Louisiana State University
LSU Digital Commons

2009

Ecological risk models for visceral leishmaniais [sic] in Bahia, Brazil and diagnosis of Trypanosoma cruzi infection in dogs in south central Louisiana

Prixia Nieto

Louisiana State University and Agricultural and Mechanical College, pnieto1@lsu.edu

Follow this and additional works at: https://digitalcommons.lsu.edu/gradschool_dissertations

Part of the Veterinary Pathology and Pathobiology Commons

ECOLOGICAL RISK MODELS FOR VISCERAL LEISHMANIAIS IN BAHIA,
BRAZIL AND DIAGNOSIS OF *TRYPANOSOMA CRUZI* INFECTION IN DOGS IN
SOUTH CENTRAL LOUISIANA

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
In partial fulfillment of the
Requirements for the degree of
Doctor of Philosophy

In

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

By
Prixia Nieto
DVM., La Salle University, Colombia, 2001
May 2009

ACKNOWLEDGEMENTS

TABLE OF CONTENTS

ABSTRACT

Three predictive models were developed within a geographic information system using earth observing satellite remote sensing (RS), the Genetic Algorithm for Rule-Set Prediction (GARP) and the growing degree day-water budget (GDD-WB) concept to predict the distribution and potential risk of visceral leishmaniasis (VL) in the State of Bahia, Brazil. The objective was to define the environmental suitability of the disease as well as to obtain a deeper understanding of the eco-epidemiology of VL by associating environmental and climatic variables with disease prevalence. The RS, the GARP model and the GDD-WB model, using different analysis approaches and with the same human prevalence database, predicted similar distribution and abundance patterns for the *Lutzomyia longipalpis-Leishmania chagasi* system in Bahia. When applied to the ecological zones of Bahia, all three approaches indicate that the highest VL risk is in the interior region of the state, characterized by a semi-arid and hot climate known as Caatinga, while the risk in the Bahia interior forest and the Cerrado ecological regions is lower. The Bahia coastal forest was predicted to be a low-risk area due to unsuitable conditions for the vector and VL transmission.

In a second study in Louisiana, dogs considered to be at high risk of infection with *Trypanosoma cruzi*, were tested serologically using the indirect fluorescent antibody test (IFAT). Serum samples obtained from a total of 122 dogs from three kennels, and from client dogs from local veterinary practices tested by IFAT revealed a prevalence rate of 22.1%. Fifty randomly selected samples from this group were also tested using two rapid experimental immunochromatographic assays designed as alternative or complementary diagnostic tests for *T. cruzi* infection. Of the fifty samples tested thirteen

animals tested positive using rapid assay A and eleven animals tested positive using rapid assay B. In the same group, 11 animals tested positive by IFAT. The sensitivity of rapid assay A and B were 100%; the specificity of rapid assay A was 95%, and rapid assay B was 100% as compared to the IFAT, the test standard. Clinico-pathological reports revealed that cardiac signs are the main indicators of Chagas disease.

INTRODUCTION

Leishmania spp and *Trypanosoma spp* are related hemoflagellate protozoan parasites (Kingdom: Protista; Subkingdom: Protozoa; Order: Kinetplastida; Family: Trypanosoma-tidae). The two genera, *Leishmania* and *Trypanosoma,* infect both humans and animals. *Leishmania spp* causes leishmaniasis (cutaneous, mucocutaneous, defuse cutaneous and visceral) and is transmitted by the bite of infected female sandflies of the family Phletominae. *Lutzomyia longipalpis* (Lutz and Neiva, 1912) is the most important vector of visceral leishmaniasis (VL) in the new world. *Lu. longipalpis* is the major vector of *Leishmania chagasi*, (Cunha and Chagas, 1937) the most significant etiologic agent in Latin America of VL, a generalized infection of the reticulo-endothelial system. It is the most severe form of leishmaniasis and if left untreated can cause death.

Trypanosoma cruzi causes Chagas diseases, or American trypanosomiasis, and is transmitted among mammalian hosts by insect triatomine vectors of the family Reduviidae. Infection with *T. cruzi* progresses in three consecutive phases; acute, indeterminate and chronic. This disease affects mainly the heart, although serious pulmonary, ascites, hepathomegaly, splenomegaly, megacolon, megaesophagus can occur. The sources of *T. cruzi* infection for humans and animals are by insect bite, ingestion of contaminated bugs, consumption of food or water contaminated with vector feces containing metacyclic trypomastigotes, by contamination of mucus membranes or breaks in the skin with parasites. Other potential routes of infection include congenital transmission, blood transfusion, laboratory accident and organ transplants (Barr et al., 1995; Collins and Kennedy, 1999).

The two genera *Leishmania* and *Trypanosoma* infect humans as well as a variety of rodents, primates, dogs, and other small mammals. Visceral leishmaniasis is

geographically and ecologically widespread, occurring in tropical and subtropical regions on all continents except Australia. Chagas disease, or American trypanosomiasis, occurs through North, Central, and South America.

Leishmaniasis and Chagas are very important zoonotic vector-borne diseases. Leishmaniasis accounts an estimated 1.98 million disability adjusted life-years and 57,000 deaths annually (Reithinger et al., 2001). Chagas disease affects an estimated 13 million people in Latin America (UNICEF/UNDP/World Bank/WHO, 2007). More than 90% of the VL cases reported from Latin America are located in the northeastern part of Brazil.

Public health concerns associated with leishmaniasis and American trypanosomiasis reveal the need for investigation in endemic areas on control, prevention, prediction, treatment and diagnosis of these zoonotic diseases. Information regarding prevalence and geographical distribution of infections is essential for developing and monitoring strategic control measures (Rosypal et al., 2007). Geographic information systems and remote sensing technologies have been applied to a number of public health problems caused by environmentally sensitive diseases including malaria, onchocerciasis, rift valley fever, fascioliasis and African trypanosomiasis (Malone et al., 1995, 2005, 2003). The use of these technologies has permitted the evaluation of spatial and temporal landscape features to stratify regions and define the target populations as well as studying the behavior of the diseases and vectors. In this way information is available for appropriate decisions to be made to implement effective control measurements (Malone et al., 2004). American trypanosomiasis and VL affect mainly populations in rural areas, often in unsanitary housing, and in poorly nourished populations with decreased immunocompetence. Climate change, deforestation, existing agrarian practices,

2

exploitation of the land and increased international travel have contributed to the spread of these two diseases. In the present study we link the prevalence of VL with environmental features that influence the presence of the disease, by creating environmental risk models that predict the distribution and potential risk of transmission of VL in the state of Bahia, Brazil.

For Chagas disease, an increase in cases of *T. cruzi* reported to the Louisiana Animal Disease Diagnostic Laboratory in dogs in south central Louisiana prompted further field investigation. An information database was developed through a seroprevalence study on *T. cruzi* in dogs in south central Louisiana using the indirect immunofluorencence antibody test (IFAT) as the standard diagnostic method to estimate the prevalence of infection in groups of dogs considered to be at high risk of infection. Two experimental, easy to perform, easily read assays were tested as alternative diagnostic or alternative methods that could be used as screening tests for use at veterinary clinics, or in the field, for rapid diagnosis (while confirmatory tests are being processed in a specialized laboratory). Early detection of *T. cruzi* infection may prevent cardiac complications through effective early treatment, especially in the acute phase of the disease. In this study we also describe pathological and clinical findings of natural *T. cruzi* infected dogs in Louisiana. Limited information on the infection in the United States suggests that Chagas disease is commonly undiagnosed, misdiagnosed and/or mistreated. A definite diagnosis of Chagas disease requires a combination of tests (at least 2), testing for cross reactivity with *Leishmania spp* and consideration of the clinical picture and exposure history of tested dogs.

Efforts to control both VL and Chagas disease require focus on the concurrent availability of clinical pathologic evaluations, rapid and reliable non-invasive diagnostic

techniques, accurate prevalence data, and robust epidemiological assessment by computerized systems that can be used for disease data collection and analysis as well as a tool to evaluate vector control (WHO, 1995 -2006).

I.1 References

Barr, S.C., Van Beek, O., Carlisle-Nowak, M.S., Lopez, J.W., Kirchhoff, L.V., Allison, N., Zajac, A., de Lahunta, A., Schlafer, D.H., Crandall, W.T.,1995. *Trypanosoma cruzi* infection in Walker hounds from Virginia. Am. J. Vet. Res.56, 1037-1044.

Collins, C.H., Kennedy, D.A., 1999. Exposure, sources and routes of infection. In: Laboratory-acquired infections: history, incidence, causes and preventions. 4th ed. Oxford, U.K: Butterworth-Heinemann Ltd. pp. 38-53.

Cunha, A.M., Chagas, E., 1937. New species of protozoa of the genus *Leishmania* pathogenic to man *Leishmania chagasi* n. sp previous note. Hospital (Rio de Janeiro) 11: 3-9.

Lutz, A., Neiva, A., 1912. Contribução para o conheciemento das espécies do gênero Phlebotomus existents no Brasil. Mem. Inst. Oswaldo Cruz. 4, 84 –95.

Malone, J.B., Poggi, E., Igualada, F., Sintasath, D., Ghebremeeskel, T., Corbett, J., McCarroll, J., Chinnici, P., Shililiu, J., McNally, K., Downer, R., Perich, M., Ford, R., 2003. Malaria environmental risk assesment in Eritrea. International Geoscience and Remote Sensing Symposium 2003. pp 1000-1003.

Malone, J.B., 2005. Biology-based mapping of vector-borne parasites by Geographic Information Systems and Remote Sensing. Parassitologia.47, 27–50.

Malone, J.B., 1995.The geographic understanding of snail borne disease in endemic areas using satellite surveillance. Mem. Inst. Oswaldo Cruz. 90, 205-209.

Malone, J.B., Soulsby, E.J., Roncalli, R., 2004. History of the World Association for the Advancement of Veterinary Parasitology (WAAVP).Vet. Parasitol. 125, 3-18.

Reithinger, R., Teodoro, U., Davies, CR., 2001. Tropical insecticide treatments to protect Dogs from sandfly vectors of Leishmaniasis. Emerg. Inf. Dis. 7, 872-876.

Rosypal, A.C., Cortes-Vecino, J.A., Gennari, S.M., Dubey, J.P., Tidwell, R.R., Lindsay, D.S., 2007. Serological survey of *Leishmania infantum* and *Trypanosoma cruzi* in dogs from urban areas of Brazil and Colombia. Vet. Parasitol. 149, 172-177.

UNICEF/UNDP/World Bank/WHO. TDR Web site. Tropical disease research: progress 2003-2004. Special Programme for Research & Training in Tropical Diseases. Seventeenth Programme Report. Available at: http://www.who.int/tdr/publications/publications/pr17.htm. Acceded Sep 14, 2007.

World Health Organization. Executive Board 118th Session. Provisional agenda Item 5.1 Geneva, Switzerland: World Health Organization May, 2006.

World Health Organization. Report of the second WHO meeting on emerging Infectious diseases. Document WHO/CDS/BVI95.2. Geneva, Switzerland: World Health Organization January 1995.

CHAPTER 1 REMOTE SENSING AND ECOLOGICAL NICHE MODELS TO PREDICT VISCERAL LEISHMANIASIS IN THE STATE OF BAHIA, BRAZIL

1.1 Visceral Leishmaniasis

In tropical America, visceral leishmaniasis (VL) is mainly caused by *Leishmania chagasi,* an intracellular protozoan that causes a chronic infectious disease characterized by weight loss, cough, fever, diarrhea, hepatosplenomegaly, and lethargy (Arias et al,. 1996). *L. chagasi* is transmitted from one mammalian host to another by the bite of a sandfly that has fed on an infected human or animal. Occasional non-vector transmission has also been reported including congenitally, and through blood transfusions and organ transplants (Low et al., 1926; Elamin et al., 1992; Boehme et al., 2006).   In the Americas, the principal sandfly vector is *Lu. longipalpis* (Lainson et al., 1978).

Because of a complex array of environmental and social factors, an increasing number of new and reemerging infectious diseases are being recognized in both industrialized and developing countries in the Americas (WHO, 1995). Cholera, plague, AIDS, dengue hemorrhagic fever and urban/periurban visceral leishmaniasis are examples of such new and reemerging diseases in the region. Exploitation of new areas for human settlement and agriculture increases the likelihood that new infectious diseases will emerge. Visceral leishmaniasis was previously known as a rural disease, but large outbreaks and epidemics of VL have recently been reported in large cities in Brazil because of the favorable epidemiologic conditions associated with the reduction of the natural ecologic space ordinarily occupied by this zoonotic parasite (Arias et al., 1996).

1.1.1 History and Geographical Distribution

Leishmaniasis has a long history. Designs on pre-Colombian pottery and the existence of thousand-year-old skulls with evidence of leishmaniasis prove that the

disease has been present in the Americas for a long time. It is known to have been present in Africa and India since at least the mid-eighteen century (Allison, 1993). Human dwellings have been evolving in various forms in Latin America ever since people first colonized central and South America some 12,000 years ago. It has been hypothesized that one of the reasons the Inca civilization built its cities so high was to escape sandflies and other haematophagous insects (Shaw, 2002). Visceral leishmaniasis, also known as calazar or Kala azar, was first described in the western hemisphere in 1913 by Migone in Paraguay (Deane, 1956). This disease exists in many other countries, with major concentrations typically found semi arid regions of the world. The spread of the disease to urban settings has become a complicating factor in the control of VL. At present, 90% of the cases of VL cases in the world are Bangladesh, India, Nepal, Sudan and Brazil. India has the biggest focus of VL in the world (Daba, 2002). In the Americas VL is widely distributed from the southern part of Mexico to northern part of Argentina (Grimaldi et al., 1989). In Latin America VL causes more than 16,000 new clinical infections annually and fatalities are common among children (Thompson et al., 2002). Guyana, Paraguay, Bolivia, Argentina, Colombia, Venezuela and Brazil are the countries in South America where the disease is endemic, although more than 90% of VL cases reported in South America have occurred in Brazil (Grimaldi et al., 1989). In Brazil, VL is endemic in the northeastern part of the country, particularly in the states of Bahia, Piauí, Maranhão and Ceará (Mutebi et al., 1999), and it sporadically occurs in the southeastern region and in the lower Amazon basin.

1. 1. 1.1 Visceral Leishmaniasis in the State of Bahia, Brazil

The beginning of the devastation of the Atlantic forest dates from 1500, at the start of the Brazilian colonization by the Portuguese. The agricultural expansion, urban

7

occupation and industrial development in the state of Bahia, up until the 20th century, caused the diminution of vegetation resources. Human intervention in natural environments for extraction of wood, for the cultivation of cocoa plant *Theobroma cacao,* and for use as pasture has intensified in the last few decades, radically modifying the vegetation cover. In the same way the Caatinga brushlands have also suffered accentuated degradation. These environmental alterations caused modifications in the vegetation pattern, changes in the composition of sandfly fauna and spread of leishmaniasis (Dias-Lima., 2003). In the first description in America of VL by Penna (1934), the geographical distribution of the disease in the state of Bahia was limited to the central island plateau "Chapada Dimantina" between 10 and 15°S and 40 and 43°W. This early affected area had a hot and dry climate and about 550 mm of annual rainfall. The vegetation was predominantly xerophilous (plant adapted for life with a limited supply of water). Visceral leishmaniasis did not occur in the humid zones with broad leaf forest or in Atlantic coastal areas with tropical littoral forest. More recently, the disease has since been spreading rapidly through the state even reaching localities in the coastal zone and in the periphery of big cities where previous ecological conditions were inadequate to support the life cycle (Sherlock, 1996). In several Brazilian cities, canine visceral leishmaniasis has become a serious zoonotic problem, and in localities where the diseases are endemic, domestic dogs represent the main reservoir host for *Leishmania* infection Courtenay et al., 2002.

1. 1. 2 Epidemiology

Transmission of leishmaniasis is highly dependent on ecology, and, consequently, the conditions under which humans become infected vary considerably in time and space. Many of the leishmaniasis are zoonosis, and the intrusion of humans into a sylvatic cycle

may have resulted in a greater exposure to sandflies that are part of the sylvatic cycle and, hence, in a higher risk of infection. In large parts of both the Old and New World, transmission can be domestic or peridomestic. In many villages and cities of various countries, the infection rate has been reported to be highest among people living at the edge of natural foci (e.g., forest, deserts), close to the sylvatic cycle (WHO, 1990). Epidemiological studies of leishmaniasis in the New World have revealed that the genus *Leishmania*, (protozoa: Trypanosomatidae) includes a number of different species that infect a wide variety of mammalians hosts. Some species are associated with disease in humans, but others appear to be restricted to lower orders of mammals, such as rodents and edentates (Grimaldi, 1989).

Fig. 1. Geographic distribution of Old and New World Visceral Leishmaniasis.
Data source: www.vet.uga.edu/.../leishmania/Eng/Leish04.htm access date: 11-16-08

1.1.3 *Leishmania* Species

Leishmania spp. are protozoa belonging to the order kinetoplastida and the family Trypanosomatidae (Daba, 2002). Until recently, the taxonomy of New World *Leishmania* was based largely on the clinical and epidemiologic features of the disease produced in

humans and on the biological characteristics of the parasites in laboratory animals and sandflies. Two main forms of disease have been generally recognized, visceral leishmaniasis and tegumental leishmaniasis. The latter form can be further characterized by clinical appearance as cutaneous, mucocutaneous or diffuse forms.

This system of classification has now been supplemented by a variety of biochemical and immunologic methods. Newer molecular criteria define intrinsic characteristics of the parasites themselves that are not modified or obscured by host or environmental factors (Grimaldi et al., 1989). Quantitative comparisons of nDNA fragment patterns indicate that *Le. chagasi* (main etiologic agent of American visceral leishmaniasis) and *Le. infantum* are very closely related and may actually represent the same species. There is increasing evidence that *Le. chagasi* may represent strains of *Le. infantum* that were introduced into the new world by earlier human migrants or their dogs from the Mediterranean region (Killick-Kendrick et al., 1987).

The ecological changes that have affected the distribution of vectors and reservoirs are natural phenomena that have occurred during the past 80 million years, the estimated time span of evolution of *Leishmania* (Fernandes et al., 1993; Shaw, 1997) from a single ancestor that begun with bifurcation into two major groups known today as the subgenera *Le. (leishmania)* and *Le.(viannia).* Old world visceral leishmaniasis is caused by the parasite *Le. donovani* in India; whereas *Le. major* was reported in Israel and *Le. infantum* in the Mediterranean (Grimaldi et al., 1993). Although there are several species of *Leishmania* causing different pathologies (Table 1), in the new world the etiological agent of visceral leishmaniasis is principally *Le. chagasi* (WHO, 1990).

1. 1. 4 The *Leishmania* Life Cycle

Leishmania spp have a host life cycle involving an invertebrate host, the sandfly vector, and a vertebrate mammal host. Parasites of the genus *Leishmania* exhibit, during their life cycle, two well-known forms: 1) the flagellated and motile promastigote form, which multiplies in the gut of phlebotomine sandfly vectors, and 2) the intracellular non-motile amastigote form normally found within macrophage (phagolysosomes) of the vertebrate host (Killick-Kendrick., 1990). There are approximately 70 different species of sandfly vectors for the three forms of the disease complex, with more than 20 recognized *Leishmania* species that serve as etiological agents. The parasite tends to colonize different sections of the fly's gut so that the parasite is classified as: 1) suprapylorian (thoracic and abdominal mid gut), 2) peripylorian (abdominal gut and pylorus), or 3) hypopylarian (hind gut). In recent years it has become increasingly clear that the behaviour and life cycles of different species of *Leishmania* in the invertebrate host are not uniform, that defined sequences of morphological types occur within the vector and that life cycle development in the gut sections suprapylaria and peripylaria vary as to the initial sites of establishment in the sandfly (Killick-Kendrick and Peters., 1987). Behavior of the parasite in the sandfly's gut also differs somewhat among the different *Leishmania* species (Killick-Kendrick., 1977). Lainson and Shaw (1979) introduced the concept of the sections hypopylaria, suprapylaria and perypilaria to separate biologically dissimilar groups of *Leishmania* parasites and introduce a sub-generic classification based on differences in the site of development in the sandfly and other biological criteria.

It is considered that all the members of genus *Phlebotomus* are vectors for leishmanial parasites, since their gut environment is favorable for the survival of all *Leishmania* parasites (Daba et al., 2002).

11

Table1. The Neotropical *Leishmania* species found in South America and their pathologies. The data in this table are taken from review articles by Grimaldi et al., 1989; Lainson and Shaw, 1979; Shaw., 1997. CL: cutaneous leishmaniasis. MCL: mucocutaneous leishmaniasis. DCL: diffuse cutaneous leishmaniasis. VL: visceral leishmaniasis. Source: Shaw JJ. "New world Leishmaniasis: The ecology of leishmaniasis and the diversity of Leishmanial species in Central and South America, In: World class parasites, Vol. 4. *Leishmania*, Ed. J.P. Farrell, 2002.

Country	Leishmania Species	Pathology
Argentina	*Le. (L.) infantum chagasi*	VL
	Le. (V.) braziliensis	CL
Bolivia	*Le. (L.) amazonensis*	CL, DCL
	Le. (L.) infantum chagasi	VL
	Le. (L.) lainsoni	CL
	Le .(V.) braziliensis	CL, MCL
Brazil	*Le. (L.) amazoniensis*	CL, DCL, MCL, VL
	Le. (L.) forattinni	No records
	Le. (L.) infantum chagasi	VL, CL
	Le. (L.) major-like	CL
	Le. (V.) braziliensis	CL, MCL
	Le. (V.) guyanensis	CL, MCL
	Le. (V.) lainsoni	CL
	Le.(V.) naiffi	CL
	Le. (V.) shawi	CL
	Le. deanes	No records
		No records
	Le *. enrietti*	
Colombia	*Le. (L.) amazonensis*	CL, DCL
	Le. (L.) infantum chagasi	VL
	Le. (L.) mexicana	CL, DCL
	Le. (V.) brasiliensis	CL, MCL
	Le. (V.) guyanensis	CL
	Le. (V.) panamensis	CL, MCL
	Le. colombiensis	CL
Ecuador	*Le. (L.) major like*	CL
	Le. (L.) mexicana	CL
	Le. (V.) brasiliensis	CL, MCL
	Le. equatoriensis	No records
French Guyana	*Le. (L.) amazonensis*	CL, DCL
	Le. (V.) braziliensis	CL, MCL
	Le. (V.) guyanensis	CL
	Le. (V.) naiffi	CL

Table 1 Continued

Guyana	*Le. (V.) guyanensis*	CL
Paraguay	*Le. (L.) amazonensis*	CL, DCL
	eL. (L.) infantum chagasi	VL
	Le. (L.) major like	CL
Peru	*Le. (L.) amazonensis*	DCL
	Le. (V.) braziliensis	CL, MCL
	Le. (V.) guyanensis	CL
	Le. (V.) lainsoni	CL
	Le. (V.) peruviana	CL
	Le. (V.) braziliensis	CL, MCL
	Le. (V.) peruviana	
	Le. colombiensis	CL
Suriname	*Leishmania sp.*	CL
Venezuela	*Le. (L.) infantum chagasi*	VL
	Le. (L.) garnhami	CL
	Le. (L.) pifanoi	CL, DML
	Le. (L.) venezuelensis	CL
	Le. (V.) braziliensis/	CL, MCL
		CL
	Le. (V.) guyanensis	VL
	Le. colombiensis	

1. 1. 4.1 Life Cycle in the Mammalian Host

The infection process in the mammalian host commences with transmission of several hundred metacyclic promastigotes into the host dermis by a female infected sandfly while taking a blood meal. When blood feeding, sandflies inject saliva that has a variety of compounds that are vasodilators, anti-inflammatory, anti-histaminic which together enable the insect to feed, minimizing the perception of the vertebrate host and hemostasis (Titus and Ribeiro., 1990). It has been shown that the salivary glands of the sandfly contain potent inflammatory agents, which enhance the infectivity of promastigotes. Finally, infected macrophages have been shown to produce colony-stimulating factors, which stimulate precursor cells, thereby providing new target cells (monocytes, histiocytes and macrophages) for the parasite to infect (WHO, 1990). It has recently been reported that, in the transition from the sandfly to the mammalian host,

promastigotes face two major environmental changes, a temperature shift to 35-37°C and a pH change to 5 (Antoine et al., 1998). Walters et al., 1993 stated that the organisms detect the change in the new environment and transform into the obligatory intracellular amastigote, with the loss of the flagellum and reduction in size. Spread of the infection within the host takes place when heavily parasitized cells calls rupture and infect other macrophages, or when infected macrophages divide and share their parasites among the daughter cells. In this way, the parasites preferentially infect macrophage-rich organs like the spleen and bone marrow. The amastigotes return to the dermis via macrophages in the blood where long-lived mononuclear phagocytes migrate into affected tissues to replace other macrophages (Walters et al., 1993).

1.1.4.2 Life Cycle in the Sandfly

The sandfly gut is an environment specialized for blood digestion that serves as the location where *Leishmania* parasites replicate and develop into the infective metacyclic promastigote form that invades the vertebrate host (Tang et al., 1998). Sandflies have mouthparts adapted to cutting the skin of the vertebrate host, forming a tiny pool of blood which is sucked up into the midgut by a muscular pharynx. If the amastigotes of *Leishmania* are in the skin (dermis) or peripheral blood, they are taken up with the blood meal and, in the stomach of the fly, liberated amastigotes parasites elongate to form promastigotes. Depending on the species, the promastigotes move to the fore or midgut, where multiplication occurs. Later the promastigotes move cranially to the esophagus where the parasites reside until the vector feeds on an animal and the parasites invade the host, multiply in it and complete the life cycle (Bates, 1997).

14
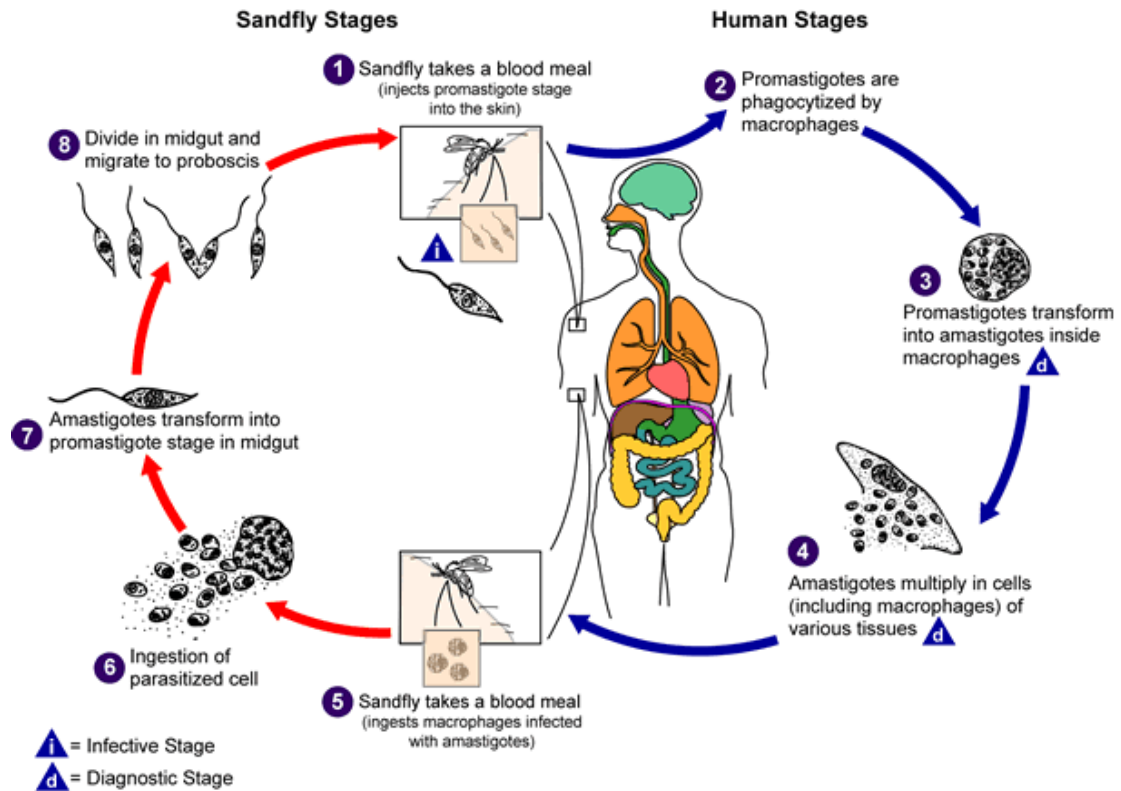
Fig. 2. *Leishmania* life cycle. © CDC's web site for laboratory identification of parasites. Leishmaniasis is transmitted by the bite of female phlebotomine sandflies. The sandflies inject the infective stage, promastigotes, during blood meals❶. Promastigotes that reach the puncture wound are phagocytized by macrophages ❷and transform into amastigotes❸. Amastigotes multiply in infected cells and affect different tissues, depending in part on the *Leishmania* species❹. This originates the clinical manifestations of leishmaniasis. Sandflies become infected during blood meals on an infected host when they ingest macrophages infected with amastigotes (❺,❻). In the sandfly's midgut, the parasites differentiate into promastigotes ❼, which multiply and migrate to the proboscis ❽. http://www.dpd.cdc.gov/dpdx/HTML/Leishmaniasis.htm. access date: 12-14-08.

