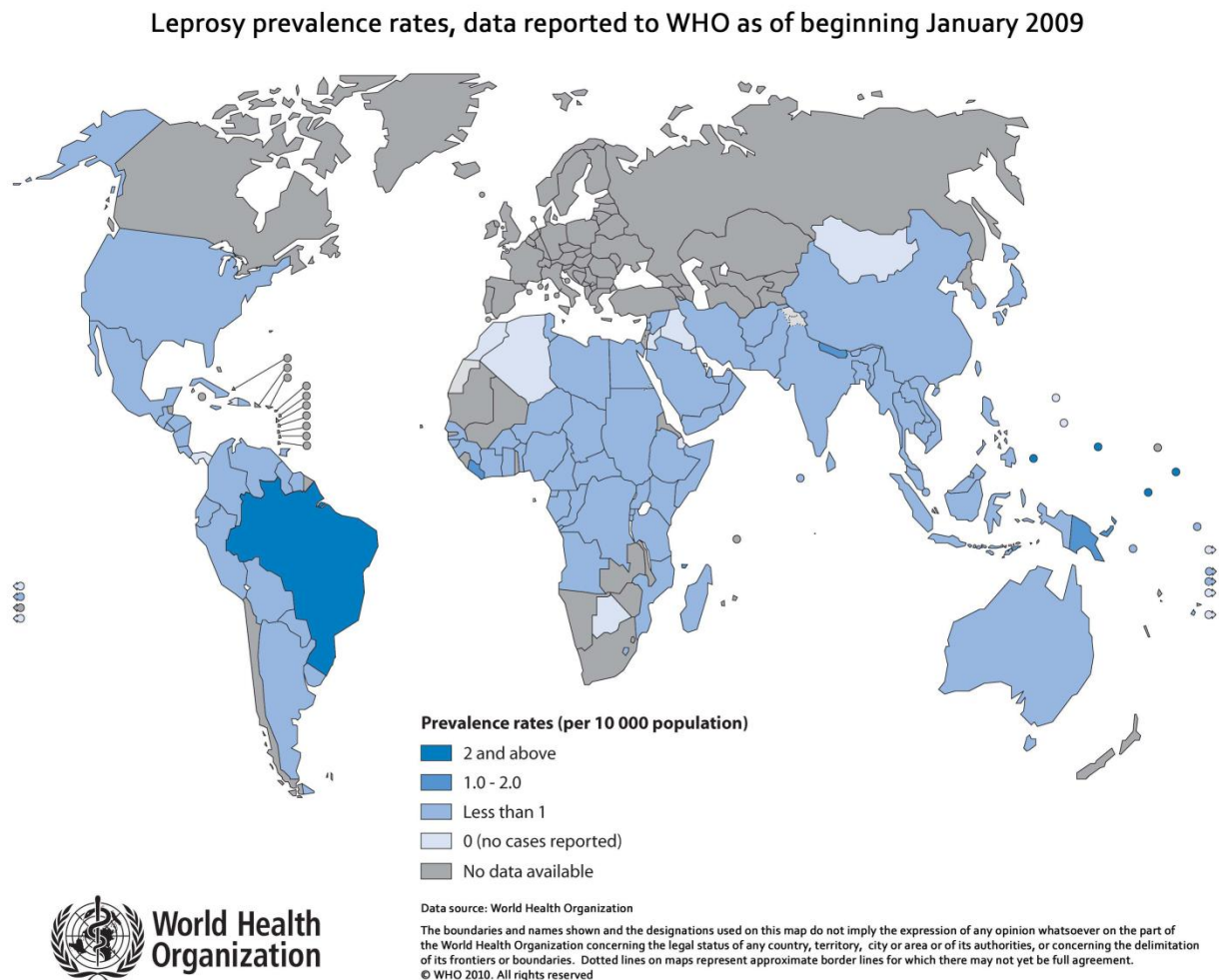


Figure 1: Geographic Distribution of Leprosy: Courtesy of the World Health Organization

Accessed from <http://www.who.int/lep/situation/LEPPRATEJAN2009.pdf>

Leprosy was first reported in 1600 in Rio de Janeiro, followed by reports in the states of Bahia and Para (Brazilian Ministry of Health 1989). Molecular research on single nucleotide polymorphisms indicate that leprosy was first brought to the Americas by European immigrants and African slaves (Truman 2005). The majority of the leprosy cases occurring in Latin America and the Caribbean (LAC)

occur in Brazil (93%) (Araujo 2003). Leprosy in Brazil has decreased in the last twenty years from a rate of 16.4 cases per 10,000 residents in 1985 to 1.5 cases per 10,000 residents in 2005, but it is still above the target set by the world health organization of less than 1 case per 10,000 population (Araujo 2003, Opromolla 2005, Penna 2008).

The distribution of the disease is unevenly dispersed throughout the country, with very low levels of disease in the South and Southeastern regions and highly endemic areas in all other regions (Lindoso 2009). In 2007, the Northern, Northeastern and Central Western were responsible for 63.5% of all of the reported leprosy cases (Penna 2009b). Known prevalence rates per 10,000 inhabitants are 6.23 in the Central West region, 5.21 in the Northern region, 3.16 in the Northeastern regions and 0.89 and 0.53 for the Southeastern and Southern regions respectively.

2.2 Epidemiology

The exact mechanism of transmission is not yet understood, but is primarily thought to spread through nasal secretions or droplets. It is theorized that infectious nasal droplets may be able to enter the body through broken skin as well as the nasal mucosa. Large numbers of bacilli can be shed by leprosy patients into the environment through sneezing, coughing and talking (Davey 1974, Rees 1974, Huang 1980, Fine 1982)

Susceptibility to the disease is linked to genetics, and family clustering of the disease is well documented (Ooi 2001). Disease immunity is partially controlled through genetic determinants related to host susceptibility and resistance, as well as HLA-D related immune responses (Ooi 2001, Scollard 2006), and non-HLA genes (SLC11A1, TNF α) (Abel 1998, Roy 1997). Mira found that a locus on chromosome 6q25 appeared to control partial susceptibility to leprosy in a study among 86 families with leprosy in Vietnam (2003). A similar study in India found that the locus on chromosome 10p13 was linked to paucibacillary leprosy (Siddiqui 2001). There is mounting evidence that genetic determinants play a role in disease susceptibility and presentation, but more research is needed to delineate the specifics of this relationship and its role in disease transmission.

Multibacillary patients are thought to be a more important source of disease transmission, and it is estimated that household contacts of these patients have a 5 to 10 fold greater relative risk of developing the disease as compared to the general population (Goulart 2008a). However in areas where multibacillary patients are relatively rare, subclinical transmission may have a greater role in the disease transmission cycle (Goulart 2008a). Disease risk was also found to be higher for children less than 14 years of age (Rao 1975, Fine 1997, Vijayakumaran 1998, Ranque 2007).

Leprosy cases tend to be found in clusters which may indicate that environmental factors could play a role in distribution and transmission of the disease (Sterne 1995, Fine 1997). Water and soil moisture have been associated with high prevalence in Malawi (Sterne 1995), and *M. leprae* DNA has been detected in water sources utilized by leprosy patients in Indonesia (Matsuoka 1999). A geospatial risk assessment model for leprosy in Ethiopia, found that certain thermal-hydrological regimes favor

leprosy survival in the environment in that region (Argaw 2006). Increasing molecular evidence has also implicated the nine banded armadillo (*Dasypus novemcinctus*) in human leprosy transmission in the southern United States (West 1988, Bruce 2000, Clark 2008, Truman 2011). This species is also prevalent throughout Brazil, but its relationship or lack thereof to human leprosy, is not well studied.

2.3 GIS Mapping and Modeling

The use of GIS analysis for leprosy epidemiology is promising as little is known about the role of environmental factors in leprosy transmission. This is an area in which, GIS and environmental niche modeling could prove to be informative. A geospatial risk assessment model for leprosy in Ethiopia, found that certain thermal-hydrological regimes favor leprosy survival in the environment in that region (Argaw 2006).

Small scale studies analyzing the relationship between leprosy distribution and socioeconomic variables have been carried out in the municipalities of Goiania in Goias (Martelli 1995), Manaus in Amazonas (Imbiriba 2009), Mossoro in Rio Grande do Norte (Dias 2007), Olinda in Pernambuco (Lapa 2001, Souza 2001) and Ribeirao Preto in Sao Paulo (Guay 2007). A statewide study in Ceara conducted by Montenegro, found a heterogeneous spatial distribution of leprosy with high incidence clusters in the northwest, center and southeast regions of that state (2004). On a country wide scale, Penna used GIS and spatial cluster analysis to find focal clusters of the leprosy in Brazil (2009). In this study, significant clusters were identified throughout the North, Northeast, and Centralwest regions and displayed a heterogenous pattern across those areas.

2.4 Hosts and Reservoirs

The armadillo, *Dasypus novemcinctus*, is the only animal found to date routinely naturally infected with *M. leprae*, and has been linked to human leprosy infection (West 1988, Bruce 2000, Truman 2005, Clark 2008, Truman 2011). A recent study by Truman *et al.* found a unique *M. leprae* genotype in both armadillos from the gulf coast and human leprosy cases in that area, which implicates armadillos in the transmission cycle in the United States (2011). This species of armadillo also has a wide distribution range in Brazil, and biomarkers for *M. leprae* have been reported in armadillos in the state of Espirito Santo (Deps 2002, Deps 2007, Antunes 2009.) Little to no research has been done on armadillo infection in other areas of Brazil with the exception of a few small studies in Sao Paulo where all of the armadillos sampled were negative for leprosy infection (Pedrini 2010). Several studies have found a correlation between armadillo handling and consumption to disease occurrence (Thomas 1987, Rodrigues 1993, Clark 2008, Deps 2008,) but other studies have not found such an association (Kerr-Pontes 2006, Schmitt 2010). These contradictory studies do not necessarily discount the role the armadillo in transmission, but may reflect the presence of a non infected armadillo population in those areas (Schmitt 2010).

Chimpanzees and mangabey monkeys can also develop leprosy, but their susceptibility and role in the transmission cycle is not well defined (Donham 1977, Walsh 1981, Gormus 1991, Hubbard 1991, Meyers 1992, Suzuki 2010). It is suggested that they may maintain the infection in the wild and could serve as sources of infection to humans in Africa in the same way that armadillos may serve as sources of transmission to humans in the Americas (Suzuki 2010), but this is yet to be substantiated.

2.5 Pathogenesis

M. leprae preferentially targets macrophages and peripheral Schwann cells. The disease exhibits a wide spectrum of clinical and histopathological manifestations dependent on the host's cellular immune response. Tuberculoid type or paucibacillary patients initiate a Th1 cell mediated immune response using the cytokines IL 2 and IFN γ against the bacteria, promoting granuloma formation around infected macrophages and Schwann cells, successfully limiting bacterial replication and spread. Lepromatous type or multibacillary patients initiate a Th2 cell mediated response with the aid of IL 4 and IL 10 cytokines that impede granuloma formation and allow the bacteria to rapidly replicate and infiltrate skin and nerve cells. The wide range of presentations and the different classification systems employed by different agencies work to complicate diagnosis of the disease.

In 1962, Ridley and Jopling developed a five-part classification scheme to differentiate different leprosy presentations based on bacillary load and cell-mediated response time resulting in the following classifications; tuberculoid (TT), borderline tuberculoid (BT), borderline (BB), borderline lepromatous (BL), lepromatous (LL), and indeterminant (1966). Tuberculoid type leprosy is defined by a high resistance and thus a low number of bacilli present (Ridley 1966). This type of leprosy presents as a few, well defined, hypopigmented, asymmetric lesions with a loss of sensation (Ridley 1966). Skin smears are negative or show a very small number of bacilli (Ridley 1974). Borderline tuberculoid presentation is similar to tuberculoid leprosy, with smaller, less defined, asymmetric lesions that are more numerous (Ridley 1966). Additionally there is less hypopigmentation associated with the lesions and satellite lesions are commonly seen (Ridley 1966). The center of the lesion demonstrates slow, incomplete or a complete lack of healing, resulting in broad infiltrated edges to the lesion (Ridley 1966). Skin smears are negative or weakly to moderately positive for bacilli in the active stage of the disease (Ridley 1974). Borderline leprosy presents numerous tuberculoid like symmetrical lesions, thus showing characteristics of both tuberculoid and lepromatous type of leprosy (Ridley 1966). This form is typically unstable and tends to develop into borderline tuberculoid or borderline lepromatous type leprosy (Ridley 1966). Skin smears are always moderately to strongly positive, though bacilli may not appear in large globi clusters seen with lepromatous type leprosy (Ridley 1974). Borderline lepromatous leprosy presents as numerous, moderately well defined, somewhat hypopigmented lesions that trend toward a symmetrical distribution (Ridley 1966). Various sized plaques, dome shaped lesions and nodules are all present (Ridley 1966). Skin smears are strongly positive and small globi of bacilli may be present (Ridley 1974). Lepromatous type leprosy is characterized by a low resistance and a high number of bacilli present (Ridley 1966). Indeterminant leprosy is a benign, unstable form that presents as flat, ill-defined skin lesions that may be hypo-pigmented or erythematous (Jenkins 1990). *M. leprae* bacteria are rarely

detected in the sample, and nerve involvement is absent (Jenkins 1990). Skin lesions may heal without treatment, remain without progression, or progress into tuberculoid or lepromatous type leprosy (Jenkins 1990).

The WHO defines a case of leprosy in a person with one or more of the following features: hypopigmented or reddish skin lesion(s) with a definite loss of sensation; involvement of the peripheral nerves demonstrated by skin thickening or sensation loss; or a positive skin smear for acid-fast bacilli (WHO 1998). In terms of diagnosis, the World Health Organization created a simpler system that classifies leprosy into two types based on the number of skin lesion; paucibacillary leprosy and multibacillary leprosy. Less than five skin lesions is indicative of paucibacillary leprosy while five or more lesions is indicative of multibacillary leprosy (WHO 1998). Indeterminate, tuberculoid and borderline tuberculoid leprosy typically fall under the paucibacillary leprosy classification while borderline, borderline lepromatous and lepromatous leprosy usually fall under the multibacillary leprosy classification (WHO 1998). The WHO further advised that patients showing a positive skin smear and those whose classification was in doubt should be treated as having multibacillary leprosy (WHO 1998).

Tuberculoid type or paucibacillary leprosy patients typically present with one or more light or slightly red skin patches on the trunk or extremities that exhibit a decrease or lack of touch sensation. Other symptoms include muscle weakness particularly in the extremities, skin stiffness and dryness, eye problems leading to blindness if left untreated, enlarged nerves, and loss of fingers and toes in some later stage patients.

Lepromatous type or multibacillary leprosy patients present with a symmetrical skin rash found commonly on the face, ears, wrists, elbows, knees, or buttocks. These associated skin lesions vary in terms of size, coloring and can appear flattened or raised. The bacteria spread freely through the skin and peripheral nerves but cannot spread into deeper tissues because the bacteria does not tolerate the higher temperatures. Other symptoms can include thinning of eyebrow and eyelashes, collapsing of the nose, lymphadenopathy, laryngitis, thickening of facial skin, male infertility and gynecomastia. Complications from the disease include blindness, loss of fingers or toes following injury or infection, and an increased risk for arthritis and amyloidosis.

2.6 Diagnosis

In many areas where laboratory equipment is limited or absent, this disease is diagnosed on the clinical presentation of lesions. Skin smears or biopsies are commonly done to assess the presence of acid-fast bacilli with the Ziehl-Neelsen stain (skin smear) or Fite stain (biopsy). A negative stain or one with few bacilli is classified as paucibacillary leprosy, in contrast to a sample containing numerous bacilli indicative of multibacillary leprosy (Anderson 2007). The diagnostic “gold standard” for leprosy detection is a full thickness skin biopsy sample from the most active edge of a lesion that is then stained using the Fite-Faraco method or Wade stain (Scollard 2006). Slit-smear technique is also used as a semi-quantitative measure of acid-fast organisms in infected skin lesions, but its reliability is dependent on interpretation by experienced technicians (Scollard 2006). Samples where bacilli are not detected are

classified in the paubacillary categories while samples with detectable bacilli are classified as multibacillary (Anderson 2007). Seventy percent of patients have negative smears and are classified as paubacillary leprosy (Ustianowski 2003).

The lepromin test is sometimes used to determine a patient's degree of resistance to *M. leprae*, but it is not a diagnostic test. It does however help to determine whether or not a person with leprosy has tuberculoid type or lepromatous type leprosy. People with tuberculoid type leprosy will always have a positive lepromin test.

Serologic tests such as enzyme-linked immunosorbent assays and immunoassays have been used in epidemiologic studies, but they fail to diagnose patients with moderate to high grade cellular immune responses (tuberculoid cases) because these patients do not consistently produce the specific circulating antibodies measured by these types of tests (Scollard 2006). Although they are not as useful as an initial diagnostic test, serologic tests can be useful for monitoring the usefulness of treatment, as antibody titers correlate with the bacterial index (Cho 2001).

The development of *M. leprae* specific PCR and other molecular techniques has helped to extend research into the epidemiology of the disease which has previously been stalled due to the properties of *M. leprae* as compared to other bacterium. One hundred percent specificities have been demonstrated for PCR and reverse transcription PCR based technologies for *M. leprae*, but sensitivities vary depending on the form of the disease (Scollard 2006). Multibacillary forms have sensitivities of greater than 90 percent while sensitivities for paucibacillary forms range from 34 to 80 percent (Scollard 2006). PCR is ideal for initial detection in multibacillary forms of leprosy, but it does not distinguish between dead and living bacilli, which makes it unsuitable for treatment monitoring and relapse detection (Katoch 2002).

2.7 Treatment

To date no highly effective vaccine has been developed for the disease. Multidrug therapy with multiple antibiotics is commonly used to treat the disease because monotherapy for leprosy has been shown to lead to drug resistance (Noordeen 1995). Rifampin and clofazimine are given in supervised monthly doses of 600mg and 300mg respectively for adults, 450mg and 150 mg for children 10-14, and 300mg and 100mg for children under 10 (Ishii 2003). The WHO has recommended a 12 month treatment period for multibacillary patients, though an additional 12 month treatment regimen may be needed for some patients (Ishii 2003).

Paucibacillary patients are given a dual drug combination of rifampin and dapsone in the same dosage as that given to multibacillary patients for a duration of six months (Ishii 2003). Single lesion paucibacillary leprosy is treated with a three drug combination of rifampin, ofloxacin, and minocycline (Ishii 2003). Rifampin is given at a monthly dosage of 600mg for adults and 300 mg for children 5 to 14 years of age (Ishii 2003). Ofloxacin is given at a first day dose of 300mg for adults and 150mg for children, while the dose for minocycline is 100mg for adults and 50 mg for children (Ishii 2003).

Rifampin is bactericidal against *M. leprae* and is capable of killing 99.9 percent of viable bacterium (Ishii 2003). Rifampin is typically well tolerated, but common side effects include anorexia,

vomiting, abdominal pain, diarrhea, and orange discoloration visible in tears, sweat and urine (Ishii 2003). Dapsone has bacteriostatic and weakly bactericidal effects on *M. leprae* (Ishii 2003). Side effects include anemia, hemolysis and methemoglobinemia, which is a serious consideration in glucose-6-phosphodehydrogenase deficient patients (Ishii 2003). The use of dapsone is contraindicated in pregnant and breast feeding women. Clofazimine works to inhibit *M. leprae* growth and exerts a slow bactericidal effect through preferential binding to mycobacterial DNA (Morrison 1976, Schaad-Lanyi 1987). Typical side effects include diarrhea, stomach pain, vomiting, eye irritation, itchy skin, high blood sugar, and darkening of urine, sweat and feces. Ofloxacin is a synthetic fluoroquinolone that has bactericidal effects on *M. leprae* by inhibition of a specific bacterial DNA gyrase in the bacterium (Nakashima 1992). Side effects include nausea, headache, insomnia, dizziness, diarrhea, and ear and eye discomfort. The use of this drug is contraindicated in children. Minocycline is a semisynthetic tetracycline that induces bacteriostasis in *M. leprae* by inhibiting protein synthesis (Fajardo 1995, Ishii 2003,). Typical side effects include nausea, fever, diarrhea, vomiting, anorexia, skin photosensitivity, itchy skin, fatigue, and dizziness.

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Chapter 3: Introduction to Schistosomiasis in Brazil

3.1 History and Geographic Distribution

Schistosomiasis was first observed and characterized by Theodore Bilharz in 1851 Cairo, and today remains as one of the most serious parasitic diseases along with Malaria. There are six species of *Schistosoma* known to cause disease in man, but only *Schistosoma mansoni* exists in the Americas (Amaral 2006). *S. mansoni* is the most widespread schistosome parasite and can be found not only in the Americas, but also throughout Africa and the Arabian Peninsula (Assis 1998) (Figure 2).

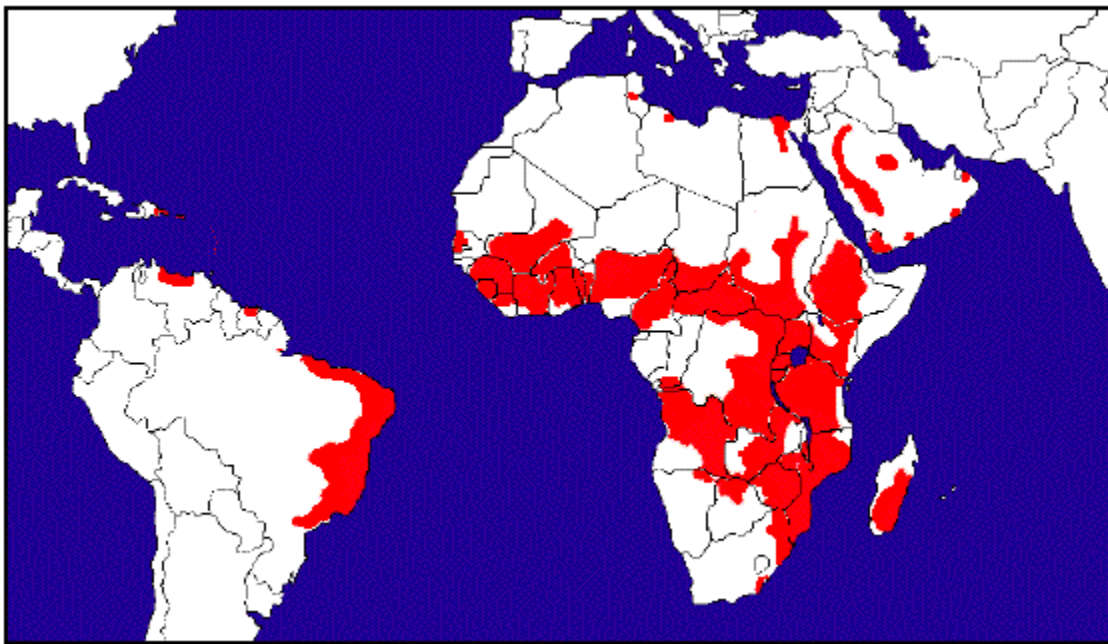


Figure 2: Geographic distribution of *Schistosoma mansoni* Courtesy of University of Cambridge Department of Pathology Accessed from: http://www.path.cam.ac.uk/~schisto/schistosoma/schisto_distribution.html

Piraja da Silva first reported the presence of *S. mansoni* in the northeastern state of Bahia, Brazil in 1902 (Doumenge 1987). The northeast region of Brazil has historically been endemic for *S. mansoni* infection, and a 1940s study by Meira found high levels of the disease in the states of Alagoas, Bahia, Ceara, Pernambuco, Rio Grande do Norte and Sergipe(1949).

In the Southeast region, the states of Minas Gerais and Espirito Santo have historically been highly endemic for the disease (Meira 1949). In Minas Gerais alone, 519 of 853 municipalities are prevalent for the disease (Drummond 1994) with these areas primarily in the northern, eastern and central regions of the state (Pellon 1950, Lambertucci 1987, Guimaraes 2008, Martins-Bede 2010). The

northwestern and southern parts of the state are non-endemic for the disease (Martins-Bede 2010). Transmission in Rio de Janeiro and Sao Paulo is associated with sugarcane, rice and watercress irrigated cultivation areas (Schall 1985, Baptista 1993, Filho 2010). The Southern region of Brazil is considered non-endemic, but areas of low endemicity have been observed in Rio Grande do Sul (Graeff-Teixeira 2004), Santa Catarina (Coura 2004, Borda 2010), and Parana (Borda 2010).

In the Northern region, Para is the only state where schistosomiasis transmission is known to occur. Cases of schistosomiasis do occur in the state of Rondonia, but these are related to migration to the area and not due to areas of transmission as none of the three snail vector species are found there (Brillet 2000, Coura 2004). Other species of *Biomphalaria* snails do occur there, including *B. amazonica* which has been found to be experimentally susceptible to *S. mansoni* (Paraense 1985). In the Central West region, only Brasilia in the Federal District, and Goiana in the state of Goias have a high number of schistomiasis cases, but like Rondonia disease is thought to be related to migration to the area and not actual disease transmission (Brillet 2000).

3.2 Epidemiology

An estimated 200 million people, in 76 countries are infected with pathogenic schistosomes (WHO 2002). In Brazil, control programs have helped to decrease the disease from an estimated 10-12 million people in the 1970s (WHO 1983), to an estimated 2.5 million (Passos 1998) to 6.3 million (Katz 2000) through the activities of the national schistosomiasis control program. This program, implemented in 1975, built up sanitation infrastructure, promoted health education, implemented improved large scale case detection and mass chemotherapy programs, and implemented snail control measures (WHO 1983, WHO 2000).

