LANDSCAPE EPIDEMIOLOGY OF HANTAVIRUS IN THE ATLANTIC FOREST OF PARAGUAY

by

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B.S., University of Wisconsin-Madison, 1991
M.S., Kansas State University, 2002

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

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Abstract

Hantaviruses are zoonotic, RNA viruses that are harbored by muroid rodents of the families Muridae and Cricetidae. While the virus is endemic, and mostly non-symptomatic in its rodent reservoirs, when humans contact the virus it can result in serious disease. My purpose in this dissertation is to investigate the effect that landscape patterns and land cover condition can have on pathogen prevalence in a hantavirus reservoir species (*Akodon montensis)* within the Atlantic Forest region of Eastern Paraguay and to investigate ways to analyze those patterns using remotely sensed data. The first component to this research is to test potential improvements to image classifications on land use/land cover classifications useful for the study of small mammal communities. An object-based classification produced the best results with seven classes: Forest, Wet Cerrado, Dry Cerrado, Latifundia, Minifundia, Dry Pasture, and Wet Pasture. The classified imagery was then used to assess landscape effects on the presence of hantaviral antibodies (a 'marker' for exposure to the virus) in populations of *A. montensis*. In the overall landscape, proximity of similar habitat patches was related to seroprevalence in *Akodon.* When considering only the forest class, high amount of forest, high number of forest patches, and high diversity in forest patch sizes were all associated with seroprevalence.

Next, was an analysis of ways to distinguish understory density variables through the use of satellite imagery. Horizontal and vertical density in the understory has been associated with the presence of hantavirus in *A. montensis*.

Vertical and horizontal density measurements were correlated with NDVI and the Fourth band in the Tasseled Cap transformation. Finally, I consider the relationship between small mammal community diversity and seroprevalence, and their association with NDVI. Diverse small mammal communities are associated with low hantavirus seroprevalence. Low diversity metrics and high hantavirus seroprevalence were associated with high mean NDVI values. Many aspects of landscape patterns are important to hantavirus seroprevalence in small mammal communities in Eastern Paraguay. Several of the landscape patterns important to hantavirus seroprevalence can be studied using satellite-derived data.

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Major Professor
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List of Abbreviations

AAI – Ape Aime virus

AKMO – *Akodon montensis*

ALPA – Alto Paraguay virus

BMJ-ÑEBU – Bermejo virus strain Ñeembucu

DBH – Diameter at Breast Height

ENSO – El Niño Southern Oscillation

GIS – Geospatial Information Systems

HFRS – Hemorrhagic Fever with Renal Syndrome

HPS – Hantavirus Pulmonary Syndrome

IFA – Immunofluorescence Assay

IP37 – Itapua virus strain 37

LAN – Laguna Negra virus

LULC – Land use/land cover

LULCC – Land use/land cover change

NDVI – Normalized Difference Vegetation Index

NE – Nephropathia Endemica

PIT – Passive Integrated Transponder

RBM – Mbaracayú Biosphere (Reserva de Biosfera del Bosque Mbaracayú)

RNBM – Mbaracayú Forest Reserve (Reserva Natural del Bosque Mbaracayú)

S+ – Seropositive

TC4 – Tasseled Cap Fourth Band

WNV – West Nile Virus

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Chapter 1. Introduction

1.1. Background

Hantavirus Pulmonary Syndrome (HPS, below) is an emerging infectious disease (Despommier, 2007). Hantaviruses, the etiologic factor for HPS, are a genus of zoonotic RNA viruses that belong to the *Bunyaviridae* family (Jonsson and Schmaljohn, 2001; Plyusnin and Morzunov, 2001). Hantaviruses can cause several serious diseases in humans. In Eurasia, hantaviruses cause Nephropathia Epidemica (NE) and Hemorrhagic Fever with Renal Syndrome (HFRS) (Klein and Calisher, 2007). Throughout the Americas, the hantavirus-associated disease is Hantavirus Pulmonary Syndrome (HPS) (Klein and Calisher, 2007; Raboni et al., 2005; Butler and Peters, 1994). The first recognition of HPS was in 1993 during an outbreak in the southwestern United States (Hjelle et al., 1994). Since that time, hantaviruses have been discovered to be endemic throughout the Americas (Klein and Calisher, 2007). Cases of HPS were first discovered in Paraguay in 1995 (Williams et al., 1997), Laguna Negra virus (LNV) was the name given to this strain of hantavirus (Chu et al,. 2009).