1.1.5 Hosts and Reservoirs of Visceral Leishmaniasis in the New World

Human beings are incidental hosts of VL. Domestic dogs are considered the main reservoirs for the parasites that cause VL. The geographic spread and increasing prevalence of VL in urban areas is linked to human migration, involving the transportation of infected dogs from endemic regions to impoverished urban areas where *Lu. longipalpis* already exists. Many of the inhabitants raise chickens, pigs and other livestock in their yards, and, because of the general climate of insecurity, keep dogs

15

which act as the principal reservoir. All these factors that favor parasite transmission may be concentrated within a relatively small area (Alexander et al., 2002).

In a study in Colombia, cows and pigs were the preferred host of phlebotomine sandflies (Morrison et al., 1993). The horse and donkey may serve as a reservoir host in the urban cycle of these parasites (Grimaldi et al., 1989). Moreira et al., 2003, reported data that showed an increased likelihood of *Leishmania* infection in dogs with hen houses or other livestock raised in the yards of their dwellings. Nevertheless, chickens are unable to sustain infection with *Leishmania*, and the role different mammalian livestock may play in the transmission of the protozoan to other hosts has yet to be established (Moreira et al., 2003). Corredor et al, 1989, showed a higher prevalence of *Leishmania* skin test positivity in villages where most of the houses have stables to keep animals (mainly mules and goats), as compared with villages in which houses rarely have stables. *Didelphis albiventris*, the opossum, was found naturally infected in Colombia; this marsupial does not suffer from the pathogenic effects of *Le. chagasi*. This finding suggests the existence of an evolved host/parasite association that permits this marsupial to be one of the primary reservoirs of American visceral leishmaniasis (Sherlock, 1996). Sloths (*Choloepues hofmannii* and *Bradypus Infuscatus)*, foxes (*Cedcocyon thous* and *Lycalopex vetulus*) as well as edentates, such as the tamandua (*Tamandua didactyla*) have also been reported to be reservoirs of *Leishmania* (Grimaldi et al., 1989).

1.1.6 Vectors of Visceral Leishmaniasis in the New World

Lutzomyia longipalpis (Lutz and Neiva., 1912) is the primary and most studied vector of visceral leishmaniasis in the New World. However, observations made by Ferro and Morales (1998) in Colombia have reported *Lu. evansi* is also a vector of visceral leishmaniasis. After the description of *Le. chagasi* as the major agent of VL in the

16

Americas, the taxonomic position of this species has been controversial due to its similarity to *Le. infantum* (Nicolle, 1908), a Mediterranean species. Laison and Shaw (1979) accepted that it is a separated species, while not excluding the presence of *Le. infantum* in Brazil (Soares and Turco., 2003). In recent years, the number of studies on the biology and genetics of the phlebotomine sandfly *Lu. longipalpis* has increased tremendously. It is now thought that this sandfly is a complex of three and possible more sibling species. Some investigators have reported reproductive isolation and significant genetic divergence among strains from different geographic regions (Mutebi et al., 1999). The existing data suggest that *Lutzomyia spp.* sandflies are more susceptible to development of *Leishmania* infections than sandflies in the genus *Phlebotomus* (Killick-Kendrick., 1977).

1.1.6.1 Taxonomy

Of approximately 800 species or subspecies of phlebotomine sandflies in the World, 80 are proven or probable vectors of the 22 species of *Leishmania* that cause human disease. In some leishmaniasis foci, the vectors are unknown and it is certain that more species will be added to the list (Table 2). With the exception of *Lu. longipalpis* in the new world and *Phlebotomus papatasi*, *P. ariasi* and *P. perniciosus* in the old world, the biology of most of these vectors is poorly understood (Killick -Kendrick., 2002).

1.1.6.2 Sandfly Biological Cycle

Sandflies are holometabolous insects that proceed in development from the egg through four larvae stages, a pupae and the adult (Chanioitis, 1974). The ecology of the immature stages of most species is still unknown because of the difficulty of locating eggs, larvae or pupae and monitoring their life cycle in natural environments. The detection of immature stages has been recently reported in natural environments

17

(Ximeens et al., 2001). The larval stages (first to fourth instar) and pupal period together last 3 to 4 weeks at the optimal temperature of 23 to 28°C and 70% to 100% relative humidity (Kettle, 1990). Only female adults feed on blood, with a blood meal required prior to oviposition. Approximately 70% of the *Lu. longipalpis* females could survive up to seven days without a blood meal. For the larval stages, Soares and Turco (2003) have tested in different experiments many types of food and found that the conditions promoted by certain regimes of humidity, temperature and food quality may enhance fungal growth, killing the larvae. Feliciangeli (2004) reported that the pores present in the eggs are small and very close to the chorion. This characteristic seems important to prevent desiccation and favor the survival of the vector in dry environments. This feature does not favor the variability of the eggs in high humidity conditions, however since water can cover the pores and kill the larvae by asphyxiation. Also, the caudal setae of the larvae show transversal grooves with small and scarce pores, which probably influence its development in drier environments. The larvae of *Lu. intermedia* and *Lu. whitmani* have very small and abundant pores and those of the *Lu. migonei* are large and plentiful; these species, which are vectors of CL, live in areas where the humidity is high (Pessoa, 2001).

1.1.6.3 Larvae Morphology

Recently Leite and Williams (1996, 1997) described fourth and first instar stages of *Lu. longipalpis* pupae using scanning electron microscopy (SEM). Using the fourth instar larva, Fausto et al, (1998), described the structure of the larvae spiracular system in eight *Lutzomyia* species, including *Lu. longipalpis* using light microscopy and SEM. In *Lu. longipalpis* as well as some other diptera, the fourth instar larva is amphipneustic, having two pair spiracles in the thorax and abdomen (Soares and Turco., 2003). These

structures can assume a great variety of forms and therefore can be used as a taxonomical tool for grouping different species. Additional data on external morphology have been reported for the posterior spiracles and external sensory structures (Pessoa et al., 2001). Two morphological forms have been described based on the number of pale patches (one or two spot phenotypes) observed in the abdomen of *Lu. longipalpis*. These patches consist of secretory glands and were suggested to produce sex pheromones after an SEM study (Lane and ward., 1984). Ward and Morton (1991) showed that different Brazilian populations of adult *Lu. longipalpis* were able to react to male pheromones in a conspecific way in Jacobina state of Bahia. Another population from Sobral, Ceará State reacted after stimulation with pheromone from Jacobina, Bahia State sandflies, but preferentially selected conspecific sexual partners.

Table 2. Proven (*) and probable vectors of *Leishmania* in the New World. Source: Jeffrey J. Shaw "New world Leishmaniasis: The ecology of leishmaniasis and the diversity of Leishmanial species in Central and South America, In: World class parasites, Vol. 4. *Leishmania*, Ed. J.P. Farrell, 2002.

Causative organism *Leishmania*	Proven or probable vector *Lutzomyia*	Countries of known or suspected transmission by listed vectors
Le. infantum	*Lu. longipalpis**	Argentina; Bolivia; Brazil; Colombia, Costa Rica; El Salvador; Guatemala; Guyana; Honduras; Mexico; Nicaragua; Paraguay; Suriname.
	Lu. evansi	Colombia; Costa Rica; Venezuela.
Le.granham	*Lu. youngi*	Costa Rica; Venezuela
Le. peruviana	*Lu. peruensis*	Peru
	Lu. verrucarum	Peru
Le. lainsoni	*Lu. ubiquitali s**	Brazil; Peru
Le. shaw	*Lu. whitmani *	Brazil
Le. naiffi	*Lu. squamiventris*	Brazil
Le. colombiensis	*Lu. hartmanni *	Colombia
	Lu. gomezi	Panama; Venezuela
	Lu. panamensis	Panama; Venezuela

Table 2 Continued

Le. guyanensis	Lu. ubratilis *	Brazil; French Guiana;
	Lu. anduzei *	Colombia
		Brazil; French Guiana
Le. braziliensis	Lu. wellcome *	Brazil
	Lu. complexus *	Brazil
	Lu. intermadia	Brazil
	Lu. pessoai	Brazil
	Lu. migonei	Brazil
	Lu. amazonensis	Brazil
	Lu. paraensis	Brazil
	Lu. whitmani *	Brazil
	Lu. panamensis	Venezuela
	Lu. ovallesi *	Venezuela
	Lu. yucumensis	Bolivia
	Lu. llanosmartinsi	Bolivia
	Lu. c. carrerai *	Bolivia
	Lu. ayrozai	Bolivia
	Lu. spinicrassa	Colombia
	Lu. colombiana	Colombia
	Lu. pia	Colombia
	Lu. Townsendi	Colombia
Le. panamensis	Lu. trapidoi *	Panama; Colombia
	Lu. gomezi	Panama
	Lu. panamensis	Panama
	Lu. Ylephiletor	Panama
Le. mexicana	Lu. o. olmeca *	Belize; Costa Rica;
		Guatemala; Honduras;
	Lu. ayachuchensis *	Mexico
	Lu. ylephiletor	Ecuador
		Guatemala
Le. amazonensis	Lu. flaviscutellata *	Bolivia; Brazil; Colombia;
		Ecuador
		French Guiana; Paraguay;
		Venezuela
	Lu. olmeca nociva	Brazil
	Lu. Reducta	Brazil; Venezuela
Le. venezuelensis	Lu. olmeca bicolor	Venezuela
Leishmania sp.	Lu. anthophora	USA; Mexico
Leishmanai sp.	Lu. christophei	Dominican Republic
Le. pifanoi	Lu. flaviscutellata	Venezuela

The sexual preferences among different populations that have been reproductively

isolated may result in failure of copulation and/or viability of the offspring (Santos et al.,

1991). Even though the morphologic difference studies done by Pessoa et al, (2001)

20

suggest no intraspecific variation for Brazilian strains of *Lu. longipalpis*, Venezuela species of *Lu. longipalpis* do present notable variation (Fausto et al., 1998). Muterbi et al, (1999) studied adult flies from 11 natural populations of *Lu. longipalpis* collected from different climatic regions of Brazil (the Amazon basin, the Brazilian plateau, and the tropical east coast) and found that there was no evidence supporting the existence of more than a single species among the field populations of *Lu. longipalpis* they surveyed. *Lu. pseudolongipalpis* is the first new species in the complex that has been separated by both genetic (Arrivillaga et al., 2000) and morphological characteristics of the fourth instar larvae (Arrivillaga and Feliciangeli., 2001). This species was described from an American cutaneous leishmaniasis focus in la Rinconada, Curarigua, Lara state, Venezuela and it distribution seems, thus far, to be restricted to this area, having been found sympatric with *Lu. longipalpis* in El Paso, a community 17 Km from Curarigua (Lampo et al., 1999).

1.1.6.4 Behavioral and Environmental Requirements of *Lutzomyia Longipalpis*

The behavior of the vector obviously affects the maintenance of disease transmission; as the rate of anthropophily increases, human-vector contact increases and the risk for humans of being attacked by infected sandflies is enhanced (Agrela et al., 2002). Larvae of *Lu. longipalpis* and *Lu. oswaldoi* were detected in caves, under rocks, at the base of trees, under leaves, under decomposed materials in animal shelters, and in animal feces (Ximeens et al., 2001). In the natural environment, *Lutzomyia* larvae instars feed on organic material from the soil, while adults feed on sugar from plant sources. Knowledge of sandfly breeding remains scanty (Feliciangeli, 2004). Searching for developmental stages of sandflies in their natural biotopes is difficult, tedious and has proved to be remarkably unproductive (Deane and Deane, 1957; Killick-Kendrick, 1999).

The deficit in information on environmental sources of sandfly populations prevents the avoidance of such sites and disallows the targeting of control measures against the pre-imaginal stages of sandflies (Feliciangeli, 2004). *Lutzomyia* has been associated with dry and semi-dry zones in rural and urban areas. The species is common in mountainous places with low humidity and abundance of superficial rocks (Dias-Lima et al., 2003).

A study in an endemic focus in Jacobina, Bahia, Sherlock (1996) showed that the biting activity of *Lu. Longipalpis* in nature begins at dusk and reaches its highest peak from 9 to 11 p.m. After 11 p.m. the number of sandflies decreases until they disappear by 5-6 a.m. There are seasonal fluctuations in the density of *Lu. longipalpis*, which is most abundant during two periods, the months of June and November. The vector abundance corresponds to the wet-cold and wet-hot months. This seasonal fluctuation correlates with the occupation of new human shelters and with peridomestic frequency of wild animals. *Lu. longipalpis* abundance varies weekly according to the lunar phase; it is more frequent during both the full moon and the last quarter moon (Aguilar et al., 1987). During droughts, it is much more common to find sandflies inside houses. In the years of normal weather *Lu. longipalpis* is abundant throughout all areas. *Lu. longipalpis* has very eclectic feeding habits and may feed on humans, dogs, goats, equines, swine, bovines, chickens and wild animals such as fox, opossum, and rodents. This eclectic feeding characteristic favors its existence in ecosystems that are apparently hostile, such as the Caatinga, and the consequent dispersion to new environments such as urban areas, granting them ample ecological variance.

The eggs and larvae of *Lu. longipalpis* have peculiar characteristics that allow them to survive in dry environments and with certain types of vegetation (Sherlock, 1996). In areas in the state of Bahia covered by forest, where the humidity is high and

22

there is water retention by the soil, *Lu. longipalpis* has difficulty in completing its lifecycle cycle. Hot air fronts that move from the coast towards the plateau meet cold air fronts precisely at the Atlantic forest, causing frequent precipitation; the high humidity coupled with suitable temperature favors the maintenance of the ecosystem and allows for the survival of certain species of phlebotomines, such as *Lu. whitmani* and *Lu. intermedia*. It was thought that that *Lu. longipalpis* was restricted just to the areas of mountainous land with rocky appearance, and the existence of seasonal semidecidious forest (Costa, 1997). However it is present in the littoral zones at sea level and in the open and ventilated field, demonstrating that the species possesses ample ecological variance in tolerance and is being adaptable to environments of diverse characteristics. The vector *Lu. longipalpis* is anthropophilic and reproduces best between 23ºC and 28ºC and relative humidity of 70-100%. It completes its life cycle 5-8 weeks under ideal conditions (WHO, 1990).

1.1.6.5 Laboratory Breeding Requirements of *Lutzomyia Longipalpis*

Killick-Kendrick et al, (1977) established laboratory colonies of *Lu. longipalpis* using eggs from engorged females collected on one of the Campinha caves in Minas Gerais, Brazil. The colonies were maintained in the dark, at 25ºC and a relative humidity of 95-100%. Modi et al, (1983), reported the maintenance of *Phelotomus papatasi* colonies at 28°C, 60-70% relative humidity and a 14:10 (light: dark) photoperiod. Particular care was taken to ensure that eggs and the 1st instar did not become dry. Later instars, notably the 4th, appear to prefer somewhat drier conditions than the early instars. If pots are too wet, 4th instar larvae tend to crawl to the lid to pupate. The number of eggs produced by the *Lutzomyia spp* is directly proportional to the weight of blood meals taken. The time between bloodmeal and oviposition varies from 6-16 days (Moura-

Luitgards et al., 2000). Few survive oviposition more than 24 hr, but gravid females denied damp surfaces do not oviposit and may live for more than a month. At 25 ºC in the dark, males and females that have not had a blood meal normally live for 2 weeks to more than a month. Although many sandflies are delicate and short-lived, *Lu. longipalpis* is relatively robust. Females immobilized by cold (24 hr at +4ºC) revived when returned to room temperature, and some then took a blood meal. (Killick-Kendrick et al., 1977)

1.1.7 *Leishmania* / Human Immunodeficiency Virus Co Infection

Leishmaniasis is one of the opportunistic infectious that attacks human immunodeficiency virus (HIV) infected individuals. Most co-infections involve the visceral form of leishmaniasis. There is concern that *Leishmania*/HIV co-infection may increase the transmission of leishmaniasis, particularly the visceral form. The overlap in the geographical areas with high risk of both HIV and leishmaniasis is increasing, with the spread of leishmaniasis into urban areas and the increased spread of HIV into rural areas. Leishmaniasis patients are highly susceptible to HIV infection and in HIV–infected patients, leishmaniasis accelerates the onset of AIDS by cumulative immuno-suppression and by stimulation of the replication of the virus. HIV also may be associated with change of asymptomatic *Leishmania* infections into symptomatic infections. In addition, since visceral leishmaniasis can be spread intravenously, sharing of needles by intravenous drug users is a direct way of spreading leishmaniasis (Desjeux et al., 2000).

Since 1990, cases of co-infection have been reported from 31 countries worldwide. Most cases have been reported in southwestern Europe; where the surveillance system was first implemented. In contrast to Europe, intravenous drug users do not predominate among those exposed to HIV infection in Brazil. Changes in the epidemiological patterns of HIV and *Leishmania* infections are likely to result in a

greater degree of overlap and a greater risk of co-infection that justifies increase on alertness (Rabello, 2003). *Leishmania* HIV co-infection is not common in Brazil, however, even from regions where the overall incidence of HIV and *Leishmania* infection is both relatively high. It is reported that many cases go undetected because lack of awareness among clinicians or limited access to appropriate diagnostic methods (Rabello, 2003).

Human Immunodeficiency Virus is known to propagate mainly in T-lymphocytes because these cells express the primarily cellular receptor for viral entry into target cells, the surface molecule DC4 (Dalgleish et al., 1984; McDougal, 1986). However, the macrophage has been recognized as the predominant cell line infected with HIV in the lymph nodes, lungs and central nervous system (Meltzer et al., 1990). Monocytes and macrophages thus represent an important reservoir for HIV and serve as vehicles that disseminate the virus through the host. Bernier et al, (1995) observed that *L. donovani*, in its surface has lipophosphoglycan (LPG), a glycoconjugate, is one of the major constituents expressed on the surface of the *Leishmania* promastigote. When a promastigote is inoculated into a human host by an infected sandfly, it is engulfed into the phagolysosome of a macrophage, where it rapidly differentiates into an amastigote. During its engulfment, the parasite loses most of its structural LPG at the surface of the phagocyte, and retains only the intramembrane component. This component, the phosphatidyl inositol core (core-PI), is present on the surface of the amastigote. The LPG surface molecule has been recognized as favoring the intracellular survival and establishment of the parasite (Turco, 1999).

Humans co-infected with HIV and *Le. infantum* are able to mount a T-cell response against the parasite after anti-leishmanial treatment but this response is lost as

the viral infection progresses and is almost always followed by relapse of the *Leishmania* infection (Fig. 3). Co-infection may thus induce both an uncontrollable spread of the parasite and an increase in viral replication. Treatment against leishmaniasis that are effective but do not promote HIV replication need to be developed (Oliver et al., 2003).
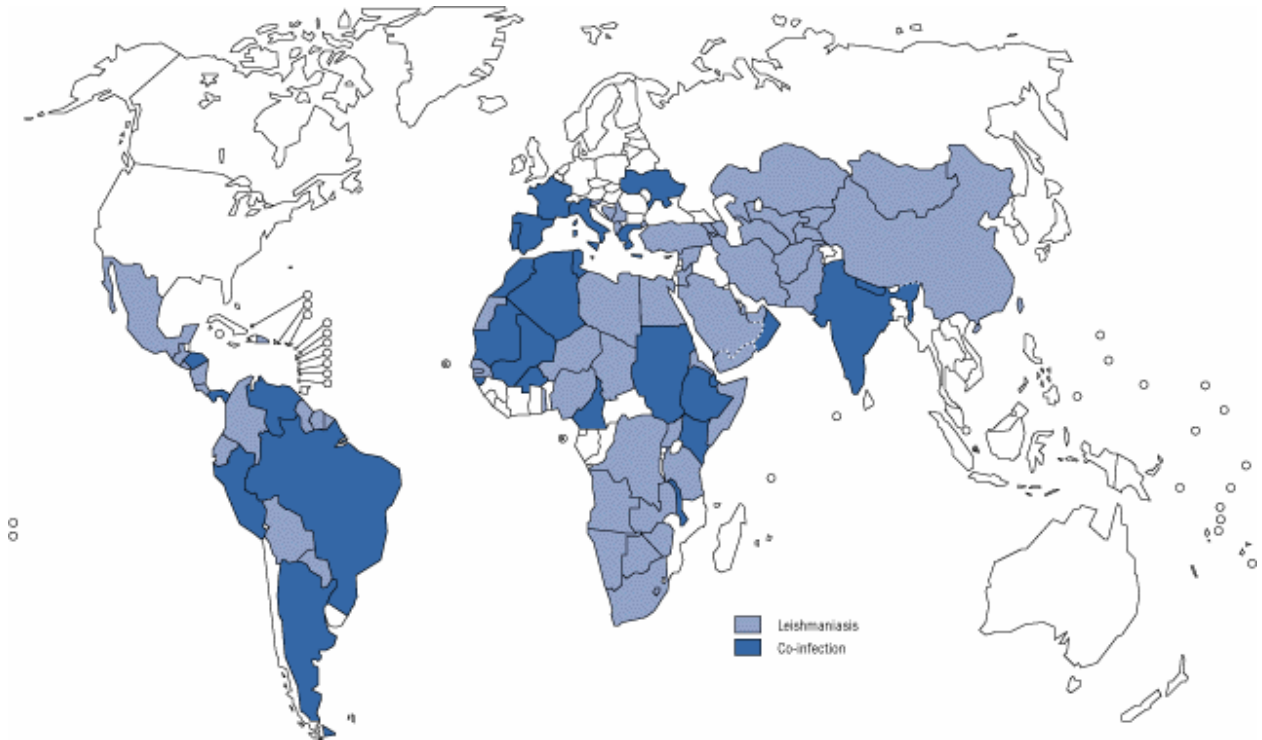
Fig. 3. Countries reporting *Leishmania*/HIV co-infection worldwide.
Source: WHO/CSR/EDC-UNAIDS **Map production:** Public Health Mapping Group Communicable Diseases (CDS) World Health Organization, October 2003. http://www.who.int/leishmaniasis/leishmaniasis_maps/en/index.html Access date: 12-14-08.

1.1.8 Congenital Visceral Leishmaniasis

Congenital visceral leishmaniasis was first described in 1926 by Low and Cooke. The course of the disease seems to be identical for congenital transmission or otherwise acquired kala-azar. Most congenitally infected children developed disease symptoms in the first year of life. In congenital cases, the route of transmission remains unclear; most likely the infection occurs during labor via blood exchange from mother to the child. Transplacental transmission during pregnancy before birth is improbable, because no

parasites were found in the organs of an aborted fetus of five-month's gestational age born to a 30 –year-old infected mother in Sudan even though the placenta showed large number of amastigotes (El-Toum et al., 1992).

The human impact of visceral leishmaniasis is significant. Some 500,000 new cases are reported annually world wide, and epidemics may decimate local populations. A more complete understanding of the human immune response and its pathological consequences are needed for the design of vaccines and for improvements in therapy. Epidemiological data suggests that greater than 95% of exposed immunocompetent individuals show no clinical signs of infection (Herwaldt et al., 1999). There are no reports of the early kinetics of the immune response, nor of the precise mechanisms that control parasite multiplication and/or allow for the establishment of subclinical infection. Genetics, age, malnutrition and concurrent infection are contributing factors for disease progression.

1.1.9 Control

Following the discovery of canine visceral leishmaniasis in Tunisia by Nicole and Conte in 1908, dogs have been implicated as important reservoir for visceral leishmaniasis. These findings suggested that the dog might serve as a reservoir for human leishmaniasis. Since these early findings, several studies have described the presence of canine seropositivity in areas of endemic Kala-azar (Ashford et al., 1998). As a result of these literature reports, control programs for visceral leishmaniasis often include elimination of seropositive dogs or other suspected reservoirs along with treatment of infected animals and humans. Reithinger et al, (2001) suggest that treating dogs is not practical control policy, not only because of the prohibitive cost involved but also because of high relapse rates among treated and clinically cured dogs. Moreover, high

proportions of clinically cured dogs remain parasitologically positive and are therefore infectious to the sandfly vectors.

Although elimination of dogs has been associated with a decrease in the incidence of visceral leishmaniasis, transmission has been not eliminated probably because VL has been associated with several other mammalian species (Ashford et al., 1998). Sherlock et al, (1996) concluded that VL is possible to control by three simultaneous measures: treatment of human cases, elimination of positive dogs and spraying of insecticides to kill the vector.

Control methods for sandflies include chemical control by the application insecticides and environmental management, e.g. eliminating breeding sites. Insecticides play an important role especially in domestic and peridomestic situations where people live in rural areas. Before choosing a chemical method it is important to establish the sandfly vector species present, the behavior of adults and any reported case of insecticide resistance. Spraying houses with insecticides is the most widely used intervention for controlling sandflies that are endophilic (rest mostly indoors after feeding) (Davies et al., 2003). Insecticides used are DDT (insecticide resistance has been reported in India), malathion, fenitrothion, propoxur and synthetic pyrethroids such as deltamethrin. Studies have shown that curtains and bed nets impregnated with deltamethrin are effective in protecting people from sandfly bites (Kroeger et al., 2002).

Alternative mechanisms have been used, such as lotions or insecticide-impregnated dog collars with deltamethrin, permethrin or fenthion (Reithinger et al., 2001). For the control of exophilic sandflies (peridomestic vector species), surfaces, shelters, stones, walls and other potential outdoor resting sites should be spayed as well. Knowledge of the vector's flying and resting habits can reduce not only the amount of

insecticide and the extent of the operations but also the expense involved (WHO, 1990). Malaria and Chagas disease control programs have had important side-benefits for the control of leishmaniasis by sandfly control along with mosquito and triatomine control.

The aim of personal protection is to protect individuals from infective sandfly bites through the use of mesh nets, screened doors and windows, protective clothing, insect repellents, coils and vaporizers.

A standardized inoculum of culture promastigotes as a vaccine was developed by Israeli scientists and used in several trials. This process, known as leishmanization, have been proven to be efficacious against Old World cutaneous leishmaniasis (Nadim et al., 1997). However several theoretical and logistical problems have precluded the widespread use of this procedure to prevent cutaneous leishmaniasis. Some of these problems include: 1) difficulty in standardizing the virulence of the vaccine, and 2) side effects such as the severe and long lasting lesions that occur in many vaccinated individuals (Modabber, 1989). Moreover, there is no evidence of the effectiveness of this *Leishmania* vaccine against either new world tegumental leishmaniasis or against human visceral leishmaniasis.

Passive case detection requires that staff of health centers in endemic areas be trained to recognize possible cases of leishmaniasis and obtain samples for confirmation and notification of suspected cases. Surveillance can considerably reduce individual suffering and at the same time serve as a rough indicator of the local prevalence of the disease. Passive surveillance relies on awareness among the population or early symptoms and of the importance of early treatment; health education campaigns can considerably improve compliance. Regular systematic screening of the population at risk

29

is the most effective system for active medical surveillance and early case detection (WHO, 1990).

1.1.10 Diagnosis

An early diagnosis is important, as leishmaniasis is a serious infection for the individual and the community. No single method is the gold standard for the diagnosis of leishmaniasis. In endemic areas a provisional diagnosis can be made on the basis of clinical signs and epidemiological data. A definitive diagnosis depends on demonstration of *Leishmania* parasites.

The most commonly affected age group is 1-4 years, but the disease also occurs among teenagers and adults. Cases of VL are occurring in increasing number in people with immunosuppression due to acquired immunodeficiency syndrome (AIDS), drug addiction or chemotherapy. Visceral leishmaniasis infections due to *L. chagasi* are apparently often asymptomatic; the typical incubation period is 2 to 4 months but varies from 10 days to 34 months or longer (Evans et al., 1992).

Common symptoms of VL are irregular fever (for longer than 2 weeks), malaise, abdominal swelling, headache, vomiting, bleeding (especially nosebleeds) shivering or chills, weight loss, anorexia and discomfort in the left hypochondrium (TIH, 2000). The following signs may be found upon clinical examinations in suspected VL cases: splenomegaly, hepatomegaly, cachexia, anemia, edema, lymph node enlargement, jaundice, and/or skin hyperpigmentation (WHO, 1990). These signs and symptoms, alone or in combination, are not sufficiently specific to be diagnostic of Kala-azar and should be differentiated from other systemic infections like schistosomiasis, typhoid fever, Chagas disease, malaria or prolonged multi etiological septicemia (Silveira el al., 1997). Symptoms of VL develop after a 2-4 month incubation period. It is common that people

30

experience asymptomatic infection. The second form is cutaneous in nature with ulcers and dermal reactions in the absence of visceral complications and is known as post kala-azar dermal leishmaniasis (PKDL). Post kala- azar dermal leishmaniasis is characterized by hypopigmented (India) or hyperpigmented (Africa) macules and nodules on any part of the body or the face (WHO, 1990), changes in skin color and a previous history of VL (TIH, 2000). The dermal lesions are usually not seen with American visceral leishmaniasis. Clinical suspects of visceral leishmaniasis should be referred for parasitological confirmation and serological examination.