Schistosomiasis disproportionately affects the impoverished and is related to areas lacking basic health services and health education. People acquire the infection through water-based activities including personal hygiene, laundry, fishing, and recreation (Coura-Filho 1994, Silva 1997, Lima e Costa 1998, Bethony 2004). Other studies have associated poor quality housing (Lima e Costa 1991), absence of piped water (Lima e Costa 1987, Coura-Filho 1996, Gazzinelli 1998, Gazzinelli 2001), and lower levels of education (Bethony 2001, Gazzinelli 2001) with schistosomiasis infection. The lifecycle of the parasite is maintained in freshwater bodies through indiscriminate human defecation in these water sources. Measures to improve sanitation and create a safe water supply, in conjunction with treatment of infected individuals, have been shown to reduce *S. mansoni* transmission and to prevent new infections (Kloetzel 1987, Coura 1995, Katz 1998, Kloos 1998, Ximenes 2003, Gazzinelli 2006, Enk 2010). Control of the disease in Brazil is directed towards treatment for infected individuals and eradication of vector snail hosts, *B. glabrata*, *B. straminea*, and *B. tenagophila*. Health education programs and increased sanitation measures have been promoted in the country, but implementation has been variable (Schall 1995). Some health education programs have also been ineffective because they have relied on pamphlets to provide health information in areas where most adults are illiterate and thus cannot

benefit from them (Schall 2001). Additionally the information in these pamphlets is somewhat inaccurate and unclear to the population they are attempting to help (Uchoa 2000, Schall 2001).

Children and adolescents are particularly important in the epidemiology of the disease, as they tend to have higher prevalence rates, higher rates of *S. mansoni* egg elimination, and higher rates of resistance to treatment when compared to adults in this region (Katz 1978, Firmo 1996). This is related to water related leisure activities and indiscriminate defecation habits more commonly associated with this age group than in adults (Schall 1995). Some studies have also found that males have a higher risk for infection than females, but this is thought to be more related to female activities and occupations that keep them away from contaminated water sources than due gender differences alone in relation to susceptibility to infection (Coutinho 1997, Kloos 1998, Enk 2010)

Although schistosomiasis has traditionally been a disease of poor rural areas, increased urbanization is leading to an increasing number of urban cases, some even in upscale neighborhoods (Barbosa 2004, Araujo 2007, Kloos 2008, Filho 2010, Igreja 2010). Rapid urbanization leads to increased settlement of peripheral areas typically lacking in basic sanitation and infrastructure, which can establish new transmission sites (Barbosa 2004, 2010, Filho 2010, Igreja 2010).

3.3 GIS Mapping and Modeling

Municipal level spatial studies for Schistosomiasis have been conducted in Pernambuco (Paredes 2010), Sergipe (Rollemberg), and Minas Gerais (Gazzinelli 1998, Bethony 2001) that have analyzed the relationship of the disease to socioeconomical factors such as lack of sanitation, water usage and lack of improved housing. State wide studies have been conducted by Bavia (2001) in the state of Bahia, and in Minas Gerais (Guimaraes 2006, Guimaraes 2008). Both of these states have the largest prevalence to schistosomiasis, and the focus of all of these studies was the evaluation and improvement of GIS in order to better estimate and forecast schistosomiasis risk.

3.4 Life Cycle

Schistosomiasis is a complex waterborne disease caused by digenetic blood trematodes. Adult schistosomes live in male and female pairs in the superior mesenteric veins draining to the large intestine. Females deposit eggs in of the small venules of the portal, which migrate toward the lumen of the intestine where they are excreted with feces. If the feces is excreted into a suitable water source, the eggs hatch and release miracidia, which swim and penetrate specific snail intermediate hosts (*B. glabrata*, *B. straminea*, and *B. tenagophila* in Brazil). Within the snail intermediate host, the parasite undergoes asexual reproduction producing cercariae, which are eventually released from the snail. These cercariae swim through the water, and upon locating a human host, penetrate the skin, shedding their forked tail to become a schistosomulae. The schistosomulae migrate through the host tissues entering the lymphatics and venules and eventually the lungs. From the lungs, it enters the circulatory

system through the left side of the heart to the hepatportal circulation and finds a mate. The two worms develop into sexually mature adults and migrate together to the mesenteric veins. (Figure 2)

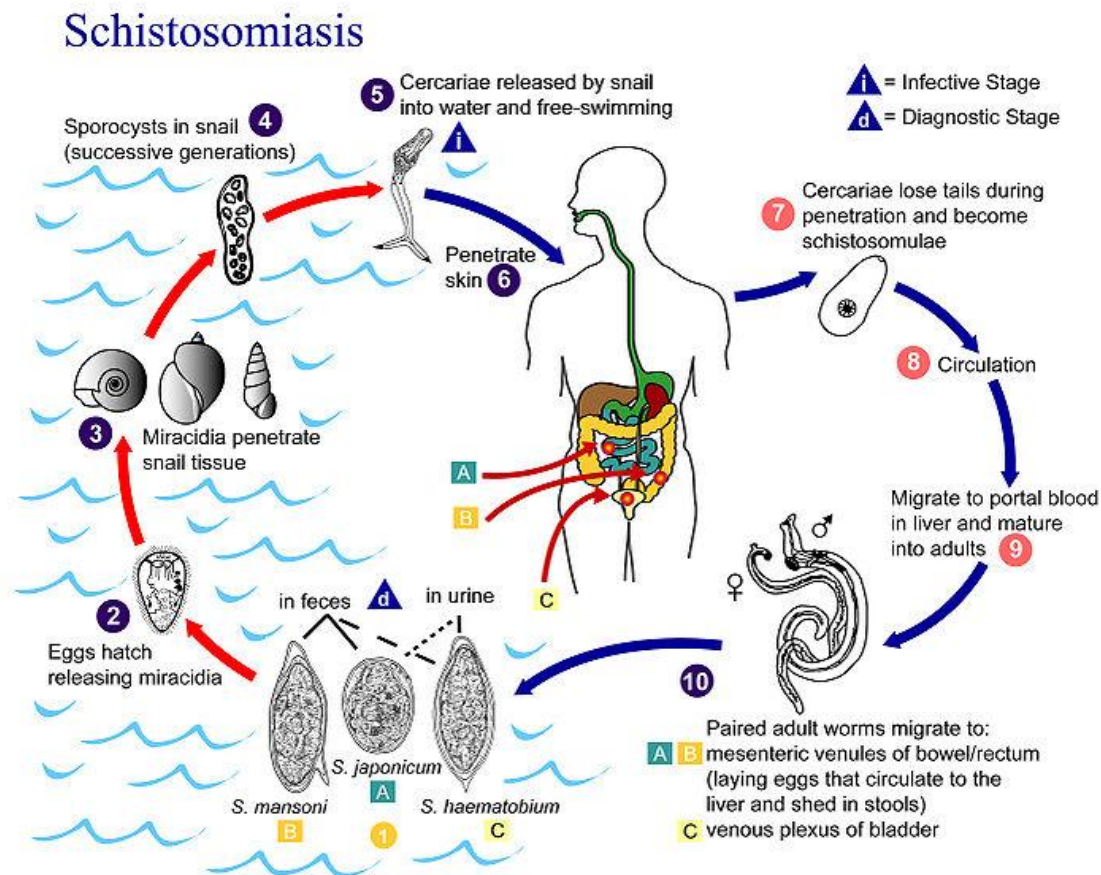


Figure3: Schistosomiasis life cycle Courtesy of the Centers for Disease Control (CDC). Accessed from <http://www.dpd.cdc.gov/dpdx/HTML/Schistosomiasis.htm>

3.5 Host and Reservoirs

Humans are considered the main hosts of *Schistosoma mansoni*, though studies have indicated that some primate and rodent species are found to be naturally infected with the disease. In Brazil, the “water rat” *Nectomys squamipes* and the “marsh rat” *Holochilus brasiliensis* are considered to be important non-human hosts for the parasite because of their high susceptibility to infection and lifelong shedding of viable parasite eggs (Picot 1992, Rodrigues-Silva 1992, Souza 1992, Gentile 2006). Their relationship to the human disease is currently being evaluated. Recently Modena discussed the reservoir potential of cattle in the maintenance of *Schistosoma mansoni* in Brazil due to their high susceptibility to experimental infection and high environmental contamination potential (2008).

3.6 Vectors

There are three snail vectors for *Schistosoma mansoni* in Brazil, *B. straminea*, *B. glabrata*, and *B. tenagophila* (Carvalho 1992, Doumenge 1987). Of these species, *B. glabrata* is the most important because of its large distribution and its efficiency in disease transmission (Carvalho 1992). Paraense found that the species distribution is from the southeast of Bahia, the eastern half of Minas Gerais and Espirito Santo and throughout the coastal areas of the states of Rio Grande do Norte, Paraiba, Pernambuco, Alagoas, and Sergipe (1986). Additionally it can be found in the states of Para, Maranhao, Bahia, Goias, Rio de Janeiro, Sao Paulo and Parana (Paraense 1986). *S. mansoni*'s development in *B. glabrata* is limited by water temperature from 16°C to 32°C (Plorin 1983).

B. tenagophila typically is found in Brazil with low rates of infection, but is the primary snail vector for schistosomiasis in the southern and southeastern regions: Vale Paraiba (Correa 1956) and Peruibe on the Sao Paulo coast (Bernardini 1981), Sao Francisco do Sul in Santa Catarina (Bernardini 1981), Grande Vitoria in Espirito Santo (Pereira 1972) and in Rio de Janeiro (Deane 1953). It is found throughout coastal area from southern Bahia to Rio Grande do Sul and in the interior of Sao Paulo (Paraense 1986). Isolated populations of this species occur in Minas Gerais and the Distrito Federal (Paraense 1986).

B. straminea has the widest distribution of the three vector snail species and can be found in almost all water basins in the country but is most dense in the northeast, south of Bahia and the northeast of Minas Gerais (Paraense 1986). In nature, this species has low levels of infection and is less important for transmission in areas where its distribution overlaps with *B. glabrata* (Carvalho 1992). Its main importance in schistosoma transmission occurs towards the interior of the country (Lucena 1950) and has been implicated in disease transmission in Fordlandia in the state of Para (Machado 1951) and Goiania in the state of Goias (Cunha Neto 1967).

General features of snail vector habitat include shallow water with moderate light penetration, low turbidity, an organic matter mud substrate and submerged or partially submerged aquatic vegetation (Bavia 1999). Long periods of drought restrict snail populations in habitats at lower elevations, and heavy seasonal rains can eliminate snails from waterways (Jordan 1982a, Kvale 1981, Richards 1967). In short droughts, snails can escape the drying conditions through burrowing into the organic substratum, aestivating until the onset of the rainy season when they can quickly repopulate the habitat (Bavia 1999).

3.7 Control of Intermediate Hosts

Intermediate host control falls into three categories: chemical, biological and environmental. Chemical methods involve the use of chemical molluscicides to kill snail hosts. Biological methods include vector snail displacement by biologically similar snails, and the use to natural enemies to limit vector snail populations. Environmental modifications can reduce snail populations and habitats by making the area no longer compatible for snail survival.

Niclosamide (Bayluscide) is the only molluscicide currently recommended by the World Health Organization for use in the control of intermediate hosts of *S. mansoni*, and functions by uncoupling oxidative phosphorylation at the mitochondrial level within the snail host (Andrews 1983). This action disrupts the respiratory function within the snails leading to mortality. In addition to snail mortality, the compound is also toxic to fish, amphibians, and crustaceans which makes it less ideal for disease control in large areas (Parashar 1990, Sukumaran 2004). Nicotinanilide is a related compound that also has molluscicidal and cercaricidal properties, but has less detrimental impacts on non-target aquatic species (Tang 1986, Parashar 1990). In a study by Sukumaran, nicotinanilide required a higher dose and longer time period to lymnaeid snails when compared to Niclosamide (2004). In addition to chemical molluscicides, some plants have similar snail toxic properties. *Phytolacca dodecandra* and *Jatropha curcas* are the most promising, but their effects are not snail specific and long-term toxicological studies still need to be conducted (WHO 1985, WHO 1993). Due to the expense and the toxicity to non-target species, the use of molluscicides is most useful in relatively arid areas where foci of transmission are relatively small and seasonal (Sukumaran 2004).

Biomphalaria snail populations can be reduced through competition with other species especially those of the *Marisa* and *Helisoma* genera (Jobin 1977, Jobin 1979, WHO 1985, Pointier 2004). *Marisa cornuarietis* preys on eggs and juveniles of *Biomphalaria* (Demian 1965) in addition to competing for food in Puerto Rico (WHO 1982) and Guadeloupe (Pointier 2004). *Thiara granifera* has also been shown to outcompete *Biomphalaria* species in Saint Lucia (WHO 1993). Predatory fish such as Tilapia, can also be used to reduce snail populations, but will not completely eliminate them (WHO 1993).

Irrigation schemes utilizing overhead sprinklers and trickle irrigation reduce the number of open canals and drains and thus reduce human contact with water (WHO 1993). Additionally these systems improve water management through reduction in water usage and waste through evaporation (WHO 1993). Periodic removal of canal vegetation and periodic canal drainage are also methods to environmental control that have been shown to reduce vector snail populations (WHO 1993, Boelee 2004).

3.8 Pathogenesis

Acute schistosomiasis is typically asymptomatic, but can include nausea, headache, fever, cough, abdominal pain, and rarely diarrhea and is associated with egg laying by the female parasite (Elliott 1996). Common pathologies associated with chronic infection include hepatosplenomegaly, portal hypertension, and esophageal bleeding (Assis 1998). These effects are the result of the host's immune response to the accumulation of eggs lodging within the tissues over months and years of infection (Elliott 1996, Assis 1998). Granulomas form around the trapped eggs in the intestinal wall or liver, leading to fibrosis and splenic hypertension (Elliott 1996). Granuloma formation is initiated through antigens secreted through the microscopic pores within the shell of the egg, and function to trap and isolate these hepatotoxic components (Elliott 1996). Thus the severity of the disease typically

worsens as the duration of infection increases (Assis 1998). Hepatosplenic schistosomiasis or ascites is common in advanced cases as fluid accumulates in the peritoneal cavity (Elliott 1996). This condition can also occur as soon as 18 months in individuals with large parasite loads (Elliott 1996).

Most human infections are light to moderate intensity and disease effects are thought to be minimal or not adequately defined (Assis 1998). Severe morbidity has been correlated with the intensity of infection in adults (Lehman 1976) and school age children (Barreto 1984). Additionally several studies have indicated that *S. mansoni* infection contributes to nutritional deficiencies and stunted growth in adults (Mikhal 1982) and children (Assis 1998, Corbett 1992, de Lima e Costa 1988, Parraga 1996). Treatment can reduce the affect of malnutrition and subsequent adolescent stunting due to schistosomiasis infection, even when *S. mansoni* infections are of light to moderate intensity (Parraga 1996, Assis 1998).

3.9 Diagnosis

Diagnosis is typically made by the detection of *S. mansoni* eggs in stool samples using the thick-smear Kato-Katz method. This method can yield qualitative results 30 minutes after slide preparation. *S. mansoni* eggs are 140µm by 60µm and have a characteristic lateral spine that differentiates them from other schistosome species. Several authors have questioned the sensitivity of the Kato-Katz method in areas with low to moderate levels of infection, but the sensitivity was found to be improved by testing multiple stool specimens from an individual (Barretto 1990, Rabello 1992, Firmo 1996, Goncalves 2006, Enk 2008). Liver and spleen enlargement is known to be correlated with infection intensity, and egg count categories are defined as light (24-96 eggs per gram of feces), moderate (120 – 792 eggs per gram of feces) and heavy (> 816 eggs per gram of feces) (WHO 1985). Liver and spleen enlargement is almost always seen in infections characterized as heavy and frequently seen in those classified as moderate (WHO 1985).

Serologic tests such including ELISA, are more sensitive testing methods that can be valuable in detecting light infections where eggs are not routinely shed (WHO 1993). While these tests are useful in the initial detection of infected individuals, they cannot detect between past and current infections and are not useful in follow up testing of individuals successfully treated in the past (WHO 1993).

3.10 Treatment

Praziquantel is a heterocyclic pyrazino-isoquinoline derivative that causes tegument changes (Redman 1996) and a reduction in the glutathione concentration in the parasite (Ribeiro 1998). The standard dosage is a three day dose of 60 mg/kg for children and 50 mg/kg for adults (Coutinho 1984). Praziquantel is the preferred drug to treat schistosomiasis and is highly effective against larval and adult worms but has less efficacy against juvenile stages (2-4 week old parasites) (Shaw 1990). A study by

Ferrari, found that Praziquantel was more effective than oxamniquine in curing the disease (2003). The drug is well tolerated, but reported side effects include abdominal pain, diarrhea, dizziness and sleepiness (WHO 1985).

Oxamniquine, the only other current treatment for the *S. mansoni*, is a 2-aminomethyltetrahydroquinoline derivative, inhibits nucleic acid synthesis in the parasite (Pica-Mattoccia 1989). The compound is effective against early stages of *S. mansoni* as well as the adult male schistosomes, but adult females are less susceptible (WHO 1985). A single oral dose of 15 mg/kg for adults and two daily doses of 10mg/kg for children, is routinely used and has been demonstrated to effectively reduce egg shedding (Silva 1975, Katz 1976). The drug is well tolerated, with dizziness, drowsiness and headaches being the most frequently observed side effects (WHO, 1985). Rarely reported side effects include hallucinations, psychic excitement, and convulsions (WHO, 1985)

3.11 References

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Chapter 4: Introduction to Chagas Disease in Bolivia and Brazil

4.1 History and Geographic Distribution

Chagas disease was first discovered by Brazilian physician Carlos Chagas in 1909. He was able to recognize the link between the flagellated protozoan parasite in the triatomine insect and the flagellated protozoan parasite in the blood of a child. He incorrectly believed that the infection was transmitted through the bite of the insect. Later work by Emile Brumpt, Silveira Dias and Cardoso showed that parasite was not transmitted through the bite of the insect but through the fecal material. It has also been theorized by many that Charles Darwin may have been a victim of the disease. Darwin reported in his diaries, encounters with triatomine like insects and experienced Chagasic like symptoms later in life. The disease is endemic throughout Central and South America, but migration from these areas has spread the disease to sizable populations of infected individuals in several nonendemic countries such as Spain and (Figure 4).

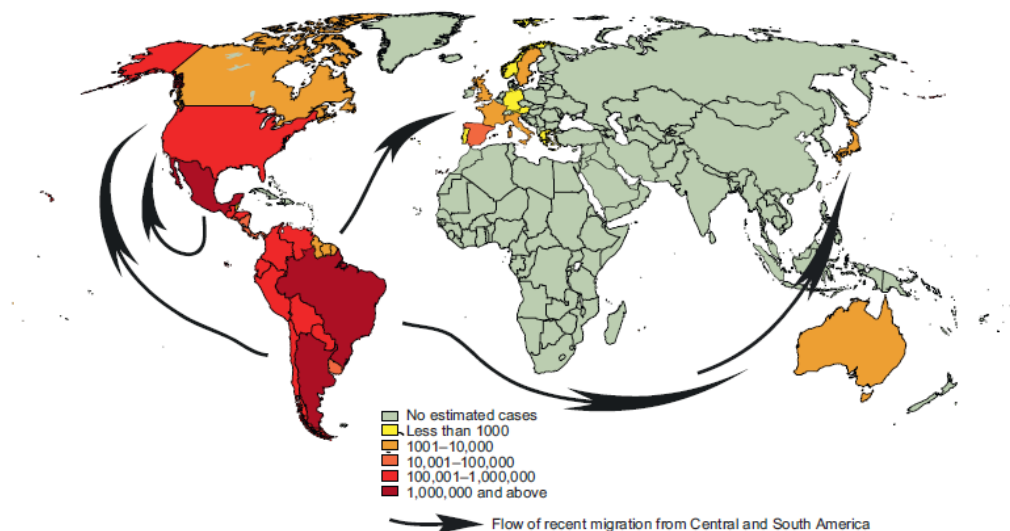


Figure 4: Geographic distribution of Chagas disease. Current estimated global population infected by *Trypanosoma cruzi*. Schmunis (2007).

4.1.1 Chagas in Bolivia

Bolivia has the highest prevalence of Chagas disease in the Americas (Guillen 2002 Araujo-Jorge 2009), and in some endemic towns, seroprevalence rates approach 100% in older adults (Arata 1994). Vectorial transmission, infection through blood transfusion, and congenital transmission are all important modes of transmission for the disease in Bolivia.

Control activities in Bolivia started in the mid 1980s, but have not been completely successful due to a number of factors including sylvatic vector populations, vector resistance to insecticides, and inconsistent monitoring and control activities (Guillen 1997). *Triatoma infestans*, the main vector in this country, has been found in seven of the nine departments in Bolivia; Beni Chuquisaca, Cochabamba, La Paz Potosi, Santa Cruz, Tarija (Albarracin-Veizaga 1999, WHO 2002). Vectorial transmission in this country is found at altitudes between 300 and 3,500 meters above sea level (Zuna 1985). Domestic animals have been implicated in the peridomestic and domestic transmission cycle in this country due to the close proximity of these animals to human dwellings particularly in rural areas (Pizarro 2007). Cows and guinea pigs in this area are kept in close proximity to homes and dogs typically sleep close to human bedrooms (Pizarro 2007). Pizarro found that all three of these animals play an important epidemiologic role in disease transmission in this country (2007).

A 1985 blood bank study in a city in Santa Cruz found an infection incidence rate of 47.6% (Zuna 1985) A systematic survey of blood banks a few years later found seropositive rates of 35% in Chuquisaca, 28% in Cochabamba, 4.9% in La Paz, 6.0% in Oruro, 24% in Potosi, 51% Santa Cruz, and 45% in Tarija, and a country wide rate of 25% (Carrasco 1990). From 2002 through 2006, Doctors without Borders began a mass treatment program in the cities of Entre Rios (Tarija department) and Sucre (Chuquisaca department) (Yun 2009). Children under 15 in Entre Rios and children under 18 years of age in Sucre, were tested and treated if seropositive (Yun 2009). Seroprevalence rates for these areas were 19.4% and 5.9% respectively (Yun 2009). Congenital transmission in this region varies from 4% up to 9.5% in Bolivia (Azogue 1985, Freilij 1994, Torrico 2004, Brutus 2007, Brutus 2008) and infections can be asymptomatic or present with hepatosplenomegaly and low birth weight (Torrico 2004).

4.1.2 Chagas in Brazil

Chagas disease is rarely reported in the Amazon region of the country and is typically related to food borne related outbreaks associated with the consumption of assai palm juice contaminated with infected triatomine bugs (Shikanai-Yasuda 1991, Coura 1994, Valente 1997, Beltrao 2009). Most of the vectors in this area are in the *Rhodnius* (*R. brethesi*, *R. nasutus*, *R. neglectus*, *R. paraensis*, *R. pictipes*, *R. prolixus*, and *R. robustus*) which are associated with palm trees in the area (Coura 2002, Briceno-leon 2007). *Panstrongylus geniculatus* and *P. lignarius* are also present in the area in small quantities.

The disease has historically been worst in the northeastern region of Brazil. Overall prevalence decreased from 3.05% in a survey from 1978 to 1980, to 0.08% from 1997 to 1998 (Dias 2000). The northeastern states of Bahia (7.4%), Alagoas (5.1%), Sergipe (4.8%) and Piaui (3.7%) had the highest

prevalence rates in the initial 1978 survey (Dias 2000). In the follow up survey the states of states of Rio Grande do Norte (0.2%), Sergipe (0.19%), and Paraiba (0.16%) had the highest prevalence rates, though all the states in the northeastern region showed significant reductions in overall prevalence (Dias 2000).

Chagas associated mega syndromes are predominantly reported in the states of Bahia, Goias, Minas Gerais, and Sao Paulo (WHO 2002). Blood bank prevalence in Brazil decreased from 7.0% in 1980 to 0.73% in 1998 (WHO 2002).

4.2 Epidemiology

The majority of disease cases are related to contact with the triatomine vector. The insect draws a bloodmeal from a sleeping host, typically on the face or other exposed areas of the body such as the arms, and during the bloodmeal defecates. The sleeping host then will inadvertently scratch the bite site introducing the parasite into the open wound or contaminate their hands from scratching and then rub their eyes introducing the parasite into the conjunctiva. This is also known as stercorarian transmission. When the parasite is introduced into the eye, it can produce a unilateral edema of the eyelid called Romaña's sign.

Blood transfusion, organ transplantation and congenital transmission are additional methods of human transmission that are especially important in endemic countries. The risk of acquiring infection from blood transfusion increases with the number of transfusion received and is typically asymptomatic. Clinical manifestations appear days to several weeks after transfusion, but most cases are thought to be asymptomatic (WHO 2002). The most frequent clinical signs are fever, splenomegaly and polylymphadenopathy (WHO 2002). Transmission through organ transplantation carries a more immediate serious effect because the immunosuppression of the patient leads to severe cases of myocarditis, meningoencephalitis and ultimately death if not diagnosed quickly (WHO 2002). This type of transmission has increasingly become a concern in areas non endemic for the disease due to immigration of Chagas infected individuals (Diaz 2007, Fores 2007).

Orally acquired Chagas can potentially occur through the ingestion of infected mother's milk (Mazza 1936, Ferreira 2001), the ingestion of food or drinks contaminated with infected triatomines and/or their feces, and through the consumption of food or drinks contaminated with infected anal gland secretions from some marsupials (Deane 1984, Janson 1999). Oral transmission is of increasing concern and importance in the Brazilian Amazon region where triatomine vectors are typically sylvatic. Oral infection results in worsened Chagas symptoms and acute death in several cases related to the increased parasite load and possibly greater parasite infectivity through the oral route (Pereira 2009). Some recent studies have indicated the increased pathogenicity associated with this mode of infection is related to the effect of gastric juices on the infective potential of the parasite (Holt 1996, Cortez 2006). The first oral outbreak occurred in Brazil in 1965 in Estrela, Rio Grande de Sul and was associated with consumption of sugar cane juice (Shikanai-Yasuda 1991, Totto 2007). Oral outbreaks of acute Chagas disease typically occur in the northern states of Amapa, Amazonas, and Para, the northeastern state of

Paraíba, and the southern states of Rio Grande de Sul and Santa Catarina (Pereira 2009). These outbreaks have been linked to Acai consumption in the states of Amapa and Para (Pinto 2003, Nobrega 2009), sugar cane juice consumption in Bahia and Santa Catarina (SVS 2007), and bacaba consumption in Para (SVS 2007).