Taxonomically, hantaviruses are composed of numerous serotypes or 'strains'. Over twenty different serotypes have been identified throughout the Americas, each of these has a different rodent host species or reservoir (Klein and Calisher, 2007). In Paraguay (where the analysis in this dissertation was done), there are at least five serotypes (Figure 1.1), these types with their reservoirs are: Laguna Negra virus (LAN) with *Calomys laucha*, Alto Paraguay virus (ALPA) with *Holochilus chacarius*, Ape Aime virus (AAI) with *Akodon montensis*, Itapua virus strain 37 (IP37) with *Oligoryzomys nigripes*, and Bermejo - Ñeembucu (BMJ-ÑEBU) with *Oligoryzomys chacoensis* (Chu et al., 2003; Chu et al., 2009).

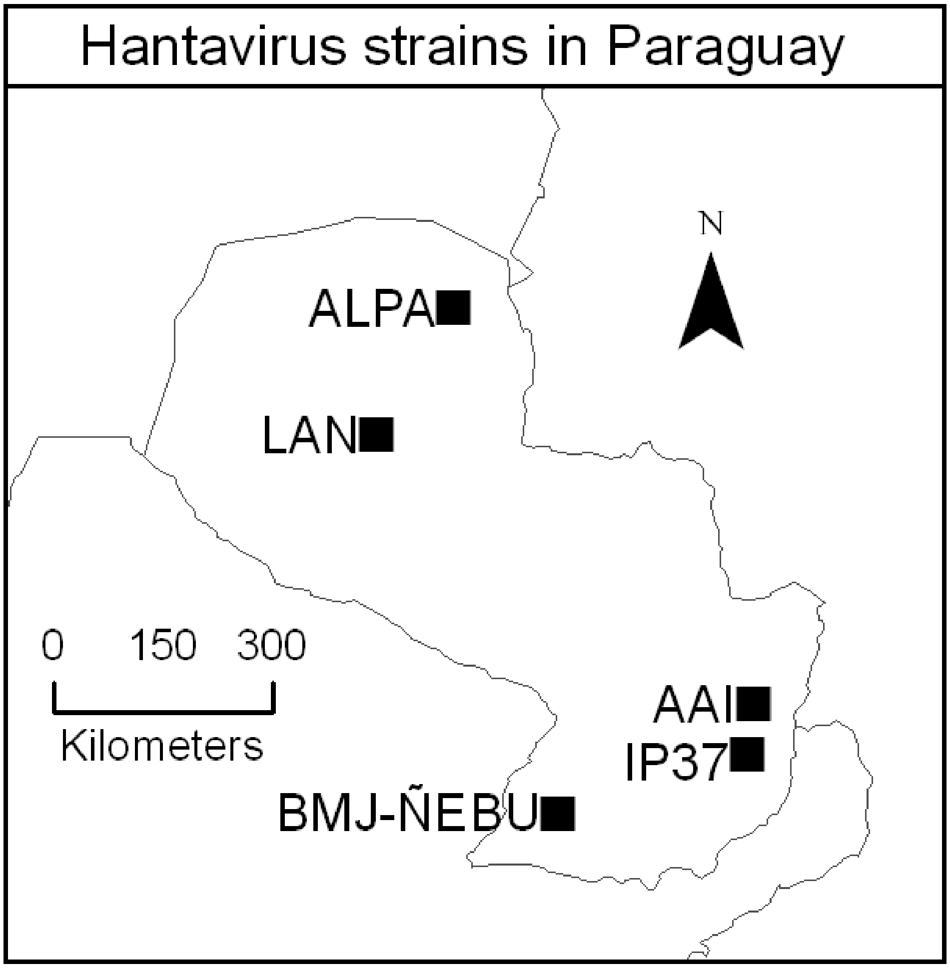

Figure 1.1. Map of general locations of strains of hantavirus in Paraguay. All strains have dispersed habitat, locations are approximate location of initial discovery (Chu et al., 2003).