The ideal method for diagnosing visceral leishmaniasis is microscopic parasite identification in tissue smears of splenic, bone marrow (usually from iliac crest) or lymph nodes aspirates stained with Giemsa; it is simple, rapid, low cost and it is applicable to field conditions (TIH, 2000). If culture medium NNN (consists of a solid phase or blood agar and a liquid water or balances salt solution), or Schneider's medium (supplemented with 10% calf serum) is available, carefully inoculate culture tubes with one or two drops of the aspirate for later identification. However, difficulties in obtaining and examining tissues mean that serological methods are increasingly being used (Davies et al., 2003). Other techniques used are: animal inoculation of macerated infected organs, macerated infected sandflies, injections of culture promastigotes or by feeding infected sandflies on dogs, opossums, hamsters or rats and subsequent parasitological confirmation three to six moths later (Sherlock, 1996).

Serological examinations are the most efficient screening procedures. Serological tests are based on detection of circulating antibody. High levels of antibody characterize visceral leishmaniais. The antibody is of two types non-specific (IgG and IgM) and specific antileishmanial antibody. Available serological procedures are; 1) the aldehyde

formol test (detect raised levels of IgG), 2) direct agglutination by serum antibodies (DAT) (highly sensitive; >90% of VL cases can be successfully diagnosed by detection of antileishmanial antibody), 3) the indirect immunofluorescence test (IFAT) for detection of circulating antibodies and, 4) enzyme-linked immunosorbent assay (ELISA; a highly sensitive test to quantify antileishmanial antibody) (TIH, 2000). Weak responses in some patients, persistence of antibodies after cure, and presence of antibodies in some healthy individuals are inherent limitations to antibody tests (Davies et al., 2003). The K39 antigen derived from the cell wall of *Le. donovani* amastigotes is used in a chromatographic strip test. Detection of leishmanial antigen in urine using a latex agglutination test (katex) seems to be promising for both diagnosis and prognosis (Davies et al., 2003).

Other laboratory tests like the polymerase chain reaction (PCR), which is potentially highly sensitive and specific, and immune-florescent tests are techniques that are less suitable for field use in terms of cost, laboratory facilities and user skills required.

1.1.11 Treatment

The parenteral administration of pentavalent antimonials has been the mainstay of treatment for all forms of leishmaniasis since the 1940's (TIH, 2000). The treatment should achieve clinical and parasitological cure, use an appropriate drug regime, prevent relapse and avoid drug resistance. The following are antimonials available: Pentostam (sodium stibuglonate) 100 mg pentavalent antimony/ml and Glucantime (meglumine antimoniate) 85 mg pentavalent antimony/ml. Treatment may be given by intramuscular injections and larger volumes of pentavalent antimony require intravenous injection or infusion. Second-line drugs include; Amphotericin B, and aminocidine sulfate (Davies et al., 2003). Amphotericin B is an alternative first line treatment where antimonials are

contraindicated (pregnancy, obesity or underlying cardiac, renal or pancreatic diseases) and in areas of antimonial drug resistance eg. in India. Drugs used in repeated relapses and unresponsive cases are: pentamidine isethionate, pentavalent antimony and allopurinol (Martinez, 1992), liposomal amphotericin B (less toxic than conventional amphotericin B), pentavalent antimony + gamma interferon and Miltefosine (Davies et al., 2003). Patients who are unresponsive to a course of pentavalent antimony or who relapse after treatment should never be treated without parasitological confirmation.

Leishmaniasis is a highly complex disease in which response to treatments varies between the different clinical forms, between and within *Leishmania* species, geographical regions, and with the status on the host cellular immunity. Because of the complexities of the disease, management of each case should be individualized. It may be also necessary to treat concurrent illnesses such as pneumonia, and the most common and most lethal complicating infections, diarrhea, malaria, and anemia (TIH, 2000).

1.2. Goals and Objectives of the Present Study

1.2.1 Long Range Goal

Studies in Latin America (Thompson et al.,2002; Thompson, 1998; Fuentes et al., 2004), Africa (Malone et al., 2001, 2003) and elsewhere have shown promise that emerging geospatial health technologies can be used to create comprehensive digital map databases on the biology, epidemiology and control of VL using Geographic Information Systems (GIS) and remote sensing (RS). Little has been reported on use of computer-based geospatial analysis tools in previous studies on VL in Brazil. The long range goal of the present study is to develop GIS/RS methodologies that can be integrated into state and national control programs for leishmaniasis in Brazil.

1.2.2. Specific Objectives:

1. Use landscape epidemiological theory and analysis tools to define climatic determinants of the distribution and abundance of VL in Bahia state in Brazil.

2. Create digital databases and maps based on an 11-year Ministry of Health database on VL prevalence in Bahia to relate long-term climate and annual climate records to the geographic range of the disease and annual variation in risk of VL infections.

1.3 Hypothesis

Climatic and environmental factors influence the *Lu. longipalpis- Le. chagasi* vector-parasite complex, determining the presence of visceral leishmaniasis in the state of Bahia.

1.4 Materials and Methods

1.4.1 Study Area

The area of study comprised the state of Bahia located in the northeastern part of Brazil. Bahia has an area is 567,295 km², approximately the size of France, and is the largest state in Northeast Brazil. Its coastline is 1,188 Km long, which represents 13.2% of the national coastline. The neighbor states are: Sergipe, Alagoas, Pernambuco and Piauí in the north; Minas Gerais and Espírito Santo in the south; Goiás and Tocantins in the west. Ninety percent of Bahia is above 200m; the highest point is Serra do Barado (2033 m). The main rivers are: São Francisco, Paraguaçu, Jequitinhonha, Itapicuru, Capivari, de Contas. The state of Bahia is located within the parallels 8˚00' and 18˚ 30' South Latitude and between the meridians 36˚ and 46˚ west longitude.

In March 29, 1549, Thomé de Souza, arrived with his Portuguese fleet at Porto da Barra, and named the city Salvador, which became the capital of the Portuguese empire in the Americas. In 1763, Brazil moved its capital from Salvador to Rio de Janeiro in a reaction to the gold industry that was growing rapidly in Brazil. Salvador is the capital of

the state of Bahia and the third largest city in Brazil. Bahia as the fourth most populated state of Brazil, with more than 13 million inhabitants, it is the leader in the North-East, and its capital Salvador is the largest city in this region. Bahia state is fifth in territorial area, corresponding to 36.3% of the total area of Brazil's northeastern region. Bahia is Brazil's most historic state, and has retained strong links with West African as well as Portuguese, and Spanish heritage.

The Bahian economy has undergone major changes the last 30 years, with the growth of the industrial activity and the modernization of the commercial and service sectors. With the exception of a few centers of development in the interior, this forward thrust in the economy is concentrated in greater Salvador. The main sectors in the industry are chemical, petrochemicals, metallurgy, foodstuffs, nonmetallic minerals and drinks. Bahia is one of the most mineral-rich states in Brazil, with deposits of gold, copper concentrate, magnesite, chromite, rock-salt, barite and manganese. Commerce and agriculture continue to be strong within the total economy of Northeast Brazil. Tourism is a strong contributor in the economic profile of Bahia; it is the second port of entry for tourists after Rio de Janeiro.

1.4.2. Climate

The climate of North Brazil is classified as dry arid to arid tropical. The notable characteristic of the climate in northeast Brazil is the huge variation in inter-annual rainfall with notorious droughts, which occur periodically (Rao et al., 1993). Situated between the equator and the Tropic of Capricorn, it enjoys a tropical climate in coastal areas and semiarid inland with annual average temperatures between 19º and 27º C. Rainfall ranges from 2,000 mm in the coastal plain region to 360 mm in the northern lowlands of the São Francisco basin. Tropical forest is the dominant vegetation within

coastal areas and mangues (bushes and other species of trees adapted to salinity and poorly oxygenated soils) occur in littoral areas; cerrado and caatinga occur inland (Bavia, 1996).

Fig. 4. Location of the state of Bahia, Brazil.

The climate of Bahia is very hot nearly the entire year. The maximum precipitation is during the summer months of December, January and February. During the winter months of June, July and August, and during the spring months of March, April and May, minimum annual rainfall is registered in most areas of Bahia state. Rainfall in Bahia is characterized by high concentration during few months with the exception of the coastal area and Reconcavo (the region embracing All Saints' Bay, parallel to the coastal margin from Salvador along 150 Km of coastline) (Bavia, 1996). The hottest days are in the summer (January-February) when the highest temperature

ranges from 30°C to 33°C. In the interior of the state where the altitude is higher (excluded semi-arid regions), temperature is somewhat lower than in the coastal areas. The annual mean temperature varies between 26 $^{\circ}$C and 28°C (Secretaria da cultura e turismo Estado da Bahia). In the coastal area of Bahia the dry season occurs during summer (Fig. 5). In the West of Bahia the dry periods occurs during both the winter and summer, major variations in the seasonality and duration in the rainy periods is observed in different parts of Bahia. In all of the Bahia littoral and extending to the reconcavo, the dry season occurs during the summer. From east to west to the border with Piaui, both winter and summer are dry. In the San Francisco area, which extends from the north to the south of the state, the dry season includes the winter, spring and summer. Overall, the number of dry months varies from 0 to 11 in various parts of Bahia**.**

1.4.3 Demographics

The estimated population of Bahia is 13,026,171 (census 2000, Instituto Brasileiro de Geografia e Estadistica IBGE **http://www.ibge.gov.br/**) (Table 3), distributed through 415 municipalities with a median altitude of 400 m above sea level. Almost 20% live in the capital, Salvador. Other major cities are Feira de Santana, Ilhéus, Vitória da Conquista, ltabuna, Juazeiro, Jequié,Camaçari, Barreiras and Porto Seguro (SEAGRI, Secretaria da Agricultura, Irricacao e Reforma Agraria estado da Bahia **http://www.seagri.ba.gov.br/investir_oportunidadei.asp**). The Bahian population is divided into the 59% that live in urban areas and 40.9% that live in rural areas. Ninety percent of the municipalities have less than 50,000 inhabitants; 9% have less than 270,000 inhabitants. The two most populated municipalities are Feira de Santana with 503,900 inhabitants and a population density of 369.73 inhab/Km^2 and Salvador with a

population of 2,556,429 and a population density of 3616.9 inhab/Km^2. The population is 50.6% female and 49.4 % male (IBGE through the resolution number 5 of October 2002).

	Climate	Class	Number of dry months
	Hot	Very humid	0
	Hot	Very humid	<1
	Hot	Humid	1-2 months
	Hot	Humid	3 months
	Hot	Semi-humid	4-5 months
	Hot	Semi-Arid	6 months
	Hot	Semi-Arid	7-8 months
	Hot	Semi-Arid	9-10 months
	Hot	Semi-Arid	11 months
	Warm	Humid	1-2 months
	Warm	Humid	3 months
	Warm	Semi-humid	4-5 months
	Warm	Semi-humid	6 months
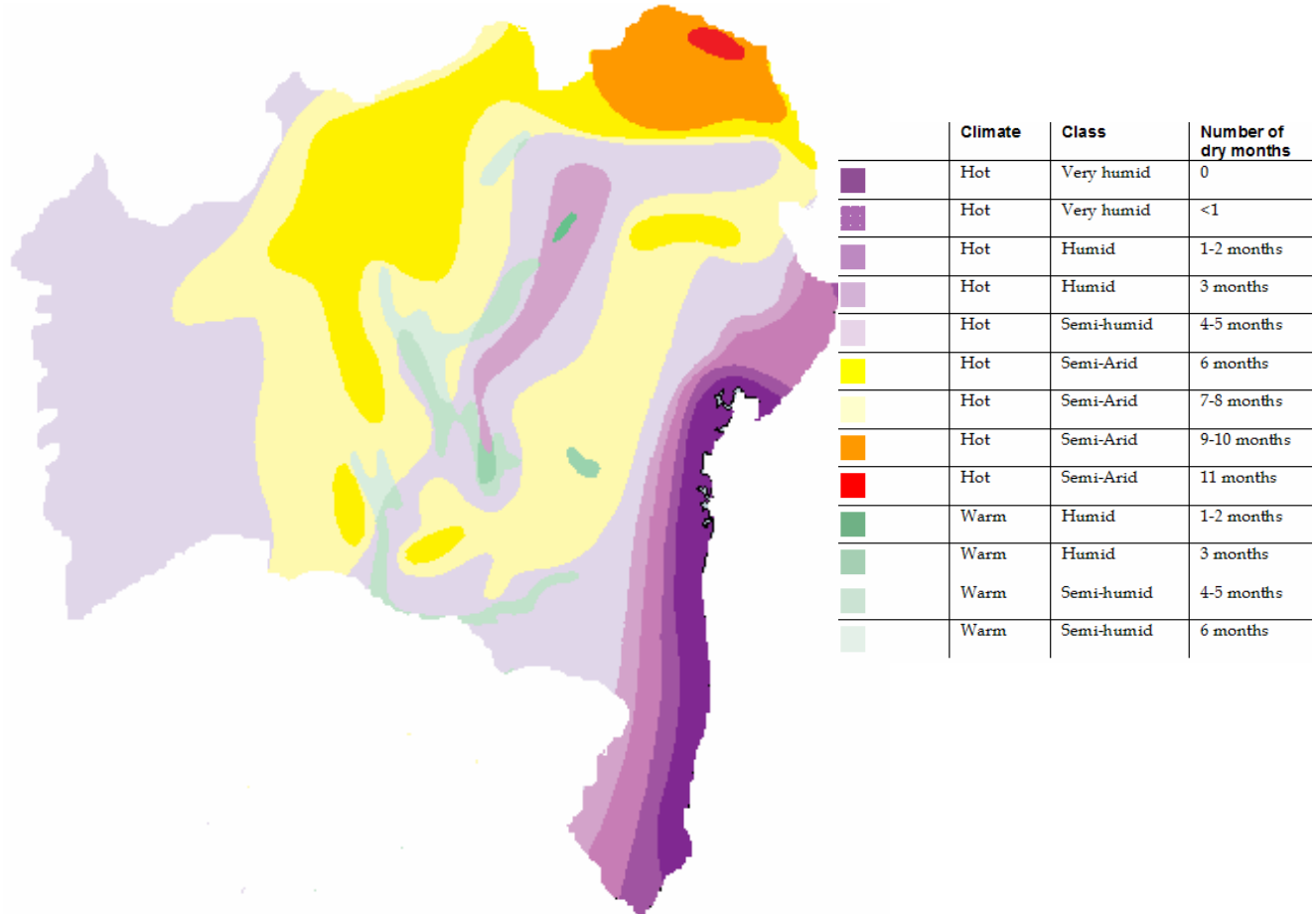
Fig. 5. Map of climate, Bahia state. Source: Instituto Brasileiro de Geografia e Estadistica IBGE.. http://www.ibge.gov.br/.accesed: 11-14-07.

1.4.4 Parasitological Data

Parasitological data consisted of annual human prevalence data from the year 1990 to 2000 for each of the 417 municipalities of the state of Bahia. Data was collected by the Fundação Nacional de Saúde, Salvador - Bahia.

1.4.5 Geographic Information Systems (GIS) and Remote Sensing (RS) Methods

Three approaches were taken to develop separate GIS risk models: 1) remote sensing models using earth observing satellite data, 2) an ecological niche model, 3) a climate based growing degree day-waterbudget model.

38

Table 3. Indicators of the state of Bahia. Source: IBGE Instituto Brasileiro de Geografia e Estatistica,*SEI; Superentêndencia de Estudos Econômicos e Sociais da Bahia http://www.sei.ba.gov.br/conheca_sei/historico/index.http.

Capital	Salvador
Area*	564,692.67 km^2
Municipalities	415
Population	13,026,171 Inhb.
Urban Population	8,025,229 Inhab
Rural population	5,000,942 Inhab
Density of population*	23.79 Inhb/Km^2
Urbanization	59.1%
Total urban households	2,038,781
Total rural households	1,088,000
Non-electrified households	620,160
Percent of rural households unelectrified	57%
Ave. no. people per household rural/urban	4.17 / 3.9
Illiteracy rate rural / urban	40.4 / 15.9

1.4.5.1 Remote Sensing Models

Satellite images from the Advanced Very High Resolution Radiometer (AVHRR) and the Moderate Resolution Imaging Spectroradiometer (MODIS) from the United States Geological Survey (USGS) and National Aeronautics and Space Administration (NASA) respectively, as well as environmental features data from the USGS Global GIS Digital Atlas of South America (ecological zones, political boundaries, populated areas) were used to develop the remote sensing models. Using ArcView GIS 8.3 and ERDAS IMAGINE 8.6 image processing software, the satellite data, environmental data and prevalence data was processed and used to develop the GIS ecological risk assessment

models (Fig. 6). Buffer zones of 5 km radius were created around prevalence points, to extract mean values within buffer areas and define ranges of climate and environmental features associated with the transmission of the parasite and the presence of the sandfly vector *Lu. longipalpis*. The ranges established were used to create models to define suitable areas for VL.

The climatic and environmental variables including mean land surface temperature (Tmax), elevation, ecological regions and normalized difference vegetation index (NDVI) were used to establish relationships of the prevalence data to the environment of the different regions in the state of Bahia. NDVI is an index that ranges from –1 to 1, but was rescaled to yield NDVI values of 0-200. The primary use of NDVI is to measure vegetation health. However, NDVI can also be used as a surrogate of soil moisture based on vegetation health and vigor (Huh and Malone, 2001). To determine the density of green on a patch of land, researchers must observe the distinct colors (wavelength) of visible and near-infrared sunlight reflected by the plants, which is related to chlorophyll activity. When sunlight strikes objects, certain wavelengths are reflected. Chlorophyll pigment in the plant leaves strongly absorbs visible light from 0.4 to 0.7 um for use in photosynthesis. The cell structure of the leaves, on the other hand, strongly reflects near-infrared light from 0.7 to 1.1 um. The wavelengths of light reflected are related to leaf area index and chlorophyll activity.

1.4.5.1.1 AVHRR Remote Sensing Model

The Advanced Very High Resolution Radiometer (AVHRR) sensor has been one of the most widely used remote sensing systems used in parasitological and epidemiological studies. This system uses 5 bands of the electromagnetic spectrum, with bands 1 and 2 being in the visible portion of the energy spectrum, bands 4 and 5 being

40

thermal infrared data, and band 3 being mid-range infrared data, all with a pixel resolution of 1.1 km^2 (Huh and Malone, 2001).
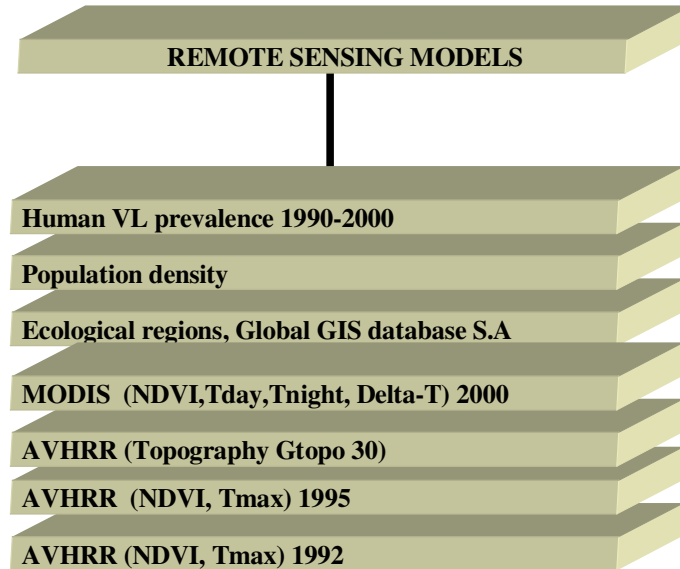
Fig. 6. Data layers that were used to construct remote sensing models to predict visceral leishmaniasis risk in the state of Bahia.

AVHRR data was obtained for 1992-1993 and 1995-1996 via the Internet from the United States Geological Survey (USGS) global 1 Km² website (http://edcdaa.usds.gov/1KM/1kmhomepage.html) for daytime land surface temperature (Tmax) and Normalize difference Vegetation Index (NDVI). Elevation data was obtained from the GTOPO 30 dataset from the United States Geological Survey (USGS) with 30 seconds resolution at earth surface (http://edcdaac.usgs.gov/gtopo30/gtopo30.asp). The NDVI and Tmax data from the AVHRR was downloaded at dekadal intervals (every 10 days) already processed by the USGS to eliminate cloud cover using an algorithm that records the highest pixel value for each pixel during the 10 day period. All the satellite images were calibrated and georeferenced to a geographical decimal-degree latitude and longitude coordinate system using ERDAS IMAGINE 8.6 image processing software

(Leica Geosystems GIS & Mapping LLC). The dekadal data was combined to create monthly composite maps by averaging images of three 10-day periods together ((Image1+Image2+ Image3)/3). Averaged monthly data was similarly averaged to create an annual composite. Composite images were incorporated into an ArcView GIS 8.3 project and converted to a grid file for further analysis. Buffer zones of 5 km diameter radius centered on the capital of each of the 415 municipalities of the state of Bahia were created to represent the health system service area basis of the annual human prevalence data for VL. These buffers were used to extract mean values of Tmax, NDVI and elevation. Since each pixel in the AVHRR and elevation grid images represents $1km^2$ ArcView GIS 8.3 calculated the mean value for all pixels within the 5km diameter buffer areas. These data provide statistically significant mean values. Based on the mean values, map queries were performed to establish ranges depending on the different prevalence for each of the three prevalence classes (high, moderate and low).

1.4.5.1.2 MODIS Remote Sensing Model

MODIS (or Moderate Resolution Imaging Spectroradiometer) is one of five sensors on-board the Terra (EOS AM) and Aqua (EOS PM) satellites of the Earth Observing System (EOS) of the National Aeronautic and Space Administration (NASA) designed to monitor the state of the Earth's environment and ongoing changes in its climate. Terra's orbit around the Earth is timed so that it passes from north to south across the equator in the morning, while Aqua passes south to north over the equator in the afternoon. Terra MODIS and Aqua MODIS view the entire Earth's surface every 1 to 2 days, acquiring data in 36 spectral bands of defined wavelength.

In this study the MODIS Terra data was used to develop the predictive models. Day and night land surface temperature (LST) as well as vegetation index (NDVI) were

used as environmental variables. MODIS data was ordered from the NASA Earth Observing System Data Gateway (http://edcimswww.cr.usgs.gov/pub/imswelcome/). The day and night thermal data was used to establish a third variable; temperature difference (dT), which is the result of the subtraction of day land surface temperature and night land surface temperature (dT= day LST – night LST). Day and night LST data was obtained as 8-day average images of 1km^2 resolution; NDVI data 16-day average composite image data with a spatial resolution of 250m.   A complete Julian calendar year of images (equivalent to the year 2000) was used in the elaboration of this model. All the satellite images were calibrated and georeferenced to a geographical decimal degree latitude and longitude coordinate system using ERDAS IMAGINE 8.6 image processing software. The 8-day composite LST and 16-day composite NDVI images were combined separately to create an annual LST and NDVI composite map by summing all the images and dividing by the number of images. The images were processed to exclude cloud cover.

Composite images were incorporated into an Arc View GIS 8.3 project and converted to a grid file for further analysis. Buffer zones of 5 km diameter were created and centered on all surveyed and geolocated points that represent the annual human prevalence of visceral leishmaniasis in the 417 municipalities of the state of Bahia. These buffers were used to extract mean values of LST (day, night and delta T) and NDVI. Arc-View GIS 8.3 was used to calculate the mean value for pixels within the entire 5 km buffer area. These data were analyzed to obtain statistically significant mean values. Based on the mean values, map queries were performed to establish ranges depending on the prevalence class (high, moderate and low).

1.4.5.2 Ecological Niche Modelling

Ecological niche models were developed using the Genetic Algorithm for Rules-set Prediction (GARP) software program http://www.lifemapper.org/desktopgarp. GARP models the ecologic niche of species based on relating point-occurrence data to the electronic maps of relevant ecological dimensions, producing a heterogeneous set of rules that describe the potential distribution of species (Peterson et al., 2002b). Previous tests of the predictive power of this modelling technique have been published elsewhere (Peterson et al., 2002a, b; Peterson et al., 2004 a, b; Stockwell, 1999; and Peterson et al., 2003).

Within GARP, input data are further divided randomly and evenly into training and intrinsic testing data sets. GARP works in an iterative process of rule selection, evaluation, testing, and incorporation or rejection: a method is chosen from a set of possibilities (e.g., logistic regression, bioclimatic rules), applied to the training data and a rule is developed or evolved (Stockwell, 1999). Rules may evolve by a number of means that mimic DNA evolution: point mutations, deletions, crossing over, etc. the change in predictive accuracy from one iteration to the next is used to evaluate whether a particular rule should be incorporated into the model, and the algorithm runs either 1000 iterations or until coverage (Peterson et al., 2004a). GARP relates ecologic characteristics of occurrence points to those of ecologic characteristics sampled randomly from the rest of the study region, developing a series of decision rules that best summarize factors associated with presence (Peterson et al., 2002b).

To generate the niche models, WorldClim data variables and elevation data (Table 4) were processed using Arc-View 3.3 Spatial Analyst and DIVA-GIS 4.0 (http://www.diva-gis.org/). GARP was run using these environmental variables with two

different groups of human leishmaniasis prevalence data. 1) places where no cases were reported and 2) sites where prevalence ranged from 1 percent to the highest observed percentage. This procedure was done for each of the eleven years (1990-2000) of available human visceral leishmaniasis data. To optimize model performance, 100 replicate models were developed based on random 50-50 splits of available occurrence points. The procedure for choosing best subsets models was based on the observation that: (1) models vary in quality (2) variation among models involves an inverse relationship between errors of omission (leaving out true distributional areas or under predicted areas) and commission (including areas not actually inhabited or over predicted areas) and (3) best models (as judged by experts blind to error statistics) are clustered in a region of minimum omission of independent points and moderate area predicted (an axis related directly to commission error); the position of the cloud of points relative to the two error axes provides an assessment of the relative accuracy of models (Costa et al., 2002; Peterson et al., 2004a).

To choose the best subset models in this study the following procedure was done: (1) Eliminate all models but those that had no omission (intrinsic) error based on independent tests points. Intrinsic omission is the percentage of the training points that are omitted from the prediction area; that is, those that are predicted to be absent but have presence records; (2) select 20 models with the least values in the omission (extrinsic) error. Extrinsic omission is the percentage of the test points that are omitted from the prediction; that is, those that are predicted absent but have presence records; (3) calculate the median area predicted present among these minimum omission points; (4) identify the 10 models closest to the overall median area predicted; (5) average these 10 models to get

45

a best subset annual model (Peterson et al, 2004b); (6) Sum the 11 (year 1990-2000) final

best subset annual models (Fig. 7).

Table 4. List of bioclimatic variables, derived from the monthly temperature and rainfall values (http://biogeo.berkeley.edu/worldclim/bioclim.htm) analyzed in this study.

BIO1 = Annual Mean Temperature
BIO2 = Mean Diurnal Range (Mean of monthly (max temp - min temp))
BIO3 = Isothermality (P2/P7) (* 100)
BIO4 = Temperature Seasonality (standard deviation *100)
BIO5 = Max Temperature of Warmest Month
BIO6 = Min Temperature of Coldest Month
BIO7 = Temperature Annual Range (P5-P6)
BIO8 = Mean Temperature of Wettest Quarter
BIO9 = Mean Temperature of Driest Quarter
BIO10 = Mean Temperature of Warmest Quarter
BIO11 = Mean Temperature of Coldest Quarter
BIO12 = Annual Precipitation
BIO13 = Precipitation of Wettest Month
BIO14 = Precipitation of Driest Month
BIO15 = Precipitation Seasonality (Coefficient of Variation)
BIO16 = Precipitation of Wettest Quarter
BIO17 = Precipitation of Driest Quarter
BIO18 = Precipitation of Warmest Quarter
BIO19 = Precipitation of Coldest Quarter
ALT   = Topography (mean elevation)

1.4.5.3 Growing Degree Day-Water Budget Model

Little data is available on the environmental preferences and limits of tolerance of

the *L. chagasi-Lu. longipalpis* system in relation to climate. Thus these factors were

estimated by defining the climate regime associated with presence or absence of the

parasite-vector within a GIS in combination with the available published data. Resource

data used to generate the GDD-WB model included a 30-year average monthly climate surface grid (18x 18km) of South America dataset (Mud Springs Geographers, Temple, TX), a human visceral leishmaniasis prevalence database from the state of Bahia (Fundação Nacional de Saude, Estado da Bahia) and development data obtained in different studies where *Lu. longipalpis* colonies were established and maintained in the laboratory (Killick-Kendrick et al., 1977). The objective was to define a suitability gradient in the environment for propagation and transmission of the vector-parasite system.
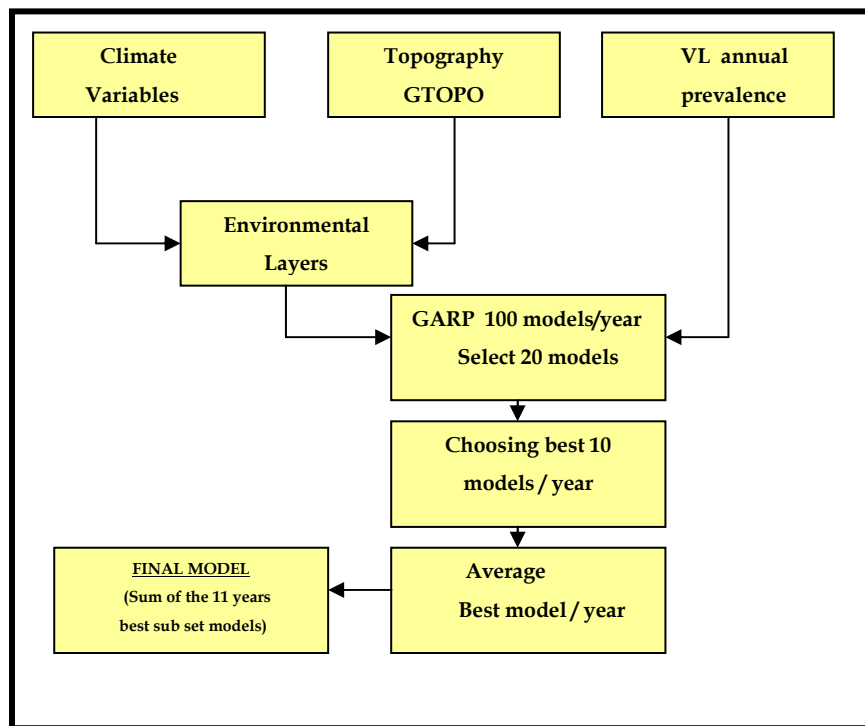
Fig. 7. Procedure to select the best subsets models to predict VL in the state of Bahia, Brazil.