4.3 GIS mapping and modeling

There are numerous mapping and modeling studies related to Chagas disease vectors in Argentina (Cecere 2004, Vazquez-Prokopec 2005, Kitron 2006), Mexico (Peterson 2002, Lopez-Cardenas 2005, Cruz-Reyes 2006, and the United States, but there is a lack of such studies in both Bolivia and Brazil. Costa did however use ecological niche modeling to characterize different populations of *Triatoma brasiliensis*, an important vector in northeastern Brazil. This study was able to evaluate the distribution of separate subspecies of this vector which is important because not all of the subspecies of *T. brasiliensis* demonstrate colonization behavior (Costa 1999). Similar studies in Bolivia on distinct populations of *T. infestans* are warranted because like the situation with *T. brasiliensis*, not all populations of *T. infestans* currently demonstrate colonization behavior (Noireau 2009).

4.4 Life Cycle

Triatomine vectors become infected from ingesting trypomastigotes circulating in the blood of infected mammalian hosts. Once inside the insect vector, the tryomastigotes travel to the insect's midgut and transform into epimastigotes and then travel to the insect's intestines. These epimastigotes will multiply by binary fission and travel to the hindgut of the insect where they will transform into infective metacyclic trypomastigotes. At this stage, the parasite will be excreted by the parasite and is infective to mammalian hosts.

Once inside a mammalian host, the parasite travels through the blood stream and enters muscle and neuron tissue where the flagellum is lost and the parasite transforms into an amastigote. During this life stage, the parasite undergoes multiple rounds of asexual reproduction and will sometimes transform into trypomastigotes that circulate in the blood stream to in hopes of getting picked up by the insect vector.

4.5 Hosts and Reservoirs

T. cruzi has been found in over 180 species of both domestic and wild mammalian species belonging to seven orders and 25 families (WHO 2002). Common reservoir hosts in LAC include armadillos, marsupials, sloths, capybaras, monkeys, porcupines and opossums (Peters 1997.) Opossums are of particular importance to the disease cycle as they carry the infective metacyclic trypomastigote stage within the anal scent gland (Deane 1984, Jansen 1999). Many mammals acquire the infection

through the ingestion of infected triatomine vectors. In Bolivia, wild rodents play an important role in the maintenance of the disease in the sylvatic cycle (Cortez 2006b). Guinea-pigs in both Bolivia and Peru are frequently found with high infection rates and are frequently reared inside dwellings or close to dwellings (WHO 2002). Domestic canines and cats are of epidemiological importance to the disease because they serve as sentinels for vector-mediated transmission and have been found to have high seroprevalence rates in some endemic areas in LAC (Mott 1989, Castanera 1998, Crisante 2006, Gurtler 2006, Pizarro 2007). Infection has also been demonstrated in a wide variety of other domestic animals including goats, sheep, alpacas, and swine though they are not as important to the domestic transmission cycle (WHO 2002).

Trypanosomiasis, American (Chagas disease)

(*Trypanosoma cruzi*)

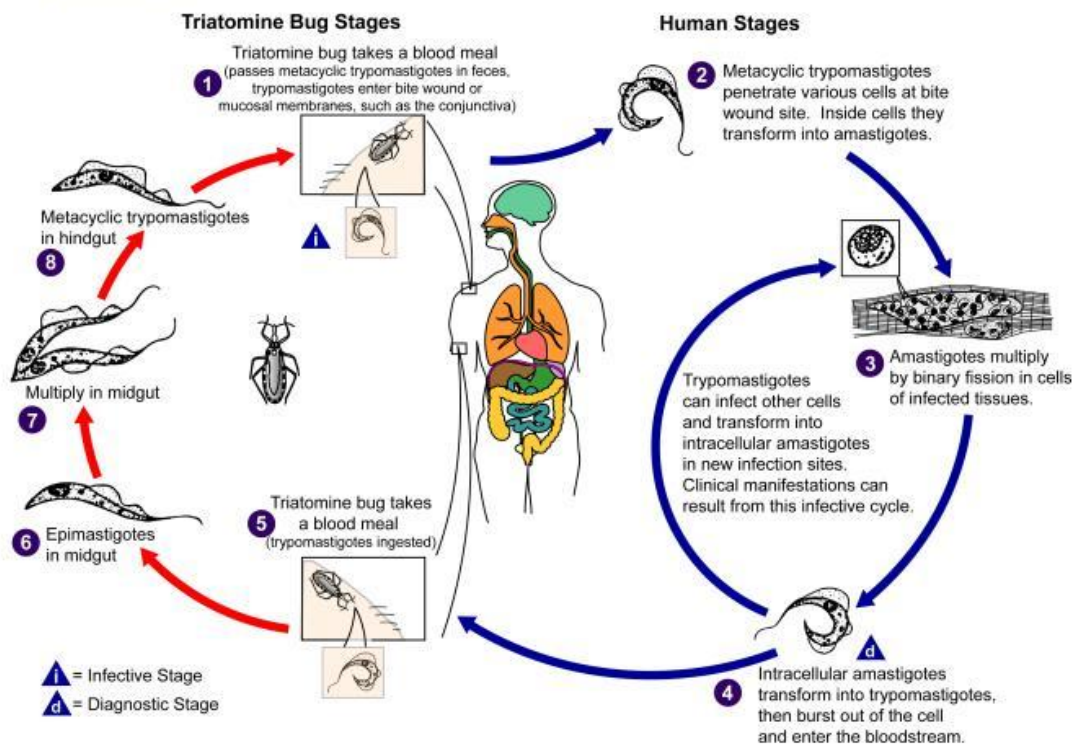


Figure 5: *Trypanosoma cruzi* life cycle. Courtesy of the Centers for Disease Control (CDC)
 Accessed from <http://www.dpd.cdc.gov/dpdx/HTML/Schistosomiasis.htm>

4.6 Vectors

T. cruzi vectors are insects in the order Hemiptera, family Reduviidae and subfamily Triatominae. All members of this subfamily are hematophagous and require vertebrate bloodmeals to complete their life cycle (Lent 1979). There are currently over 130 species of triatominae known belonging to 6 tribes; Alperporseniini, Bolboderini, Cavernicolini, Linshcosteusini (all species in this family occur in India) Rhodniini, and Triatomini (Lent 1979). *Triatoma infestans*, *Panstrongylus megistus*, *Triatoma brasiliensis*, *Triatoma sordida*, and *Triatoma pseudomaculata* are the most important vectors associated with human disease in Brazil and Bolivia (Villela 2005).

Historically, *Triatoma infestans* has been the most important vector of Chagas disease in the Americas due to its high degree of adaptation to human domiciles (Cortez 2010). Through population genetic analysis, it is believed that this species originated from Bolivia highlands and migrated outward to Peru, Chile, Paraguay, Argentina, Brazil, and Uruguay (Cortez 2010). Before vector control program implementation in Brazil, the species was found in 15 of 27 states; Alagoas, Bahia, Goias, Mato Grosso, Mato Grosso do Sul, Minas Gerais, Paraiba, Parana, Pernambuco, Piaui, Rio de Janeiro, Rio Grande do Sul, Sao Paulo, Sergipe, and Tocantins (Vinhaes 2000). Due to the Southern Cone initiative and subsequent vector control programs starting in 1975, Brazil was declared vector free in 2006 (Araujo 2009). In Bolivia this vector was found in every state except Oruro and Pando (Albarracin-Veizaga 1999). This vector is extremely important in Bolivia due to populations of domestic, peridomestic, and sylvatic populations (Noireau 2009) and evidence of insecticide resistance in some populations in this area (Orihuela 2008, Toloza 2008).

Panstrongylus megistus is considered to be very epidemiologically important in Brazil due to its broad distribution, susceptibility to *T. cruzi* infection, food source generality and its capacity for domesticity (Barbosa 2006). This species is widespread throughout Brazil and found in 20 of 27 states; Alagoas, Bahia, Ceara, Espirito Santo, Goias, Maranhao, Mato Grosso, Mato Grosso do Sul, Minas Gerais, Para, Paraiba, Parana, Pernambuco, Piaui, Rio de Janeiro, Rio Grande do Norte, Rio Grande do Sul, Santa Catarina, Sao Paulo, and Sergipe (Dias 2000, Guilherme 2001, WHO 2002, Barbosa 2006). This species occurs in humid climates, and during the rainy months, when competition for food is high, this species is more likely to search for new habitats and colonize new dwellings (WHO 2002). This species predominantly lives in sparse woods and gallery forests but in some areas of Brazil it is the only species found in houses (WHO 2002).

Triatoma brasiliensis is found throughout the Northeast region in Alagoas, Bahia, Ceara, Maranhao, Paraiba, Piaui, Rio Grande do Norte, and Sergipe as well as the northern part of Minas Gerais and the state of Tocantins (Guarneri 2000, Panzera 2000, WHO 2002). A 1997 study found that this was the predominant species captured in the states of Ceara, Paraiba, Piaui and Rio Grande do Norte (Dias 2000). Similar to *P. megistus*, this species migrates during the rainy season and is more likely to colonize human habitats during this period. This species is commonly found in rocky environments associated with rodents, but has increasingly colonized houses even in areas previously sprayed to control triatomines (Silveira 2000, WHO 2002, Costa 2003).

Triatoma pseudomaculata typically lives under bark, in the hollows of dead trees or in birds nest and is thought to be introduced through passive transport in firewood (WHO 2002). A 1997 study found that this was the predominant species captured in the state of Alagoas, Pernambuco, and Sergipe, but it tends to favor peridomestic environments and is poorly adapted for homes (Dias 2000, WHO 2002).

Triatoma sordida is found predominantly under the bark of dead trees and is considered mainly ornithophilic (WHO 2002). It is highly adaptive to unstable environments due to the sporadic availability of feeding sources within its ecotope, which leads it increasingly into peridomestic environments (WHO 2002). A 1997 study found that this was the predominant species captured in that state of Bahia from 1992 to 1997 (Dias 2000). Unlike other triatomine species, *T. sordida* tend to have low levels of infection with *T. cruzi* and are unable to form large domiciliary colonies (Diotaiuti 1995, Herrera 2003).

Sylvatic vectors are typically found associated with rodent burrows, hollow trees, palm trees, and rocky outcroppings. In impoverished and rural parts of the Americas, adobe, mud, wood and palm associated housing materials provide attractive alternatives for these insects providing both shelter and bloodmeal sources (Albarracin-Veizaga 1999, Dias 1999). Deforestation also helps to push triatomine insects away from their native habitat and towards human dwellings increasing the likelihood of a shift towards peridomesticity and domesticity in these species.

4.7 Control

The organochlorides dieldrin and gamma-BHC (BHC) were the first synthetic insecticides found to be useful in controlling Chagas vector populations (Romana 1948, Dias 1948). New synthetic pyrethroid insecticides became available in the 1980s and outperformed BHC and dieldrin at lower doses and for a longer period of time (Dias 2002). Additionally these new insecticides were more cost effective and did not have the negative property effects associated with the bioaccumulation of organochloride agents (unpleasant smell, property markings) (Dias 2002).

At the time that Brazil implemented a campaign against Chagas disease in 1975, 711 municipalities had dwellings infested with *T. infestans* (Moncayo 2009). In 1986 the focus shifted away from this disease and towards reemerging yellow fever and dengue outbreaks which affected urban areas as well as rural areas in contrast to Chagas disease (Dias 2002). In 1990, Chagas disease came back to the forefront as a problem not just for Brazil but throughout South America. This resulted in the formation of the Southern Cone Initiative, an agreement between the governments of Argentina, Bolivia, Brazil, Chile, Paraguay, Uruguay, and later Peru to control the disease through targeted elimination of the primary vector *Triatoma infestans* (Dias 2002). This seemed achievable as *Triatoma infestans* was thought to be primarily domestic with the exception of isolated populations in Bolivia that appeared to be isolated and strictly sylvatic (Dujardin 1998). This initiative resulted in a marked decline in childhood infection rates in those born after the initiative, as well as a large scale reduction in the range of *T. infestans* and by 1993 only 83 infested municipalities remained (Moncayo). Uruguay and

Chile were declared free of human Chagas transmission in 1997, and 1999 respectively, and Brazil followed in 2006 (Araujo 2009).

Biological control agents are also gaining attention in regards to vector control, most notably the fungus *Beauveria bassiana*. *B. bassiana* has been shown to reduce populations of both *T. infestans* (Lecuona 2001, Lazzarini 2006, Pedrini 2009) and *T. sordid* (Luz 2004) and has promise in areas where *T. infestans* have become resistant to insecticides (Panzera 2004, Orihuela 2008, Toloza 2008).

Physical control measures are also useful in preventing household colonization or preventing reinfestation. Chagas disease has been tied to poverty and inadequate housing that provides suitable habitat for the insect such as thatched roofs and adobe wall material (WHO 2002). Improvement of housing material has long been suggested and has been successful in some studies, but the costs are high and in rural areas it is hard to target homes that need improvement (Albarracin-Veizaga 1999, Dias 1999). However, a 2009 study by Monroy, found that cost effective housing improvements can be achieved. In that Guatemalan study, improved plastering formulations were derived from materials already utilized by Jutiapo residents, which was shown to not only dramatically decrease the number of intradomiciliary triatomines in that village but also led to greater community involvement other triatome control measures (Monroy, 2009). Also peridomestic structures such as animal shelters should be moved far from homes to discourage colonization (WHO 2002).

4.8 Pathogenesis

T. cruzi infection has three stages, the acute stage, the indeterminate stage, and the chronic stage. The acute phase is asymptomatic in the majority of infected people, but is characterized by swelling at the entrance of the parasite into the body (Brener 1997, Rassi 2009). Romaña's sign is the most recognized sign of the disease and occurs when contaminated vector fecal material is inadvertently rubbed into the eye (WHO 2002). Other generalized symptoms of this phase include fatigue, fever, splenomegaly, hepatomegaly, lymphadenopathy, rash, anorexia, diarrhea and vomiting. This phase lasts from 4-8 weeks after infection (WHO 2002). Symptoms in this phase are recognized in 1-2% of all cases (WHO 2002). In 3% of acute cases, young children or adults will develop severe myocarditis or meningoencephalitis which is potentially fatal (WHO 2002). Infections in immunosuppressed individuals are a notable exception, and typically present with acute chagasic cardiomyopathy or meningoencephalitis.

The indeterminate stage is completely asymptomatic and any acute phase symptoms completely disappear (WHO 2002). The majority of people infected with the disease remain in this stage, showing no outward signs of infection. These individuals remain in the disease cycle acting as natural reservoirs where vectorial transmission still occurs and can still contribute to congenital disease transmission (WHO 2002).

Ten or more years after infection, approximately 20-30% of infected individuals will develop symptoms related to the chronic stage of the disease (Brener 1997, Marin-Neto 2007, Albareda 2006).

Most individuals developing symptoms in this phase suffer from chagasic cardiomyopathy, featuring a range of clinical manifestations (Rassi 2009). Chronic Chagas cardiomyopathy is the leading cause of cardiac disease and cardiac related death in impoverished and rural populations in Latin America (Rassi 2009). Early manifestations of Chagas cardiomyopathy involve right bundle branch block or left anterior fascicular block and segmental left ventricular wall motion abnormalities (Maguire 1987). This is followed by ventricular tachycardia, sinus node dysfunction, high-degree heart block, pulmonary and systemic thromboembolic phenomena and progressive dilated cardiomyopathy and congestive heart failure (Hagar 1995). Abnormal ECG readings are useful in early detection of chronic Chagas cardiomyopathies in suspect patients.

In some parts of South America, affected individuals suffer from mega-syndromes that include enlargement of the esophagus and colon in addition to heart involvement. Mega-syndrome disease is the result of damage to intramural neurons and presents with a range of manifestations (Mota 1984, de Oliveira 1998, WHO 2002). Mega esophagus effects include dysphagia, esophageal reflux, aspiration, cough, achalasia and weight loss (Atlas 1994, de Oliveira 1995, WHO 2002). Mega colon symptoms include abdominal pain, chronic constipation, fecaloma and an increased risk for both volvulus and bowel ischemia (WHO 2002).

4.9 Diagnosis

Parasitological diagnostic tests include blood smear, buffy coat analysis, hemaculture and xenodiagnosis are all methods for detecting the parasite within a host. Hemaculture involves taking a sample of the host's blood, adding growth media, and monitoring for the presence of trypanosomes. Xenodiagnosis is a technique where uninfected triatomine insects are allowed to feed on a person suspected to have the disease. The feces of these insects are examined for one to two months to look for the presence of trypomastigotes indicating that the person is infected. All of these methods are highly specific as they can recover the actual parasite but their sensitivity is variable depending on the infection stage of the host. As the length of the disease increases in humans, less circulating trypomastigotes are found in the blood making these techniques much less effective in later infections (Portela-Lindoso 2003, Gomes 2009). While specificity of these techniques is 100%, sensitivities are a very low 30% to 50% (Chiari 1989).

Immunodiagnostic tests are useful for disease detection in indeterminate and chronic stages when circulating parasitemia is low. During the chronic phase, IgG class antibodies are produced which can be detected by serological tests. Conventional serological tests widely used today include indirect haemagglutination (IHA), Immunofluorescence Assay (IFA), and Enzyme Linked Immunosorbent Assay (ELISA). Due to variability in sensitivities and specificities between tests, it is recommended that a specimen undergo at least two types of assays to be confirmed positive for the disease (WHO 2002). The IHA and ELISA both utilize a complex mixture of parasite antigen. The IHA is the simplest test as it involves no special equipment or technical skills and results can be obtained in 2 hours (WHO 2002).

The WHO did a comparative analysis of five IHA tests from 3 countries and found sensitivities ranging from 88.09% to 100% and specificities ranging from 59.92 to 97.33% (Otani 2009).

The IFA uses the entire parasite for antibody reaction and binding. This test has a sensitivity of 99%, but requires specialized equipment, technical expertise, and readings are subjective (WHO 2002). This type of testing is conducive for small numbers of samples, but is too cumbersome and time consuming for a large volume of samples such as blood bank screening (WHO 2002).

ELISA, like IFA requires both technical expertise and several hours to perform, but is objectively read by a spectrophotometer and not a human technician (WHO 2002). These tests have excellent sensitivity and specificity, but can yield borderline results making disease interpretation difficult (WHO 2002). A WHO sponsored study compared several ELISA assays from several different countries and found sensitivities ranging from 94.04% to 100% and specificities ranging from 96.56 to 100% although no single ELISA test had a 100% sensitivity and specificity (Otani 2009). It carries an advantage over the IFA in that it can simultaneously screen multiple samples at the same time. In 2006, the US Food and Drug Administration approved the Ortho *T. cruzi* ELISA test system for blood bank screening in the US (CDC 2007). Confirmatory tests commonly used include the radioimmunoprecipitation assay (RIPA), western blot and recombinant immunoblot which can be used to differentiate Chagas infection from other relation cross reactive organisms such as *Leishmania*. The RIPA test is currently used to confirm blood donor samples testing positive by the Ortho *T. cruzi* ELISA in the United States (CDC 2007).

PCR has been used to analyze samples infected with *T. cruzi*, but there is a wide variety of protocols and quality controls established by different laboratories resulting in a wide range of PCR sensitivity and specificity (Schijman 2011). Like hemoculture and microscopy, PCR has a high sensitivity in the acute stage of the infection, but the sensitivity is variable in the chronic stage (Bern 2009). Sensitivity of PCR can be improved by testing multiple blood samples.

4.10 Treatment

Currently there are only two available drugs for Chagas treatment; nifurtimox and benznidazole. Neither of these drugs meet the WHO's criteria for being a good drug based on the tenets of parasitological cure of acute and chronic cases of infection, effectiveness in a single dose or a few doses, accessibility to patients, no side effects or teratogenic effects, no need for hospitalization for treatment, and no resistance demonstrated in the etiological agent (Coura 2009). Historically, only the acute phase of the disease and congenital infection were treated because the chronic symptoms were thought to be the result of autoimmune disease and not from the parasite. With new scientific advances in PCR revealing the presence of *T. cruzi* DNA in individuals with chronic infections, treatment of chronic cases is being reconsidered. Subsequent long term studies of Benznidazole treatment in individuals with chronic cardiomyopathy showed a significant decrease in clinical deterioration when compared to nontreated patients but the cure rate was still low (Coura 1997, Viotti 2006, Sosa-Estani 2006).

Nifurtimox is a 5-nitrofuran tetrahydro-4H-1,4-thiazine 1,1-dioxide that produces nitro-anion radicals, which in the presence of oxygen, leave the *Trypanosoma cruzi* parasite incapable of detoxifying free radicals (Do Campo 1986). The recommended dosage is 8-10 mg/kg/day, divided into 2 or 3 doses per day for a total of 60 days (Coura 2009). The most frequently observed side effects are anorexia, weight loss, psychological changes, excitability, muscle tremors, somnolence, hallucinations and digestive manifestations such as nausea and diarrhea (Bern 2009, Coura 2009). Convulsions are rarely observed and can be controlled by diazepam and other medications (Bern 2009, Coura 2009).

Benznidazole is a 2-nitroimidazole that inhibits protein and ribonucleic acid synthesis in the parasite (Polack 1978, Diaz de Taranzo 1988). The recommended dosage is 5 mg/kg/day, divided into 2 or daily doses for 60 days (Coura 2009). The most frequently observed side effects are hypersensitivity, bone marrow depletion (neutropenia, thrombocytopenic purpura, and agranulocytosis) and peripheral polyneuropathy (Bern 2009, Coura 2009). These can be controlled with antihistamines and corticosteroids, but in the case of severe side effects such as agranulocytosis, treatment should be suspended (Bern 2009, Coura 2009).

Allopurinol, a hypoxanthine analogue, is incorporated into RNA and leads to the formation of a non-physiological nucleotide that blocks de novo synthesis of purines (Coura 2009). Lauria-Pires showed that the compound was ineffective during the acute phase of the disease (1998), but the drug has shown promise in treating chronic infections such as infection reactivation due to post surgical immunosuppression (Tomimori-Yamashita 1997). A drug study by Gallerano found that 75- 92% of patients treated with the drug became negative by xenodiagnosis (1990), but in a study by Apt the cure rate was only 44% in allopurinol treated patients (1998).

Additionally the antifungal agent ketoconazole, fluconazole, itraconazole and posaconazole have been shown to be active in vitro against *T. cruzi* (Coura 2009). Ketoconazole, a derivative of imidazole, was the first of these drugs to show in vitro activity against *T. cruzi* in acute stage infection, but it has since been shown to be ineffective against the parasite in chronic infections (De Castro 1993). Posaconazole is showing the most promise of these antifungal agents and is in initial human drug trial testing (Ferraz 2007, Coura 2009). This compound has shown a 50-100% cure rate in the acute phase in animals, and a 50-60% cure rate in chronically infected animals (Ferraz 2007).

4. 11 References

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Chapter 5: Ecological Niche Modeling Methods and Methodology

5.1 Data Collection

Geographic information system (GIS) is a system designed to capture, store, manipulate, analyze, and manage georeferenced data to support planning, management and decision making processes. Remote sensing (RS) is defined as the ability to acquire information about an object or phenomenon without physical contact with the object or phenomenon of interest. In regards to epidemiologic research, remote sensing coupled with geographic information systems has been used to obtain environmental information such as elevation, temperature, and moisture. GIS and RS have been used to create risk models for a variety of diseases including malaria, schistosomiasis, and African trypanosomiasis (Bavia 1999, Malone 2001, Hay 2000, Courtin 2005, Odiit 2006, Zhou 2008).

Moderate Resolution Imaging Spectroradiometer (MODIS) is a sensor that captures data in 36 spectral bands at 250m, 500m, and 1km spatial resolutions. MODIS sensors are onboard the Terra Earth Observing System satellite launched in 1999 and the Aqua Earth Observing System satellite launched in 2002. The two sensors are able to provide measurements on changes in the Earth's cloud cover, temperature, and vegetation for the entire Earth every 1 to 2 days. From these measurements, many different MODIS products can be derived such as Land Surface Temperature (LST), Normalized Difference Vegetative Index (NDVI), and land cover type.

Land surface temperature measures how hot the land is to the touch and provides monthly land surface temperature data for daytime and nighttime. Land surface temperature can be influenced by many factors including land use and land cover.

Normalized Difference Vegetative Index is used to measure and monitor greenness or plant growth cover. Plants have an influence on how high the surface temperature can rise. Plants absorb visible red-light during photosynthesis and reflect near-infrared light, which results in a high NDVI value. Non-vegetated areas such as soil, water bodies, snow and cloud cover reflect visible red light and absorb the near infrared light and thus have a low or sometimes negative NDVI value. The equation for determining NDVI is

$$NDVI = (NIR - VIS) / (NIR + VIS)$$

Where VIS and NIR stand for visible red spectrum and near infrared regions, respectively. VIS covers the spectral range of 350 to 1000 nanometers, while NIR obtains wavelengths from 1100 to 2200 nanometers.

The WorldClim database provides altitude, 19 bioclimatic (BioClim) variables, monthly precipitation (prec), monthly maximum temperature (tmax), monthly minimum temperature (tmin) and mean monthly temperature (tmean). Data layers in this database were derived through interpolation of average monthly climate data from a wide variety of weather stations from the time period of 1950 – 2000, and had a spatial resolution of 1km.