The 30-year average monthly climate surface grid (18 x 18 km cells) of South America included data on maximum temperature, minimum temperature, precipitation and potential evapotranspiration (PET). These parameters were used to calculate mean

monthly temperature ((max temp - min temp)/2) and water budget (PPE = (precipitation / PET)). Growing-degree-days (GDD) is defined as the number of the degrees over a base value (base temperature) below which no development of a species occurs, in this case the sandfly vector *Lu. longipalpis*. GDD can be accumulated for months in which conditions were within the suitability threshold range temperature and water budget threshold values to derive annual GDD values.

The water budget (PPE) threshold value was defined by GIS analysis, as a condition where PPE, the ratio of rain/potential evapotranspiration (R/PET), was >0.7, (ie. the soil moisture content was 70% saturated). The PPE threshold was determined by extracting mean climate attribute data values from grid cells in which annual human prevalence data points were located for two infection prevalence ranges; moderate prevalence (1-5%) and high prevalence (5-14%) of VL. GIS map query functions were performed to confirm the PPE threshold of the presence of suitable amount of surface water or soil moisture; no positive sites were found if PPE > 0.7, suggesting a preference of the vector for drier environments.

The base temperature for development of *Lu. longipalpis* has not been determined. Thus a best estimate of the base temperature value was made by GIS query analysis using known endemic site data in combination with data from the following literature reports on temperature requirements for reproduction and development of *Lu. longipalpis*:

-A temperature of 25°C is the optimal temperature for reproduction and development in the laboratory of *Lu. longipalpis* (Killick- Kendrick et al., 1977; Mody and Tesh., 1983; Rangel et al., 1996).

-The potential generations that can be completed in one year at 25°C for *Lu. longipalpis* is 7.7 generations per year (Killick-Kendrick et al., 1977).

-At 25°C, 40 days are required from engorgement of female *Lu. longipalpis* to first emergence of adults of the next generation (Killick-Kendrick et al., 1977).

-The average annual mean minimum temperature recorded in the state of Bahia for sites of >1% prevalence was 14.7^{o}C, based on point-polygon extractions and GIS map queries on the 30-year-average monthly climate surface grid.

The base temperature of *Lu. longipalpis* was estimated using the reported laboratory development requirements (40 days for one generation, 7.7 generations per year at 25 ^{o}C, 80% humidity) and the average annual mean minimum temperature value (>14.7^{o}C), in a two-step calculation:

- The number of growing degree days that must be accumulated to complete one generation was estimated to be 412 GDD. For this estimation the following parameters were used: the optimum mean temperature in laboratory (25 ^{o}C) minus the minimum mean temperature registered at positive sites for *L. chagasi* in the state of Bahia (14.7 ^{o}C) times days required from engorgement to first emergence of adults (40 days):

$$[(25^oC – 14.7\,^oC) \times 40\ days] = 412$$

- The number of growing-degree-days to complete one generation (412) and the potential generations that can be completed in one year (7.7) was used to solve for the base temperature (X): $[(25\,^oC – X) * 365]/412 = 7.7;\ X = 16^oC$

A base temperature value of 16°C was accepted as the best estimate, pending more detailed laboratory +/or field studies, of the minimum temperature in which no development of the sandfly vector *Lu. longipalpis* proceeds in the state of Bahia.

Derivative climate variables were calculated, using 16°C as the base temperature, for each cell of the 18 x 18 km climate grid, including monthly and annual values for:

- *Monthly GDD*. Monthly GDD was calculated as the mean temperature minus 16 $^{\circ}$C times days of the month (eg. Jan= 31, Feb = 28, June= 30); the annual GDD is the sum of monthly GDD values.

-*GDD* times water budget *(PPE)*, *if PPE < 0.7*. Using the formula (GDD*(PPE< 0.7)), a thermal-hyrdrological gradient value was calculated that considered *both* thermal regime (GDD) and the influence of variable moisture regime for each month. The moisture threshold value <0.7 was estimated by a GIS map query of the PPE value in grid cells that included positive *L. chagasi* points. This procedure was performed for each month, then summed to obtain the 'gradient model' and divided by the number of growing degree days to complete one generation (412) to get the annual potential generations.

1.4.6 Statistical Analysis

The 11-year prevalence average data for VL in Bahia were analyzed with environmental data by stepwise logistic regression and by multiple regression for the remote sensing models (AVHRR, MODIS) and the GDD-WB climate based model to establish significant (*p*<0.05) associations between environmental variables and the prevalence of visceral leishmaniasis. Analysis was done using SAS software (Cory, NC). GARP is a statistical analysis program.

1.5 Results

1.5.1 AVHRR and MODIS Remote Sensing Models

AVHRR satellite derived NDVI and LST data for the years 1992 and 1995 independently, and GTOPO30 elevation data were significantly related to prevalence data when analyzed using stepwise logistic regression. For MODIS satellite data from the

year 2000, day temperature and NDVI were significantly related to prevalence data. (Table 5). The multiple regression analysis was done to identify the environmental variables that may have impact on the distribution of the disease in Bahia.

The AVHRR variables that were significantly related to prevalence were elevation, NDVI 1995 and day LST (1992 and 1995). For analysis of MODIS data from the year 2000, the significant variables were NDVI and temperature difference (DeltaT) (Table 6). The data ranges of suitability used to create the climate model that represented the high risk area for visceral leishmaniasis are shown in (Table 7). In the three models (Fig. 8, 9, 10) the high and medium prevalence points were found in regions predicted as high risk area. The low prevalence or negative points were mainly distributed in coastal zone regions, with a few in the Cerrado ecological zone.

Table 5. Logistic regression analysis for AVHRR 1992, 1995 and *MODIS 2000 satellite data related to VL prevalence data. (OR: odd ratios; CI confidence intervals; NDVI: normalized difference vegetation index; LST: land surface temperature).

Variables	OR	95%CI	P
Elevation	0.997	0.996-0.998	<.0001
NDVI 1992	1.028	1.009-1.048	0.0038
LST 1992	0.812	0.760-0.868	<.0001
NDVI 1995	1.031	1.013-1.050	0.0007
LST 1995	0.864	0.824-0.906	<.0001
LST 2000*	0.892	0.806-0.986	0.0256
NDVI*	186.361	7.008->999	0.0018

Table 6. Multiple regression analysis for AVHRR 1992, 1995 and *MODIS 2000 satellite data related to VL prevalence data. (NDVI: normalized difference vegetation index; LST: land surface temperature; Delta T (day temperature – night temperature)).

Variables	Error	Type II	F value	Pr>F
Elevation	0.00102	808.492	30.47	<.0001
LST 1992	0.08519	629.3438	23.71	<.0001
NDVI 1995	0.03550	205.61924	7.32	0.0071
LST 1995	0.06994	482.525	17.19	<.0001
Delta T 2000*	0.04769	12.9262	4.43	0.0360
NDVI 2000*	1.3136	25.0852	8.59	0.0036

Table 7. Ranges used for queries to develop the AVHRR 1992, 1995 and MODIS 2000 remote sensing models to predict VL. (NDVI: normalized difference vegetation index; LST: land surface temperature; Delta T (day temperature – night temperature)).

Variable	Satellite	Year	Ranges
NDVI	AVHRR	1992	131 – 148
LST	AVHRR	1992	19 - 25 ^{o}C
Elevation	AVHRR		475 - 963 mts
NDVI	AVHRR	1995	128 - 145
LST	AVHRR	1995	17.5 - 24.5 ^{o}C
NDVI	MODIS	2000	0.44 - 0.69
Day temp	MODIS	2000	28 - 37 ^{o}C
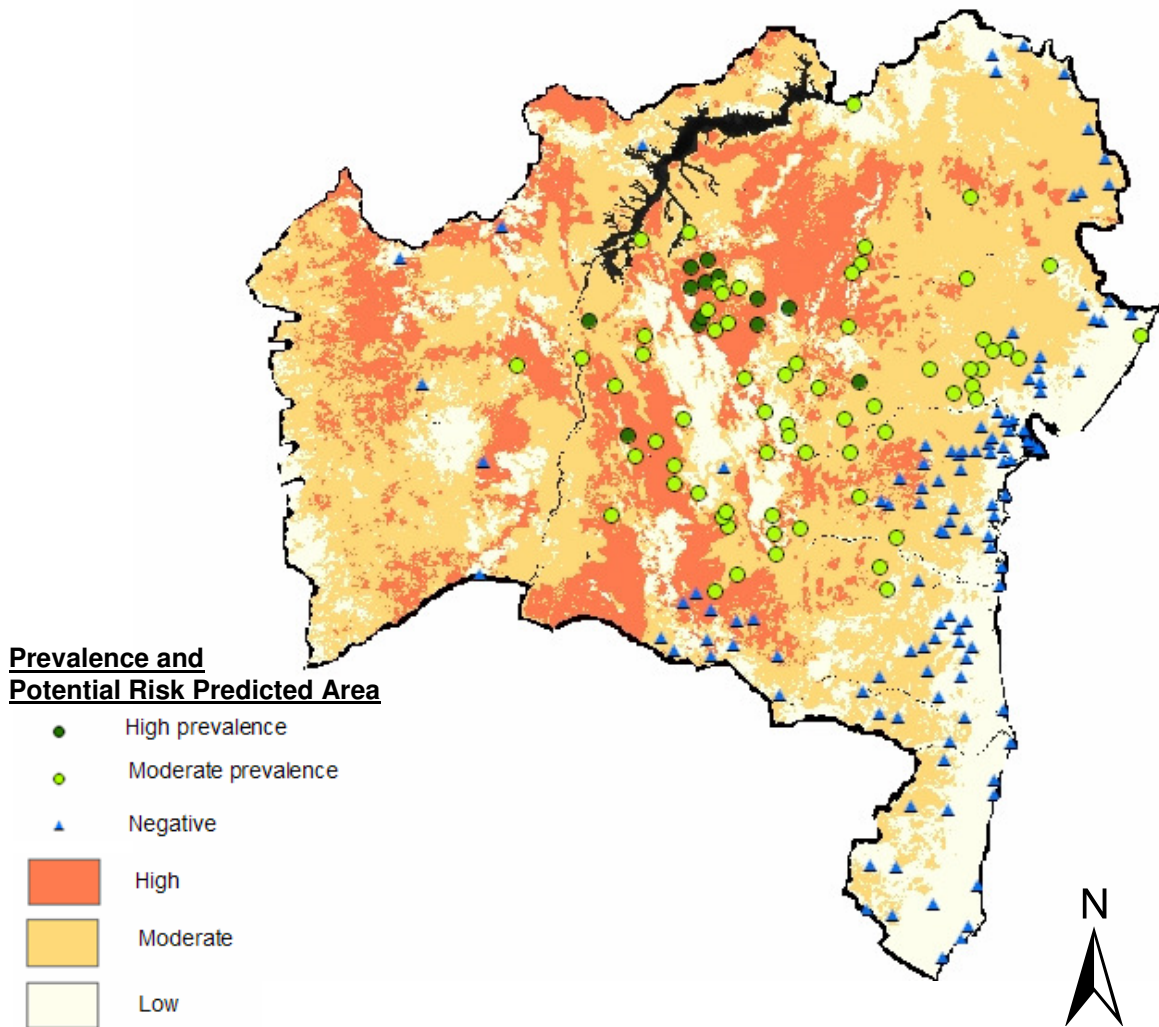
Fig. 8. AVHRR 1992 Model. High and moderate prevalence points were found in regions predicted as high and or moderate risk areas. Negative points were mainly distributed in the costal area of the state of Bahia predicted as a low risk area. ▲ Negative: no reported VL cases.

**Prevalence and
Potential Risk Predicted Area**

○   Medium Prevalence

●   High Prevalence

▲   Negative

▮   High Risk Predicted Area
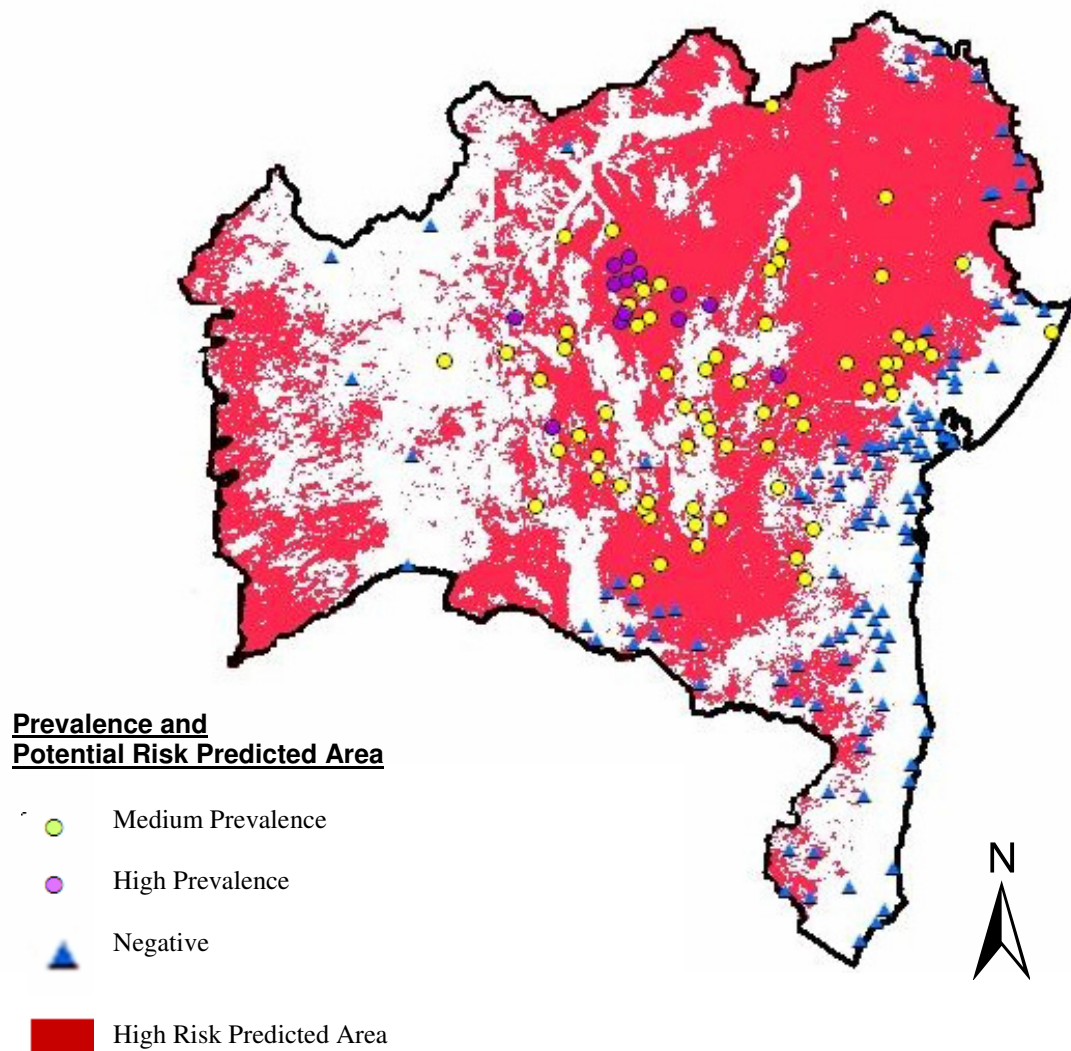
Fig. 9. AVHRR 1995 model. High and medium prevalence points are mainly found in regions predicted as high risk areas. Negative points are mainly distributed in the costal area of the state of Bahia. ▲ Negative: no reported VL cases.

1.5.2 Ecological Niche Model

The ecological niche model using the GARP analysis, based on the WorldClim bioclimatic data and topographic data was processed using Arcview 3.3 software to create and classify an eleven-year composite risk map (Fig. 7) The 11 best annual models were summed and ranked by the criteria of how many times each model predicted the same pixel within the endemic area and classified as: High, 11 times; Moderate, 6-10; Low, 1-5 and Negative, where none of the final models predicted the disease. All of the

high prevalence points fell in to the predicted high risk area. All the middle prevalence points fell into the high or moderate risk area, and (Number); 32% of the negative prevalence points were predicted within the low risk areas. The remaining negative points (Number; 68%) fell in the area predicted as negative risk. The final model predicted that the ecological region known as Bahia coastal forest was uniformly negative (Fig. 11).

Prevalence and Potential Risk Predicted Area

○ Medium Prevalence

● High Prevalence

▲ Negative

■ High Risk Predicted Area
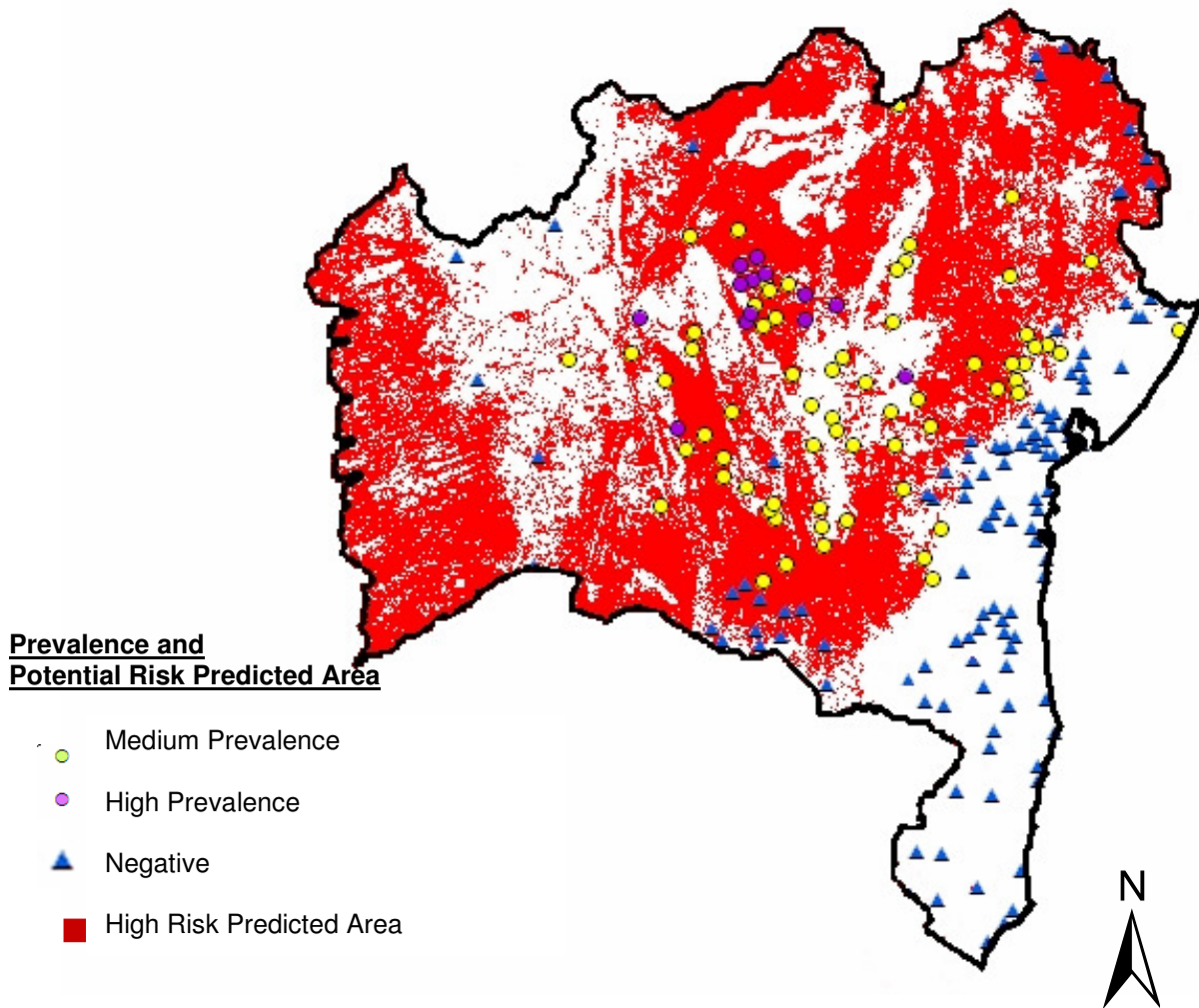
Fig. 10. MODIS model for year 2000. High and medium prevalence points are mainly found in regions predicted as high risk areas. Negative points are mainly distributed in the costal area of the state of Bahia. ▲ Negative: no reported VL cases.

Prevalence and Potential Risk
- Medium Prevalence
- High Prevalence
- Negative
- Low
- Moderate
- High

N
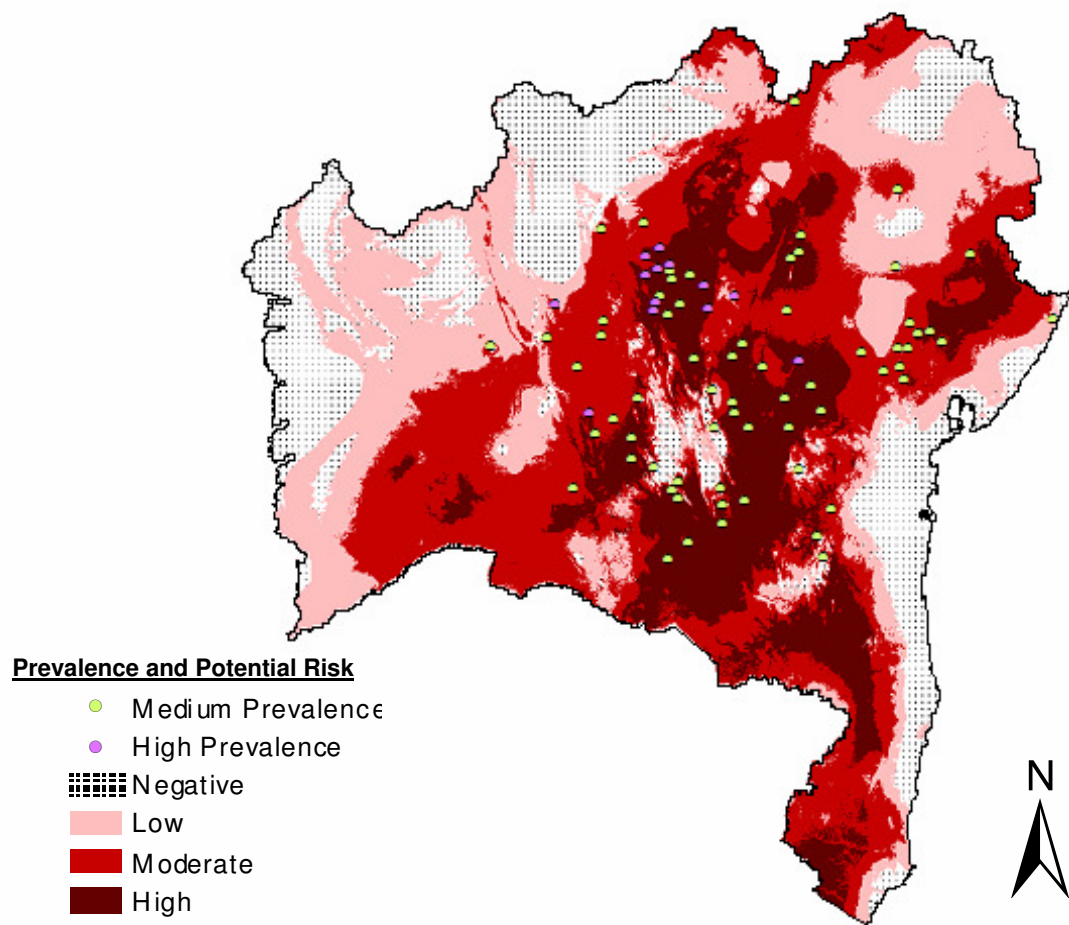
Fig. 11. GARP, ecological niche model. The best annual models were summed and ranked by the criteria of how many times each model predicted the same pixel within the endemic area, classified as: High, 11 times; Moderate, 6-10;Low 1-5 and Negative where none of the final models predicted VL. Negative: no reported VL cases.

1.5.3 Growing Degree Day – Water Budget Model

The predicted potential generations per year that can occur in various locations in Bahia ranged from 0 to 9 generations per year. The high and medium prevalence points were all found in regions predicted to have more than 5 generations per year (Fig. 12). The Caatinga ecological region was characterized as having at least 5 potential generations per year; it was the region with highest number of predicted potential generations per year (Range). The Caatinga zone is characterized as a hot and semi-arid region, suggesting its characteristics as suitable for the vector *Lu. longipalpis*. The

Cerrado ecological region was predicted to have 2-4 potential generations per year and the Bahia coastal region was predicted to have 0-3 generations per year. The number of potential generations decreased at sites of greater proximity to the east and southeast. GIS query analysis showed the maximum annual mean temperature for the development of *Lu. longipalpis* was 28°C and the minimum annual mean temperature was 16°C. All variables analyzed by stepwise logistic regression were significantly related to the occurrence of the disease (Table 8). Multiple regression analysis was done to determine which environmental factors had the highest impact on the distribution and abundance of *Lu. longipalpis.* Environmental factors that showed a significant influence in the presence and the distribution of *Lu. Longipalpis* were water budget (rain/potential evapotranspiration = PPE); annual potential generations (APG); and ecosystem (Table 9).

Table 8. Results of logistic regression analysis for the growing degree days – water budget model relating climate and VL prevalence data. PPE: potential evapotranspiration, APG: annual potential generations. OR: odd ratios, CI confidence intervals.

Variables	OR	95% CI	*P*
PPE	0.997	0.996-0.998	< 0.0001
APG	0.812	0.760-0.868	< 0.0001

Table 9. Results of multiple regression analysis for the growing degree days – water budget model relating climate and VL prevalence data. PPE: potential evapotranspiration, APG: annual potential generations and Ecoregions: ecological regions.

Variables	Error	Type II	F value	Pr>F
PPE	0.37586	13.39	4.87	0.0277
APG	0.00084	32.68	11.87	0.0006
Ecoregions	0.04266	14.92	5.42	0.0202
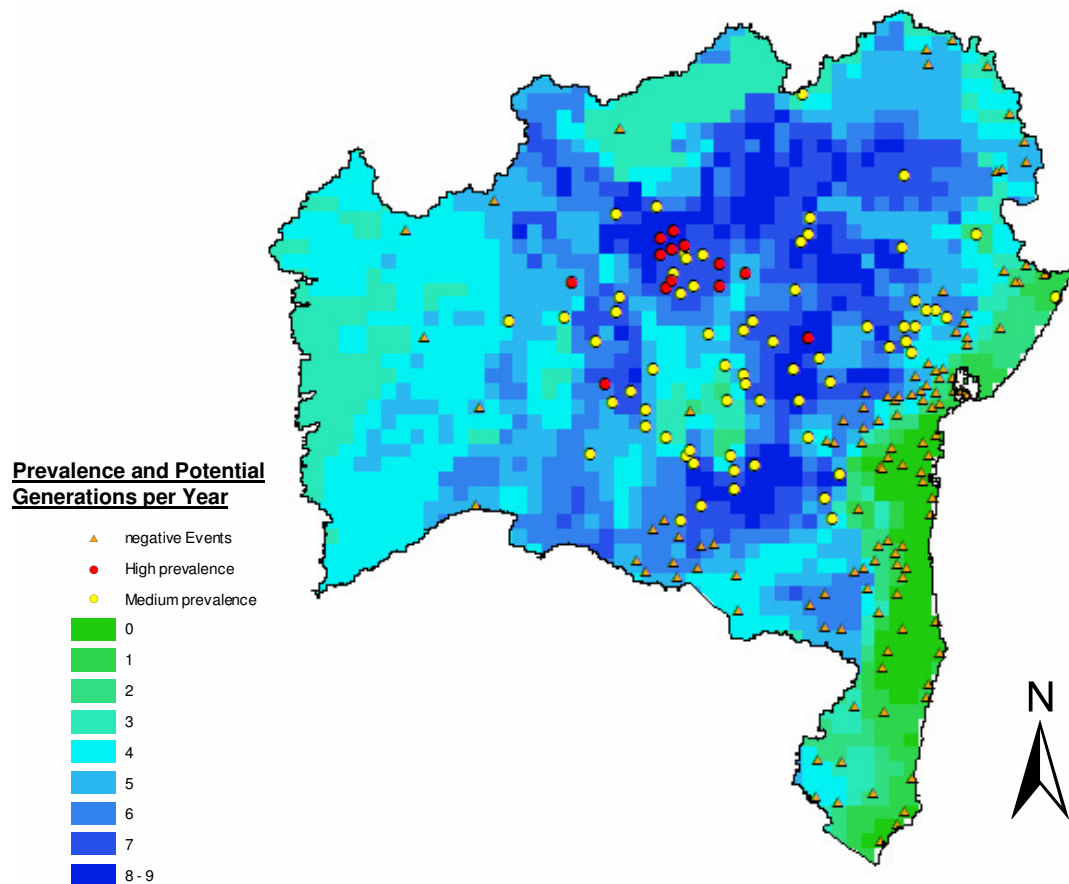
Fig. 12. Growing degree day-Water Budget model. The predicted potential generation per year of the vector *Lutzomyia longipalpis*, that can occur in various locations in Bahia ranged from 0 to 9 generations per year. The high and medium prevalence points were all found in regions predicted to have more than 5 generations per year.

1.5.4 Ecological Zones

Comparison of the GARP ecological risk model prediction and the GDD-WB model prediction of the distribution of VL in the state of Bahia to the ecological zone map of Bahia suggests that the Caatinga ecological zone, a zone characterized by a hot and semi-arid environment, is the highest risk area and that the coastal region and the Cerrado ecological regions are lower risk areas (Fig. 13). The Northeast Brazil Dry Forest and the Bahia Interior Forest zones may be intermediate transitional zones. Both the final GARP model and the GDD-WB generations per year model predicted major areas in the ecological region known as Bahia Coastal Forest as negative. Table 10,

shows the municipalities with the highest average prevalence in eleven years the ecological regions and climatic typology.

Using the GDD-WB model, the Caatinga ecological region was characterized as having more than 5 potential generations per year; it was the region with highest number of predicted potential generations per year. The Cerrado ecological region was predicted to have 2-4 potential generations per year and the Bahia coastal region was predicted to have 0-5 generations per year. The number of potential generations per year decreased at sites of greater proximity to the east and southeast.

Table10. Municipalities in the state of Bahia, Brazil with the highest average prevalence over eleven years, elevation, population, ecological regions, and climatic typology (Thornthwaite).

Munipality	Altitude (M)	Population	11years average Prev	Highest prev in 11 years	Ecological region	Climatic typology (Thornthwaite)
Jussara	615	13,111	13.5	51	Caatinga	Semi-arid
Barra do Mendes	700	12,827	12.5	41.3	Caatinga	Semi-arid, Sub-humid, dry
Uibaí	600	13,341	12.5	21.7	Caatinga	Semi-arid
Morpará	415	8,496	10.8	41.6	Caatinga	Semi-arid, Sub-humid-dry
Central	697	14,200	10.3	19	Caatinga	Semi-arid
Presidente Jânio Quadros	680	12,858	9.9	22.5	Caatinga	Semi-arid Sub-humid-dry
Cafarnaum	750	14,600	9.2	25.3	Caatinga	Semi-arid, Sub-humid-dry
América Dourada	660	14,879	8.6	23.5	Caatinga	semi-arid
Ruy Barbosa	360	28,804	8.3	17.7	Northeast dry forest	Semi-arid
Boquira	600	20,828	7.5	19.2	Caatinga	Sub-humid to dry semi-dry
São Gabriel	680	18,211	6.8	14.8	Caatinga	Semi-arid
Ibipeba	700	14,413	6.3	19.4	Caatinga	Semi-arid
Morro do Chapéu	1040	31,979	5.5	16.9	Caatinga	Sub-arid to arid

Prevalence and Eco-regions

- ● High Prevalence
- ● Medium Prevalence
- ● Negative Events
- ■ Bahia coastal forests
- ■ Bahia interior forests
- ■ Brazilian Atlantic coast restingas
- ■ Caatinga
- ■ Campos Rupestre montane savanna
- ■ Cerrado
- ■ Northeast Brazil dry forests
- ■ Northeast Brazil segment 5 mangroves
- ■ Northeast Brazil segment 6 mangroves
- ■ Pernambuco interior forests
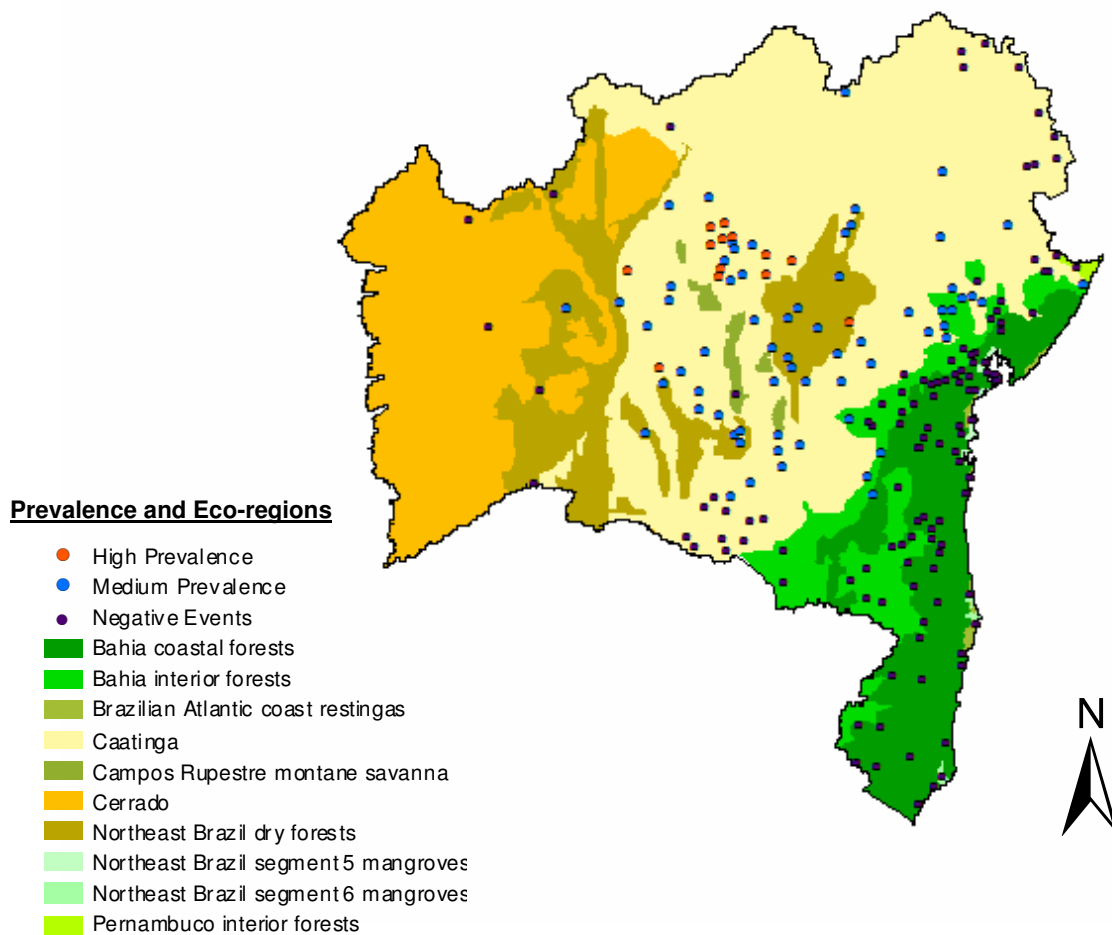
Fig. 13. Ecological Regions Bahia State, Brazil. The final models growing degree days – water budget and Ecological risk model predicts as negative an ecological region known as Bahia coastal forest. The high risk area corresponds mostly to the interior region of the state, matching with the ecological region known as Caatinga.

1.6 Discussion

Three climate-based risk models were developed for VL in Bahia; a remote sensing image data model, a model based on GARP statistical software and a model using GDD-WB analysis. The three models, using different approaches in their analysis, predicted a similar distribution and abundance pattern for the *Lu. Longipalpis-L chagasi* system in Bahia. Temperature and moisture are fundamental determinants of the distribution and abundance of species and are an essential part of any attempt to assess environmental risk factors of the diseases' agents and vectors. Each species has unique

thermal-hydrological regime requirements that determine the biological and habitat preferences, limits of tolerance and optimum conditions for development that determine where it is found (Pavlovski. 1954). Where these requirements are unknown, it is possible, within a GIS, to use site records of known positive and/or negative occurrence to define biological niche requirements using automated statistical packages (e.g. GARP).

Alternatively, it is possible to infer niche requirements by iterative step-by-step 'range-finding' of individual environmental feature layers extracted from known sites in a GIS, followed by extrapolation to areas with similar environmental features or combinations of features by GIS query analysis of layers of statistically important environmental features (Malone, 2005). Previous studies have reported the temperature, humidity and generations per year that can completed by *Lu. longipalpis* under laboratory conditions. Little data is available on the environmental preferences of the *Lu. longipalpis - Le. chagasi* system, and the eco-epidemiology of VL in the state of Bahia. In this study, previously reported laboratory data was used to create models that extrapolate laboratory conditions to natural habitats. The models were used to define the thermal hydrological niche of the *Lu. Longipalpis-L chagasi* system. The potential number of generations per year that can be completed by the vector *Lu. longipalpis* was calculated using GDD-WB climate analysis and GIS query analysis based on known VL prevalence point records in the state of Bahia. Environmental niche models, using the GARP analysis and GIS-RS models, show the potential risk and distribution of the disease in Bahia. These analysis also highlighted important environmental variables that influence the presence of the *Lu. longipalpis - Le. chagasi* system. The geospatial analysis tools using GIS methodologies make possible the study of the distribution, risk, and environmental factors that affect

disease presence and transmission. This unique approach is also important as a tool for disease prevention, prediction and control measurement.

1.6.1 AVHRR and MODIS Remote Sensing Models

The AVHRR and MODIS remote sensing models showed very similar results in the predicted distribution pattern of *L. chagasi* disease risk. For both the AVHRR and MODIS models, a large part of the positive prevalence points fell into the predicted risk area. Comparison of the remote sensing models, the growing degree day-water budget (GDD-WB) model and the GARP ecological niche model, revealed that all three methods resulted in similar predicted risk areas and showed the suitability of dry areas for the vector. Results indicate remote sensing climate surrogate data of temperature and moisture regime, LST and NDVI respectively, as well as elevation are all measures of the fundamental climatic determinants of the distribution and abundance of *Lu longipalpis*. Previous studies in schistosomiasis, leishmaniasis and malaria (Guimarãesa et al., 2008; Bavia, 1996; Malone et la., 2003; Thompson et al., 2002; Sudhakar et al., 2006) have found that climatic and environmental variables, such as NDVI, temperature and elevation, are important factors for the presence and transmission of diseases.

A similar study has been performed using GIS to predict distribution of VL vectors. Teshome et al., 2004 studied the distribution of two principal vectors of kala-azar in East Africa (*Phlebotomus martini* and *Phlebotomus orientalis)* using GIS with variables as NDVI, LST, FAO climate and soil data. Annual, wet season and dry season models were also constructed. Logistic regression analysis indicated that: LST dry season composite, average altitude, mean annual temperature and soil moisture are the best ecological determinants for *P. martini* while LST annual composite was the only important ecological determinant for *P. orientalis*. Other studies performed in the state of

Bahia indicate that NDVI is one of the most important risk factors and low NDVI values are related to high numbers of sand flies and high numbers of human and canine VL positive cases (Bavia et al., 2005). The present study agrees that temperature, moisture and vegetation are fundamental determinants for the distribution and abundance of the vector in this case (*Lu. longipalpis*) and therefore the risk of VL transmission.

A potential drawback for the future use of remote sensing data in risk prediction models could be the difficulty of working with AVHRR and MODIS images due to the effects of cloud cover on the image. This is an issue particularly in zones of high rainfall such as the tropical broadleaf forests that are subject to frequent cloud cover. Data from the Tropical Rainfall Measuring Mission (TRMM), a joint mission between NASA and the Japan Aerospace Exploration Agency designed to monitor and study tropical rainfall, may offer an alternative or complimentary method with the potential to adjust for the rainfall-cloud cover issue. TRMM utilizes a combination of data from five sensors, including radar and microwaves, that are able to penetrate through cloud cover.

1.6.2 Ecological Niche Model

Ecological niche modeling using GARP generated accurate models of climate suitability. The final 11-year-composite map of high, moderate, low or no risk of VL significantly corresponded to actual disease prevalence records. Importantly, all of the high prevalence points (14) fell into the predicted high risk area, all of the middle prevalence points (58) fell into the high or moderate risk area, and (38) of the negative points were predicted within the low risk areas. Prevalence points with less than 1% were not considered in the development of the GARP model as sites with <1% were considered to represent potential migratory or sporadic cases.

The variation in area covered by annual GARP risk prediction maps over the eleven year study period, as compared to reported cases of VL, suggests that annual variation in geospatial risk may occur in individual years in endemic areas due to annual climate variation. Further study in Bahia and other VL endemic areas is warranted to confirm and extend this observation for use in the development of annual risk prediction methods. The GARP risk map result, shown in Fig. 10, is compatible with the fundamental principles described for the distribution and abundance of species where there is a zone of stable presence. Outside the zone of stable presence there is a transitional zone of patchy presence, or absence, in marginally suitable conditions. This zone is surrounded by an unsuitable zone where the species is absent (Andrewartha and Birch., 1954).

Previous studies have used the GARP as a tool to predict potential geographic and ecological distributions of *Lutzomyia* sand flies vectors that transmit leishmaniasis in South America (Peterson et al., 2004; Peterson and Shaw, 2003). This new tool in disease prediction provides a large-scale perspective on species' geographic distributions based on ecological and or climatic factors that determine the presence of the disease. No studies on VL using the GARP have been carried out in the state of Bahia; in this study we incorporate a new approach for the prediction of risk areas of VL in the state of Bahia.

1.6.3 Growing Degree- Water Budget Model

Development of climate risk models using GDD-WB analysis depends on availability of data on the environmental preferences and limits of tolerance of the *L. chagasi-Lu. longipalpis* system in relation to climate. Similar studies have used the growing degree day-water balance spatial analysis in malaria, based on climate suitability for the *Plasmodium falciparum-Anopheles arabien-sis* system in Eritrea (Malone et al.,

2003) and snail-borne diseases (Malone et al., 2004), to demonstrate the use of the unique thermal-hydrological preferences and limits of tolerance of individual parasite -vector systems. These studies were based in known parasite- vector temperature requirements and limits of tolerance. For the *Le. chagasi-Lu. longipalpis* system, only incomplete published data from the field or laboratory studies were available (Table 11).Thus these factors were estimated from available published data in combination with GIS query analysis to define the thermal-hydrological regime associated with presence or absence of VL. The GDD base temperature, below which no development progresses, was estimated to be 16°C through review of literature reports and GIS query of conditions at positive site records. No recorded cases were found where average annual monthly water budget values of >0.7 were seen or where average annual mean temperature exceeded 28°C.

The GDD-WB model indicated that the highest prevalence of the disease corresponded to areas with the highest predicted number of potential generations per year (Fig. 12). The GDD-WB predictive model was based on data acquired from GIS analysis and literature review of parameters of the reproduction in the laboratory of the VL vector *Lu. longipalpis*. Further studies on the temperature requirements of *Lu. longipalpis* in natural environments and in the laboratory are needed to more accurately define the distribution of the vector and potential risk of VL transmission based on the GDD-WB potential generations per year concept.

1.6.4 Ecological Zones

Comparison of the remote sensing models, the GARP ecological niche model and the GDD-WB models that predict the risk of VL in the state of Bahia to the ecological zone map of Bahia suggests that the Caatinga ecological zone, a zone characterized by a hot and semi-arid environment, is the highest risk area and that the coastal region and the

Cerrado ecological regions are lower risk areas (Fig. 13). The Northeast Brazil Dry Forest and the Bahia Interior Forest Zones may be intermediate transitional zones. Results of analysis by the GDD-WB model revealed the western part of the Bahian Cerrado had low number of generations. The Bahia coastal forest that is part of the Bahian Mata Atlantica showed a lower number of generations per year indicating low development of the vector and consequently low risk for VL in moist zones.

Literature reports support our findings that *Lu. longipalpis* is found most often in brush land and not in open savannah, grasslands or heavily forested areas with broadleaf forest or on the Atlantic border with tropical littoral forest. Sherlock (1996) describes the distribution of the disease in the state of Bahia as limited to the central Plateau, where the vegetation is predominantly xerophilous. Although the Caatinga has been predicted as the most suitable ecological region for the disease to occur (Bavia et al., 2005), Sherlock (1996) also concludes that major modification of sylvatic ecosystems caused by deforestation, causes different animals (wild and domestic) to encroach on places near human dwellings and changes the distribution of the disease as well as the distribution of the vector. Moreover, Equatorial-semi-arid areas of poor soils predispose children to malnutrition, increasing the risk of becoming infected with VL (Thompson et al., 2002).

In the present study, ecological risk models were developed within a GIS using GARP analysis, GDD-WB generations per year concept and the remote sensing models to predict the distribution and potential risk of VL in the state of Bahia, Brazil based on thermal-hydrological climate regime. The remote sensing models, GARP ecological niche analysis, and the GDD-WB models using different analysis approaches and the same 11-year VL prevalence database, predicted similar distribution and abundance patterns for the *Lu. longipalpis -L chagasi* system in Bahia, and disease prevalence

65

records were shown to be related to major ecological zone maps. At macrohabitat scales, the three approaches (RS model, GARP niche model and the GDD-WB generations-per-year model) showed that climate and thermal-hydrological regime are key determinants of VL disease risk potential in Bahia. The prevalence classifications (high, moderate and low) coincided with the predicted areas of the GDD-WB model and the GARP ecological niche model. Additional studies to validate and apply the remote sensing, GARP and GDD-WB prediction models of VL risk in Bahia and elsewhere in Brazil as control program management tools are warranted. Further development of biology-based disease risk models for VL will be conditional on availability more detailed data from laboratory and field studies on the biological requirements and distribution at both macrohabitat and microhabitat scales of the *Lu. longipalpis-Le. chagasi* vector-parasite system.

Table 11. Reported parameters for maintenance and productivity of *Lu. longipalpis*[1]Killick-kendrick et al, 1977; [2]Rangel et al, 1986; [3]Modi and Tesh, 1983; [4]Aquino-Teixeira et al, 2002.

Developmental Times	At room temperature $25^{\circ}C$[1], relative humidity 80%[2]. From engorge to the first emerge of adults is 35[3]-40[1] Breed in less constant conditions (48-54)[1] (25-42)[2].
Hatching	4-9 days[1] and 6-9 days[2].
Larvae *First instar* *Second instar* *Third instar* *Fourth instar*	Larvae (four instars)14-19) days[2] 3-5 days (first week)[1] 2-4 days (second week)[1] 1-5 days (send week)[1] 3-9 days (third week)[1]
Pupae	Emergence from pupae on day 10[1] (most of the adults) and 8-9 days[2]. Few males emerges as early as 7 days[1]
Adults longevity	From 2 weeks-one month (adults are robust in rough conditions). In females that have taken blood is determined by when eggs are laid (few survive 24 hours after ovoposition). Most live for more than one month[1].
Productivity of the colony	23 generations in 36 months[1]
Cultures of promastigotes and amastigotes	Promastigotes at $22^{\circ}C$, amastigotes at $34^{\circ}C$, reversion from amastigotes to promastigotes at $28^{\circ}C$[4]

1.7 References

Agrela, I., Sánchez, E., Gómez, B., Feliciangeli, M.D., 2002. Feeding behavior of *Lutzomia pseudolongipalpis* (Diptera: Psychodidae), A Putative vector of Visceral Leishmaniasis in Venezuela. J. Med. Ent. 39, 440-445.

Aguilar, G.M., Vilela, M., 1987. Aspects of the ecology of sandflies at the Serra dos Órgãos, National park, State of Rio de Janeiro. VI-Shelters and breeding places (Diptera, Psychodidae, Phblebotominae). Mem. Inst. Oswaldo Cruz. 82, 585-586.

Alexander, B., Lopes de Carabalho, R., McCallum, H., Pereira, M.H., 2002. Role of the doemstic Chicken (Gallus gallus) in the epidemiology of urban visceral leishmaniasis in Brazil. Em. Inf. Diseases. 8, 1480-1485.

Allison, M.J., Leishmaniasis. In: Kiple KF.ED. The cambrige History of human disease, Cambridge, Cambridge University press, 1993.

Andrewartha, H.G., Birch, L.C., 1954. The Distribution and Abundance of Animals. The University of Chicago Press, Chicago, Illinois.

Antoine, J.C., Prina, E., Lang, T., Courrent, N., 1998. The biogenesis and properties of the parasitophorous vacuoles that harvour Leishmania in murine macrophages. Trends Microbiol. 7, 392-401.

Aquino Teixeira, M.C., De Jesus Santos, R., Barreto Sampaio, R ., Pontes-de-Carvalho, L., Washington Dos-Santos, L.C., 2002. A simple and reproducible method to obtain large numbers of axenic amastigotes of different *Leishmania* species. Paras. Research. 88, 963 – 968.

Arias, J.R., Monteiro, P., Zicker, F., 1996. The Reemergence of Visceral leishmaniasis in Brazil. Em. Inf. Diseases. 2, 145-146.

Arrivillaga, J., Rangel, Y., Oviedo, M., Felliacialgelli, M.D., 2000. Genetic divergence among Venezuelan populations of *Lutzomyia longipalpis* (Diptera: Psychodidae: Phlebotomine). J. Med. Entom. 37,325-330.

Arrivillaga, J.C., Feliciangeli, M.D., 2001. *Lutzomyia pseudolongipalpis*: the first new species within the *longipalpis* (Diptera: Psychodidae: Phlebotominae) complex from La Rinconada, Curarigua, Lara State, Venezuela. J. Med. Entomol. 38,783-790.

Ashford, D.A., David, J.R., Freire, M., David, R., Sherlock, I., Eulalio, M.C., Sampaio, D., Barado, R., 1998. Studies on control of visceral leishmaniasis: impact of dog control and human visceral leishmaniasis in Jacobina, Bahia, Brazil. Am. J Trop. Med. Hyg. 59, 53-57.

Bates, P.A., 1997. Infection of the phebotomine sandflies with Leishmania. In: the molecular Biology of insect disease vectors. A methods manual. Eds: Crampton, JM; Beard, CD, Chapman and hall, London 27-47. Chapman and Hall, London.

Bavia, M., 1996. Geographic Information Systems for Schistosomiasis in Brazil. Ph.D. Thesis, Louisiana State University pp 25.

Bavia, M.E., Carneiro, D.D., Gurgel Hda, C., Madureira, C., Barbosa, M.G., 2005. Remote Sensing and Geographic Information Systems and risk of American visceral leishmaniasis in Bahia, Brazil. Parassitologia. 47, 165-169.

Bernier, R., Turco, S.J., Olivier, M., Tremblay, M.J., 1995. Activation of human immunodeficiency virus type 1 in monocytoid cells by the protozoan parasite *Leishmania Donovani*. J. Virology. 69, 7282-7285.

Boehme, C., Hain, U., Novosel, A., Eichenlaub, S., Fleischmann, E., Löscher, T., 2006. Congenital visceral leishmaniasis. Em. Inf. Diseases. 12, 259-366.

Chaniotis, B.N., 1974. Sugar-feeding behavior of *Lutzomyia trapiodi* (Diptera: Psychodidae) under experimental conditions. J. Med. Entomol. 11, 73-79.

Corredor, A., Gallego, J.F., Tesh, R.B., Pelaez, D., Diaz, A., Montilla, M., Palau, M.T., 1989. *Didelphis marsupialis*, an apparent wild reservoir of *Leishmania donovani chagasi* in Colombia, South America. Trans. R. Soc. Trop. Med. Hyg. 83, 195.

Costa, A.I., Casanova, C., Rodas, L.A., Galati, E.A., 1997. Atualização da distribuição geográfica e primeiro encontro de *Lutzomyia longipalpis* em área urbana no Estado de São Paulo, Brasil. Rev. Saúde Pública. 31, 632-633.

Costa, J., Peterson, T., Beard, C.B., 2002. Ecologic niche modeling and differentiation of populations of *Triatoma brasiliaensis* Neiva, 1991, the most important Chagas' disease vector in Northeastern Brazil (*Hemiptera, Reduviidae, triatominae*). Am. J. Trop. Med. Hyg. 67, 516-520.

Courtenay, O., Quinnell, R.J., Garcez, L.M., Shaw, J.J., Dye, C., 2002. Infectiouness of a cohort of Brazilian dogs: why culling fails to control visceral leishmaniasis in areas of high transmission. J. Infect. Dis. 186,1314-1320.

Daba, S., Youssel, F.G., Sterling, L.M., El Sawaf Bhira, M., 2002. Vector-host parasites inter relationships in the Leishmaniasis: A new concept. Egiptian J. Biolog. 4, 157-164.

Dalgleish, A.G., Beverlesy, P.C., Clapham, P.R., Crawford, D.H., Greaves, M.F., Weiss, R.A., 1984. The CD4 (T4) antigen in an essential component of the receptor for the AIDS retrovirus, Nature. 321, 763-767.

Davies, C.R., Kaye, P., Croft, S.L., Sundar, S., 2003. Leishmaniasis: New approaches to disease control. JBM. 326, 377-382.

Deane L, Deane M.P, 1957. Observações sobre abrigos e criaderos de flebotomos no Noreste do Estado do Ceará. Rev. Bras. Malariologia E Doenças Tropicais. 9: 588-589.

Deane, L., De Mello, L., 1956. Leishmaniose Visceral no Brasil. Serviço Nacional de Educação Sanitária. Rio de Janeiro.

Desjeux, P., Meert, J.P., Piot, B., Alwar, J., Medrano, F.J., Portus, M. Leishmania/ HIV co-infection, South Western Europe 1990-98. Document WHO/LEISH/2000. 42 Geneva: World Health Organization.

Dias-Lima, A.G., Silva, M.L., Sherlock, I.A., 2003. Horizontal stratification of the sandfly Fauna (diptera: Psychodidae) in transitional vegetation between Caatinga and tropical rain forest, State of Bahia, Brazil. Mem. Inst. Oswaldo Cruz. 98, 7333-737.

Elamin, A., Omer, M.I., 1992. Visceral leishmaniasis in a 6-week –old infant: possible congenital transmission. Trop. Doct. 22, 133-135.

Eltoum, I.A., Zijlstra, E.E., Ali, M.S., Ghalib, H.W., Satti, M.M., Eltoum, B., el-Hassan, A.M., 1992. Congenital kala-azar and leishmaniasis in the placenta. Am. J. Trop. Med. Hyg. 46, 57–62.

Evans, T.G., Thomas, G., Teixeira, M.J., McAulifee, I.T., Vasconcelos, I.A., Vasconcelos, A., de Queiroz Susa, W.A., de Oliveira, W.L., Jose; Pearson, R.D., 1992. Epidemiology of visceral lesihmaniasis in the Northeast Brazil. J. Infect. Dis. 166, 1124-1132.

Fausto, A.M., Feliciangelli, M.D., Maroli, M., Mazzini, M., 1998. Morphological study of the larval spiracular system in eight *Lutzomyia* species (Diptera: Psychodidae). Mem. Inst. Oswaldo Cruz. 93, 71-79.

Feliciangeli, M.D., 2004. Natural breeding places of Phlebotomine sandflies. Med. Vet. Ent. 18,71-80.

Fernandes, A.P., Nelson, K., Beverley, S.M., 1993. Evolution of Znuclear RNAs in kinetoplastids protozoa: prospective on the age and origins of parasitism. Proc. Natl. Acad. Sci. USA. 90, 11608-11612.

Ferro, C., Morales, A., Flebótomos de Colombia. Estudios realizados por el Laboratorio de Entomología. INS, 1965-1997. En: Toro G, Hernández CA, Raad J, editores. Instituto Nacional de Salud 1917-1997. Una historia un compromiso. Santa Fe de Bogotá: Instituto Nacional de Salud; 1998.

Fuentes, M.V., Sainz – Elipe, S., Nieto, P., Malone, J.B., Mas-Coma, S., 2005. GIS risk assessment for zoonotic fasciolasis in South America. Parassitologia. 47, 151-6.

Grimaldi, G.Jr., Tesh, R.B., 1993. Leishmaniases on the new world. Current concepts and implications for future research. Clin. Microbiol. J. 6, 230-250.