Disease data was compiled by municipality for 2001 to 2009 from the National Information System for Notifiable Diseases in Brazil (SINAN) for leprosy, schistosomiasis and acute chagas disease. SINAN is a national notifiable disease information system database introduced in 1994 that provides disease case information for a wide range of diseases, by municipality, for each year. Bolivian Chagas prevalence data for 2007 – 2009 was provided by municipality from the Bolivian ministry of health. The average prevalence was calculated for the three year period for analysis.

Disease data was stratified on a case/10,000 population basis. Socioeconomic data was compiled by municipality from the Brazilian Institute of Geography and Statistics (IBGE) (Table 1) and the Bolivian National Institute of Statistics (INE). Environmental remote sensing data was obtained from the MODIS website (<http://modis.gsfc.nasa.gov/>) and included normalized difference vegetation index (NDVI), daytime land surface temperature (LSTd) and nighttime land surface temperature (LSTn). Worldclim data was downloaded from the Internet (<http://www.worldclim.org>) to provide long term normal monthly data on minimum temperature, maximum temperature, and precipitation, and 19 derived Bioclim variables, including altitude (Table 2). Environmental and Socioeconomical data were kept in separate databases for statistical analysis. This was due to the inherent differences in the data types as well as to improve the multiple regression modeling step.

TABLE 1:
Environmental Variables used in Regression Analysis

Normalized Difference Vegetation Index (NDVI)	Mean Temperature of Coldest Quarter (BIO11)
Daytime Land Surface Temperature (LSTD)	Annual Precipitation (BIO12)
Nighttime Land Surface Temperature (LSTN)	Precipitation of Wettest Month (BIO13)
Annual Mean Temperature (BIO1)	Precipitation of Driest Month (BIO14)
Mean Diurnal Range (BIO2)	Precipitation Seasonality (BIO15)
Isothermality (BIO3)	Precipitation of Wettest Quarter (BIO16)
Temperature Seasonality (BIO4)	Precipitation of Driest Quarter (BIO17)
Max Temperature of Warmest Month (BIO5)	Precipitation of Warmest Quarter (BIO18)
Min Temperature of Coldest Month (BIO6)	Precipitation of Coldest Quarter (BIO19)
Temperature Annual Range (BIO7)	Monthly Precipitation
Mean Temperature of Wettest Quarter (BIO8)	Monthly Mean Temperature (tmean)
Mean Temperature of Driest Quarter (BIO9)	Monthly Minimum Temperature (tmin)
Mean Temperature of Warmest Quarter (BIO10)	Elevation

TABLE 2:
Brazil Socioeconomic Variables used in Regression Analysis

Domestic gross product at current prices (R\$)	Percentage of houses with electricity availability
Domestic gross product per capita	Unemployment rate
Poverty incidence	Life expectance when born
Percentage people with 1 UBN	Adults literacy rate
Percentage of people with 4 UBN	School attendance rate
Percentage of people with 5 UBN	Income per capita R\$
Percentage of people with UBN education	Longevity index
Percentage of people with UBN overcrowding	Education index
Percentage of people with UBN subsistence	Income index
Percentage of people with UBN sanitation	Human development index
Percentage of houses with plumbing	Life expectance when born
Percentage of Houses with water availability	Houses with sanitation availability %

TABLE 3:
Bolivia Socioeconomic Variables used in Regression Analysis

Infant Mortality Rate	Human Development Index 2005
Brick,block, or concrete wall material (% of population)	Corrugate metal roofing material (% of population)
Adobe wall material (% of population)	Cement or clay roofing material (% of population)
Tabique-quinche wall material (mud mixed with straw or cane) (% of population)	Concrete slab roofing material (% of population)
Stone or rock wall material (% of population)	Straw, reed or palm roofing material (% of population)
Cane, palm, or trunk wall material (% of population)	Piped drinking water into the household (% of population)
Dirt floors (% of population)	Public tap water source (non household source) (% of population)
Hardwood floors (% of population)	Pumped well drinking water (% of population)
Wood paneled floors (% of population)	Non-pumped well drinking water (% of population)
Carpet floors (% of population)	River or stream collection drinking water (% of population)
Cement floors (% of population)	Lagoon or lake collection drinking water (% of population)
Tile or ceramic floors (% of population)	% of population with NBI in education
% of population with 1 neglected basic	% of population with overcrowding
% of population with 2 NBI	% of population with inadequate housing materials
% of population with 3 NBI	% of population without sanitation or inadequate sanitation
% of population with 4 NBI	% of population with subsistence
% of population with a low quality of life	% of population with indoor plumbing
% of population with a medium quality of life	% of population with in home drinking water
% of population with a high quality of life	% of homes with indoor toilets
Unemployment rate	% of homes with electricity

5.2 Variable Selection

Each database was analyzed statistically through multiple stepwise regression modeling. Multiple regression was chosen to reduce the number of variables used in the final modeling process as both the environmental and socioeconomical variable databases contained a large number of variables. The Models were selected through analysis of F statistic, Mallows Cp and R^2 value. Stepwise regression modeling was done on areas with high levels of disease in Brazil (defined as having a new case detection rate of ≥ 100 cases per 10,000 population in the cumulative period of 2001 to 2009 for leprosy and schistosomias and ≥ 1 case per 10,000 population in the cumulative period of 2001 to 2009 for chagas

disease. For Bolivia, prevalence of $\geq 1\%$ was set as the cutoff for regression modeling and MaxEnt analysis.

To eliminate multicollinearity between variables, variance inflation factors (VIF) were calculated for each variable in the model and those with VIFs greater than ten were sequentially removed until all remaining variable VIF values were less than ten.

5.3 MaxEnt Modeling

An ecological niche is defined by Grinnell as the limited range of ecological variables that can maintain a population without the influx of immigration into that population (1917). Hutchinson further defined an ecological niche as a product of all environmental factors acting upon an organism (1944). Ecological niche modeling is therefore a technique to identify environments that can sustain the organism of interest to the researcher (Blackburn 2010). Ecological niche model programs such as MaxEnt, accomplish this through pattern matching species occurrence points with environmental or socioeconomic data layers through the use of algorithms (Blackburn 2010).

MaxEnt estimates the target distribution of the species of interest by finding the distribution of maximum entropy (the distribution closest to uniform) with the constraints that the expected value of each variable constraint matches its empirical average (Phillips 2004). Many possible distributions may satisfy the variable constraints, but MaxEnt chooses from among these the one of maximum entropy or the one closest to uniform (Phillips 2004). This program became available in 2004 and has become extensively used for species distribution modeling due to its high predictive performance (Elith 2006). MaxEnt only requires presence samples and performs well even with few samples and gives a smooth gradient of least to most suitable conditions (Phillips 2004). MaxEnt models are also more easily interpreted than models generated through GARP (Phillips 2004). A potential disadvantage seen with MaxEnt and other ecological niche programs is that there is a potential for sampling bias since distributions are weighted towards areas and environmental conditions that have been better sampled (Phillips 2004). In this study, all diseases were reportable to the ministry of health, and it was from this agency that the data was collected. The potential sampling bias was therefore decreased because of disease as opposed to formal survey data.

Variable relationships with occurrence points were evaluated within MaxEnt through three separate analyses; jackknife analysis of variables, variable response curves and variable percent contributions to the model. Jackknife analysis evaluates the creation of the model with in three ways; exclusion of each variable in turn, with each variable in isolation, and by using all variables (Phillips 2004). From this analysis, bar graphs are created which show the gain of model for each of these scenarios. A significant loss of gain from the exclusion of a specific variable, or a large gain from a single variable in exclusion indicate increased importance of that variable to the model (Phillips 2004). Variable response curves reveal upward or downward trends to occurrence data, or variable ranges related to occurrence data (Phillips 2004). Variable percent contributions show how important each variable was in the creation of the model (Phillips 2004).

Bayes' theorem is used to determine the conditional probability of an event based on prior knowledge about some other event that has already occurred. For example, if someone tested positive for HIV, what is the probability that they actually have the disease. Given prior knowledge about the sensitivity and specificity of the test then a probability of a correct diagnosis can be predicted. Bayesian modeling has been developed for schistosomiasis (Beck-Worner 2007, Raso 2007, Wang 2008, Vountasou 2009, Clements 2010), leprosy (Souza 2001), African trypanosomiasis (Wardrop 2010), and filariasis (Boyd 2005, Gambhir 2010).

One of the biggest disadvantages associated with Bayesian models involves the difficulty in specifying a priori and there is no correct way to do this. Also the use of an incorrect prior can result in the generation of a misleading and erroneous final product. In many cases of disease mapping, prior knowledge is vague or incomplete which makes specifying a unique prior distribution problematic (Ferson 2005, Gelman 2008). Using different priors can lead to different outcomes, which has led many to question bayesian technique (Ferson 2005, Gelman 2008). Another main argument concerns the subjectivity of Bayesian technique which involves the subjective application of a priori (Ferson 2005, Gelman 2008).

Ecological niche models (ENM) were developed to understand the relationship between the geographic distribution of leprosy in Brazil, and environmental and socioeconomic factors related to disease. MaxEnt is a maximum entropy approach to presence-only distribution modeling that has shown both a high predictive power for large as well as very small sample sizes (Hernandez 2006, Phillips 2006). From 2001 to 2009, 314 Brazilian municipalities had leprosy new case detection rates of ≥ 100 cases per 10,000 people, 304 Brazilian municipalities had schistosomiasis new case detection rates of ≥ 100 cases per 10,000 people, and 241 Brazilian municipalities had acute case Chagas new case detection rates of ≥ 1 case per 10,000.

Bolivia prevalence data was calculated from household surveillance data compiled by municipality for 2007, 2008 and 2009 from the Bolivian Ministry of Health. An average prevalence was calculated for each municipality from the years provided. To improve the quality of the data, municipalities with less than 100 households surveyed in a given year were deleted from consideration for that year. Additionally, data from the municipality of Reyes in the department of El Beni was deleted because the validity of data from that specific municipality is questionable. Prior literature indicates this area is non-endemic for Chagas disease (Aguliar 2007). This municipality was only surveyed by the health department in 2008 and it had an extremely high prevalence (60 %).

These municipalities were geocoded and input into MaxEnt. Variable data was projected, resampled to 1km spatial resolution and converted to a uniform ASCII format to run within MaxEnt. The MaxEnt modeling procedure assessed the importance of the variable data contributing to Leprosy distribution through jackknife analysis of variable contribution to the model, the average values of area under the curve (AUC) of 10 model iterations, and the average percentage contribution of each variable to the model.

5.4 Model Evaluation

To validate the accuracy and power of the model, SINAN disease data from 2010 was compiled and stratified by population. Municipalities with new case detection rates of ≥ 11 cases per 10,000 population were culled and used to validate the model for leprosy and schistosomiasis. This level corresponded to the 100 cases per 10,000 that were used in the creation of the model. For Chagas in Brazil, 2010 cases were compiled by municipality and sorted by mode of transmission (vectorial, oral, or unknown). Disease data from 2010 was not available for Chagas disease for Bolivia. Ten iterations of the model were run with 75% of the points used as training data, and the 25% of the points set aside to test the model. The test performance of the model was evaluated by deriving the area under the curve (AUC) of the receiver operator characteristic plot analysis done in MaxEnt (Hernandez 2006, Phillips 2006, Phillips 2008). AUC values range from 0.5 which indicates a model no different from chance, and 1; 0.5-0.6 = No discrimination; 0.6-0.7 = Discrimination; 0.7-0.8 Acceptable; 0.8-0.9 Excellent; 0.9-1.0 Outstanding (Phillips 2006).

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Chapter 6: Results

6.1 Leprosy

6.1.1 Statistical Analysis

A total of 314 Brazilian municipalities were classified as hyperendemic and used in multiple linear regression to determine the best socioeconomic and environmental models for leprosy in Brazil. From this preliminary modeling the following environmental variables were identified as being associated with disease; mean temperature of the coldest quarter (BIO 11), precipitation during the driest quarter (BIO 17), daytime land surface temperature, July precipitation, June minimum temperature. Life expectancy was the best socioeconomic variable selected from regression modeling (Table 4).

TABLE 4:
Final Leprosy Multiple Regression Models

VARIABLE	PARAMETER ESTIMATE	STANDARD ERROR	T-VALUE	Pr t	VARIANCE INFLATION FACTOR	PERCENT CONTRIBUTION
MEAN TEMPERATURE DURING THE COLDEST QUARTER (BIO 11)	-14.892	4.685	-3.18	0.0016	6.627	58
PRECIPITATION DURING THE DRIEST QUARTER (BIO 17)	-0.252	0.161	-1.56	0.1196	2.972	12.4
JUNE MINIMUM TEMPERATURE	9.308	3.823	2.43	0.0155	7.174	4.5
JULY PRECIPITATION	0.388	0.178	2.17	0.0305	2.718	9.5
DAYTIME LAND SURFACE TEMPERATURE	4.861	2.252	2.16	0.0317	1.337	10.6
LIFE EXPECTANCY	-0.95413	0.36413	2.6	0.0092	N/A	5.1

6.1.2 MaxEnt Ecological Niche Modeling Analysis

Socioeconomical and environmental variables were combined in MaxEnt and compared to the city center of the original 314 Brazilian municipalities. Table 4 shows the percent contribution of each variable to the MaxEnt model and Figure 6 shows the final MaxEnt model.

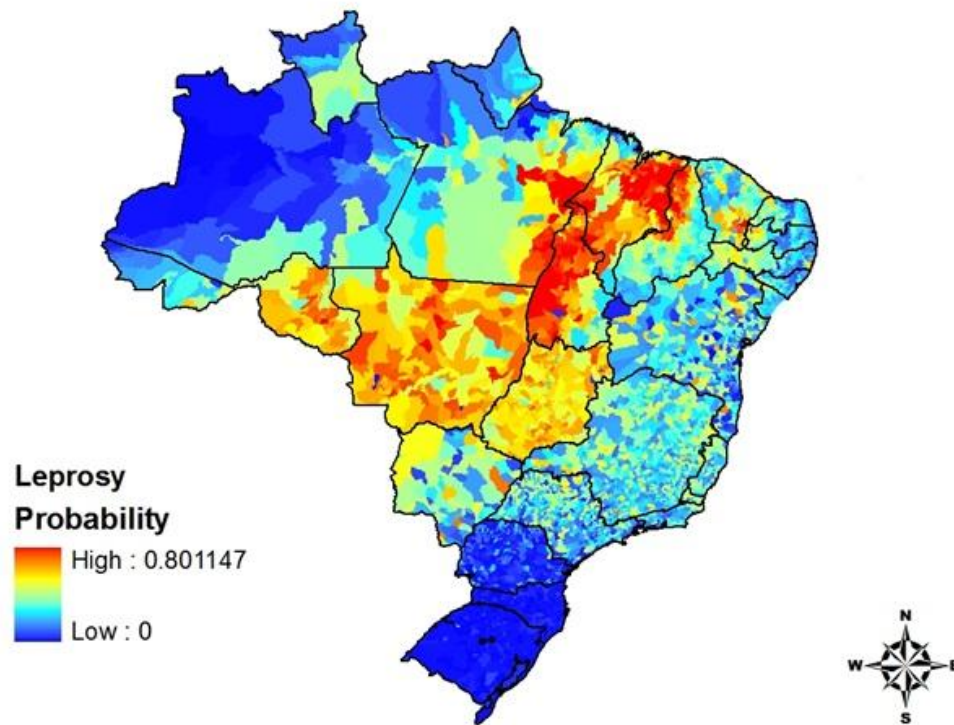
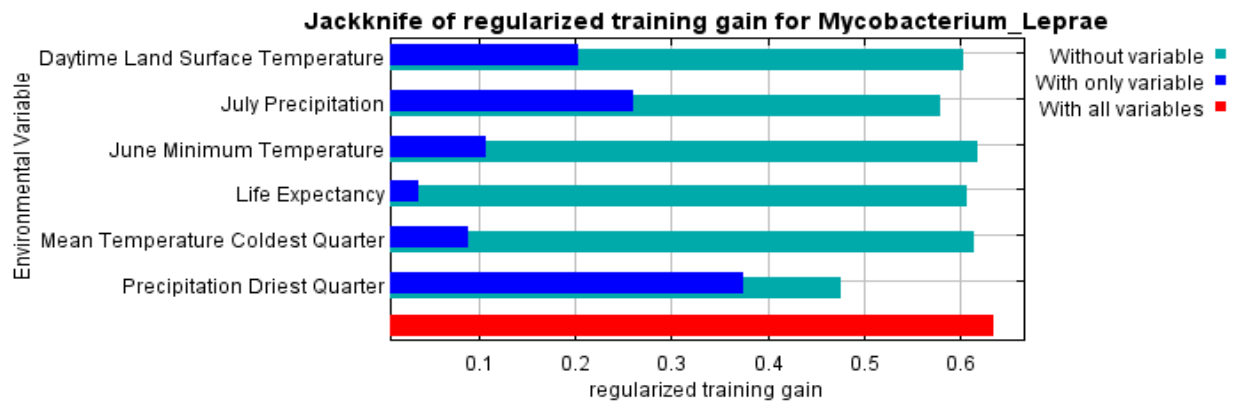


Figure 6: MaxEnt Leprosy Prediction Model. MaxEnt predictive model showing the distribution probability of leprosy occurrence. Red indicates a higher probability of occurrence, while blue indicates a low probability of occurrence.

The relative importance of each variable to the hyperendemic municipalities was evaluated by jackknife plots of training gain, test gain and area under the curve (AUC) (Figure 7). Precipitation during the driest quarter was the single most important factor influencing the model as demonstrated in both the jackknife analysis and percent contribution to the model (Figure 7 and Table4). This particular variable had the highest gain when used in isolation and decreased the gain the most when omitted from the model indicating that it was more informative than the other variables.

A



B

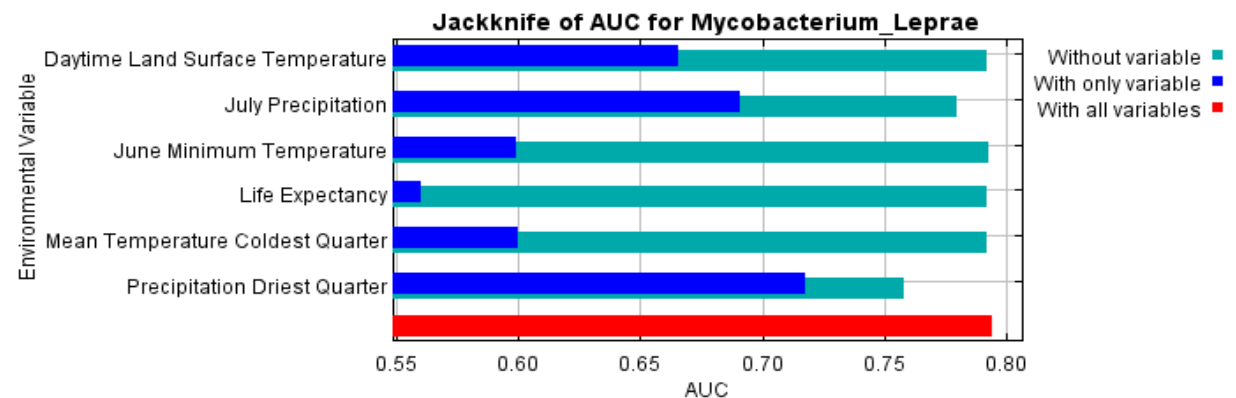
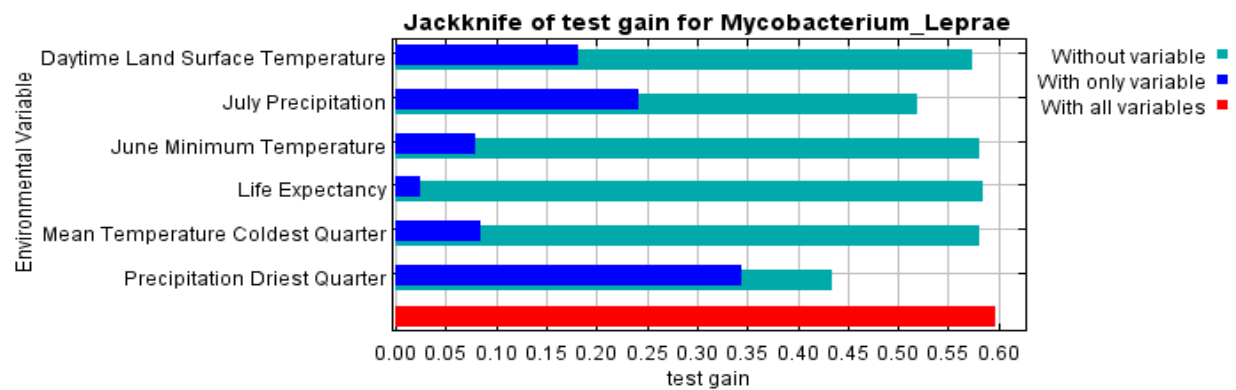


Figure 7: Leprosy MaxEnt Jackknife Analysis. Jackknife analysis results of training gain, test gain, and area under the curve (AUC). The blue, light blue and red bars represent results of the model created with each individual variable, all the remaining variables and all variables respectively.

The response curve for precipitation during the driest quarter favored a relationship between low moisture and leprosy, which was also echoed in July precipitation variable (Figure 3B and 3C). The three temperature measures important in the model, mean temperature in the coldest quarter, daytime land surface temperature and June minimum temperature showed similar response curves which leprosy increasing with temperature (Figure 8A, 8D, and 8E). The response curve for life expectancy showed a high degree of variability and did not appear to be as responsive to the disease as the other variables (Figure 8F). During the statistical process, regression modeling created a weaker socioeconomic model in comparison to the environmental regression model. This could be due to the coarseness of municipal level data and may indicate a need for a finer level of socioeconomic data, perhaps at a census tract level.

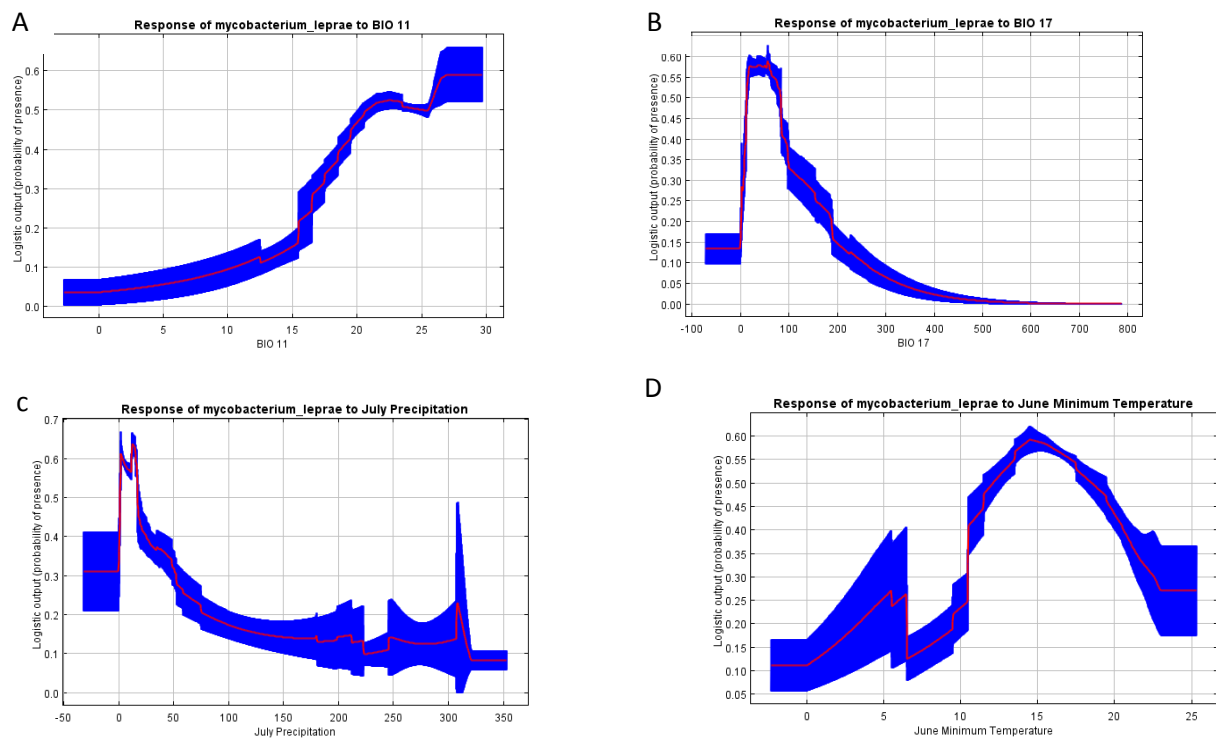


Figure 8: Leprosy MaxEnt response curves. Response curves for the variables related to leprosy presence in Brazil. Red lines are mean values for the 10 MaxEnt iterations and the blue bars represent ± 1 standard deviation

(Figure 8 continued)

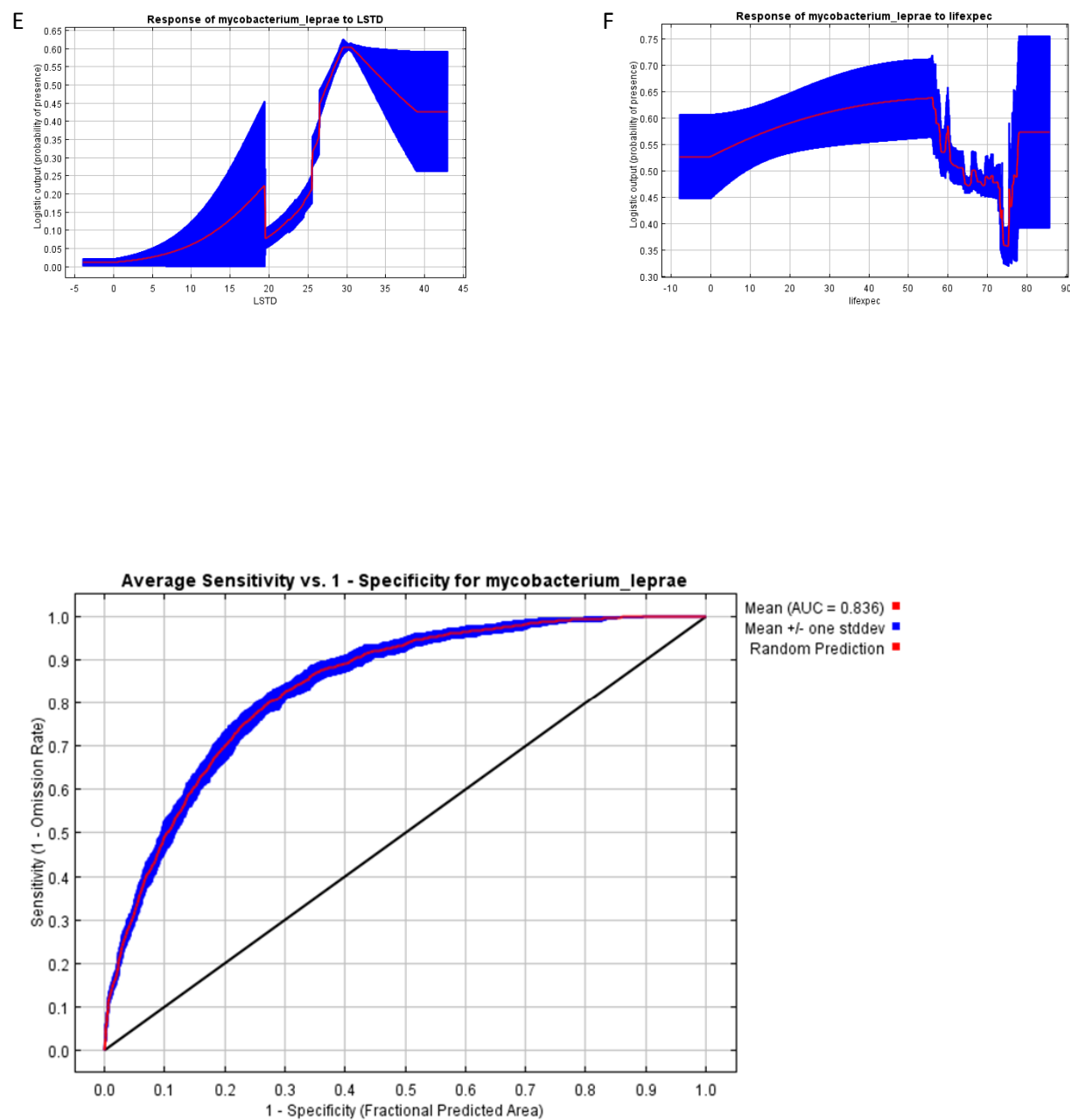


Figure 9: Area under the curve (AUC). Red line indicates the mean value for 10 MaxEnt runs and blue line indicates ± 1 Standard deviation.