Grimaldi, G.Jr., Tesh, R.B., McMahon-Pratt, D., 1989. A review of the geographical distribution and epidemiology of Leishmaniasis in the New World. Am. J. Trop. Med. Hyg. 40, 687-725.

Guimarãesa, RJ., Freitas, C.C., Dutra, L.V., Mourad, A.C., Amaral, R.S., Drummondf, S.C., Scholtea, R.G.,Carvalhoa, O., 2008. Schistosomiasis risk estimation in Minas Gerais State, Brazil, using environmental data and GIS techniques. Acta Trop. 108, 234-241.

Herwaldt, B.L., 1999. Leishmaniasis. Lancet. 354,1191- 1199.

Hug, O.K., Malone, J.B., 2001. New tools: potential medical applications of data from new and old environmental satellites. Acta Tropica. 79, 35-47.

Kettle, D.S., 1990. Trypanosomiases and Leishmaniases. Kettle DS, ed. Medical and Veterinary Entomology. Wallingford,United Kingdom: CAB International, 580–587.

Killick-Kendrick, R., Leaney, A.J., Ready, P.D., 1977. The establishment, maintenance and productivity of a laboratory colony of *Lutzomyia longipalpis* (Diptera: Psychodidae). J. Med. Ent. 13, 429-440.

Killick-Kendrick, R., Peters, W.,1987. The Leishmaniasis in biology and medicine. Vol 1 Biology and Epidemiology. Academic press. London.

Killick-Kendrick, R., 1990. Phlebotomine vectors of the leishmaniases: a review. Med Vet entomol. 4, 1-24.

Killick-Kendrick, R., 1999. The biology and the control of phlebotomine sandflies. Clinics in Dermatology. 17, 279-289.

Killick-Kendrick, R., Rioux, J.A., 2002.Mark-release-recapture of sandflies fed on leishmanial dogs: the natural life-cycle of *Leishmania infantum* in Phlebotomus ariasi. Parassitologia. 44, 67-71.

Kroeger, A., Villegas-Avila, E., Morison, L., 2002. Insecticide impregnated curtains to control domestic transmission of cutaneous Leishmaniasis in Venezuela: Cluster randomized trial. BMJ. 325: 810-813.

Lainson, R., Shaw, J.J., 1979. The role of animals in the epidemiology of South American Leishmaniasis. In: Lumden WHR and Evans DA (eds) Biology of kinetoplastida, London, New York and San Francisco: Academic Press, P 1-116.

Lainson, R., Shaw, J.J., 1978. Epidemiology and ecology of Leishmaniasis in Latin America. Nature. 273, 595-600.

Lampo, M., Torgerson, D., Marquez, L.M., Rinaldi, M., Garcia, C.Z., Arb, A., 1999. Occurrence of sibling species of *Lutzomyia longipalpis* (Diptera: Psychodidae) in Venezuela: First evidence from reproductively isolated sympatric populations. Am. J. Trop. Med. Hyg. 61, 1004-1009.

Leite, A.C., Williams, P., 1997. The First Instar Larva of *Lutzomyia longipalpis* (Diptera: Phlebotomidae). Mem. Inst. Oswaldo Cruz. 92, 197-203.

Leite, A.C., Williams, P., 1996. Description of the Fourth Instar Larva of *Lutzomyia longipalpis*, under Scanning Electron Microscopy. Mem. Inst. Oswaldo Cruz. 91,571-578.

Lane, R.P., Ward, R.D., 1984. The morphology and possible function of abdominal Patches in males of two of the Leishmaniasis vector *Lutzomyia longipalpis* (Diptera: Phlebotominae). Entomol. Med. Parasitol. 22, 245-249.

Low, G.C., Cooke, W.E., 1926. A congenital infection of kala azar. Lancet. 1209-1211.

Lutz, A., Neiva, A., 1912. Contribução para o conheciemento das espécies do gênero Phlebotomus existents no Brasil. Mem. Inst. Oswaldo Cruz. 4, 84 –95.

Malone, J.B., 2005. Biology-based mapping of vector-borne parasites by Geographic Information Systems and Remote Sensing. Parassitologia.47, 27–50.

Malone, J.B., McCarroll, J.C., Kristensen, T.K., Yilma, J.M., Erko, B., El- Bahy, MM.,Corbett, JD., 2001. Minimum Medical Database Spatial Decision Support System for the Inter Governmental Authority on Development –Nile Basin Region (IGAD/Nile). Manual and CD rom, 49.

Malone, J.B., McNally, K.L., McCarroll, J.C., Corbett, Mkoji, G., 2004. Modeling the Biocoenose of Parasitic Diseases Using Remote Sensing and Geographic nformation Systems. Parassitologia. 46, 59-61.

Malone, J.B., Poggi, E., Igualada, F., Sintasath, D., Ghebremeeskel, T., Corbett, J., Mccarroll, J., Chinnici, P., Shililiu, J., McNally, K., Downer, R., Perich, M., Ford, R., 2003. Malaria environmental risk assesment in Eritrea. International Geoscience and Remote Sensing Symposium. 2003. pp 1000-1003.

Martinez, S., Marr, J.J., 1992. Allopurinol in the treatment of American cutaneous leishmaniasis. N.E journal of Medicine. 326, 741-744.

McDougal, J.S., Nicholson, J.K., Cross, D.G., Cort, S.P., Kennedy, M.S., Mawle, A.C., 1986. Binding of the human retrovirus HTLV-III/LAV/ARV/HIV tot eh CD (T4) Molecule; conformation dependence, epitope mapping, antibody inhibition and potential for idiotypic mimicry. J. Immun. 137, 2937-2944.

Meltzer, M.S., Skillman, D.R., Gomatos, P.L., Klater, D.C., Gendelman, H.E., 1990. Role of mononuclear phagocytes in the patogenesis of human immunodeficiency virus infection. Annual. Rev. of Immun. 8, 169-194.

Modabber, F., 1989. Experiences with vaccines against cutaneous leishmaniasis: of men and mice. Parasitol. 98, S49-S60.

Modi, G.B., Tesh, R.B., 1983. A simple technique for mass rearing *Lutzomia longipalpis* and *Phlebotomus papatasi* (Diptera: Psychodidae) in the laboratory. J. Med. Entomol. 20, 568-569.

Moreira, D.E., Verena de Souza, M.M., Sreenivasan, M., Lopes, N.L., Barreto, B.R., De Caravalho, L.P., 2003. Peridomestic risk factors for canine leishmaniasis in urban dwellings: new findings from a prospective study in Brazil. Am. J. Trop. Med. Hyg. 69, 393-397.

Morrison, A.C., Ferro, C., Tesh, R.B., 1993. Host preferences of the sandfly *Lutzomyia longipalpis* at the endemic focus of America cutaneous leishmaniasis in Colombia. Am. J. Trop. Med. Hyg. 49, 68-75.

Moura-Luitgards, J.F., Bermudes, E.G, Rosa-Freitas, M.G., 2000. Aspects related to productivity for four generations of *Lutzomyia longipalpis* Laboratoy colony. Mem. Inst .Oswaldo Cruz. 95, 251-257.

Mutebi, J.P., Alexander, B., Sherlock, I.A., Wellington, J., Sousa, A., Shaw, J., Rangel, E., Lanzaro, G., 1999. Breeding structure of the sandfly *Lutzomia longipalpis* (lutz & Neiva) in Brazil. Am. J. Trop. Med. Hyg. 61, 149-157.

Nadim, A., Javadian, E., Mohebali, M., 1997. The experience of leishmanization in the Islamic Republic of Iran. Eastern Mediterranean Health J. 3,284-289.

Nicolle, C., 1908. Sur trios cas d`infection splenique infantile a corps de Leishman observes en Tunisis. Arch. Inst. Pasteur 3: 1-26.

Oliver, M., Barado, R., Medrano, F.J., Moreno, J., 2003. The pathogenesis of the *leishmania*/HIV co-infection:cellular and immunological mechanisms. Annals of Tropical Medicine and Parasitology. 97, 79-98.

Pavlovsky, E.N., 1966. Natural nidality of transmissible diseases. Urbana: University of Illinois Press, 261.

Penna, H.A., 1934. Leishmaniose visceral no Brasil. Brasil Médico. 18, 940-950.

Pessoa, F.A., Guerra de Quiroz, R., Ward, R.D., 2001. External morphology of sensory structures of fourth instar larvae of neotropical species of phlebotomine sandflies (Diptera: Psychodidae) under scanning electron microscopy. Mem. Ins. Oswaldo Cruz. 96, 1103-1108.

Peterson, A.T., Ball, L.G., Cohoon, K.P., 2002a. Predicting distributions of Mexican birds using ecological niche modeling methods. Ibis. 144, E27-E32.

Peterson, A.T., Baue,r J.T., Mills, J.N., 2004a. Ecologic and geographic distribution of filovirus disease. Emerging Infectious Diseases. 10, 40-46.

Peterson, A.T., Pereira, R.S., Camargo Neves, VF., 2004b. Using epidemiological survey data to infer geographic distributions of leishmaniasis vector species. Revista da Sociedade Brasileira de Medicina Tropical. 37, 10-14.

Peterson, A.T., Shaw, J., 2003. *Lutzomyia* vectors for cutaneous leishmaniasis in Southern Brazil: ecological niche models, predicted geographic distributions, and climate change effects. Int. J. Parasit. 33, 919-931.

Petereson, A.T., Stockwell, D.R., Kluza, D.A., 2002b. Distributional prediction based on ecological niche modeling of primary occurrence data. In: Scott JM, editor. Predicting species occurrences: issues of scale and accuracy. Washington: Island Press. 617-23.

Peterson, A.T., Vieglais, D., Andreasen, J., 2003. Migratory birds modeled as critical transport agents for West Nile Virus in North America. Vector-Borne and Zoonotic Diseases. 3, 27-37.

Rabello, A., Orsini, M., Disco, J., 2003. *Leishmania*/HIV co-infection in Brazil: an appraisal. Anns Trop Med and Parasit. 97, 17-28.

Rangel, E.F., Lainson, R., Souza, A.A., Ready, P., Azevedo, A.C., 1996. Variation between geographical populations of *Lutzomyia (Nyssomyia) whitmani* (Antunes & Coutinho, 1939) *sensu lato* (Diptera: Psychodidae: Phlebotominae) in Brazil. Mem. Inst. Oswaldo Cruz. 91, 43-50.

Rao, V.B., de Lima, M.C., Franchito, S.H., 1993. Seasonal and interannual variations of rainfall over eastern Northeast Brazil. J. Climate. 6, 1754-1763.

Reithinger, R., Teodoro, U., Davies, CR., 2001. Tropical insecticide treatments to protect Dogs from sandfly vectors of Leishmaniasis. Emerg. Inf. Dis. 7, 872-876.

Rosypal, A.C., Cortes-Vecino, J.A., Gennari, S.M., Dubey, J.P., Tidwell, R.R., Lindsay, D.S., 2007. Serological survey of *Leishmania infantum* and *Trypanosoma cruzi* in dogs from urban areas of Brazil and Colombia. Vet. Parasitol. 149, 172-177.

Santos, M.C., Williams, P., Ferreira, M., 1991. Changes in sex ratio during attempts to establish a laboratory colony of *Lutzomyia longipalpis* (Diptera: Psychodidae). Parasitologgia. 33, 169-176.

Shaw, J.J., 2002. New world Leishmaniasis: The ecology of leishmaniasis and the diversity of Leishmanial species in Central and South America, In: World class parasites, Vol. 4. Leishmania, Ed. JP Farrell.

Shaw, J.J., 1997. Ecological and evolutionary pressures on leishmanial parasites. Braz. J Gen. 20, 123-128.

Sherlock, I.A., 1996. Ecological interactions of visceral leishmaniasis in the state of Bahia, Brazil. Mem. Inst. Oswaldo Cruz. 91, 671-683.

Silveira, F.T., Tobias, F.,1994: Parasitic and infectious diseases epidemiology and ecology. Scott ME, and Smith, G (editors). Academic press, San Diego. 395 pages.

Soares, R.P., Turco, S.J., 2003. *Lutzomyia longipalpis* (Ditera: Psychodidae: Pble-botominae); a review. Anais Acad Brasileira de Ciências. 75, 301-330.

Stockwell, D.R., 1999. Genetic algorithms II. In , AH fielding machine learning methods for ecological applications. Kluwer Academic publishers, Boston. 123-144.

Sudhakar, S., Srinivas, T., Palit, A., Kar, S.K., Battacharya, S.K,. 2006. Mapping of risk prone areas of kala-azar (Visceral leishmaniasis) in parts of Bihar State, India: an RS and GIS approach. J. Vector. Borne. Dis. 43, 115-122.

Tang, Y., Anez, N., Bates, P.A., 1998. Phenol red method for measuring the ph of the gut contents in the *Lutzomyia longipalpis* (Psichodidae: Diptera). Chin. J. Parasitol. Paras. Dis. 16, 62-66.

Teshome, G.M., Malone, J. B., Balkew, M., Ali, A., Berhe, N., Hailu, A., Herzi, A. A., 2004.Mapping the potential distribution of *Phlebotomus martini* and *P. orientalis* (Diptera: Psychodidae), vectors of kala-azar in East Africa by use of geographic information systems. Acta Tropica. 90, 73-86.

Thompson, A.R., 1998. Association of environmental and climatic factors in the epidemiology of American Visceral leishmaniasis in northeast Brazil using remote sensing and Geographic Information System methods. Dissertation.

Thompson, A.R., Lima De Oliveira, J.W., Maguire, J.H., Braud, D.H., Scholl, D.T., 2002. Climatic and demographic determinants of American Visceral Leishmaniasis in northeastern Brazil using remote sensing technology for environmental categorization of rain and region influences on Leishmaniasis. Am. J. Trop. Med. Hyg. 67, 648-655.

TIH., 2000. Topics in international Health series (Leishmaniasis) Multimedia CD Review. Welcome Trust, 2000. http://www.wellcome.ac.uk/About-us/Publications/CD-ROMs/Topics-in-International-Health/WTX048879.htm. (Accessed: January, 2008).

Titus, R.G., Ribeiro, J.M., 1990. The role of Vector saliva in transmission of arthropod-born diseases. Parasitol. Today 6: 157-160.

Turco, S.J., 1999. Adverse relationship between the Leishmania lipophosphoglycan and protein kinase C of host macrophages. Parasite Immunology. 21, 597-600.

Walters, L.L., Irons, K.P., Chaplin, G., Tesh, R.B., 1993. Life cycle of *Leishmania major* (Kinotoplastida: Trypanosomidae) in the neotropical sandfly *Lutzomyia longipalpis* (Diptera:psychodidae). J. Med. Entom. 30, 699-718.

Ward, R.D., Morton, I.E., 1991. Pheromones in mate choice and sexual isolation between siblings of *Lutzomyia Longipalpis* (Diptera: Psychodidae). Parasitologia. 33, 578-533.

World health Organization. Control of the Visceral Leishmaniases: report of a WHO expert cometee. Technical Report Series 793. Geneva, Switzerland: World health Organization, 1990.

World Health Organization. Report of the second WHO meeting on emerging Infectious diseases. Document WHO/CDS/BVI95.2. Geneva, Switzerland: World Health Organization January 1995.

Ximeens de Freire, M.F., Cunha-Maciel, J., Jeronimo, S.M., 2001. Characteristics of the biological cycle of *Lutzomyia evandroi* Costa & Antunes , 1936 (Diptera: Psychodidae) under experimental conditions. Mem. Ins. Oswaldo Cruz. 96, 883-886.

CHAPTER 2 DIAGNOSIS AND CLINICAL-PATHOLOGICAL FINDINGS OF CHAGAS DISEASE *(TRYPANOSOMA CRUZI)* IN DOGS IN SOUTH CENTRAL LOUISIANA

2.1 Chagas Disease

Chagas disease is a zoonotic disease that affects an estimated 13 million people in Latin America (UNICEF/UNDP/World Bank/WHO, 2007). Its wide distribution (18 endemic countries), high transmission rates and the severity of the disease have major socio-economical impact in endemic countries. Economical losses are caused by disability or death; this causes billions of dollars per year in lost productivity and high medical costs to treat chagasic patients (Cubillos-Garzon et al., 2004). With increased migration of people from endemic areas and travel, Chagas disease may become more important in the United States. Six autochthonous human cases have been reported in the United States in the states of Texas (Woody and Woody, 1955; Ochs et al, 1996), Tennessee (Herwaldt, 2000), California (Schiffler et al., 1984), and Louisiana (Dorn et al., 2007).

2.1.1 Cause and Vectors in the USA

Chagas disease is caused by the protozoan hemoflagellate, *Trypanosoma cruzi,* a parasite transmitted among mammalian hosts by insect triatomine vectors of the family Reduviidae. The most important species of triatomines that occur in the U.S. are *Triatoma sanguisuga* in the eastern United States including Louisiana, *T. gerstaeckeri* in arid regions of Texas and New Mexico, and *T. rubida* and *T. protracta* in Arizona and California (Lent and Wygodzinsky, 1979). However, these species may not be as effective in transmitting Chagas disease as the Latin American reduviids due to delayed defecation relative to feeding activity (Zeledón, 1974). Reduviids have specific requirements for their survival. *Triatoma* species are robust in cold temperatures; few

species died in temperatures of -6°C (Silva and Silva, 1986). Reduviids can adapt to external temperatures developing in a microclimate warmer than the external environment (Carcavallo, 1999). Geographical areas with higher temperatures have accelerated embryonic periods, increased number of generations, shortened life cycle, increased feeding frequencies, increased metabolic rate and decrease in the time it takes for Triatomine's eggs to hatch when compared with temperate areas (Hack, 1955; Carcavallo and Martínez, 1972; Carcavallo et al., 1998).

2.1.2 Hosts

Tyipanosoma cruzi is maintained in the wild in the United States in at least 18 mammalian species (reviewed in John and Hoppe 1986) including raccoons, opossums, armadillos, primates, rats, skunk, ferrets and squirrels as well as domestic animals including dogs and cats (Wisnivesky-Colli et al., 1992; Bradley et al., 2000; Diosque et al., 2004). The metacyclic trypomastigotes infective form, usually occurring in the intestine of the triatomine vector, also takes place in the anal odoriferous glands of the opossums (Urdaneta-Morales and Nironi, 1996). Previous studies indicate that it is prevalent in wild animals, for example 29% in armadillos, 37.5% in opossums and 66% in raccoons (Yeager, 1998; Jansen et al., 1991; Yabsley et al., 2001; Paige et al., 2002).

2.1.3 Transmission

It is possible that species of triatomine bugs in the United States can serve as sources of infection for humans and animals following ingestion of contaminated bugs, consumption of food or water contaminated with vector feces containing metacyclic trypomastigotes, by contamination of mucus membranes or breaks in the skin. Other potential routes of infection include congenital transmission, blood transfusion, laboratory accident, organ transplants (Barr et al., 1995; Collins and Kennedy, 1999).

2.1.4 *Trypanosoma Cruzi* Infection in Dogs

Dogs are common victims of Chagas disease in endemic areas. Infection in dogs is strongly associated with levels of infected triatomine vectors found in houses, dogs are very important for transmission to bugs and humans (Gurtler et al., 2007). Studies done in Argentina found that infection in dogs precedes that of children in households, dogs are more likely to contract the parasite than are humans (Gurtler et al., 2005), likely because of their habit of eating the bugs (Zeledón, 1974), and they are 12-100 times more susceptible to infection by triatomines than are humans (Gurtler et al., 1996; Gurtler et al., 2005). In high risk areas where human cases have been found, the family dog is often infected as well (Castañera et al., 1998; Diosque et al., 2004; Wisnivesky-Colli et al, 1992). Thus, dogs are useful as sentinels of human infection risk in endemic areas (Castañera et al., 1998). In the United States, there have been no direct studies of Chagas disease among people that live in close association with infected dogs and no current evidence of the role of dogs as reservoirs - hosts in the local transmission cycle, although humans and dogs shared the same environment. Seroprevalence studies in the United States have detected *T. cruzi* infection in dogs in the southern states including: Texas (Burkholder et al., 1980; Shadomy et al., 2004; Kjos et al., 2008), California (Navit et al., 1985), Oklahoma (Bradley et al., 2000), Georgia (Tomlinson et al., 1981), Virginia (Barr et al., 1995) and Louisiana (Barr et al.,1991).

2.1.5 Pathogenesis of *Trypanosoma Cruzi* Infection

Infection with *T. cruzi* progresses in three consecutive phases: acute, indeterminant, and chronic phase. *T. cruzi* infected dogs develop the acute and chronic phases that are compatible to clinical and pathological findings of the human disease (Bittencourt et al., 1981; Macedo and Pena, 1998). The course of the acute phase is

characterized by detectable parasitemia and is frequent mild clinical signs, although serious pulmonary, myocarditis, ascites, hepatomegaly, and splenomegaly can occur. Young, immunodeficient and old animals represent a group at greatest risk of death in the acute phase (Williams et al., 1977; Montenegro et al., 2002). The indeterminate phase of Chagas disease may last indefinitely or progress into the chronic phase. Most of the individuals will remain in the indeterminate phase of the infection throughout the rest of their lives (Dias and Coura, 1997). Clinical manifestations of the chronic phase may range from an absence of clinical signs to severe disease with cardiac arrhythmias, exercise intolerance, mainly with cardiovascular involvement can also present megacolon and megaesopahgus. Significant variations in the course of the disease have been identified in different geographic regions. Both host and parasite factors are involved in the differing pathogenesis of Chagas disease. These differences are thought to be caused by genetic diversity of the vectors, genetic differences in hosts and heterogeneity of *T. cruzi* (Macedo et al., 2004).

2.1.5.1 Pathogenesis Hypotheses

The pathogenesis has been explained by two hypotheses; one is the autoimmunity hypothesis and the other is the non-autoimmune hypothesis of direct tissue damage caused by the parasite *T. cruzi.* These hypotheses are postulated to explain the variation in the symptomatology that ranges from asymptomatic to the development of a severe illness that includes cardiac, nervous and/or digestive symptoms.

2.1.5.1.1 Hypothesis 1: Autoimmunity

Autoimmunity is provoked by cross reaction between *T. cruzi* antigens and host tissue antigens. *T. cruzi* and molecular mimicry refers to the similarity in either amino acid sequence or structural confirmation between molecules of *T. cruzi* and its host as the

cause of misdirected immune response (Kierszenbaum, 1999). Mesri et al., 1990 used *T. cruzi* DNA clones that expressed a recombinant protein found to react predominantly with serum of cardiac patients with chronic Chagas disease. Synthetic peptides comprising the carboxyl-terminal residues R-13 (used as a possible marker of chronic Chagas disease) of the JL5 protein were used to study the specificity of Chagas disease antibodies. The prevalence of high anti-R-13 antibody titers in cardiac patients with chronic Chagas disease supports the hypothesis of the existence of autoimmune disorders in Chagas heart disease. Aznar et al, 1995 found that in patients with the acute form, only IgM anti-*T. cruzi* was observed. Both IgM and Ig G anti-*T. cruzi* antibodies were detected in sera from congenitally infected newborns. Antibodies against R-13 were present in a large proportion of cardiac chagasic patients but were totally absent in patients with the digestive form of Chagas disease. Anti-R-13 positive responses were detected in congenitally infected newborns.

Other studies hypothesized autoimmunity as the cause in the rejection of heart tissues grafted into mice chronically infected with *T. cruzi* (Ribeiro dos Santos et al., 1992). Chemotherapy would not be useful if a drug that kills the parasite does not affect immune responses maintained though continuous stimulation by host tissues antigens. Attempts to develop an anti *T. cruzi* vaccine would have to demonstrate that the antigens selected are not going to incite anti-host tissue response. A vaccine under these criteria could put people at high risk of acquiring the disease because of the long time that the patient manifests the symptoms (Kierszenbaum, 1999) or the vaccine would provide the antigens that by molecular mimicry would cause the body to react against it self.

2.1.5.1.2   Hypothesis 2: Direct Damage

Tissue and cell damage caused by *T. cruzi* presence is due to intracellular replication, causing cell rupture. In this way, the parasite infects other cells. Some researchers believe that this hypothesis is not sufficient to explain the tissue damage that occurs in Chagas disease. Once the metacyclic trypomastigotes are introduced to the host by the triatomine bug, vasoactive and chemotactic factors trigger a local inflammatory response. *T. cruzi* has a layer in the surface membrane of mucin-like glycol-sylphosphatidyl inositol GPI-anchored glycoproteins. These mucins from the bloodstream trypomastigotes stage of the parasite are extremely potent inducers of pro-inflammatory cytokines. During this process the permeability in the capillaries increases, allowing entry of substances and cells to the affected area. The inflammation process attracts phagocytic cells (macrophages, dendritic cells, and neutrophils) that destroy the trypomastigotes. This process causes severe inflammation, and damage of cardiac tissues (Tarleton and Zhang, 1999). The direct parasite damage theory implies that effective antiparasitic treatment can lead to regression of the inflammatory heart lesions and fibrosis in experimental animals and stop the progression of the disease in the host (Urbina,1999).

2.1.6 Diagnosis

Diagnosis of Chagas disease is usually made through parasitological and/or serological methods such as the indirect hemagglutination test (IHA), indirect immunofluorescence test (IFAT), enzyme-linked immunosorbent assay (ELISA) or by molecular methods, such as the polymerase chain reaction PCR (Duarte et al., 2006). It is recommended that at least two different methods be used for diagnosis both in animals and humans. As a consequence of the variable sensitivities among the different tests,

lack of standardization as well as the pathological progression and the stages of the disease (acute, indeterminate and chronic), available diagnostic methods often yield variable results (Ferreira and Borges, 2002; Oelemann et al., 1998).

In the acute phase of the disease when there is high parasitemia, diagnosis relies on hemoculture, direct parasite observation, xenodiagnosis and molecular procedures. For the chronic and indeterminate phases, parasitemia is low or difficult to demonstrate, but patients show specific antibodies (López-Antuñado at al., 2000). The IFAT, IHA and ELISA have been used in human and animals (Kirchhoff et al., 1993; Avila, 1993; Portela-Lindoso and Shikanai-Yosuda, 2003). In areas where *T. cruzi, T. rangeli* or *Leishmania spp* are suspected, it is recommended to use a specific purified antigen (López-Antuñado at al., 2000) to avoid cross reactive results.

Current procedures are technically and operationally demanding. The ideal serological technique to detect *T. cruzi* infection should be easy to perform, rapid, reliable and unexpensive (López-Antuñado at al., 2000). To improve the practicality and accuracy of serological diagnosis for Chagas disease in dogs, easy, rapid, sensitive and specific diagnostic methods have been developed. These methods may offer an alternative diagnostic method for practicing veterinarians to screen for canine Chagas infection and to define more precise regional seroprevalence rates for *T. cruzi* infections. Early detection of *T. cruzi* infection may prevent cardiac complications through an effective early treatment, especially in the acute phase of the disease.

2.1.7 Treatment

Current therapy for Chagas disease is not always effective and is limited by frequent and severe side effects. There are two approaches to therapy in humans and dogs: antiparasitic treatment, to kill the parasite; and symptomatic treatment, to manage

the symptoms and signs of infection. Nifurtimox (Andrade et al., 1980) and benznidazole (Da Matta Guedes et al., 2002) have been used in dogs mainly in acute cases. In the U.S., acute and chronic Chagas disease can be treated with benznidazole under an Investigational New Drug protocol from the CDC Drug Service (mainly for human use). In the chronic stage, treatment is mainly symptomatic e.g. to control arrhythmias by diuretics and digitalic drugs. Deltamethrin-treated collars have also been shown to protect dogs from being bitten by triatomines (Reithinger et al., 2005). To date, no vaccine exists against *T. cruzi,* but a Chagas disease vaccine is being developed using one of the parasite genes as the basis for a DNA vaccine. When injected into the patients, the vaccine, which contains fragments of DNA, causes the recipients cells to produce a protein that would ordinarily be produced only by the parasite. This protein triggers an immune response to the protein protecting the patient of later infections by the parasite.

2.2 Goals and Objectives

2.2.1 Long Range Goals

The long range goal of these studies is to define the prevalence of Chagas disease in dogs using IFAT and to test a rapid immunochromatographic assays that can be used as screening tests for use at the veterinary clinic level to improve detection and control of this zoonotic disease.

2.2.2 Specific Objectives

1. Survey dogs in southcentral Louisiana for *T. cruzi* that are considered to have a high exposure to the vector *T. sanguisuga* using an indirect immunofluorescence antibody test (IFAT) as our standard to estimate the prevalence of the infection in groups of dogs considered to be at high risk.

86

2. Compare two experimental commercial rapid immunochromatographic assays as alternative or complementary diagnostic tests to the IFAT.

3. Report clinical and pathological findings of natural *T. cruzi* infections in dogs that local veterinarians have referred to the Louisiana Animal Disease Diagnostic Service (LADDS), the Louisiana State University School of Veterinary Medicine Veterinary Teaching Hospitals and Clinics, Baton Rouge, LA (VTH&C), and the Iberia Animal Clinic, New Iberia, LA (IAC).

2.3 Hypothesis

In Louisiana and other areas with high prevalence of *T. cruzi* infection, easy and rapid diagnostic screening methods are accurate alternatives to the IFAT for diagnosis of Chagas disease in dogs.

2.4 Materials and Methods

2.4.1 Index Case

On May 2, 2005 a one-year-old Labrador Retriever dog died acutely with no previous clinical signs. The animal was kept in a kennel housing Labrador Retrievers being trained to hunt in a bottomland hardwood forest in the Atchafalaya basin of Louisiana. At necropsy (at LADDL) gross findings included right ventricular dilatation and severe diffuse granulomatous myocarditis. Serology examination using IFAT, revealed a *T. cruzi* antibody titer of 1:160, indicating a diagnosis of Chagas disease. The kennel owner had reported other dogs with sudden death in which Chagas disease was suspected. Kennel surroundings were searched for reduviid vectors. The kennel's owner found bugs and provided a reduviid that was identified as *Triatoma sanguisuga* (by Stephanie Gil, Dr. Lane Foil and Michael Becker, LSU Entomology Museum). Based on

87

case findings, further investigations were initiated at the initial case premises to investigate this and other reports of Chagas disease in the Atchafalaya basin area.

2.4.2 Serological Survey

Two groups of dogs were tested in this study. The criteria for the selection of the groups were based on: 1) presence at sites with previously reported *T. cruzi* positive dogs, or 2) dogs considered at high risk for *T. cruzi* infection since they were housed outdoors and likely had contact with the reduviid vector or wild reservoirs (opossums) in southern Louisiana.

2.4.2.1 Indirect Immunofluorescence Assay Test (IFAT)

The IFAT was used to detect anti- *T. cruzi* antibodies and was used as the standard method to diagnose Chagas disease in this study. The IFAT was carried out by the Centers for Disease Control and Prevention (CDC) in Atlanta, GA and/or to the Texas Veterinary Medical Diagnostic Laboratory (TVMDL), College Station, TX. The IFAT was considered negative if titers were < 1:32. Sample submission kits were provided to local veterinarians as well as owners and breeders who voluntarily wanted to test their dogs using samples drawn by their local veterinarian. Each kit had a box addressed to LADDL, two tubes for blood collection, a protocol to collect samples, precautions on zoonotic transmission potential to veterinarians, a questionnaire about the dogs' environment and a handout with layman's information about Chagas disease in dogs.

2.4.2.1.1 Group 1 (Kennel Survey)

In this group, three different kennels were included in a serological follow-up study based on positive cases reported to the LADDS by local veterinarians. Kennel 1 was located in the Atchafalaya basin where the original case was reported; fifteen hunting Labrador retrievers were tested. Kennel 2 in Henderson, Louisiana was located near a

sugarcane farm adjacent to the Atchafalaya basin; eight hunting Labrador retrievers were tested. Kennel 3 was located in Livonia, LA, where eight Beagles kept as hunting dogs were tested.

2.4.2.1.2 Group 2 (Practice Area Survey)

From September 2005 to June 2007, blood samples of 91 dogs from New Iberia and surrounding areas were collected. Local veterinarians had previously reported Chagas disease cases in this area. Samples were collected as part of a client survey campaign organized at a local veterinary clinic in New Iberia, Louisiana where clients collaborated voluntarily for blood collection from their pets.

2.4.2.2 Rapid Assay Tests

Fifty randomly chosen serum samples were tested using two experimental rapid assays A and B provided by the manufacturers to test against our standard method, the IFAT performed at the CDC. The fifty randomly selected samples were from the second group, the practice area survey, because it was the largest and most homogeneous group. The test procedures of the rapid assay A and rapid assay B, immunochromatographic screening tests for detection of antibodies to *T. cruzi,* were perform by adding three drops of serum samples at room temperature and the buffer solution provided with the tests to the in the designated area. After 10-15 minutes the result was read. The two rapid assays were scored based on intensity of band color, 0: negative, (no line) 1: negative (very weak line), 2: positive (medium line), 3: strong positive.

Rapid assay A, (Trypanosoma Detect™ for canines, InBios, Seattle, WA) is an immunochromatographic dipstick based on multi-epitope recombinant antigen (MRA) derived from different *T. cruzi* antigens. This immunochromatographic test was designed for the qualitative determination of antibodies against the *T. cruzi* MRA antigen. The test

is based on a proprietary gold mix containing target Gold Conjugate and its ability to bind to antibodies present in serum. Once bound, the gold antibody complex will move laterally to form a complex with immobilized *T. cruzi* derived proteins present on the membrane to form a test line. The unbound gold will continue to move upward to bind antigen at the positive control line. The test is positive when a test line is observed. The test is based on a proprietary gold mix containing multiepitope recombinant antigens ITC-6 and ITC-8.2 derived from different *T. cruzi* antigens, including peptide 2, TcD, TcF, TcLo, and SAPA (Cardinal et al., 2006).

Rapid assay B, (CHAGAS STAT-PAK[TM,] Chembio Diagnostic Systems, Medford, NY) is an immunochromatographic screening test for detection of antibodies to *T. cruzi* employs a combination of a specific antibody binding protein which is conjugated to dye particles and antigens which are bound to the membrane (solid phase). The test sample is applied to the sample well. As the sample flows laterally across the membrane, the specific antibody binding protein dye conjugate binds to the immunoglobulins in the sample. If the sample contains antibodies to *T. cruzi*, the complex binds to the antigens on the solid phase in the test area producing a line. This test also provides an internal immunoglobulin G antigen control. The sample continues to migrate along the membrane and produces a line in the positive control band demonstrating that reagents are functioning properly . It employs a unique combination of *T. cruzi* recombinant antigens (B13, 1F8, and H49/JL7) which are bound to the membrane, and a specific antibody-binding protein, which is conjugated on dye particles (Ponce et al., 2005). The characteristic of these two tests of using highly recombinant antigens for the detection of *T. cruzi* make the difference with most of the commercial serologic tests that employ epimastigote antigens and show a high number of

inconclusive and false-positive results, with high economic and social costs (Umesawa et al., 2003).

2.4.3 Medical Records Review

A database of previous cases of canines diagnosed with Chagas disease was compiled using case record archives from 1994-2007. Eleven cases of American trypanosomiasis or Chagas disease selected from records of necropsy cases submitted to LADDS, and clinical cases submitted to the Louisiana State University School of Veterinary Medicine Teaching Hospital and Clinics (VTH&C) or Iberia Animal Clinic (IAC) were used to compile historical information on infected dogs and to describe clinical and pathological findings.

2.5 Results

2.5.1 Serological Survey

2.5.1.1 IFAT Serological Test

Group 1 (kennel survey). Kennel 1: Of samples collected from the index case kennel, IFAT revealed that nine of fifteen (60%) dogs tested positive for *T. cruzi*. Kennel 2: two of eight (25%) dogs tested positive by IFAT for *T. cruzi* from Henderson, Louisiana; the kennel was located near a sugar cane farm adjacent to the Atchafalaya Basin. Kennel 3: five of eight (62.5%) of animals tested at this kennel in Livonia, LA, were found to be positive by IFAT. For group 1 a total of 16 of 31 (51.6%) dogs tested positive by IFAT in the three kennels tested.

Group 2 (practice area survey, New Iberia, LA): Eleven of 91 dogs (12%) included in the practice area survey tested positive for *T. cruzi* by IFAT. Of the total number of dogs tested in Groups 1 and 2 by IFAT serology, 27 of 122 (22.1%) were positive (Table12).

2.5.1.2 Rapid Assay Tests

Serum samples from 50 dogs were randomly selected from group 2 (practice area survey) for testing by rapid assay A and rapid assay B to compare to the IFAT done at the CDC. Sensitivity and specificity was calculated relative to IFAT (used as standard test). Rapid assay A revealed 13 positive animals and rapid assay B revealed 11 positive animals (table 13). The sensitivity of Rapid Assay A and Rapid Assay B was 100%; the specificity of Rapid Assay A was 95% and Rapid Assay B was 100%.

2.5.2 Pathological and Clinical Findings from the Medical Records Review

Eleven cases confirmed by pathology and/or serology (IFAT) from 1995 to 2007 medical records at LSU were used to compile historical information and record of pathological and clinical signs of naturally infected dogs. Most of the reported cases (81%) were young animals ≤ two-years-old, and in good body condition. Most (55%) of the dogs were Labrador Retrievers residing in southern Louisiana. Three owners reported previous sighting or collection of live specimens fitting the description of *T. sanguisuga,* the triatomine vector (Table 14).

Table 12. Results of tested dogs for *T. cruzi* infection by IFAT. Group 1: Kennel survey and Group 2: practice area survey.

Group	No. dogs tested	No. *T. cruzi* positive dogs	Total % positive
Group 1			
Kennel 1	15	9	60
Kennel 2	8	2	25
Kennel 3	8	5	62.5
Total Group 1	**31**	**16**	**51.6**
Group 2	**91**	**11**	**12**
Total	**122**	**27**	**22.1**

Table 13. Results of the three different *T. cruzi* diagnostic methods: the Rapid assays a and b and the imunofluorescence antibody test (IFAT).

Diagnostic Method	No. dogs tested	Positive	Total % positive
T. cruzi rapid assay A	50	13	26
T. cruzi rapid assay B	50	11	22
IFAT	50	11	22

2.5.2.1 Clinical Findings

Clinical signs observed in the 11 case records of confirmed *T. cruzi* infections in dogs included: fatal cardiac failure (4), lethargy (4), lameness (4), vomiting (2), jugular pulse (2), tachypnea /dyspnea (2), anorexia (2), acute death (1), seizure (1), rapid/shallow respiration (1), cough (1), diarrhea (1), keratoconjuntivitis (1), no clinical signs (1).

2.5.2.2 Pathological Findings

Necropsy findings observed in the 11 cases included: in order of frequency: intracellular amastigotes/ pseudocysts of *T.cruzi* in cardiopulmonary tissues (8), granulomatous subacute multifocal myocarditis (7), pulmonary edema (6), cardiomegaly (Fig. 14) (6), ascites (6), severe acute diffuse myocarditis (5), hepatic congestion (3), splenomegaly (2), hepatomegaly (2), hydrothorax (2), thick hypertrophied left ventricle (2), spleen congestion (1), renal infarct (1), centrilobular hepatic necrosis (1), hydropericardium (1) , dilated ventricle (1), bicuspid and tricuspid valvular endocarditis (1) and thin walled ventricle (1). The clinical and pathological findings of Chagas disease in dogs are listed in table 15 in order of the frequency observed and were mainly referable to compromised cardiac function. Seventeen additional animals in the same time period were submitted for necropsy examination to LADDS, in which the

pathologists' presumptive diagnosis was Chagas disease but the diagnosis could not be confirmed by finding the organism in histopathological sections or by serology.

2.6 Discussion

2.6.1 Serological Survey

2.6.1.1. IFAT Serological Test

The serological study on *T. cruzi* was done on groups of dogs that were thought to be at high risk of infection with *T. cruzi* (i.e. hunting dogs, dogs that live in outdoor kennels that may have contact with the reduviid vector or wild reservoirs, or dogs that live in same environment as another infected dog). Group 1 (kennel survey) included Kennel 1 in the Atchafalaya Basin where the index case was reported; Kennel 2, located near a sugarcane field in the area adjacent to the Atchafalaya basin; and Kennel 3, a beagle kennel in Livonia, LA on a property regularly used as a hunting camp near the Atchafalaya Basin. Dogs in Kennels 1 and 2 were Labrador Retrievers. The owners of Kennel 1 and 2 were professional hunting dog trainers and breeders. In total, in the three kennels, 16 of 31 (51.6%) dogs tested were positive for Chagas disease based on results of the IFAT.

Local veterinarians were made aware of the recent findings of *T. cruzi* infected dogs with Chagas disease in their area by informal publicizing of results from the index case and the first three kennel groups via the Lafayette Area Veterinary Medical Association. A Kit (Vacuutainer tubes for blood collection, protocol of blood collection and precautions, a standard questionnaire for owners about the dog's environment including a picture of the most common and important reduviid in the transmission of Chagas disease in the southeastern U.S. (*T. sanguisuga*) (Lent et al., 1979), and information on the disease in animals and humans was provided upon request to local

veterinarians who volunteered to submit blood samples to LADDS from dogs presented at their clinics that resided in high risk environments or were suspected clinical cases. One veterinarian organized a campaign to test animals in his clinic. He informed his clientele about the risk of the disease and high prevalence of Chagas disease in dogs, and invited them to test their dogs on a designated date. The samples collected in this phase, the second part of the study, comprised Group 2. In this group, 11 of 91 (12%) of the animals were positive, substantially lower than Group 1 (51.6%) presumably because clients of the veterinary practice area were aware and concerned about the danger of the disease and tested some dogs that were not at as high risk of becoming infected. Even though the animals lived in a high risk area, some of the animals did not have other risk factors for becoming infected with *T. cruzi*, such as living outside, hunting dogs, or no contact with wild animals.

Overall, for groups 1 and 2 combined, 22.1% of the dogs tested positive for *T. cruzi* by IFAT. The prevalence reported in the current study is quite similar to results from a recent study of canines in Texas. A serosurvey across Texas from 2000-2006 showed an overall *T. cruzi* seroprevalence of 20.3%; ranging from a low of 16.2% in 2004 to high of 25.1% in 2001, which was fairly evenly distributed across the study area (Kjos et al., 2008). Unlike what was seen in Texas, at least for these specific region tested Chagas risk for canines in Louisiana appears to vary considerably among localities. Earlier studies showed a lower seroprevalence, e.g. 8.8% (12 of 136) in a stray-canine population in the in the lower Rio Grande Valley of Texas (Beard et al., 2003; Burkholder et al., 1980) and 3.6% (11 of 301) in Oklahoma, between November 1996 and 1997 (Bradley et al., 2000). Previous studies in south Louisiana showed a

seroprevalence in roaming rural dogs of 4.7% (4 of 85 dogs) and the seroprevalence of urban shelter dogs of 2.3% (4 of 176 dogs)(Barr et al., 1991).

Table 14. Chagas disease cases from 1995 to 2007 and submitted to LADDS, the LSU Veterinary Medical Teaching Hospital and Clinics or Iberia Animal Clinic; (w: weeks; m: months; y: year; F: female, M: male, GC: good body condition, PN: poor body condition, ND: no data).

Year submitted	Breed	Location	Age		Sex	Nutrition status	Reported *Triatomine*
1995	Labrador retriever	(Unspecified),LA	18	w	F	GC	ND
1996	Beagle	Franklin, LA	1	y	F	GC	ND
1997	Labrador retriever	Baton Rouge, LA	10	m	M	GC	ND
2000	Mixed	Carencro, LA	6	w	M	GC	ND
2003	German Shepherd	Baton Rouge, LA	8 y		F	ND	ND
2004	Pitbull terrier	Gonzales, LA	11	m	M	GC	ND
2005	Labrador retriever	Atchafalaya basin, LA	1	y	F	GC	Yes
2005	Labrador retriever	New Iberia, LA	2	y	M	GC	ND
2005	Pitbull terrier	Jeanerette, LA	1.5	y	M	GC	ND
2006	Labrador retriever	New Iberia, LA	1	y	F	PN	Yes
2007	Labrador retriever	St. Gabriel, LA	8	y	F	GC	Yes

2.6.1.1.1 Environmental Features Associated with Chagas Disease Transmission

Kennel 1 was located in an opening in a densely-vegetated area, close to a river. The kennel owner lived in close proximity to the kennels; empty containers, debris, a woodpile and fallen trees were present nearby. A reduviid bug morphologically compatible with *T. sanguisuga* was collected from the kennel by the owner, who reported other occasions of findings of similar bugs dead on the kennel floor or in dog watering bowls. Kennel 2 was located next to the owner's residence close to a sugar cane field. Cane fronds can provide hiding places for reduviid bugs, which can be a potential source of infection to animals or people living nearby. Uncooked sugar cane juice sold as a drinking beverage has been shown to be the source of Chagas disease outbreaks in Brazil via the oral transmission route (Cardoso et al., 2006). Kennel 3, which housed beagle

hunting dogs, was located in a cleared area near a forested zone without nearby residences. All three kennels were located in rural areas with potential interaction with known sylvatic reservoir hosts, such as opossums, raccoons, armadillos and rodents, from the sylvan cycle involving *T. sanguisuga*.

Overall, results of this study suggest that the Atchafalaya basin region may have high risk of Chagas disease probably due to the unique ecology and environmental risk features present in the region. The Atchafalaya Basin is the largest bottomland hardwood forest in the United States, a protected seasonally-flooded wetland that supports a rich diversity of animals along the Atchafalaya River and its associated lakes, bayous and canals. The pattern of sediment deposition and impaired water movement during annual flooding provides an exceptional natural habitat for native plants, wildlife and insects, and may be one of the unique environmental factors that influence the establishment of *T. sanquisuga* and a resulting high prevalence of Chagas disease in the Atchafalaya Basin. However, case records indicate Chagas disease occurs throughout Southern Louisiana at lesser rates. A systematic statewide survey for Chagas disease in dogs is needed to better define high Chagas disease risk areas in the state.

2.6.1.2 Rapid Diagnostic Assays

Various approaches have been used in the diagnosis of Chagas disease, including use of combinations of tests. In human infections, a positive diagnosis of Chagas disease typically relies on two positive tests done with different methods: screening and confirmatory test. In a study testing dog sera for the diagnosis of Chagas disease by commonly used indirect hemagglutination (IHA), IFAT or ELISA showed a sensitivity of 68%, 94% and 94% and a specificity of 100%, 100% and 96.2%, respectively (Shadomy et al., 2004; Lauricella et al., 1998). These serological tests have high sensitivity, but their

specificity may vary because of the antigenic cross-reactivity with other parasitic species like *Leishmania spp.* or *T. rangeli* (Saldana and Sousa., 1996) or whether the infection is in the acute, indeterminate or chronic phase of the disease.

All samples found to be positive by IFAT were also positive on both rapid assays. In addition, rapid assay A indicated 2 dogs were positive which showed negative results in the other assays. Rapid Assay A revealed 13 positives, Rapid Assay B revealed 11 of the 50 animals tested were positive as compared to 11 positive samples using the IFAT. Both rapid assay A and rapid assay B have a 100% sensitivity, with a specificity of 95% in Rapid Assay A and 100 % in Rapid Assay B, relative to IFAT, the standard test. Previous studies made with the dipstick Rapid Assay A have reported high specificity (>94%) and high sensitivity (>96%) (Cardinal et al., 2006). Studies in humans in Central America using Rapid Assay B revealed a sensitivity of 99.6% and a specificity of 99.9 % (Luquetti et al., 2003; Ponce et al., 2005). Positive sera by the IFAT done at CDC in the current study were routinely tested for cross reactivity to *Leishmania spp* (all samples tested negative for *Leishmania spp*), thus, this was not a factor in results obtained by the two rapid assays tested. The variations in the positive tests by the two rapid survey methods may be a direct result of the individual test principle in which case a different confirmatory test type other than IFAT would be recommended. Even though these tests have high sensitivity and specificity the tests should not be used as sole criterion for the diagnosis of *T. cruzi* infection. A combination of multiple testing methods is recommended to support the diagnosis. All results should be considered with other clinico-pathologic information available to the veterinarian (Meurs et al., 1998).

Table 15. Clinical and pathological findings reported by LADDS, the LSU Veterinary Medical Teaching Hospital and Clinics or Iberia Animal Clinic, from 1995-2007.

	Findings	No. of Animals /11
Clinical	Cardiac failure as cause of death	4
	Lethargy	4
	Lameness	4
	Vomiting	2
	Jugular pulse	2
	Tachypnea /dyspnea	2
	Anorhexia	2
	Acute death	1
	Seizure	1
	Rapid /shallow respiration	1
	Cough	1
	Diarrhea	1
	Keratoconjuntivitis	1
	No clinical signs	1
Pathological	Intracellular amastigotes/pseudocysts of *T.cruzi* in cardiopulmonary tissues	8
	Granulomatous subacute multifocal myocarditis	7
	Ascites	6
	Cardiomegaly	6
	Severe acute diffuse myocarditis	5
	Hepatic congestion	3
	Diffuse chronic myocarditis	3
	Splenomegaly	2
	Hepatomegaly	2
	Hydrothorax	2
	Thick hypertrophied left ventricle	2
	Spleen congestion	1
	Renal infarct	1
	Hepatic centrolobular necrosis	1
	Hydropericardium	1
	Dilated ventricles	1
	Endocarditis of tricuspid/bicuspid valve	1
	Thin walled ventricle	1

2.6.2 Pathology / Clinical Findings

In Southern Louisiana, several Chagas disease cases were referred to the Louisiana State University School of Veterinary Medicine Teaching Hospital or Iberia

Animal Clinic with signs compatible with Chagas disease. Cases mainly presented with cardiac failure of unknown origin. In the present study we describe clinical signs of 11 cases that were confirmed pathologically and/or serologically for *T. cruzi*.

Most of the confirmed cases in dogs were in Labrador Retrievers from Louisiana less than two-years-old and in good body condition. Only two of the cases were in 8-year-old dogs. Labrador Retrievers are a popular breed in the region, where they are considered to be both good companions and accomplished waterfowl hunting retrievers when trained. Most of these dogs have contact with wild animals and live in outdoor kennels and thus have a higher probability of contact with wild hosts and the reduviid vectors. The parasite may be transmitted when vector feces containing metacyclic trypomastigotes contaminate food or water, contamination of the bite site or when the dog consumes infected animals or insects (Williams et al., 1977; Collins and Kennedy., 1999) or licking their fur which can be contaminated with Triatomine feces. Reduviids can often be found by the dogs' owners in or around the kennel, in water containers and even in houses, especially after flooding or strong rains.

The compilation of cases from Southern Louisiana is useful to describe the pathology and clinical signs of naturally infected dogs and to provide historical information that describes how Chagas disease affects dogs. The main clinical signs seen were referable to heart failure (cough, acute death, tachypnea/dyspnea, jugular pulse, vomiting, lameness, lethargy, fatal cardiac failure). At necropsy examination, the main gross pathology findings were linked with cardiac dysfunction (thin walled heart, dilated ventricle, endocarditis of the tricuspid or bicuspid valves, hydropericardium, thick hypertrophied left ventricle, hydrothorax, diffuse chronic myocardial inflammation, severe diffuse myocarditis, ascites, cardiomegaly, pulmonary edema, and granulomatous

subacute multifocal myocarditis). Upon histopathological examination, the conclusive finding for diagnosis of American Trypanosomiasis or Chagas disease was presence of intracellular *T. cruzi* amastigotes in tissues of the lung, heart or other organs. Seventeen additional animals were presumptively diagnosed with Chagas disease, but these cases were not confirmed by pathology or by serology. The pathogenesis of Chagas disease in dogs is often not fully understood among practicing veterinarians due to the different stages of the disease, and the absence of quick, convenient, same day diagnostic methods suited for use in busy practices.

Lack of an easy and rapid diagnostic method as well as limited information on the infection in the United States suggests that Chagas disease is commonly undiagnosed, misdiagnosed and/or mistreated. Clinical signs may easily be confused with severe canine heartworm disease (heart failure, coughing, ascites, hepatomegaly, dyspnea and lethargy / exercise intolerance). Even though the possibility of becoming infected with *T. cruzi* while interacting with infected animals is low, veterinarians and technicians should follow biosafety procedures, such as: wearing gloves while drawing blood samples. In other invasive procedures, personnel should wear gloves as well as skin and eye protection. Similar precautions should also be followed when working with infected wildlife or the reduviid vector. Several cases of laboratory-associated *T. cruzi* infections have been documented from handling *Trypanosoma* cultures or blood specimens from infected humans or animals, or as a result of accidental parenteral inoculation, contamination of skin or mucous membranes (Herwaldt and Juranek, 1993; Kirchhoff, 1993). A definite diagnosis of Chagas disease in dogs should include testing for cross reactivity with *Leishmania* as well as consideration of the clinical picture and exposure history of tested dogs.
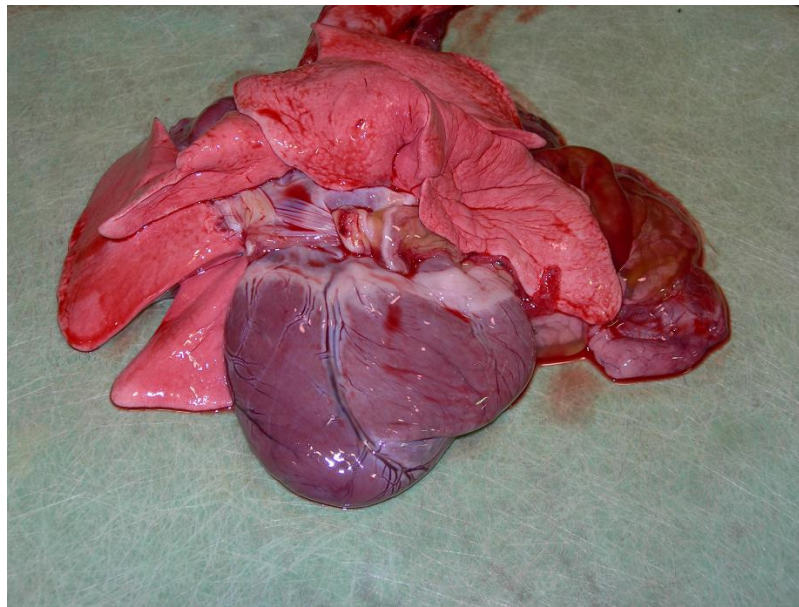
Fig. 14. Necropsy on a *T. Cruzi* infected dog. Note the severely enlarged heart with prominent rounding of the apex and bulging of the right ventricle.

[A] Trypanosoma Detect[TM] for canine, InBios, Seattle, WA.

[B] CHAGAS STAT-PAK[TM] assay, a rapid single use immunochromatographic screening test, Chembio Diagnostic Systems, Medford, NY.

2.7 References

Andrade, Z.A., Andrade, S.G., 1980. A patologia da doenc¸a de Chagas experimental no cão. Mem. Inst. Oswaldo Cruz. 75, 77- 95.

Avila, H., Borges-Pereira, J., Thiemann, O., De Paiva, E., Degrave, W., Morel, CM., Simpson, L., 1993. Detection of *Trypanosoma cruzi* in blood specimes of chronic chagasic patients by polymerase chain reaction amplification of kinetoplast minicircle DNA: comparison with serology and xenodiagnosis. J. Clin. Microbiol. 31, 2421-2426.

Aznar, C., Lopez-Bergami, P., Brandariz, S, Mariette, C., Liegeard, P., Alves, M.D., Barreiro, E.L., Carrasco, R., Lafon, S., Kaplan, D., Miguez, H., Camacho, C., Levitus, G., Levin, J.M., Hontebeyrie, M., 1995. Prevalence of anti-R-13 antibodies in human *Trypanosoma cruzi* infection. FEMS Immunol. Med. Microbiol. 12, 231-237.

Barr, S.C., Dennis, V.A., Klei, T.R., 1991. Serologic and blood culture survey of *Trypanosoma cruzi* infection in four canine populations of southern Louisiana. Am. J. Vet. Res. 52,570-573.

Barr, S.C., Van Beek, O., Carlisle-Nowak, M.S., Lopez, J.W., Kirchhoff, L.V., Allison, N., Zajac, A., de Lahunta, A., Schlafer, D.H., Crandall, W.T.,1995. *Trypanosoma cruzi* infection in Walker hounds from Virginia. Am. J. Vet. Res.56, 1037-1044.

Beard, C.B., Pye, G., Steurer, F.J., Rodriguez, R., Campman, R., Peterson, T.A., Ramsey, J., Wirtz, R.A., Robinson, L.E., 2003. Chagas disease in a domestic transmission cycle, Southern Texas, USA. Emerg. Infect. Dis. 9, 103-105.

Bittencourt, AL., Rodrigues de Freitas, LA., Galvão de Araujo, MO., Jácomo, K., 1981.Pneumonitis in congenital Chagas disease: A study of ten cases. Am. J. Trop. Med. Hyg. 30, 38-42.

Bradley, K.K., Bergman, D.K., Woods, J.P., Crutcher, J.M., Kirchhoff, L.V., 2000. Prevalence of American trypanosomiasis (Chagas disease) among dogs in Oklahoma. J. Am. Vet. Med. Assoc. 217,1853-1857.

Burkholder, J.E., Allison, T.C., Kelly, V.P., 1980. *Trypanosoma cruzi* (Chagas) (Protozoa: Kinetoplastida) in invertebrate, reservoir, and human hosts of the lower Rio Grande valley of Texas. J. Parasitol. 66, 305-311.

Carcavallo, R.U., 1999.Climatic Factors Related to Chagas Disease Transmission. Mem. Inst.. Oswaldo Cruz. 94, 367-369.

Carcavallo, R.U., Galvão ,C., Rocha, D.S., Jurberg, J., Curto de Casas, S.I., 1998. Predicted effects of warming on Chagas disease vectors and epidemiology. Entomol. Vect. 5,137.

Carcavallo, R.U., Martínez, A., 1972. Life cycles of some species of Triatoma (Hemiptera, Reduviidae). Canadian Entomol. 104, 699-704.