The average test AUC for the 10 replicate runs of the model was 0.836 with a standard deviation of 0.01 (Figure 9) which indicates that the model performance was excellent. To validate the model, municipalities with a minimum of 11 cases of leprosy per 10,000 population from 2010 SINAN were overlaid on the risk map (Figure 10). Thresholds were determined using average “maximum training sensitivity plus specificity logistic threshold” and balance training omission, predicted area and threshold value” over the 10 model iterations (Cantor 1999, Cramer 2003, Liu 2005, Wei 2011). The results gave thresholds of 0.399 and 0.10, respectively. To validate the model, 238 municipalities with high 2010 incidence rates of leprosy were overlaid on the risk map (Figure 10). For analysis, Brazil was divided into regions to determine if the model had different predictive power in the different regions of Brazil. The percentages of municipalities in predicted high, moderate, and low risk areas are listed in Table 4. The model showed the best predictive power for the North, North East and Central west regions of Brazil, where the percentage of municipalities falling into the high risk categories ranged from 71.43% to 89.77% . These areas also presented the highest prevalence for the disease. For the South East and South regions, the largest percentages of municipalities fell into the moderate risk areas (88.89% and 66.67% respectively). One explanation could be that these two regions only accounted for 8.82% of the municipalities in Brazil with incident rates of leprosy in 2010. These results are consistent with recent literature findings that leprosy rates are much higher in the North, Northeast and Central west regions of the state.

TABLE 5:
Brazil Regional Leprosy Risk Validation

BRAZIL REGION	HIGH (>0.399)	Moderate (0.100 – 0.399)	Low (<0.100)
North	66 (85.71%)	11 (14.29%)	0
North East	36 (71.43%)	13 (23.21%)	3 (5.36%)
Central West	79 (89.77%)	7 (7.96%)	2 (2.27%)
South East	2 (11.11%)	16 (88.89%)	0
South	0	2 (66.67%)	1 (33.33%)

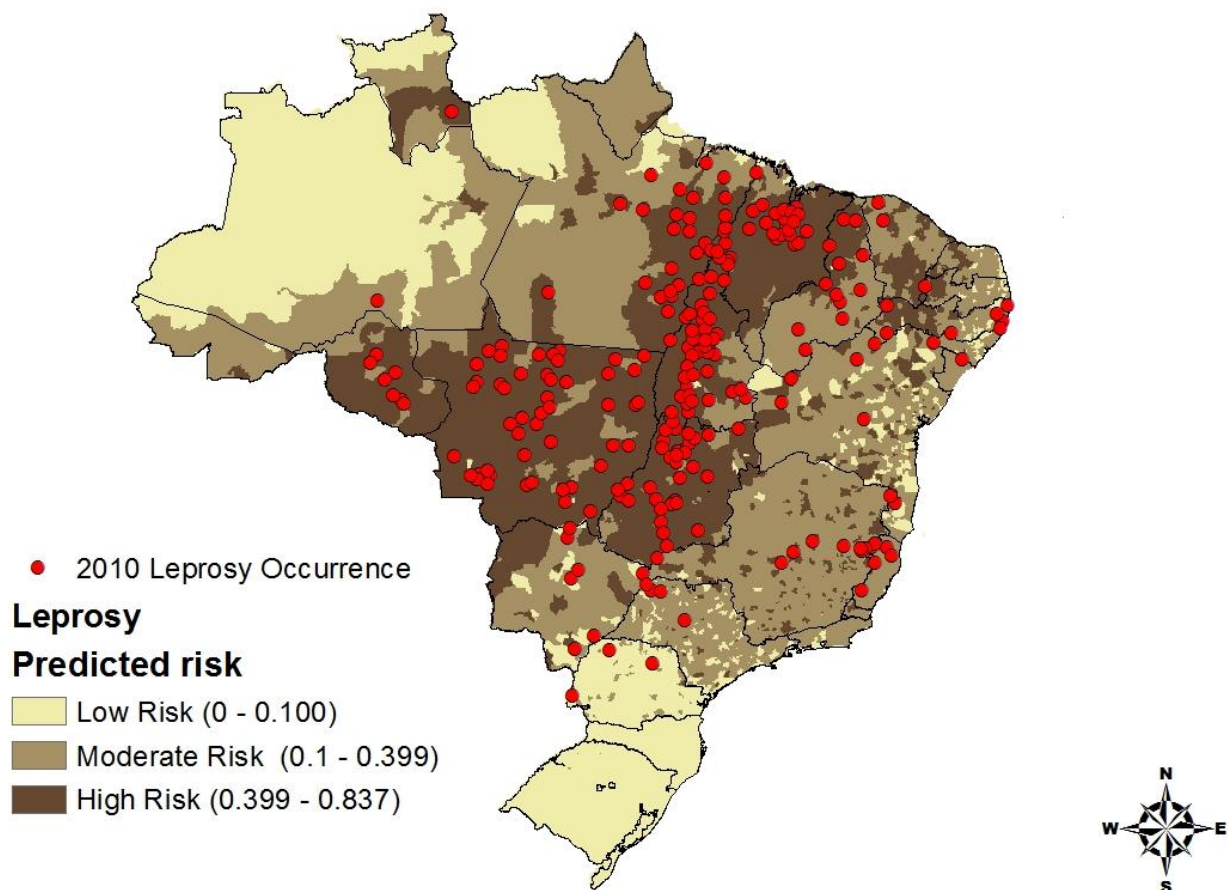


Figure 10: Leprosy risk map. The predicted risk map of leprosy overlaid with 2010 occurrence data.

6.1.3 Discussion

These data indicate a need for additional resources for leprosy control in the central part of the country, especially the states of Maranhão, Mato Grosso, Mato Grosso do Sul, Roraima, and Tocantins. Additionally, this study illuminated environmental relationships to the geographic distribution of leprosy in Brazil. More study is warranted to determine the exact nature of these environmental parameters to leprosy in this area, with particular emphasis on the potential of the nine banded armadillo (*Dasypus novemcinctus*) as a reservoir host. An ecological niche modeling study by Anacleto (2006) on armadillo distribution in Brazil yielded a distribution pattern compatible to the leprosy risk map presented in this study. These similarities coupled with increasing evidence of *Dasypus novemcinctus*'s role in potential leprosy transmission in the southern United States warrants study of this species role or lack thereof in leprosy transmission in South American and particularly Brazil.

The high risk areas of our predictive map were consistent with previous studies by Penna *et al.* that identified probable clusters in the North, Northeast and Central west regions of Brazil and shows a similar distribution (2008). Temperature and precipitation parameters were strongly correlated with the presence of high levels of leprosy in Brazil and our study indicates that drier areas with temperature ranges of 20°C to 28°C are optimum for leprosy in Brazil. One thing to note is the large amount of variability throughout the country in terms of environmental factors. This variability results in part to the large amount of variability seen in the response curves. A potential way to reduce some of this variability within the model is to reduce the analysis to regions with Brazil. This would also potentially uncover disease patterns for leprosy that may be unique to individual parts of the country. Additionally, this could increase the predictive power to areas of Brazil where leprosy is less of a problem such as the Southern region.

Correlations between environmental factors were much clearer than the correlation between socioeconomic factor and disease within our model. This would indicate that while municipal level is sufficient for environmental factors, it is not very good at adequately reflecting socioeconomic insufficiencies as they can vary greatly within a single municipality. In future studies, socioeconomic data should be collected at a level that more adequately reflects socioeconomic inequalities such as a census tract.

Statistical analysis through multiple linear regression was done first to not only cull down the variables to be used in MaxEnt, but to also find and remove multicollinearity within variables that would affect interpretation of the model in MaxEnt. It also helped to identify exactly which variables were important to the model which is not as clear when trying to cull variables by only using MaxEnt. Ecological niche models can vary in how they predict disease distributions which each run of a model (Araujo 2005, Wei 2011). To minimize this effect, we averaged the model output based on 10 random replicate data sets. Additionally, we sought to validate our model by using disease data from the following year to evaluate our model. Future work with additional disease data and more refined socioeconomic data could help to refine the model prediction of leprosy in Brazil.

6.2 Schistosomiasis

6.2.1 Statistical Analysis

A total of 304 Brazilian municipalities were classified as hyperendemic and used in multiple linear regression to determine the best socioeconomic and environmental models for leprosy in Brazil. Preliminary stepwise regression modeling indicated that isothermality (Bio 03), precipitation seasonality (Bio 15), daytime land surface Temperature (LSTD), December precipitation, and June mean temperature were the most important environmental variables to model *S. mansoni* distribution (Table 3). Education, sanitation, poverty incidence, current gross domestic product (GDP), and gross domestic product per capita were identified as the most important socioeconomic variables through the regression modeling procedure (Table 6).

TABLE 6:
Final Schistosomiasis Regression Models

Variable	Parameter Estimate	Standard Error	T Value	Pr > t 	VIF	MaxEnt Percent Contribution
Isothermality (BIO 03)	-46.434	13.962	28.71	<.0001	1.757	18.1
Precipitation Seasonality (BIO 15)	6.228	2.165	5.47	0.0201	2.174	2
Daytime Land Surface Temperature (LSTD)	-26.155	14.165	8.4	0.004	1.505	6.9
June Mean Temperature	47.225	18.35	6.12	0.0139	2.102	30.8
December Precipitation	-0.812	0.577	9.21	0.0026	2.605	19.8
GDP per capita	-0.428	0.179	-2.38	0.0179	1.106	12.3
Current GDP	10.473	3.836	2.73	0.0067	1.324	1.5
Poverty Incidence	-7.715	2.929	-2.63	0.0089	1.626	0.4
Education	14.761	7.927	1.86	0.0636	1.427	1.8
Sanitation	2.145	1.103	1.94	0.0528	1.326	6.4

6.2.2 MaxEnt Ecological Niche Modeling Analysis

Socioeconomical and environmental variables were combined in MaxEnt and compared to the city center of the original 304 Brazilian municipalities. Table 6 shows the percent contribution of each variable to the MaxEnt model and Figure 11 shows the final MaxEnt model.

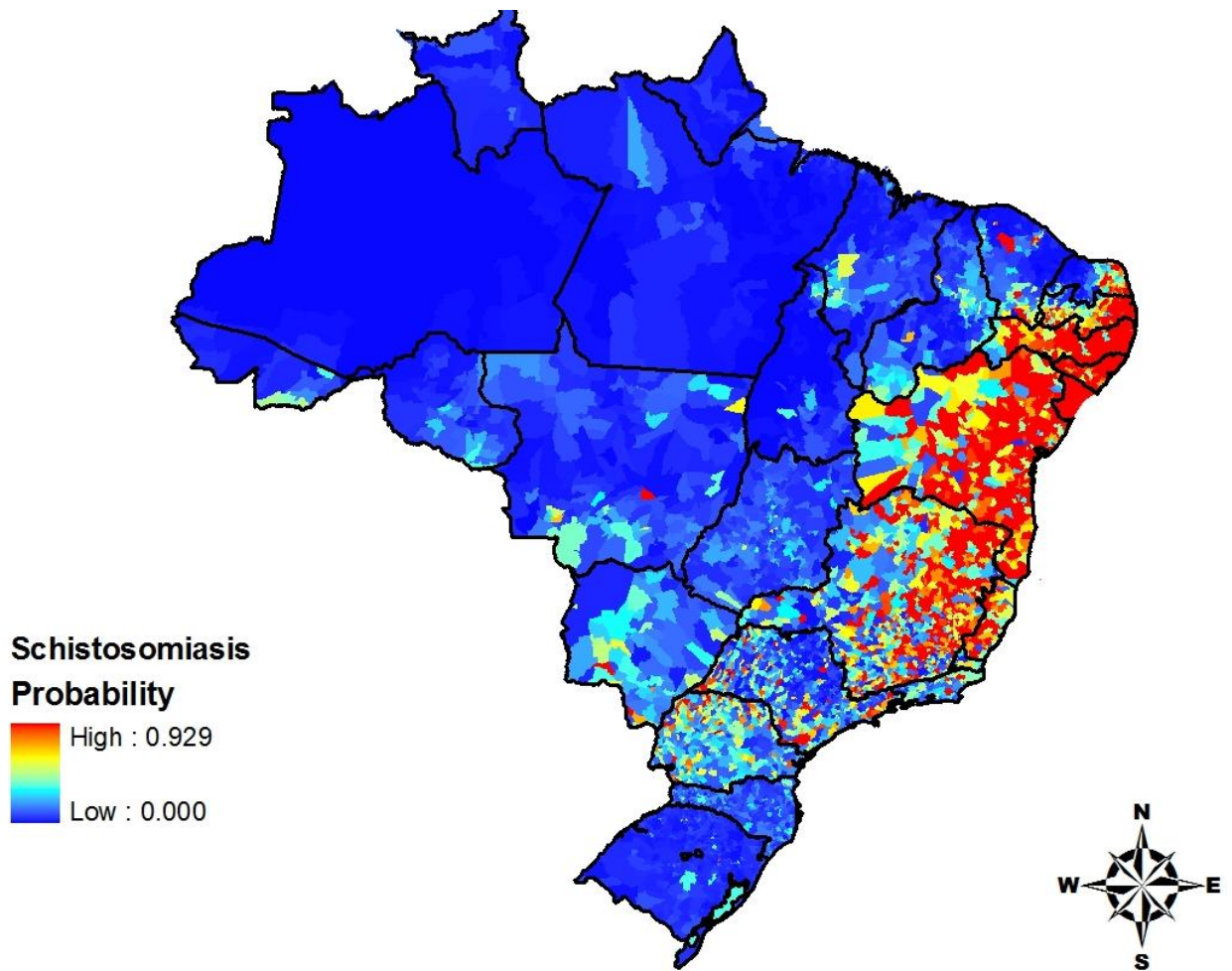
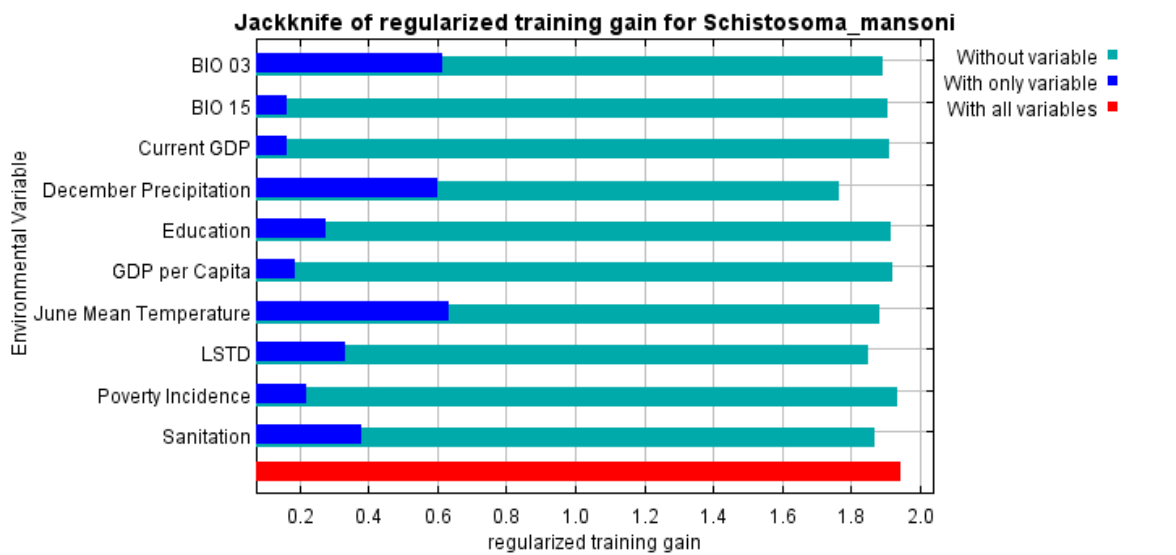


Figure 11: MaxEnt Schistosomiasis prediction model.

June Mean temperature, isothermality, and December precipitation provided the best fit to the model and each had a percent contribution to the MaxEnt model of over 20 (Table 6). Of these three variables, June mean temperature had the highest gain when used in isolation, indicating that it had the most useful information about the distribution of schistosomiasis in Brazil by itself (Figure 12). Conversely, December precipitation decreased the gain of the model the most when it was omitted, indicating that it had the most information not in the other variables (Figure 12). The probability increased with June mean temperature, and daytime land surface temperature specifically from 16° to 24° and 22° to 33° respectively (Figure 13D and 13E). Both of these ranges fall with the temperature limit for the snail vectors of schistosomiasis in Brazil (16° to 32°) (Plorin 1983). The probability also increased with isothermality from 57 to 73% indicating that the probability of the disease increased as variability in temperature during the year decreased (Figure 13A). The probability initially increased with December precipitation up to a peak of 40in and then steadily decreased with precipitation (Figure 13C). The relationship with precipitation seasonality was not as clear as there were three unequal but distinct peaks at 20, 60 and 90 (Figure 13B). The response curves for the socioeconomic variables

showed a basic relationship between poverty and an increased probability for the disease (Figures 13F, 13G, 13H, 13I, and 13J). It should be noted that both current GDP and GDP per capita showed a high degree in variability within their response curves indicating that by themselves they are not very good indicators of schistosomiasis disease distribution.

A



B

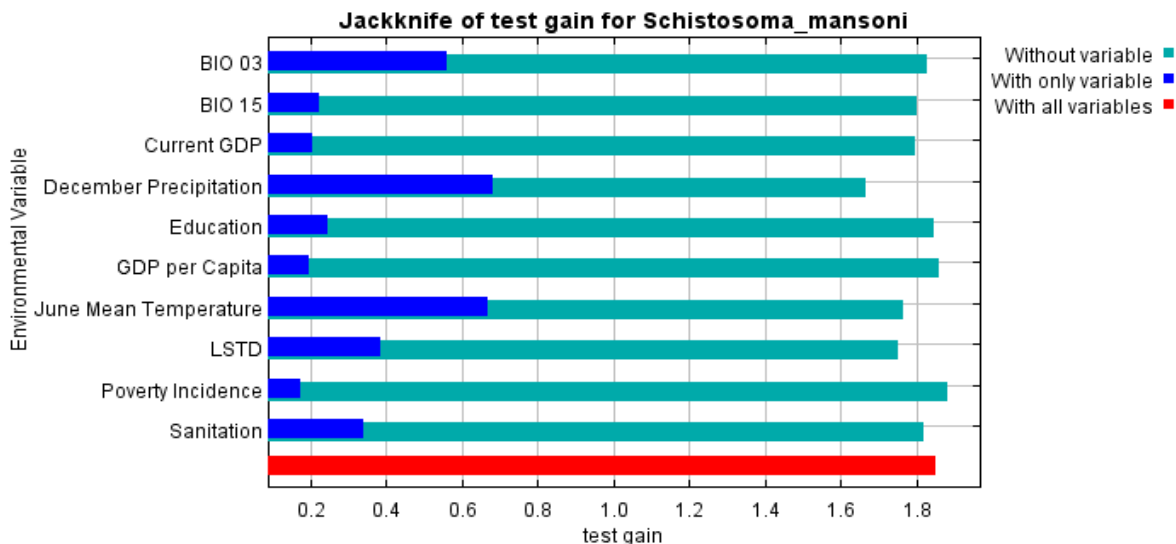


Figure 12: Schistosomiasis MaxEnt jackknife analysis. Jackknife analysis results of training gain, test gain, and area under the curve (AUC). The blue, light blue and red bars represent results of the model created with each individual variable, all the remaining variables and all variables respectively.

(Figure 12 continued)

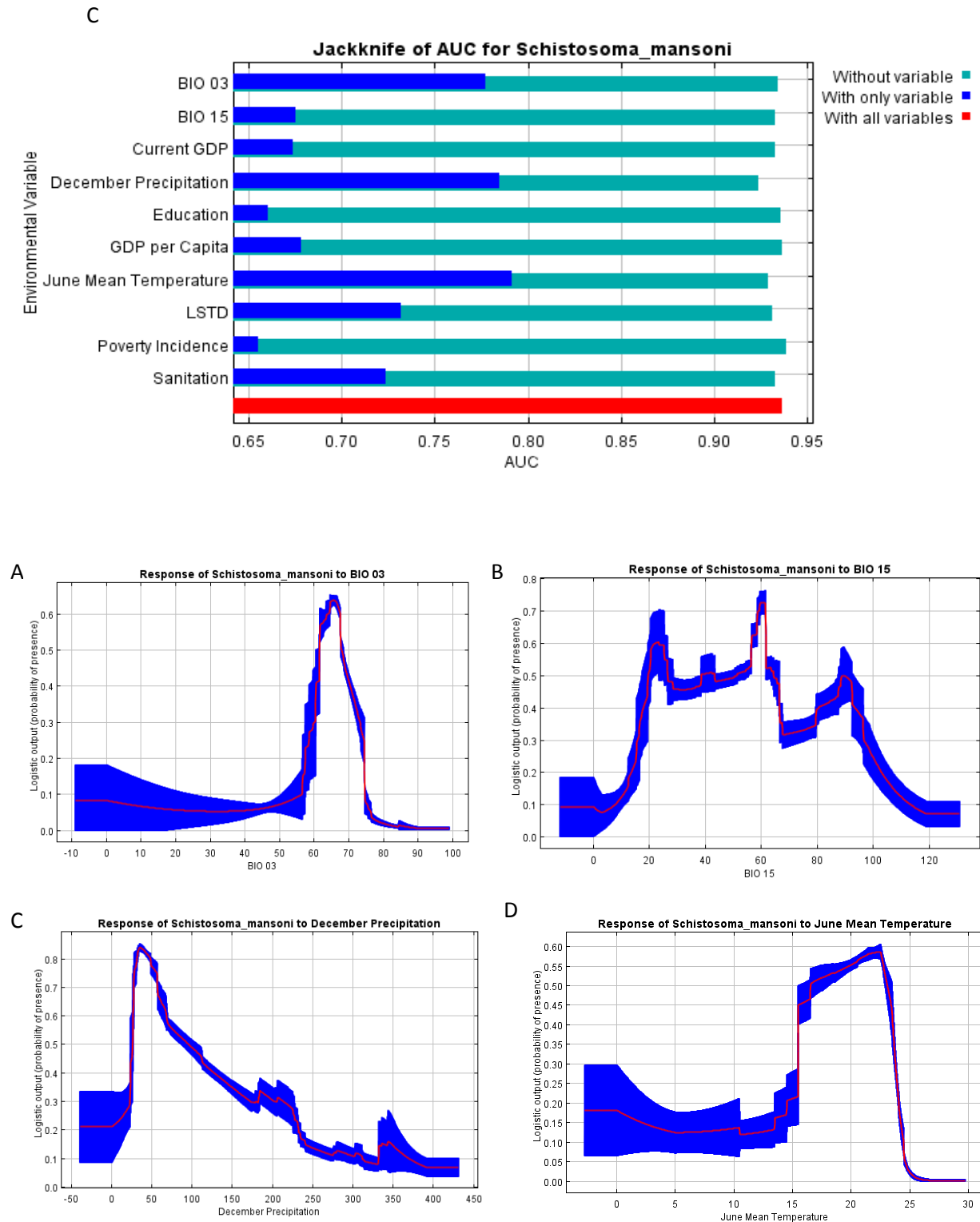
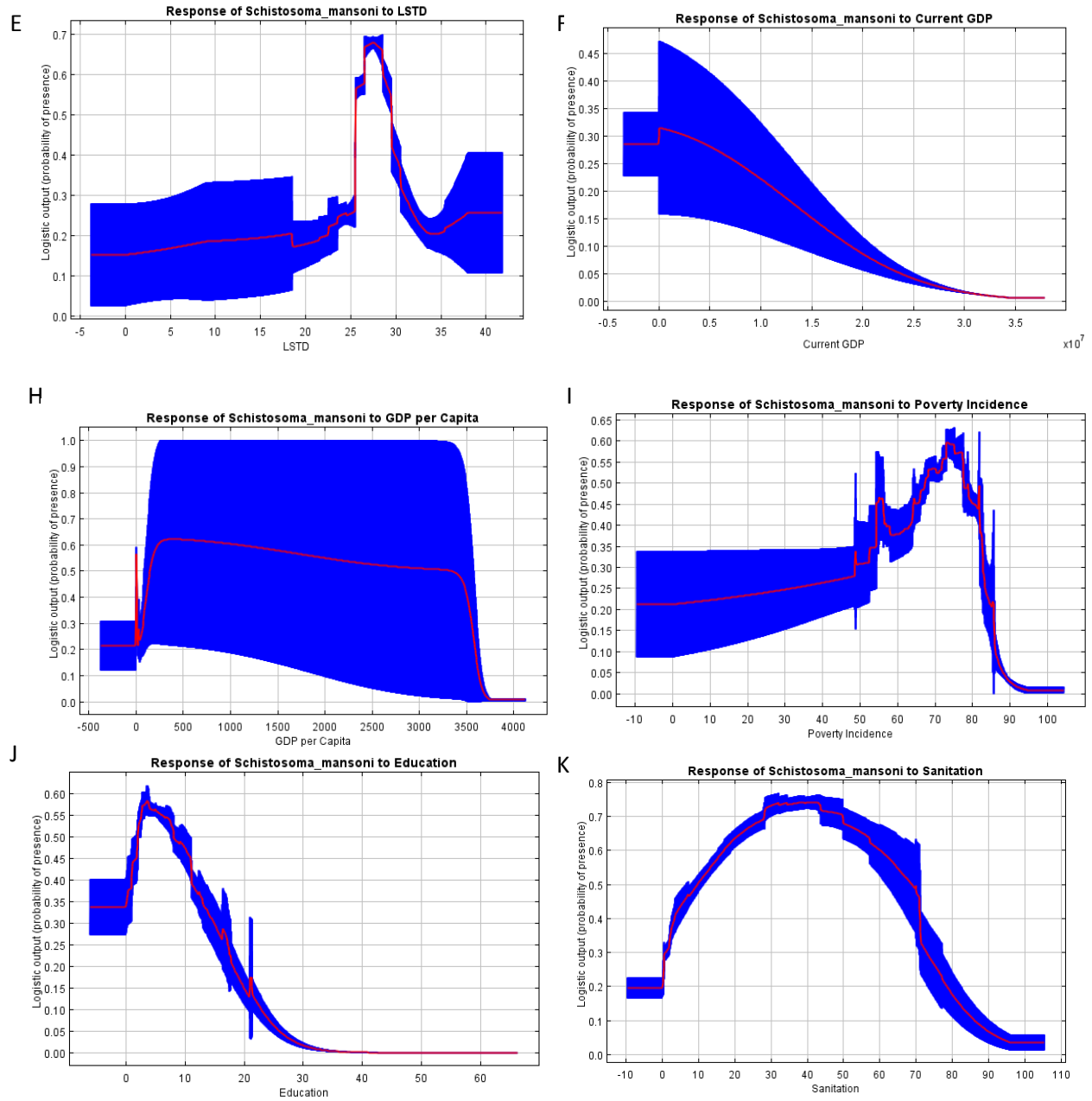


Figure 13: Schistosomiasis MaxEnt response curves. Response curves for the variables related to Chagas presence in Brazil. Red lines are mean values for the 10 model iterations and the blue bars represent ± 1 standard deviation.

(Figure 13 continued)



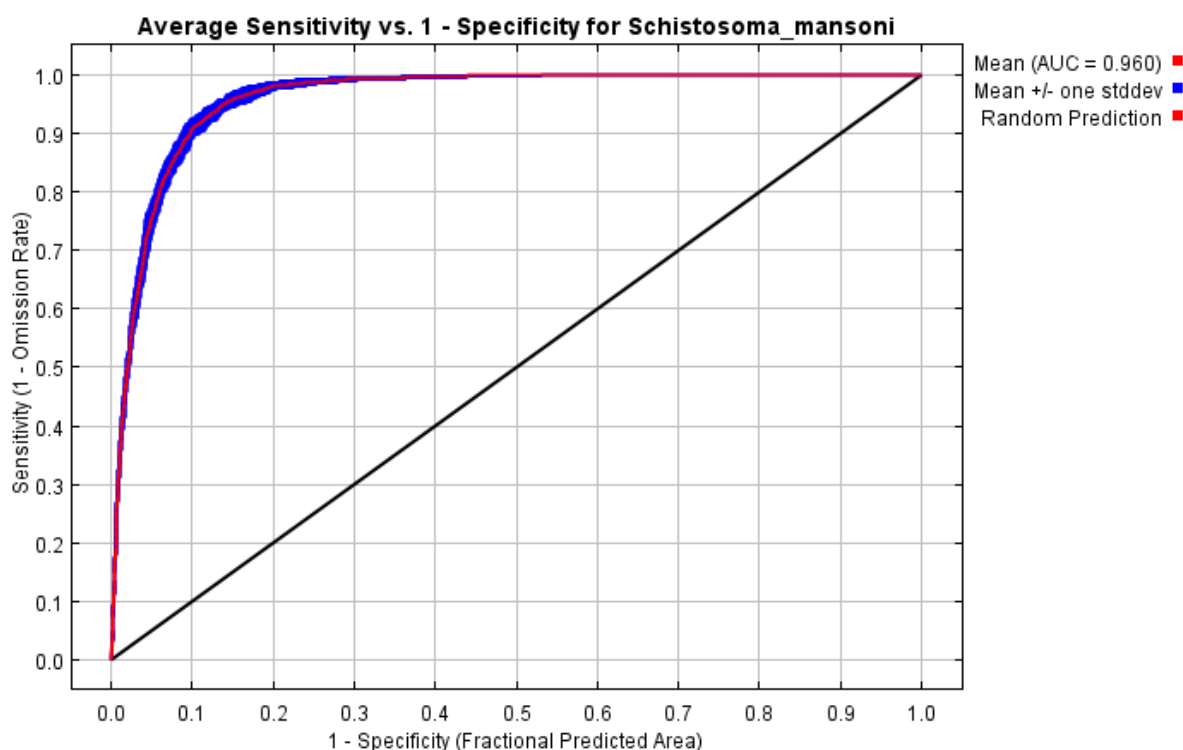


Figure 14: Schistosomiasis MaxEnt area under the curve (AUC). Red line indicates the mean value for 10 MaxEnt runs and blue line indicates ± 1 Standard deviation.

The average AUC for 10 replicate runs of the model was 0.96 with a standard deviation of 0.003 and indicates very good performance by the model (Figure 14). To validate the model, 211 municipalities with high 2010 incidence rates of Schistosomiasis were overlaid on the risk map (Figure 15). These municipalities fell in two regions of Brazil; the northeast (6.6%) and the southeast (93.4%). In the Northeast, 85.71% of the municipalities fell within the high risk area and 7.14% fell within the moderate risk (Figure 15). A single municipality fell in the low risk area (Figure 15). The vast majority of the municipalities with high 2010 incidence rates were in the state of Minas Gerais (175 total municipalities). Of these municipalities falling in the Southeast region, 77.16% fell in the high risk area, 22.34% in the moderate risk area and a single municipality in the low risk area (0.5%) (Figure 15). Thresholds were determined using average “maximum training sensitivity plus specificity logistic threshold” and balance training omission, predicted area and threshold value” which were defined by the model for each model iteration (Cantor 1999, Cramer 2003, Liu 2005, Wei 2011). Thresholds were created from the average values over the ten model iterations. The thresholds were 0.192 and 0.043 for the high and moderate ranges respectively.

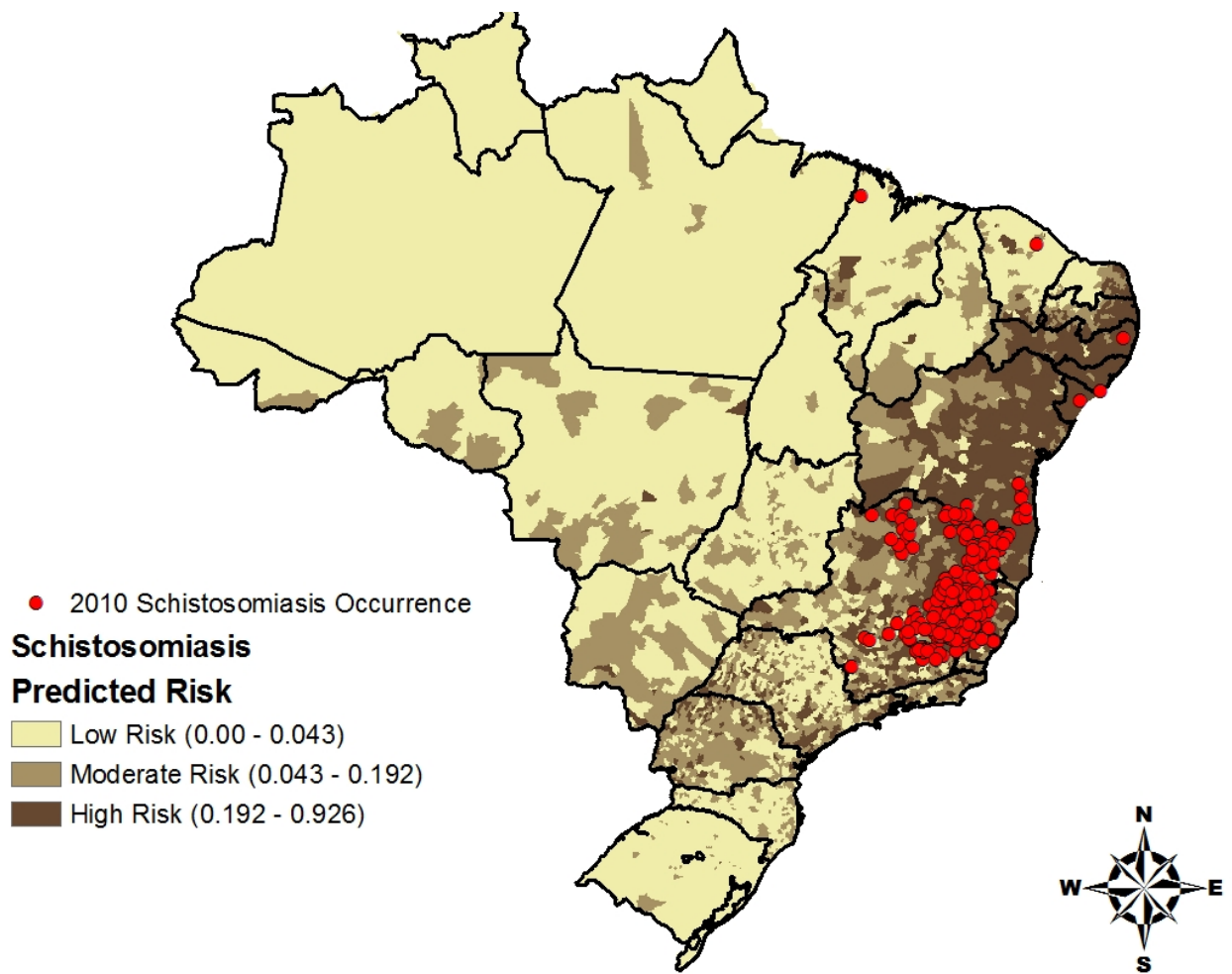


Figure 15: Schistosomiasis risk map. The predicted risk map of schistosomiasis overlaid with 2010 disease occurrence points.

Table 7:
Brazil Regional Schistosomiasis Risk Validation

Brazil Region	High	Medium	Low
Northeast	12 (85.71%)	1 (7.14%)	1 (7.14%)
Southeast	152 (77.16%)	44 (22.34%)	1 (0.5%)

6.2.3 Discussion

Environmentally, there was a relationship between temperature and the snail vector of the disease with both of the main vectors *Biomphalaria glabrata* and *B. straminea* surviving in a temperature range of 12 -40° C (Pfluger 1981). This is consistent with values seen in the response curves of both daytime land surface temperature and June mean temperature. Bavia noted that the disease in Bahia Brazil was clustered around areas with a Mediterranean seasonal rainfall pattern (1999). This same

study also found that the length of the dry season each year influenced where the disease was found with those areas having a shorter dry season also having high disease prevalence (Bavia 1999, Bavia 2001). This is reflected in our study through the importance of precipitation seasonality to the model. The response curve for precipitation seasonality showed multiple peaks and a wide range of values associated with an increased probability of disease which is probably indicative of the different rainfall patterns of the Northeast and Southeast regions. Analyzing these regions separately through MaxEnt may help clarify the relationship of precipitation seasonality to disease in these two distinct regions. The response of the other precipitation parameter, December precipitation is more easily interpreted. An appropriate amount of water is required for the snail vector to survive and breed with relates to the parasites own survival and replication. Heavy seasonal rains can flush out snail populations from their habitat affecting the disease cycle (Jordan 1982, Kvale 1981, Richards 1967).

Schistosomiasis has been related to both lack of sanitation and lack of health education in several previous studies (Barbosa 1966, Doumenge 1987, Gazzinelli 2006) and has been validated as predictive socioeconomic risk factors in this study. Schall highlighted the disparity between those most at risk for the disease and their lack of knowledge about the disease and its snail (2001). Poverty incidence, GDP per capita and current GDP were also indicated as risk factors for the disease in Brazil, though both GDP variables showed a high level of variation. This may mean that while areas endemic for the disease tend to have a low GDP, the variable is not necessarily indicative of the disease throughout the country. The high degree of variation could be reduced by breaking the country into smaller subunits for analysis such as regions. Schistosomiasis is predominantly in the Northeast and Southeast region and predictive power could be increased by only analyzing the disease in those regions where the relationship between the GDP variables may be more clear.

Statistical analysis through multiple linear regression was done first to cull the variables to be used in MaxEnt, and to find and remove multicollinearity within variables that would affect interpretation of the model in MaxEnt. It also helped to identify exactly which variables were important to the model which is not as clear when trying to cull variables by use of MaxEnt alone. Ecological niche models can vary in how they predict disease distributions which each run of a model (Araujo 2005, Wei 2011). To minimize this effect, we averaged the model output based on 10 random replicate data sets. Additionally, we sought to validate the model by based on 2001-2009 data to disease prevalence data from the following year of 2010 to evaluate our model which showed a high predictive power for both regions where high incidence rates were reported for 2010.

This work highlights the important role of socioeconomic factors to schistosomiasis in Brazil in addition to environmental parameters. Knowing that sanitation and lack of health education are repeatedly linked to the disease, governments can better implement effective strategies to by using poverty maps and poverty indicators to address these issues and better control the disease.

6.3 Chagas

6.3.1 Brazil

6.3.1.1 Statistical Analysis

A total of 139 Brazilian municipalities had new case detection rates of at least 1 case per 10,000 population and were used in multiple linear regression modeling. From this preliminary modeling the following environmental variables were identified as being associated with disease; altitude, nighttime land surface temperature, precipitation during the driest period, and February precipitation. Poverty incidence was the best socioeconomic variable selected by regression modeling (Table 8).

TABLE 8:
Final Brazilian Chagas Regression Models

VARIABLE	PARAMETER ESTIMATE	STANDARD ERROR	T VALUE	Pr > t	VARIANCE INFLATION FACTOR	MAXENT PERCENT CONTRIBUTION
ALTITUDE	0.003	0.002	1.31	0.19	1.438	5
PRECIPITATION DURING THE DRIEST MONTH (BIO14)	0.051	0.021	2.48	0.014	1.968	12.6
POVERTY INCIDENCE	0.996	0.242	4.11	<.0001	2.085	3.4
FEBRUARY PRECIPITATION	-0.018	0.006	-2.91	0.004	1.063	67.9
NIGHTTIME LAND SURFACE TEMPERATURE	0.08563	0.03726	5.28	0.0224	N/A	11.1

6.3.1.2 MaxEnt Ecological Niche Modeling Analysis

Socioeconomical and environmental variables were combined in MaxEnt and compared to the city center of the original 139 Brazilian municipalities. Table 8 shows the percent contribution of each variable to the Model and Figure 16 shows the final model.

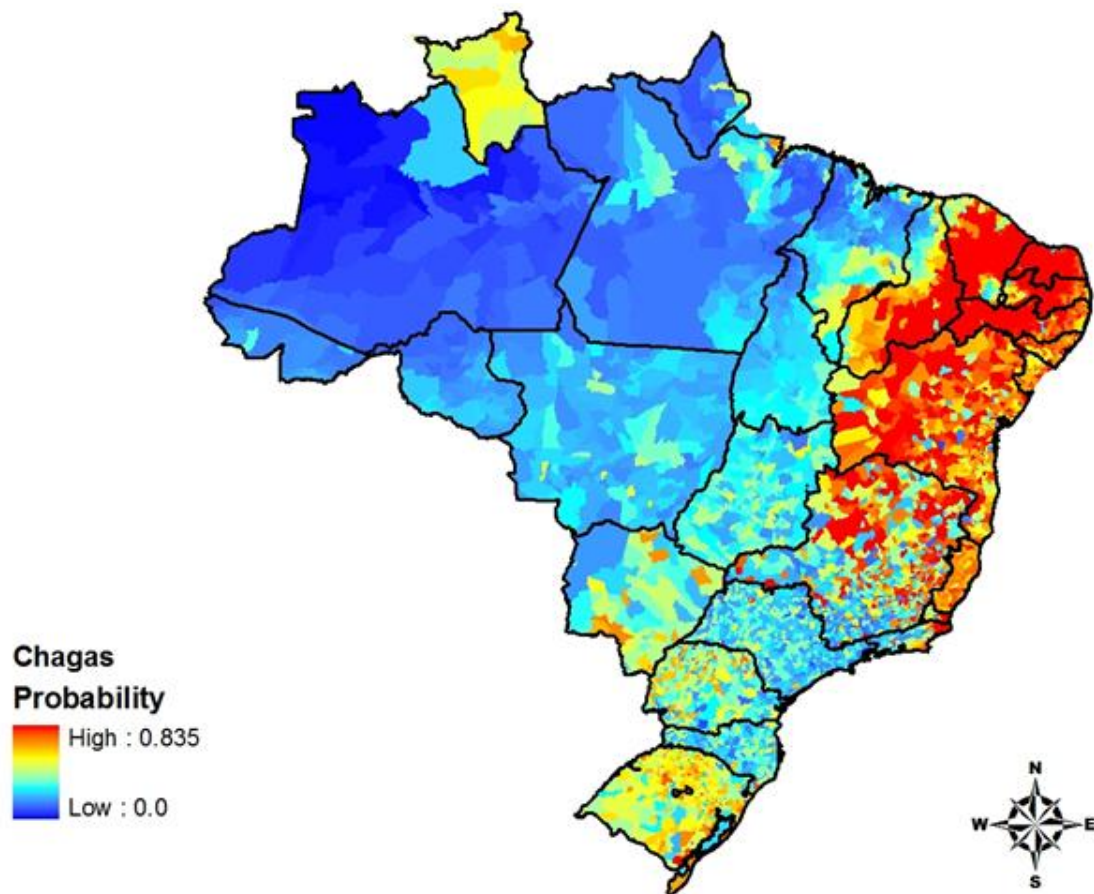
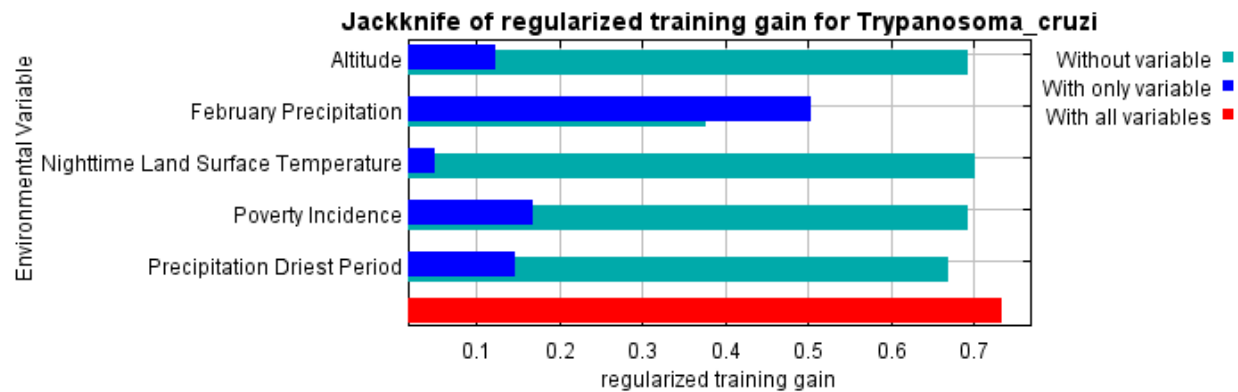


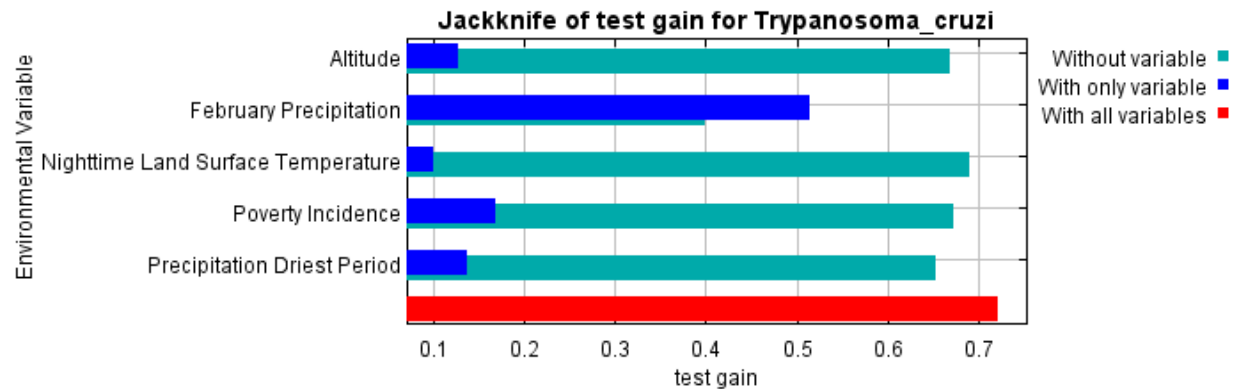
Figure 16: MaxEnt Brazilian Chagas Prediction model. Predictive model showing the distribution probability of Chagas occurrence. Red indicates a higher probability of occurrence, while blue indicates a low probability of occurrence.

The relative importance of each variable to the hyperendemic municipalities was evaluated by jackknife plots of training gain, test gain and area under the curve (AUC) (Figure 17). February precipitation, was the single most important factor influencing the model as demonstrated in both the jackknife analysis and percent contribution (Figure 17 and Table 8). This particular variable had the highest gain when used in isolation and decreased the gain the most when omitted from the model, indicating that it was more informative than the other variables.

A



B



C

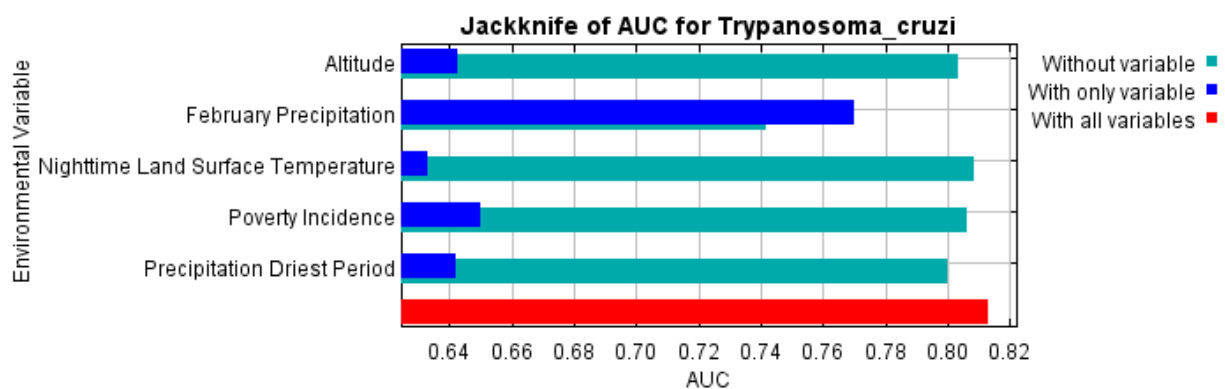


Figure 17: Brazilian Chagas MaxEnt jackknife analysis. Jackknife analysis results of training gain, test gain, and area under the curve (AUC). The blue, light blue and red bars represent results of the model created with each individual variable, all the remaining variables and all variables respectively.

The response curve for altitude revealed a relationship between altitudes above 400 meters above sea level and Chagas disease (Figure 3A). The response curves for both February precipitation and precipitation during the driest month both indicated a relationship between Chagas and lower levels of precipitation.