Cardinal, M.V., Reithinger, R., Gürtler, R.E., 2006. Use of an Immunochromatographic Dipstick Test for Rapid Detection of *Trypanosoma cruzi* in Sera from Animal Reservoir Hosts. J. Clin. Microbiol. 44, 3005-3007.

Cardoso, A.V., Lescano, S.A., Amato Neto, A., Gakiya, E., Santos, S.V., 2006. Brief communication survival of *Trypanosoma cruzi* in sugar cane used to prepare juice. Rev. Inst. Med. Trop. S. Paulo. 48, 287-289.

Castañera, M.B., Lauricella, M.A., Chuit, R., Gurtler, R., 1998. Evaluation of dogs as sentinels of the transmission of *Trypanosoma cruzi* in a rural area of north-western Argentina. Ann. Trop. Med. Parasitol. 92, 670-682.

CHAGAS STAT-PAK[TM] assay, a rapid single use immunochromatographic screening test, Chembio Diagnostic Systems, Medford, NY. Product information available at: http://www. chembio.com/products.html . Acceded Oct 25, 2006.

Collins, C.H., Kennedy, D.A., 1999. *Exposure, sources and routes of infection. In: Laboratory-acquired infections: history, incidence, causes and preventions.* 4th ed. Oxford, U.K: Butterworth-Heinemann Ltd. pp. 38-53.

Cubillos-Garzon, L.A., Cassas, J.P., Morillo, C.A., Bautista, L.E., 2004. Congestive heart failure in Latin America: the next epidemic. Am. Heart. J. 147, 412-417.

Da Matta Guedes, P.M., Veloso, V.M., Tafuri, W.L., Da Cunha Galvão, L.M., Martins Carneiro, C., De Lana, M., Egler Chiari, E., Soares, K.A., Bahia, M.T., 2002. The Dog as Model for chemotherapy of the Chagas disease. Acta Trop. 84, 9-17.

Dias, C.P., Coura, J., 1997. Clínica e terapêutica da doença de Chagas: uma abordagem prática para o clínico geral, Rio de Janeiro: FIOCRUZ, 486.

Diosque, P., Padilla, A.M., Cimino, R.O., Cardozo, R.M.,Sanchez Negrette, O., Marco, J.D., Zacca, R., Meza, C., Juarez, A., Rojo, H., Rey, R., Corrales, R.M., Nasser, J.R., Basombrio, M.A., 2004. Chagas disease in rural areas of Chaco Province, Argentina: epidemiologic survey in humans, reservoirs and vectors. Am. J. Trop. Med. Hyg. 71, 590-593.

Dorn P.L., Perniciaro, L., Yabsley, M.J., Roellig, D.M., Balsamo, G., Diaz, J., Wesson, D,.2007. Autochthonous Transmission of *Trypanosoma cruzi*, Louisiana. Emerg. Infect. Dis. 13, 603-607.

Duarte Vieira, A.M., Monteiro de Andrade, H., Hadad do Monte, S.J., Peixoto de Toledo, V.P., Dabés Guimarães, T.M., 2006. Assessment of chemiluminescence and PCR effectiveness in relation to conventional serological tests for the diagnosis of Chagas disease. Rev. Soc. Bras. Med. Trop. 39,385-387.

Ferreira, MS., Borges, A.S., 2002. Some aspects of protozoan infections in immuno-compromised patients–a review. Mem. Inst. OswaldoCruz. .97, 443–457.

Gürtler, R.E., Cecere, M.C., Castañera, M.B., Canale, D., Lauricella, M.A., Chuit, R., Cohen, J.E., Segura, E.L., 1996. Probability of infection with *Trypanosoma cruzi* of the vector *Triatoma infestans* fed on infected humans and dogs in northwest Argentina. Am. J. Trop. Med. Hyg. 55, 24-31.

Gürtler, R.E., Cecere, M.C., Lauricella, M.A., Petersen, R.M., Chuit, R., Segura, E.L., Cohen, J.E., 2005. Incidence of *Trypanosoma Cruzi* infection among children fo-llowing domestic reinfestation after insecticide spraying in rural northwestern Argen-tina. Am. J. Trop. Med. Hyg.73, 95-103.

Gürtler, R.E., Kitron, U., Cecere, M.C., Segura, E.L., Cohen, J.E., 2007. Sustainable vec-tor control and management of Chagas disease in the Gran Chaco, Argentina. PNAS.104, 16194-16199.

Hack ,W., 1955. Estudios sobre biología del Triatoma infestans (Klug, 1834) (Hemiptera, Reduviidae). An. Inst. Med. Regional. 4, 125-147.

Herwaldt, B.L., Grijalva, M.J., Newsome, A.L., McGhee, C.R., Powell, M.R., Nemec, D.G., Steurer, F.L., Eberhard, M.L.,2000. Use of polymerase chain reaction to diagnose the fifth reported US case of autochthonous transmission of *Trypanosoma cruzi*, in Tennessee. J. Infect. Dis.181, 395-399.

Herwaldt, B.L., Juranek, D., 1993. Laboratory-acquired malaria, leishmaniasis, trypano-somiasis, and toxoplasmosis. Am. J. Trop. Med. Hyg. 48,313-323.

Jansen, A.M., Leon, L., Machado, G.M., da Silva, M.H., Souza-Leão, S.M., Deane, M.P., 1991. *Trypanosoma cruzi* in the opossum *Didelphis marsupialis*: Parasitological and serological follow-up of the acute infection. Exp. Parasitol.73, 249-259.

John D.T, Hoppe K.L., 1986. *Trypanosoma cruzi* from wild raccoons in Oklahoma. Am. J. Vet. Res. 47, 1056-1059.

Kierszenbaum, F., 1999. Chagas disease and the Autoimmunity Hypothesis. Clinical Microbiology reviews. 12,210-224.

Kirchhoff, L.V., 1993. Chagas disease. American trypanosomiasis. Infect. Dis. Clin. North. Am. 7, 487- 502.

Kjos, S.A., Snowden, K..F, Craig, T.M., Lewis, B., Ronald, N., Olson, J.K., 2008. Distribution and characterization of canine Chagas disease in Texas. Vet. Parasitol. 152, 249-256.

Lauricella, M.A., Castañera, M.B., Gürtler, R.E., Segura, E.L., 1998. Immunodiagnosis of *Trypanosoma cruzi* (Chagas™ Disease) Infection in Naturally Infected Dogs. Mem. Inst. Oswaldo Cruz. 93, 501-507.

Lent, H., Wygodzinsky, P., 1979. Revision of the Triatominae (Hemiptera, Reduviidae) and their significance as vectors of Chagas disease. Bulletin of the American Museum of Natural History. 163,123–520.

López Antuñano, F.J., Mota, J., 2000.Development of immunizing agents against dengue. Pan American Journal of Public Health.7, 285-292.

Luquetti, A.O., Ponce, C., Ponce, E., Esfandiari, J., Schijman, A., Revollo, S., Añez, N., Zingales, B., Ramgel-Aldao, R., Gonzalez, A., Levin, M., Umezawa, E.S., da Silveira, J.F., 2003.Chagas disease diagnosis: a multicentric evaluation of Chagas Stat-Pak, a rapid immunochromatographic assay with recombinant proteins of *Trypanosoma cruzi.* Diagn. Microbiol. Infect. Dis.46, 265–271.

Macedo, A.M., Machado, C.R., Oliveira, R.P., Pena, S.D., 2004. *Trypanosoma cruzi*: genetic structure of populations and relevance of genetic variability to the pathogenesis of Chagas disease. Mem. Inst. Oswaldo Cruz. 99, 1-12.

Macedo, A.M., Pena, S., 1998. Genetic variability of *Trypanosoma cruzi*: implications for the pathogenesis of Chagas disease. Parasitol. Today. 14, 119-124.

Mesri, E.A, Levitus, G., Hontebeyrie-Joskowicz, M., Dighiero, D., Van Regenmortel, M.H., Levin, M.J., 1990. Major *Trypanosoma cruzi* antigenic determinant in Chagas' heart disease shares homology with the systemic lupus erythematosus ribosomal P protein epitope. J. Clin. Microbiol. 28, 1219-1224.

Meurs, K.M., Anthony, M.A., Slater, M., Miller , M.W., 1998. Chronic *Trypanosoma cruzi* infection in dogs: 11 cases (1987-1996). J. Am. Vet. Med. Assoc. 213, 497-500.

Montenegro, V.M., Jimenez, M., Pinto Dias, J.C., Zeledon, R., 2002. Chagas disease in dogs from endemic areas of Costa Rica. Mem. Inst. Oswaldo Cruz. 97, 491-494.

Navin, TR., Roberto, R.R., Juranek, D.D., Limpakarnjanarat, K., Mortenson, EW., Clover, JR., Yescott, R.E., Taclindo, C., Steurer, F., Allain, D., 1985.Human and sylvatic *Trypanosoma cruzi* infection in California. Am. J. Public. Health. 75, 366–369.

Ochs, D.E., Hnilica, V.S., Moser, D.R., Smith, J.H., Kirchhoff, L.V.,1996. Postmortem diagnosis of autochthonous acute chagasic myocarditis by polymerase chain reaction amplification of a species-specific DNA sequence of *Trypanosoma cruzi*. Am. J. Trop. Med. Hyg. 54, 526 -529.

Oelemann, W.M., Teixeira, M.G., Verissimo Da Costa, G.C., Borges-Pereira, J., De Castro, J.A., Rodrigues Coura, J., Peralta, J.M.,1998. Evaluation of three commercial enzyme-linked immunosorbent assays for diagnosis of Chagas disease. J. Clin. Microbiol. 36, 2423-2427.

Paige, C.F., Scholl, D.T., Truman, R.W., 2002. Prevalence and incidence density of *Mycobacterium leprae* and *Trypanosoma cruzi* infections within a population of wild nine-banded armadillos. Am. J. Trop. Med. Hyg. 67, 528-532.

Ponce, C., Ponce, E., Vinelli, E., Montoya, A., de Aguilar, V., Gonzalez, A., Zingales, B., Rangel-Aldao, R., Levin M.J., Esfandiari, J., Umezawa, E.S., Luquetti, L.O., Silveira, J.F**.,** 2005. Validation of a rapid and reliable test for diagnosis of Chagas disease by detection of *Trypanosoma cruzi*-specific antibodies in blood of donors and patients in Central America. J. Clin. Microbiol. 43, 5065-5068.

Portela-Lindoso, A.A., Shikanai-Yasuda, M.A., 2003. Chronic Chagas's disease: from xenodiagnosis and hemoculture to polymerase chain reaction. Cad. Saude Pública 37**,**107-115.

Reithinger, R., Ceballos, L., Stariolo, R., Davies, C.R, Gürtler, R.E., 2005. Chagas disease control: deltamethrin-treated collars reduce *Triatoma infestans* feeding success on dogs. Trans. R. So.c Trop. Med. Hyg. 7, 502-508.

Ribeiro dos Santos, R., Rossi, M.A., Laus, J.L., Silva, J.S., Savino, W., Mengel, J., 1992. Anti-CD4 abrogates rejection and reestablishes long-term tolerance to syngeneic newborn hearts grafted in mice chronically infected with *Trypanosoma cruzi*. J. Exp. Med. 175, 29-39.

Saldana, A., Sousa, O.E., 1996.*Trypanosoma rangeli*: epimastigote immunogenicity and cross-reaction with *Trypanosoma cruzi*. J. Parasitol. 82, 363-366.

Schiffler, R.J., Mansur, G.P., Navin, T.R., Limpakarnjanarat, K., 1984.Indigenous Chagas' disease (American trypanosomiasis) in California. JAMA. 251, 2983-2984.

Shadomy, S.V., Waring, S.C., Chappell, C.L., 2004. Combined Use of Enzyme-Linked Immunosorbent Assay and Flow Cytometry To Detect Antibodies to *Trypanosoma cruzi* in Domestic Canines in Texas. Clin. Diagn. Lab. Immunol. 11, 313-319.

Silva, I.G., Silva, H.G., 1986. Influência da temperatura na biologia de Triatomíneos. IV. Triatoma infestans (Klug, 1834) (Hemiptera, Reduviidae). An. Soc. Entomol. Brasil. 17, 443-455.

Tarleton, R.L., Zhang, L., 1999. Chagas disease etiology: Autoummunity or parasite persistence?. Parast. Today.13, 94-99.

Tomlinson, M.J., Chapman, W.L., Hanson, W.L., Gosser, H.S.,1981. Occurrence of antibody to *Trypanosoma cruzi* in dogs in the southeastern United States. *Am. J. Vet. Res*. 42, 1444-1446.

Trypanosoma Detect[TM] test for canine, InBios, Seattle, WA. Product information available at: http://www.inbios.com/products.php?code=rapidTryp . Acceced Feb 21, 2006.

Umezawa, E.S., Bastos, S.F., Coura,. JR., Levin, M.J., Gonzalez, A., Rangel-Aldao, R., Zingales, B., Luquetti, A.O., da Silveira, J.F., 2003. An improved serodiagnostic test for Chagas' disease employing a mixture of Trypanosoma cruzi recombinant antigens. Transfusion. 43, 91-97.

UNICEF/UNDP/World Bank/WHO. TDR Web site. Tropical disease research: progress 2003-2004. Special Programme for Research & Training in Tropical Diseases. Seventeenth Programme Report. Available at: http://www.who.int/tdr/publications/publications/pr17.htm. Acceded Sep 14, 2007.

Urbina, J. A., 1999.Chemotherapy of Chagas disease; the how and why. J. Mol. Med. 77, 332-338.

Urdaneta-Morales, S., Nironi, I., 1996. *Trypanosoma cruzi* in the Anal Glands of Urban Opossums.I- Isolation and Experimental Infections. Mem. Inst. Oswaldo Cruz. 91, 399-403.

Williams, G.D., Adams, G., Yaeger,. RG., McGrath, R. K., Read, W. K., Bilderback, W. R., 1977. Naturally ocurring Trypanosomiasis (Chagas disease) in dogs. J. Am. Vet. Med. Assoc. 15, 171-177.

Wisnivesky-Colli, C., Schweigmann, N.J., Alberti, A., Pietrokovsky, S., Conti, O., Montoya, S., Riarte, A., Rivas, C., 1992. Sylvatic American Trypanosomiasis in Argentina. *Trypanosoma cruzi* infection in mammals from the Chaco Forest in Santiago del Estero. Trans. R. Soc. Trop. Med. Hyg. 86, 38-41.

Woody, N.C., Woody, H.B., 1955. American trypanosomiasis (Chagas' disease): first indigenous case in the United States. JAMA. 159, 676-677.

Yabsley, M.J., Noblet, G.P., Pung, O.J., 2001.Comparison of serological methods and blood culture for the detection of *Trypanosoma cruzi* infection in raccoons (*Procyon lotor*). J. Parasitol. 87, 1155-1159.

Yeager, R.G., 1988.The prevalence of *Trypanosoma cruzi* infection in armadillos collected at a site near New Orleans, Louisiana. Am. J. Trop. Hyg. 38, 323-326.

Zeledón, R., 1974. Epidemiology, modes of transmission and reservoir hosts of Chagas' disease. In Trypanosomiasis and Leishmaniasis with Special Reference to Chagas Disease, Ciba Foundation Symposium. 20, 51-77.

CHAPTER 3   CONCLUSIONS

3.1 Remote Sensing and Ecological Niche Models to Predict Visceral Leishmaniasis in the State of Bahia Brazil.

The present study was done in the state of Bahia, the largest state in the north east of Brazil, where environmental change associated with deforestation, agrarian practices, exploitation of the soil, combine with poverty, malnutrition and decreased immunity to increase risk of inhabitants to several infectious diseases, including VL (Sherlock, 1996). Most VL infections occur in remote geographical areas where health facilities are not well established. Visceral leishmaniasis can be multifocal with remarkable difference among regions. Many clinical cases are not reported and go un-treated, and many cases are subclinical. The result is that health authorities have insufficient information to implement efficient control campaigns.

Geographic information systems and remote sensing technologies enable health workers to evaluate the relationship between the environment and disease agents as well as to define risk in areas where surveillance data is not readily available. The latter is possible by extrapolation of GIS epidemiological models within endemic areas to guide appropriate control and prevention strategies.

Three separate approaches (RS, GARP, GDD-WB) were used in this study to develop environmental risk assessment models that predict the distribution and potential risk areas of VL transmission in the state of Bahia. Previous studies using GIS for risk assessment of schistosomiasis, leishmaniasis, malaria and other diseases have indicated that climatic and environmental variables, such as NDVI, temperature, elevation, rainfall and humidity are important determinants of the presence and potential transmission of diseases. Similar approaches by RS technology have been used to model potential distribution and risk for leishmaniasis and schistosomiasis (Guimarãesa et al., 2008;

110

Bavia et al., 1996; Malone et al., 2003; Thompson et al., 2002; Sudhakar et al., 2006; Teshome et al., 2004; and Bavia et al., 2005). GARP statistical analysis has been used to model geographic distribution of leishmaniasis vectors (Peterson et al., 2004; Peterson and Shaw, 2003). GDD-WB models have been also used to predict potential risk of schistosomiasis and malaria (Malone et al., 2003, 2004). This is the first time where these three approaches have been used in the same area where the result shows high agreement in predicting geospatial risk zones of VL.

Results of the three models developed indicate that the interior region of the state known as Caatinga, poses the highest risk for VL. The Bahia interior forest and the Cerrado ecological regions are lower risk areas. The Bahia coastal forest was predicted as a very low risk area due to the unsuitable moisture conditions for the vector and VL transmission. High prevalence of the disease was found in the central plateau, decreasing in proximity to the east and west part of the state.

Visceral leishmaniasis was more likely to be present in areas in Bahia where the mean temperature ranged from 17.5 to 27.5 °C and elevation from 436-950 meters above sea level. For the satellite remote sensing models, data for NDVI ranged from 0.35 to 0.65 (MODIS data) or from 127 to 149 (AVHRR data) and day-night temperature difference ranged from 11-19 °C. For the GDD-WB model, annual potential generations ranged from 0 to 9 generations per year. High and medium prevalence zones had more than 5 generations per year. The Caatinga ecological region has at least 5 potential generations per year whereas the Cerrado was predicted to have 2-4 potential generations per year and the Bahia costal forest was predicted to have 0 to 3 generations per year. The GARP model was used for the first time to predict VL risk over an 11 year period by iterative statistical software analysis of the association of known VL cases with

WORLDCLIM environmental database variables. Results of the three models reported in the present study can be used as the basis for development of a GIS spatial decision support system for VL in the state of Bahia and for design of improved health planning, decision-making and ongoing surveillance efforts.

3.2 Diagnosis and Clinical-Pathological Findings of Chagas Disease *(T. cruzi)* in Dogs in South Central Louisiana.

Chagas disease, or American trypanosomiasis, is a parasitic disease caused by the protozoan hemoflagellate *T. cruzi*. This zoonotic illness is a cause of cardiomyopathy in dogs and primates. Dogs are common victims and develop pathological changes similar to those detected in humans characterized by the acute, indeterminate and chronic phases. *Trypanosoma cruzi* infection can be diagnosed by serological methods (eg. IHA, IFAT, ELISA), molecular methods (eg. PCR) or parasitological methods (eg. direct smear, xenodiagnosis and hemoculture).

In the present study, dogs considered to have a high risk of exposure to the vector *T. sanguisuga* were tested using IFAT to estimate the prevalence of *T. cruzi* infection in south central Louisiana. Two experimental immunochromatographic rapid assays were tested as alternative or complementary diagnostic tests. Clinical and pathological findings in case records of natural infected dogs in Louisiana were described.

A total of 22.1% (27of 122) dogs tested were found positive for *T. cruzi* by IFAT. The prevalence reported in the current study is quite similar to results from a recent study of canines in Texas where the seroprevalence was of 20.3% (Kjos et al., 2008). Other studies in the USA showed a lower seroprevalence rates, e.g. 8.8% (12 of 136) in the lower Rio Grande Valley of Texas (Beard et al., 2003; Burkholder et al., 1980) and 3.6% (11 of 301) in Oklahoma, (Bradley et al., 2000). Previous studies in south Louisiana showed a seroprevalence in roaming rural dogs of 4.7% (4 of 85 dogs) and a

seroprevalence in urban shelter dogs of 2.3% (4 of 176 dogs) (Barr et al., 1991). In the current study results suggest that a high percentage of *T. cruzi* infected dogs can be found in the area in and around the Atchafalaya Basin in Louisiana.

The IFAT is a commonly used laboratory test used for diagnosis of *T. cruzi* in dogs in the United States and elsewhere, although specificity may vary because of antigenic cross- reactivity with other parasite species, notably *Leishmania*. In this study all the *T. cruzi* positive cases were also tested for cross reactivity to *Leishmania.*

The two rapid immunochromatographic assays (A and B) revealed high sensitivity (100%) and a specificity of 95% in rapid assay A, and 100%sensitivity and specificity for rapid assay B, relative to the IFAT (the standard test). Previous studies reported for dipstick Rapid Assay A have reported high specificity (>94%) and high sensitivity (>96%) in dogs. The test is based on a proprietary gold mix containing multi-epitope recombinant antigens ITC-6 and ITC-8.2 derived from different *T. cruzi* antigens, including peptide 2, TcD, TcF, TcLo, and SAPA (Cardinal et al., 2006). Studies in humans in Central America using Rapid Assay B revealed a sensitivity of 99.6% and a specificity of 99.9 % (Luquetti et al., 2003; Ponce et al., 2005). Rapid assay B employs a unique combination of *T. cruzi* recombinant antigens (B13, 1F8, and H49/JL7) which are bound to the membrane, and a specific antibody-binding protein, which is conjugated on dye particles (Ponce et al., 2005). Recombinant antigens for the detection of *T. cruzi* may prove to be more accurate than available commercial serologic tests that employ epimastigote antigens and show a high number of inconclusive and false-positive results (Umesawa et al., 2003).

Our pathological and clinical findings agree with a study in Texas by Kjos et al., 2008, where most of the *T. cruzi* positive dogs were young animals that belong primarily

113

to the sporting and working groups. The pathological findings were mainly myocarditis and/or observation of intracellular amastigotes/pseudocysts of *T. cruzi* in tissue; clinical findings were acute death, cardiac failure, lethargy, enlarged heart and cardiac failure.

Our results suggest that Chagas disease in dogs is under-diagnosed in Louisiana by veterinarians due to the lack of convenient, practice-based testing methods and the difficulty of differential diagnosis of suspect cases with other conditions, such as heartworm disease. It is recommended that veterinarians test dogs considered to be at high risk of infection (i.e. hunting dogs, dogs that live in outdoor kennels that may have contact with the reduviid vector or wild reservoirs, and dogs that live in same environment as another infected dogs). The use of rapid assay screening tests that can be used in routine practice, in conjunction with confirmatory laboratory methods, other information available to the veterinarian (clinical picture, environment and exposure history of the tested dog) and testing for cross reactivity for *Leishmania* are fundamental requirements for more accurate diagnosis of suspected *T.cruzi* infection in animals in Louisiana and other states in the Southern USA.

Further studies on the effect of Chagas disease among people that live in close association with infected dogs, strain characterization of local *T. cruzi* isolates, as well as a statewide serological survey in dogs would contribute greatly to determining the true prevalence and impact of Chagas disease in dogs and to public health in the state of Louisiana.

3.3 References

Barr, S.C., Dennis, V.A., Klei, T.R., 1991. Serologic and blood culture survey of *Trypanosoma cruzi* infection in four canine populations of southern Louisiana. Am. J. Vet. Res. 52, 570-573.

Bavia, M., 1996. Geographic Information Systems for Schistosomiasis in Brazil. Ph.D. Thesis, Louisianan State University pp 25.

Bavia, M.E., Carneiro, D.D., Gurgel Hda, C., Madureira, C., Barbosa, M.G., 2005. Remote Sensing and Geographic Information Systems and risk of American visceral leishmaniasis in Bahia, Brazil. Parassitologia. 47, 165-169.

Beard, C.B., Pye, G., Steurer, F.J., Rodriguez, R., Campman, R., Peterson, T.A., Ramsey, J., Wirtz, R.A., Robinson, L.E., 2003. Chagas disease in a domestic transmission cycle, Southern Texas, USA. Emerg. Infect. Dis. 9, 103-105.

Bradley, K.K., Bergman, D.K., Woods, J.P., Crutcher, J.M., Kirchhoff, L.V., 2000. Prevalence of American trypanosomiasis (Chagas disease) among dogs in Oklahoma. J. Am. Vet. Med. Assoc. 217,1853-1857.

Burkholder, J.E., Allison, T.C., Kelly, V.P., 1980. *Trypanosoma cruzi* (Chagas) (Protozoa: Kinetoplastida) in invertebrate, reservoir, and human hosts of the lower Rio Grande valley of Texas. J. Parasitol. 66, 305-311.

Cardinal, M.V., Reithinger, R., Gürtler, R.E., 2006. Use of an Immunochromatographic Dipstick Test for Rapid Detection of *Trypanosoma cruzi* in Sera from Animal Reservoir Hosts. J. Clin. Microbiol. 44, 3005-3007.

Guimarãesa, RJ., Freitas, C.C., Dutra, L.V., Mourad, A.C., Amaral, R.S., Drummondf, S.C., Scholtea, R.G.,Carvalhoa, O., 2008. Schistosomiasis risk estimation in Minas Gerais State, Brazil, using environmental data and GIS techniques. Acta Trop. 108, 234-241.

Kjos, S.A., Snowden, K..F, Craig, T.M., Lewis, B., Ronald, N., Olson, J.K., 2008. Distribution and characterization of canine Chagas disease in Texas. Vet. Parasitol. 152, 249-256.

Luquetti, A.O., Ponce, C., Ponce, E., Esfandiari, J., Schijman, A., Revollo, S., Añez, N., Zingales, B., Ramgel-Aldao, R., Gonzalez, A., Levin, M., Umezawa, E.S., da Silveira, J.F., 2003.Chagas disease diagnosis: a multicentric evaluation of Chagas Stat-Pak, a rapid immunochromatographic assay with recombinant proteins of *Trypanosoma cruzi.* Diagn. Microbiol. Infect. Dis.46, 265–271.

Malone, J.B., McNally, K.L., McCarroll, J.C., Corbett, Mkoji, G., 2004. Modeling the Biocoenose of Parasitic Diseases Using Remote Sensing and Geographic nformation Systems. Parassitologia. 46, 59-61.

Malone, J.B., Poggi, E., Igualada, F.J., Sintasath, D., Ghebremeskel, T., Corbett, J.D., McCarroll, J.C., Chinnici, P., Shililu, J., McNally, K., Downer, R., Perich, M., Ford, R., 2003.Malaria environmental risk assessment in Eritrea. IGARSS 2003. pp 1000 – 1003.

Peterson, A.T., Pereira, R.S., Camargo Neves, VF., 2004b. Using epidemiological survey data to infer geographic distributions of leishmaniasis vector species. Revista da Sociedade Brasileira de Medicina Tropical. 37, 10-14.

Peterson, A.T., Shaw, J., 2003. *Lutzomyia* vectors for cutaneous leishmaniasis in Southern Brazil: ecological niche models, predicted geographic distributions, and climate change effects. Int. J. Parasit. 33, 919-931.

Ponce, C., Ponce, E., Vinelli, E., Montoya, A., de Aguilar, V., Gonzalez, A., Zingales, B., Rangel-Aldao, R., Levin M.J., Esfandiari, J., Umezawa, E.S., Luquetti, L.O., Silveira, J.F**.,** 2005. Validation of a rapid and reliable test for diagnosis of Chagas disease by detection of *Trypanosoma cruzi*-specific antibodies in blood of donors and patients in Central America. J. Clin. Microbiol. 43, 5065-5068.

Sherlock, I.A., 1996. Ecological interactions of visceral leishmaniasis in the state of Bahia, Brazil. Mem. Inst. Oswaldo Cruz. 91, 671-683.

Sudhakar, S., Srinivas, T., Palit, A., Kar, S.K., Battacharya, S.K,. 2006. Mapping of risk prone areas of kala-azar (Visceral leishmaniasis) in parts of Bihar State, India: an RS and GIS approach. J. Vector. Borne. Dis. 43, 115-122.

Teshome, G.M., Malone, J. B., Balkew, M., Ali, A., Berhe, N., Hailu, A., Herzi, A. A., 2004. Mapping the potential distribution of *Phlebotomus martini* and *P. orientalis* (Diptera: Psychodidae), vectors of kala-azar in East Africa by use of geographic information systems. Acta Tropica. 90, 73-86.

Umezawa, E.S., Bastos, S.F., Coura,. JR., Levin, M.J., Gonzalez, A., Rangel-Aldao, R., Zingales, B., Luquetti, A.O., da Silveira, J.F., 2003. An improved serodiagnostic test for Chagas' disease employing a mixture of *Trypanosoma cruzi* recombinant antigens. Transfusion. 43, 91-97.

VITA

Prixia del Mar Nieto was born in Bogotá, Colombia, in November, 1974. She studied veterinary medicine in La Salle University and graduated in August 2001. In January 2004 she started a doctoral program in the Department of Pathobiological Sciences in the School of Veterinary Medicine in Baton Rouge, Louisiana, and will graduate in May, 2009.