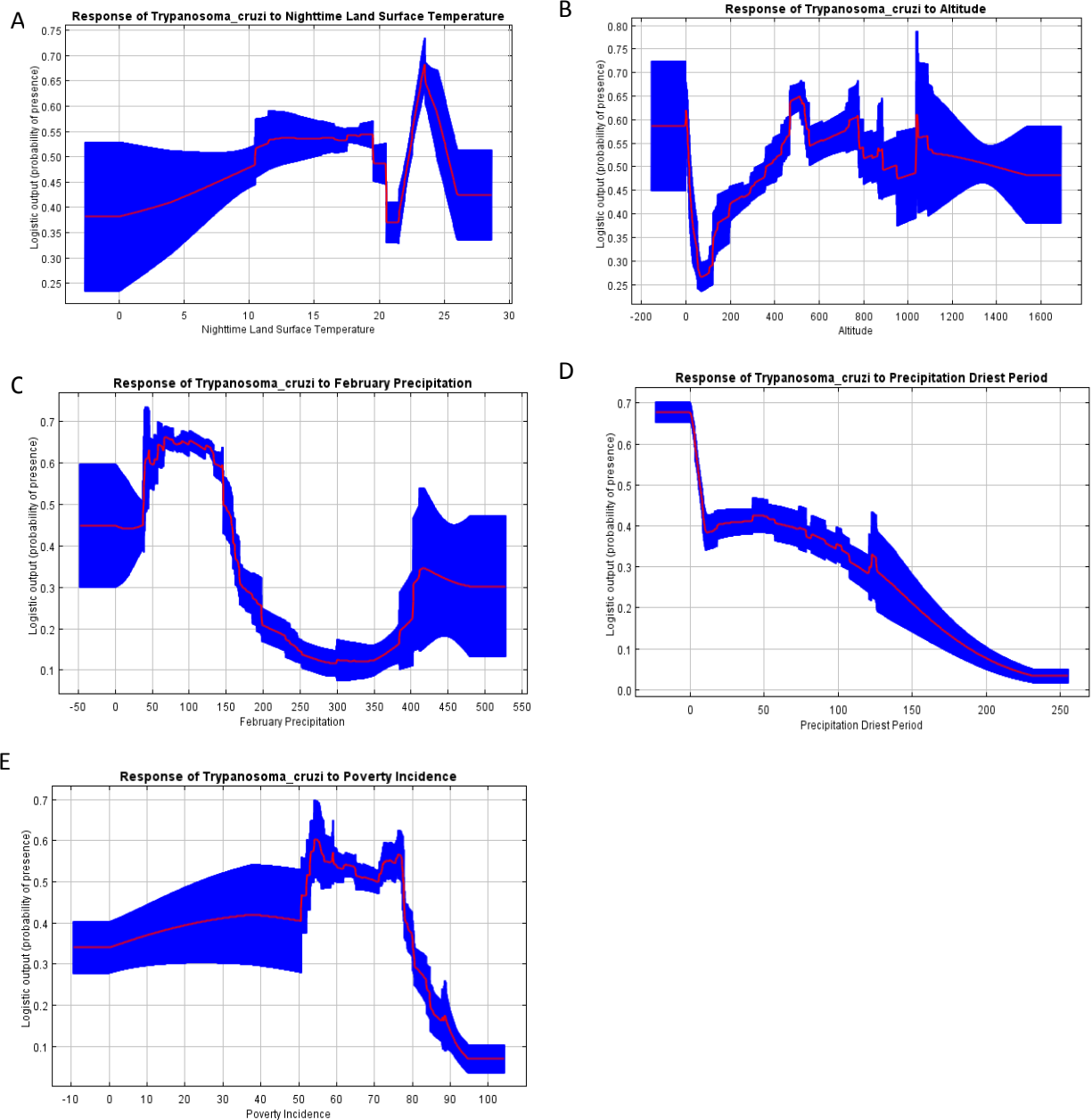


Figure 18: Brazilian Chagas MaxEnt Response Curves. Response curves for the variables related to Chagas presence in Brazil. Red lines are mean values for the 10 model iterations and the blue bars represent ± 1 standard deviation.

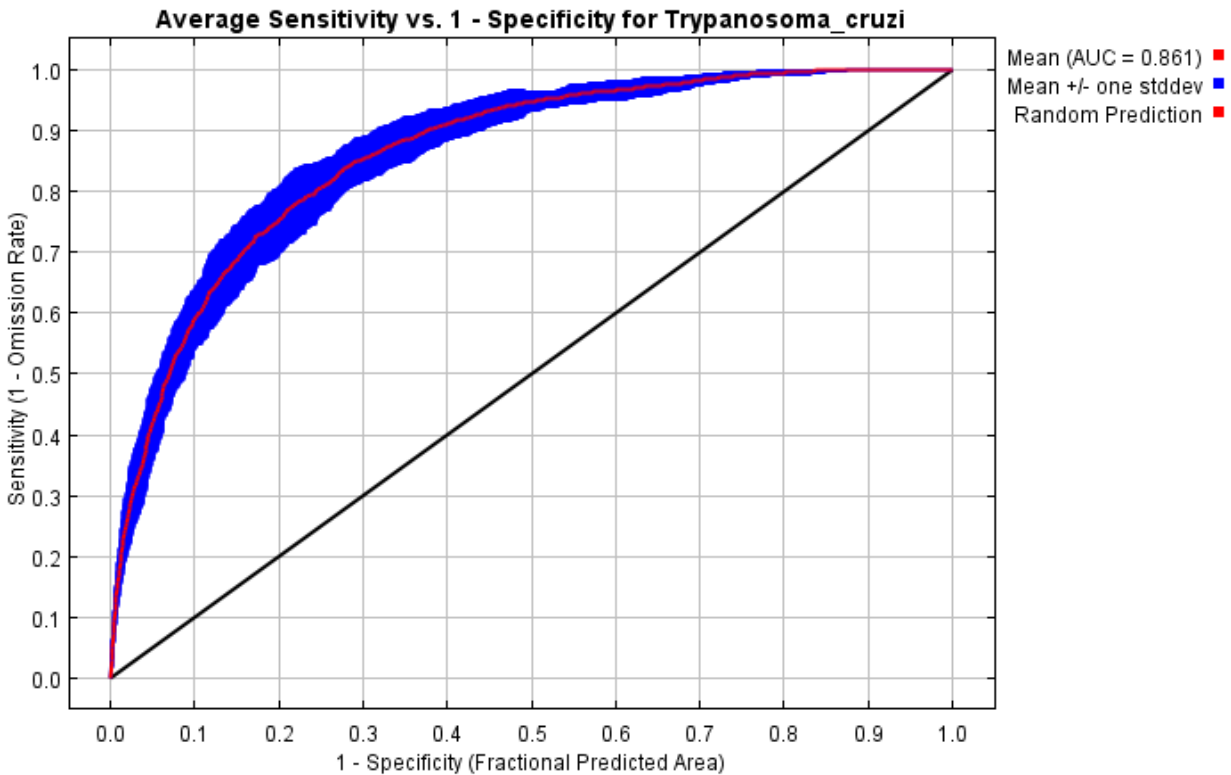


Figure 1: Brazilian MaxEnt area under the curve (AUC). Red line indicates the mean value for 10 model runs and the blue line indicates ± 1 standard deviation.

The average test AUC for the 10 replicate runs of the model developed on SINAN data from 2001-2009 was 0.861 with a standard deviation of 0.02 (Figure 19) which indicates that the model performance was excellent. To validate the model, year 2010 municipal cases of Chagas disease were compiled from SINAN, sorted by mode of transmission, and overlaid on the risk map (Figure 20). Thresholds were determined using average “maximum training sensitivity plus specificity logistic threshold” and balance training omission, predicted area and threshold” over the 10 model iterations (Cantor 1999, Cramer 2003, Liu 2005, Wei 2011). The results gave an upper limit threshold of 0.357 and a lower limit threshold of 0.108. To validate the model, 18 municipalities with vectorial transmitted Chagas cases, 9 municipalities with orally transmitted Chagas and 18 municipalities with Chagas cases of unknown origin were overlaid on the risk map (Figure 20). For analysis, Brazil was divided into 5 regions to determine if the model had different predictive power in the different regions of Brazil. The percentages of municipalities in predicted high, moderate, and low risk areas are listed in Table 9.

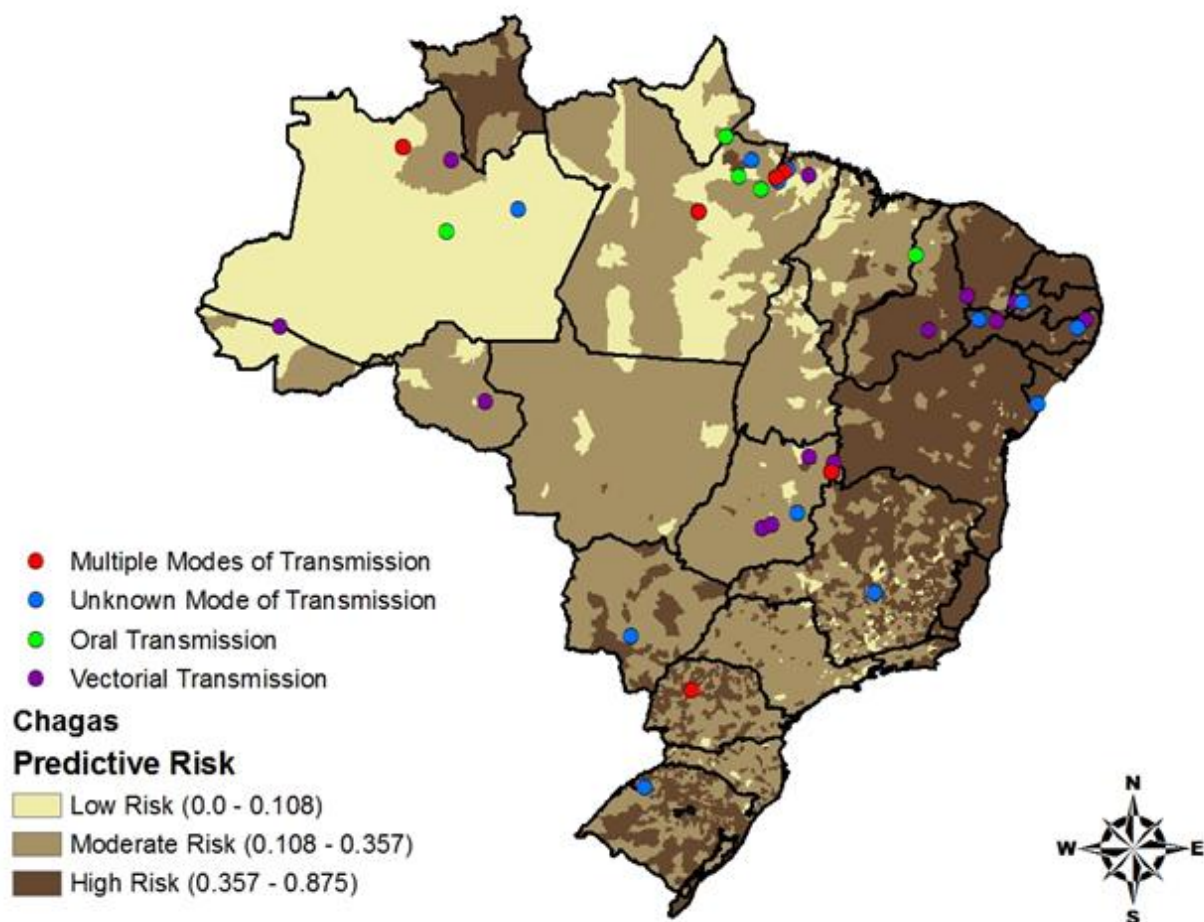


Figure 20: Brazilian Chagas Risk Map. The predicted risk map of Chagas disease overlaid with 2010 Chagas occurrence data differentiated by mode of transmission.

Table 9:
Brazilian Chagas Risk Validation

Mode of Transmission	Region	Low	Medium	High
Vectorial	North	2(28.57%)	4(57.14%)	1(14.29%)
	Northeast	0	1(20%)	4(80%)
	Central West	1(16.67%)	5(83.33%)	0
Oral	North	2 (25%)	3(37.5%)	3(37.5%)
	Northeast	0	1(50%)	1(50%)
Unknown	North	1(14.29%)	4(57.14%)	2(28.57%)
	Northeast	0	0	3(100%)
	Central West	0	3 (75%)	1(25%)
	Southeast	0	0	1(100%)
	South	0	0	1(100%)

6.3.1.3 Discussion

Statistical analysis through multiple linear regression was done as an initial step in model construction to not only cull the variables to be used in MaxEnt, but to also find and remove multicollinearity between variables that would affect interpretation of the model in MaxEnt and clearly identify which variables were important to the model. Ecological niche models can vary in how they statistically predict disease distributions with each individual run of a model (Araujo 2005, Wei 2011). To minimize this effect, we averaged the model output based on 10 random replicate data sets. Additionally, we sought to validate our model, developed using 2001-2009 data, by running it against disease data from the year 2010.

Correlations between Chagas and environmental factors were much clearer than the correlation between socioeconomic factors and disease within our model. This suggests that while the municipality level is sufficient for discrimination based on environmental factors, it does not adequately reflect socioeconomic factors since they can vary greatly within a single municipality. In future studies, socioeconomic data should be collected at a level that more adequately reflects socioeconomic inequalities such as according to census tract.

Traditionally Chagas disease has been considered a problem mainly in the Northeastern region of Brazil. The results reported here clearly indicate that Chagas is emerging in the Northern Amazon region of Brazil as well as the Central Western state of Goiás. Temperature and precipitation parameters were correlated with disease presence particularly in the Northeastern region of the state. Lower predictive power in the Amazon region is probably due to the differences in the disease cycle between these two regions. There is a large amount of variability throughout the country in terms of environmental factors. This variability results in part to the large amount of variability seen in the response curves. A potential way to reduce some of the variability within the model is to reduce the analysis to regions within Brazil. This would also potentially uncover disease patterns for Chagas that may be unique to individual parts of the country such as oral Chagas disease transmission in northern Brazil. Additionally, this could increase the predictive power in areas of Brazil where Chagas is less of a problem, such as the Northern region.

The response curve for altitude favored a relationship between altitudes above 400 meters above sea level and Chagas disease (Figure 3A). In Bolivia vectorial transmission is typically seen at altitudes between 300 and 3,500 meters above sea level (Zuna 1985). The similarities here may indicate similar niche requirements shared by the Bolivian vector *T. infestans* and important Chagas vectors in Brazil such as *T. brasiliensis* and *P. megistus*. Nighttime land surface temperature sharply increased at 23° C and then decreased at 26°C. This closely matches the temperature preferences seen in laboratory for *T. brasiliensis* (Guarneri 2003) and *P. megistus* (Pires 2002). The response curves for both February precipitation and precipitation during the driest month (BIO14) both favored a relationship between Chagas and lower levels of precipitation. Certain species of fungus such as *Beauveria bassiana*, readily kill triatomines and very wet environments such as that in the Amazon may favor these types of fungus (Lecuona 2001, Luz 2004, Lazzarini 2006, Pedrini 2009).

The model showed high predictive power for the North east region without regard to mode of transmission, with the majority of cases falling into the high risk area and no cases falling into the low risk area. Additionally the model had excellent predictive power for the few cases that occurred in the Southeastern and Southern regions, with both of the cases falling into the high risk area. Cases in the Central west region primarily fell in the moderate risk area suggesting model performance had less predictive power than that of the Northeast region. The model had the lowest predictive power in the northern region where most of the cases fell into the moderate risk category, with 28.57% of vectorial cases and 25% of orally transmitted cases falling into low risk areas. Of the four Chagas cases falling into a low risk area, three were from the state of Amazonas and one was from the state of Acre. Both of these states are part of the Amazon region where the insect vectors are considered completely sylvatic. Chagas disease is considered rare in this area, but the number of orally acquire cases has been steadily increasing (Aguilar 2007). A comparison of model performance according to transmission mode revealed similar predictive power across transmission modes, with the notable exception of cases falling in the high risk predicted area for vectorial transmission (27.7%) as compared to oral cases (40%) or unknown transmission cases (50%).

Despite tremendous success at controlling the Chagas disease through the primary vector *T. infestans*, factors are in place to allow reemergence of this disease if control measures and monitoring are not continued. MaxEnt ecological niche modeling provides a useful tool in analyzing the changing distribution of the disease and identifying emerging foci of disease.

6.3.2 Bolivia

6.3.2.1 Statistical Analysis

A total of 99 Bolivian municipalities had a prevalence of 1 or greater and was used in multiple linear regression modeling. Based on this preliminary modeling, the following environmental variables were identified as having a significant major association with disease: altitude, mean diurnal range, isothermality, and May precipitation. Socioeconomic variables selected from regression modeling included adobe wall material, wood floor material, corrugated metal roofing material, non-pumped well water for cooking, piped water for cooking, river water for cooking and subsistence (Table 10).

6.3.2.2 MaxEnt Ecological Niche Modeling Analysis

Socioeconomical and environmental variables were combined in MaxEnt and compared to the city center of the original 99 Bolivian municipalities. Table 3 shows the percent contribution of each variable to the MaxEnt Model. The relative importance of each variable to the hyperendemic municipalities was evaluated by jackknife plots of training gain, test gain and area under the curve (AUC) (Figure 22). Figure 21 shows the final MaxEnt model.

TABLE 10:
Final Bolivian Chagas Regression Models

VARIABLE	PARAMETER ESTIMATE	STANDARD ERROR	T VALUE	Pr> t	VARIANCE INFLATION FACTOR	MAXENT PERCENT CONTRIBUTION
ALTITUDE	-0.00463	0.00151	-3.06	0.0029	5.65469	62.8
MEAN DIURNAL RANGE	0.87671	0.60673	1.44	0.1519	3.27318	2
ISOTHERMALITY	-0.37227	0.2427	-1.53	0.1285	1.96893	2.1
MAY PRECIPITATION	-0.16274	0.05714	-2.85	0.0054	2.011871	18.2
ADOBE WALL MATERIAL	-0.12954	0.03791	-3.42	0.0009	1.42513	1.3
WOOD FLOORS	-1.43683	0.41331	-3.48	0.0008	1.51321	2.6
CORRUGATED METAL ROOF	0.19029	0.04562	4.17	0.0001	2.35358	0.8
PIPED WATER SOURCE	0.08781	0.07290	1.2	0.2315	4.37466	2.4
NON PUMPED WELL WATER SOURCE	0.19447	0.12622	1.54	0.1269	2.38905	4.4
RIVER WATER SOURCE	0.22818	0.07722	2.95	0.004	4.54146	0.7
SUBSISTENCE	0.07746	0.04359	1.78	0.0789	1.26674	2.6

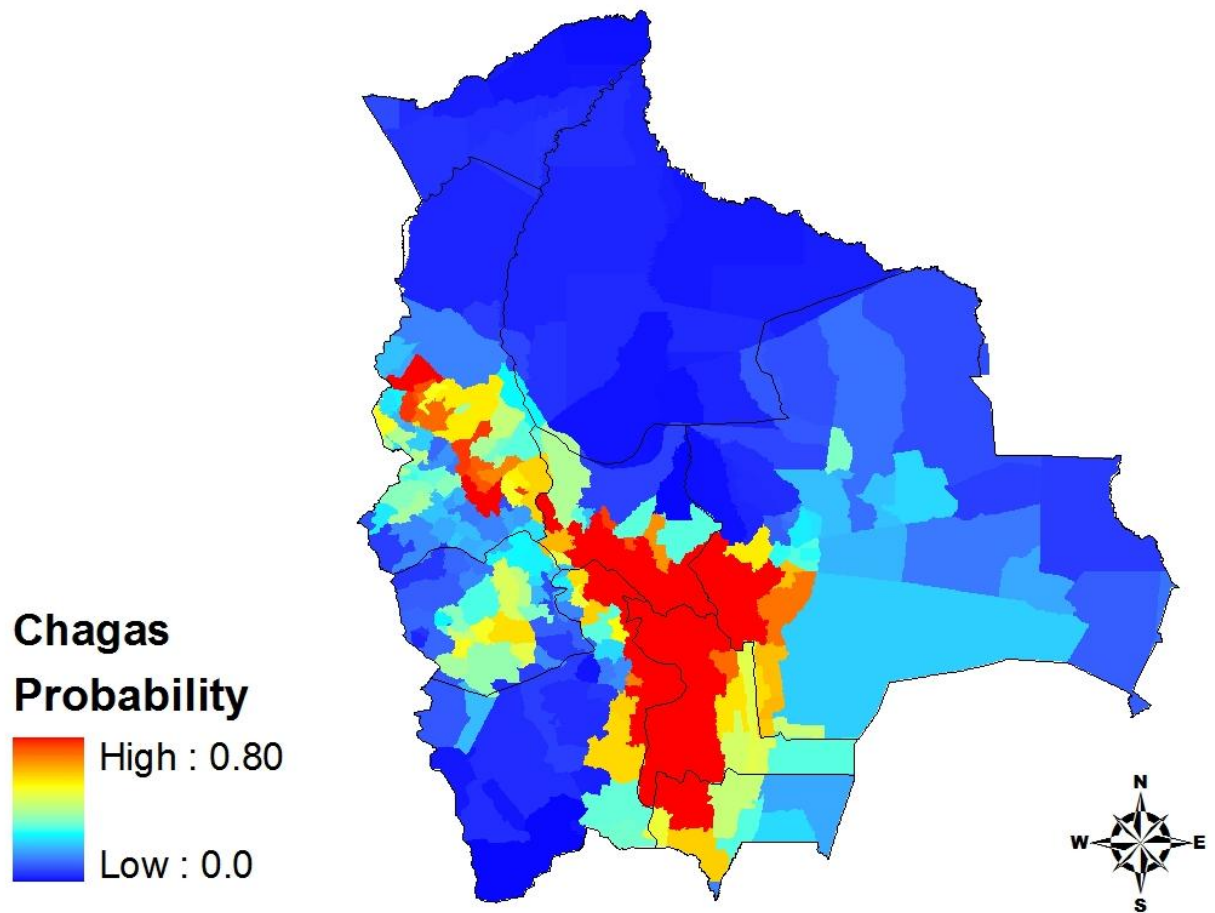


Figure 21. Final MaxEnt model for Chagas in Bolivia.

Altitude was the single most important factor influencing the model as demonstrated by both the jackknife analysis and percent contribution to the model (Figure 22 and Table 3). This particular variable had the highest gain when used in isolation and decreased the gain the most when omitted from the model, indicating that it was more informative than the other variables. The response curve

shows an increase from 500 meters above sea level to 3500 meters above sea level with a peak around 3000 meters above sea level (Figure 23A). Other reports have documented that *Triatoma infestans*, the primary vector in Bolivia, is found with human disease from 330 meters above sea level to 3500 meters above sea level (Borda Pisterna 1985, Guillen 1997, Zuna 1993).

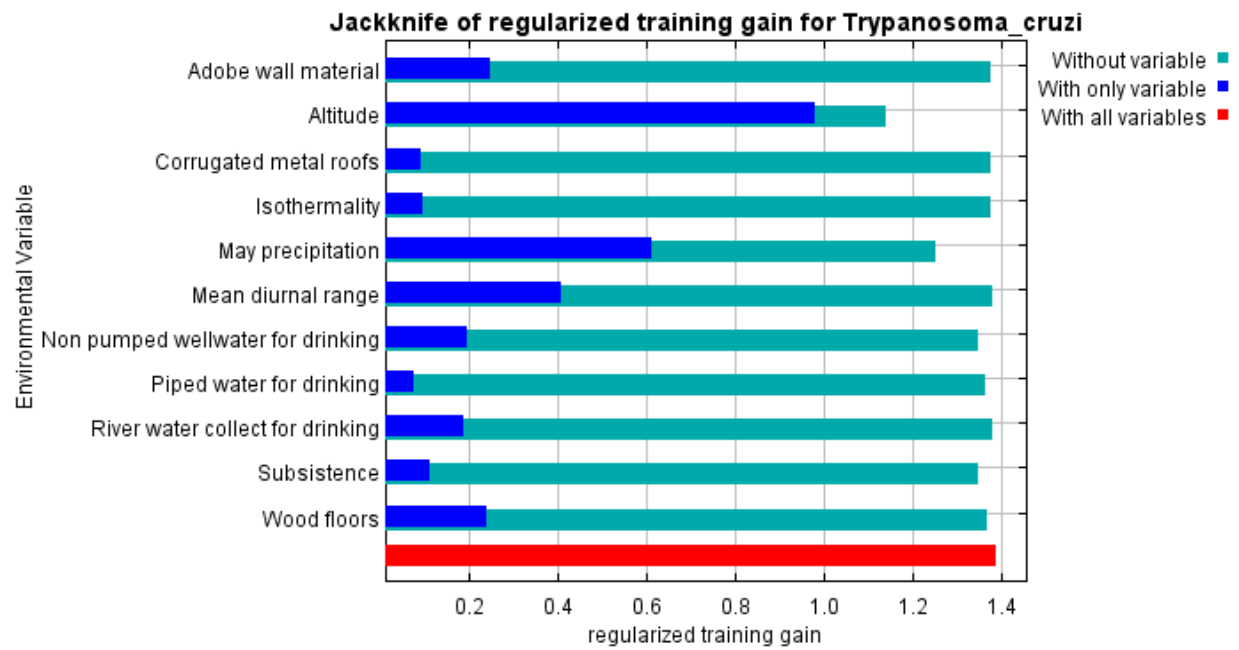
The variable May precipitation had the second largest contribution to the model both in terms of percent contribution and in the jackknife rate of gain analysis (Table 10, Figure 22). The response curve increased with precipitation from 45mm, peaked at 125 mm and then decreased to 250mm (Figure 23C). Borda Pisterna (1985) reported that the vector species, *T. infestans* was found in dry areas where the annual relative humidity does not exceed 60%, corroborating the importance of this variable to the model. Humidity acts as an ecological barrier of this species of triatomine and limits its dispersal and reproductive capacity (Borda Pisterna 1985).

Other environmental factors that had important impact on the model were mean diurnal range and isothermality. Mean diurnal range provides information about the difference in day to night temperature and can be affected by relative humidity and cloudiness. Isothermality relates the mean diurnal range to the annual temperature range and is an indicator of daily to yearly variation in temperature. The response curves for these variables indicate areas with a moderate isothermality and mean diurnal range, which explains why northern Bolivia (high isothermality and low mean diurnal range) and far western Bolivia (low isothermality and high mean diurnal range) were predicted as low risk. Northern Bolivia is found at low elevations with a very hot and humid Amazonian climate. Far western Bolivia is mountainous and has a dry, cold climate.

Adobe wall material and wood floors were the two most influential socioeconomic variables (Figure 22). The response curve for adobe wall materials shows a correlation between the percentage of people with this type of wall material and increased likelihood of disease occurrence. This relationship is well documented in the literature as this type of wall material provides the insect with shelter and access to human bloodmeals (Albarracin-Veizaga 1999, Dias 1999). The presence of corrugated metal roofing material was also correlated with disease (Figure 23F) although this correlation could be due to the predominance of this roof type in endemic areas in Bolivia. In a separate study in Cochabamba, Bolivia it was noted that 86.7% of the houses surveyed have this type of roof (Albarracin-Veizaga 1999). There was a negative correlation between hardwood floors and disease occurrence (Figure 23J). Hardwood flooring in Bolivia is rare and typically associated with higher socioeconomic conditions.

Drinking water sources also contributed to the model though at a lower rate. Non-pumped well water showed a negative relationship with disease occurrence while piped water showed the opposite relationship (Figure 23G and 23H). River water collection for drinking increased with disease occurrence up to 50% and then decreased (Figure 23 I). The relationship between piped drinking water may be related with the movement of the disease from rural areas into urban environments and would therefore be indicative of the increased number of people with piped water access and not necessarily disease. Subsistence or absolute poverty, was also positively related to disease (Figure 23K).

A



B

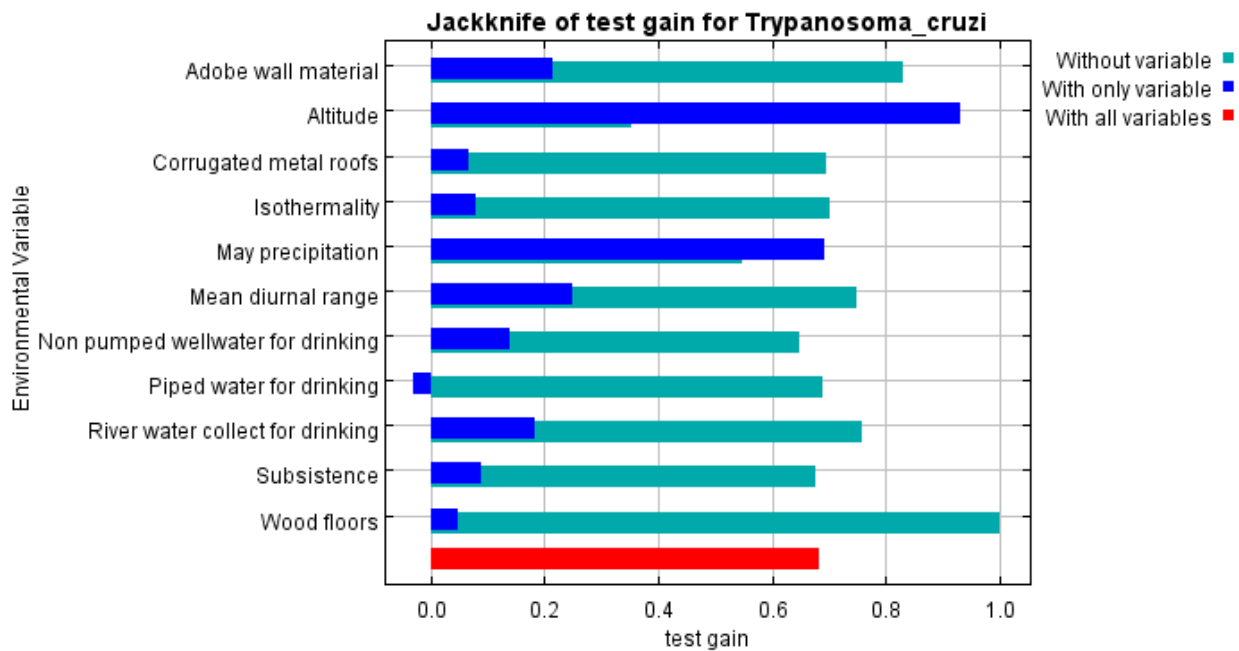


Figure 22 Bolivian Chagas MaxEnt jackknife analysis. Jackknife analysis results of training gain, test gain, and area under the curve (AUC). The blue, light blue and red bars represent results of the model created with each individual variable, all the remaining variables and all variables respectively.

(Figure 22 continued)

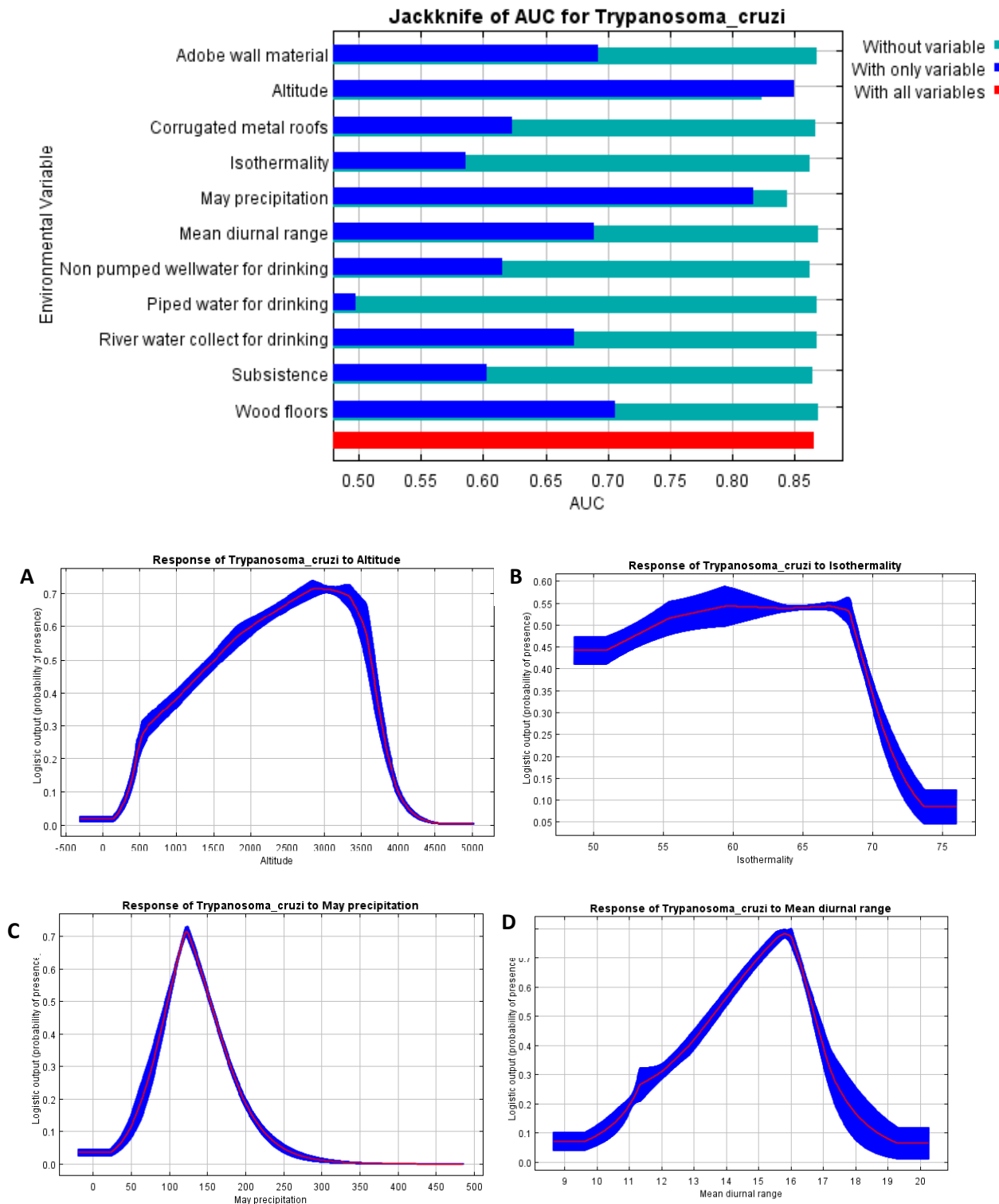
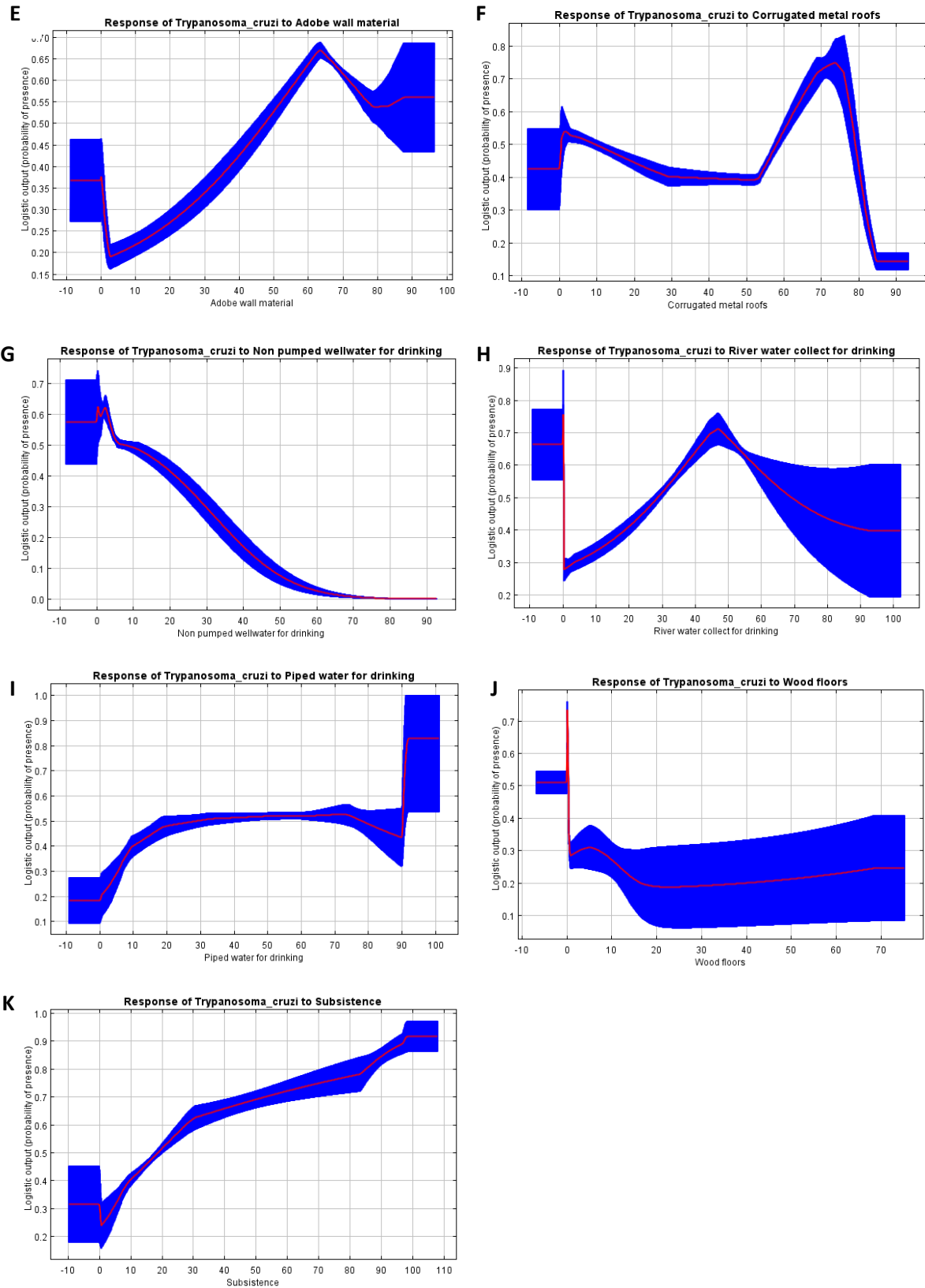


Figure 23 Bolivian Chagas MaxEnt Response Curves. Response curves for the variables related to Chagas presence in Brazil. Red lines are mean values for the 10 model iterations and the blue bars represent ± 1 standard deviation.

(Figure23 continued)



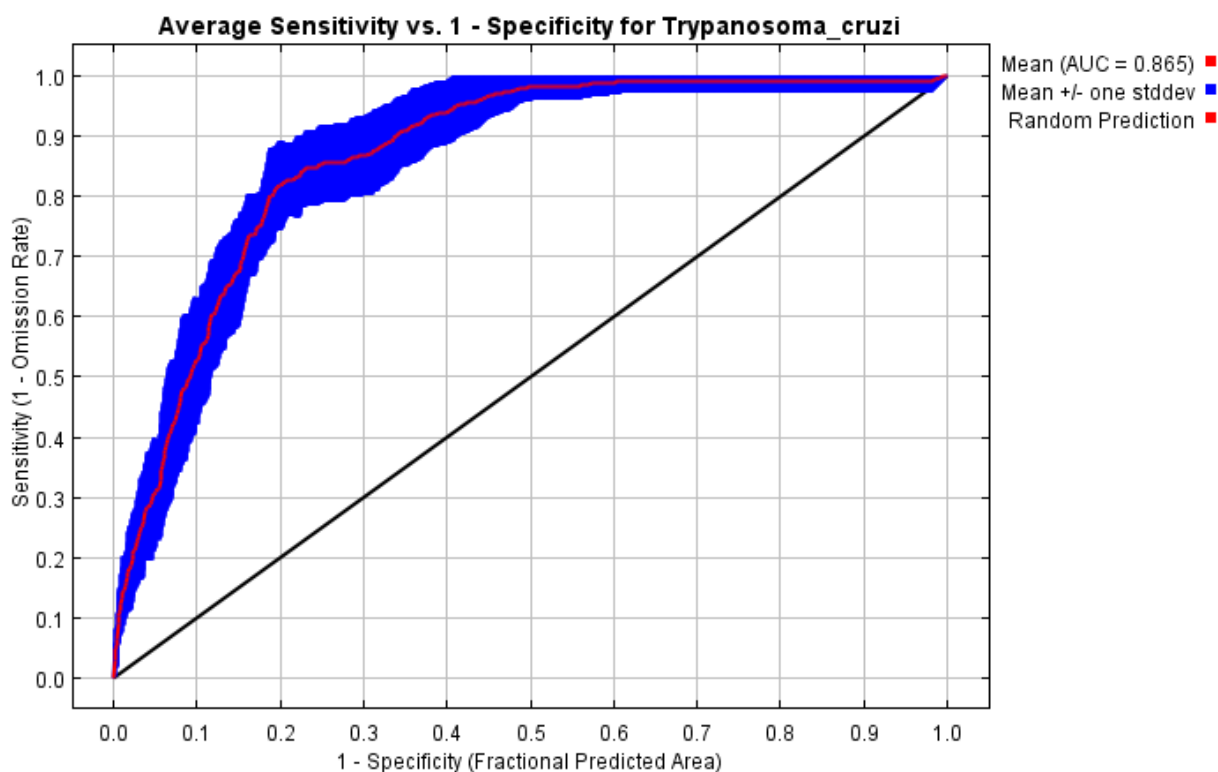


Figure 24 Bolivian Chagas MaxEnt area under the curve (AUC). Red line indicates the mean value for 10 model runs and the blue line indicates ± 1 standard deviation.

The average test AUC for the 10 replicate runs of the final model was 0.865 with a standard deviation of 0.026 (Figure 1). Thresholds were determined using average “maximum training sensitivity plus specificity logistic threshold” and “balance training omission, predicted area and threshold value” over the 10 model iterations (Cantor 1999, Cramer 2003, Liu 2005, Wei 2011). The results gave thresholds of 0.196 and 0.080 respectively.

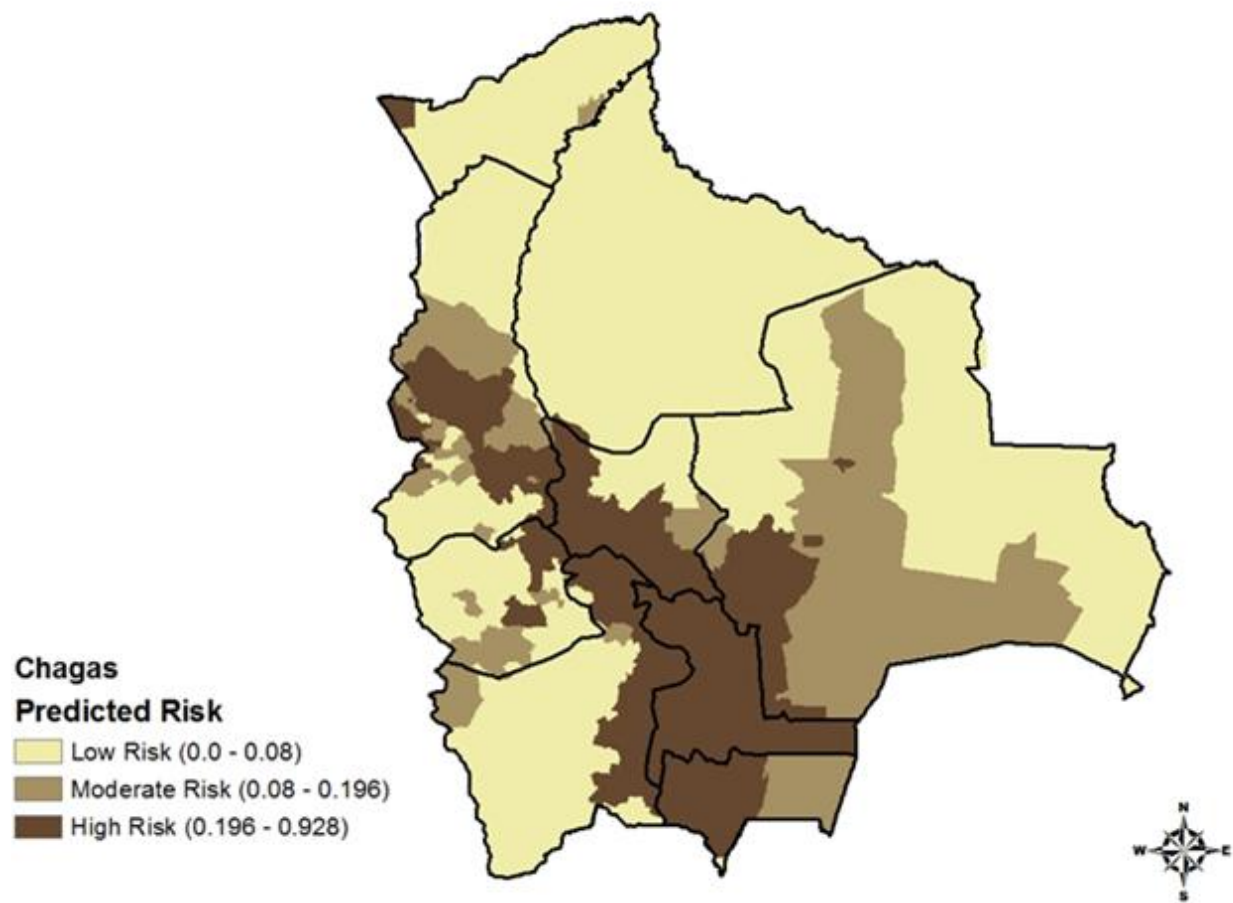


Figure 25 Bolivian Chagas risk map. The predicted risk map of Chagas disease overlaid with 2010 Chagas occurrence data differentiated by mode of transmission.

6.3.2.3 Discussion

Despite surveillance and control programs implemented in the mid 1980s, Chagas disease remains a serious public health problem in Bolivia. In this study, we combined multiple regression and remote sensing techniques to predict Chagas disease risk in Bolivia. The predicted risk map is consistent with literature case reports of the distribution of both the disease and the primary vector *Triatoma infestans* in this country. The model can be further validated by obtaining 2010 disease data and overlaying it on the risk map. Additionally the model could be further strengthened by obtaining prevalence data from a longer time span for statistical and ecological niche modeling analysis. Regression modeling was able to identify a reasonably strong socioeconomic model for the disease, but some of the relationships between variables remain unclear. One solution to clarify the role of socioeconomic factors for this disease would be to obtain measures of these variables at a finer level other than municipality where the level of a single variable can fluctuate rapidly within a single municipality. Census tract level data for socioeconomic variables would give a more accurate estimate of the rate of each variable within a given area and thus would further refine the predictive risk model.

GIS and ENM are useful tools for defining disease distributions and similar areas suitable for disease. This technology provides useful tools that can be used on a larger scale to make health planning decisions on a country wide level. This is particularly important in areas where resources are limited and better resource allocation and planning can have significant impacts on sustained disease disruption.

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Chapter 7: Discussion and Recommendations for Future Work

7.1 Discussion

Studies reported here have demonstrated both the ability and usefulness of MaxEnt in accurately defining risk areas of three neglected tropical diseases – leprosy, Schistosomiasis, and Chagas disease – in a user friendly and easy to understand manner. This makes this method particularly valuable towards disease surveillance, resource planning and allocation, and educational programs. The creation of straightforward risk models can allow better targeting of resources which can reduce wasteful spending and lead to successful disease reduction.

Overall, strong environmental models were created for all of the diseases studied. For both Chagas and Schistosomiasis, many of the environmental variables could be related to environmental ranges favored by the respective vector species. In the case of leprosy, disease occurrence was related to specific environmental conditions. This is particularly interesting because leprosy is not a vector borne disease like the others studied here, and transmission has not been previously demonstrated to be related to environmental determinants.

Results also have suggested the direction of continuing work to refine results and further improve the utility and predictive power of ecological niche models of these three diseases in the future. The role of low socioeconomic status has been demonstrated in virtually all of the neglected tropical diseases, but this study failed to create strong socioeconomic models for leprosy and Chagas disease in Brazil. These studies pointed out the overall weakness in the socioeconomic data at the municipality level. Through generalization of socioeconomic data across a municipality, areas with low socioeconomic characteristics can be masked by areas of high socioeconomic characteristics within the same municipality especially in municipalities containing larger cities. Another potential cause could be related to the quality of socioeconomic data that was acquired for Brazil. Unlike the data acquired for Bolivia, data on housing materials and water source could not be obtained for Brazil and this potentially affected several of the models, especially Chagas where the quality of housing materials has been strongly linked to the presence of the insect vector and thus disease occurrence. Leprosy susceptibility has been linked to specific genetic determinants (Ooi 2001, Scollard 2006), and it is known that household contacts of leprosy patients have a 5 to 10 fold greater risk of contracting the disease (Goulart 2008). In the case of Leprosy, alternative socioeconomic determinants such as household size may be better indicators of leprosy occurrence than the socioeconomic variables used in this study.

In addition to an insufficient level of socioeconomic data, the study results may have been affected by the large amount of variation across the Brazilian study area. This is particularly true with respect to the environmental variables and can be seen in many of the response curves. Reducing the study area to regional level or smaller could help to better demonstrate different disease mechanisms that may be specific to different areas of Brazil. The easiest disease to demonstrate this with would be Chagas in Brazil where different insect vectors are responsible for transmission in different areas of the country. Modes of disease transmission also vary across the country. Oral Chagas transmission is the major cause of disease cases in the Northern Amazon, while vectorial transmission is responsible for

most of the cases in the Central and Northeastern portions of the country. In the case of *Schistosoma mansoni*, different vector snails are associated with disease foci in different areas of Brazil. The disease mechanisms associated with the different disease vectors can more adequately be analyzed through regional studies for both diseases.

7.2 Recommendations for Future Work

Further work should evaluate the accuracy of this modeling technique on a smaller regional scale, ideally modeling unique ecologically similar regions within the country. Within the context of similar ecologic regions, disease data could be separated into one group to create models and a second group to validate the model. Modeling at this smaller spatial scale would reduce the overall variability in modeling parameters to yield increased accuracy. This is increasingly important for vector borne diseases where disease transmission is dependent on different species in separate areas of the country. In the case of Chagas disease, *Triatoma brasiliensis* is the most important vector in the Northeast of the country (), while *Panstrongylus megistus* is more important in the Southern region of Brazil (). In the Northern Amazon region other triatomine species are involved with oral transmission cases. Each species of triatomine requires a separate environmental conditions or niche which would be more adequately reflected by studies limited to the area of each separate vector.

In future studies, a larger more comprehensive database of socioeconomic factors is warranted. Socioeconomic models in Brazil tended to be inadequate and a more comprehensive array of socioeconomic factors may help to strengthen analysis and model building. The scale of municipality data was also a contributing factor to weakness in these models. Socioeconomic data collected at a census level would more accurately reflect socioeconomic conditions in the countries studied and should be used for future work. Further studies using census level socioeconomic data at a smaller regional area of study should strengthen the models for these countries.

The importance of the nine banded armadillo in leprosy transmission has gained momentum with the recent study published by Truman (2011), showing that leprosy isolates from armadillos are genetically similar to isolates from human infections occurring in the Southern United States. This species of armadillo also occurs throughout Brazil and it is reasonable to question the extent, if any, of their role in the disease transmission cycle in the country. Future work needs to address the leprosy infection rate of armadillos throughout Brazil, as well as the genetic similarity or dissimilarity between these animals and human cases in the country.

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