Despite the prevalence of virus within their reservoirs and the wide geographic distribution of hantavirus reservoirs, outbreaks of HPS are sporadic and unpredictable. This situation has resulted in efforts to understand and predict relationships between the virus, the reservoir host, human contact, and landscape and climate variables. Variation in rodent hantavirus prevalence occurs seasonally, annually (Mills et al., 1999), with varying climate conditions (Williams et al.,1997), and with varying habitat (Kuenzi et al., 1999, Root et al., 1999). In North America, HPS outbreaks have been primarily associated with precipitation patterns (Engelthaler et al., 1999; Gubler et al., 2001; Yates et al., 2002). High precipitation levels increase food availability for the reservoir, which increases population size and risk between rodents of exposure to hantavirus (Parmenter et al., 1999). Remotely sensed indices of vegetation greenness have been associated with HPS in the Southwestern U.S. (Glass et al., 2000). Similar dynamics have been noted in HPS cases in the Paraguayan Chaco region (Williams et al., 1997), another rainfall limited region. While climatological aspects play a role in the prevalence of hantavirus in rodent communities, there are additional factors that also play important roles (Calisher et al., 2007), such as landscape patterns (Langlois et al., 2001) and some anthropogenic land use/land cover changes (Goodin et al., 2006; Suzan et al., 2006). Like hantavirus, about 75% of emerging and reemerging diseases are zoonotic (Taylor et al., 2001). Climate change and increased human impacts on the landscape play a role in the emergence of hantavirus (Dearing and Dizney, 2010). Part of the evidence for the effect of climate change on hantavirus is the effect of other climate cycling patterns (e.g., the El Niño Southern Oscillation (ENSO)) and an expansion in the range of hantavirus incidences in Europe. Increased human impacts on the landscape are generally in the form of habitat fragmentation and alteration of habitat quality. Most of these changes increase hantavirus prevalence (Dearing and Dizney, 2010; Mills et al., 2006; Root et al., 1999).

My purpose in this dissertation is to investigate the effect landscape has on pathogen prevalence in a hantavirus reservoir, specifically, how changes to the Atlantic Forest system in Eastern Paraguay affect *Akodon montensis* and its association with hantavirus. There are multiple ways that landscape could affect hantavirus, including effects on inter-species interactions (Keesing et al., 2006; LoGiudice et al., 2003), intra-species interactions (Eisenberg et al., 2006; Rotureau, 2006), and individual organism health (LoGiudice et al., 2003; Ecke et al., 2002), through connectedness (Eisenberg et al., 2006; Goosem, 2001) and quality of the landscape (Lehmer et al., 2008; Ecke et al., 2002). In addition to changing presence or prevalence of active pathogens, landscape change can cause some pathogens to emerge or re-emerge (Eisenberg et al., 2007). These landscape interactions provide a tool to potentially use for the management of human exposure to pathogens through the management of landscapes.

There were a number of components to this research. These include image classification of the study region, which was done using a combination of spectral and spatial classification techniques. The classified imagery was then used to assess landscape effects on the spatial epidemiology of hantavirus.

Forested regions were then classified into different forest types and aspects of the forests that can be distinguished in remotely sensed imagery were analyzed for their impact on the presence of hantavirus seropositive rodents in rodent communities. Finally, the effect of rodent community diversity on prevalence of hantavirus within its reservoir organism was analyzed.

1.2. Research Questions

The research goals are addressed via a series of questions and hypotheses outlined below.

1.2.1. Overall Question

What roles do landscape composition and structure play in the distribution of hantavirus in *Akodon montensis* populations in Eastern Paraguay?

1.2.2. Secondary Questions

1. How can land cover mapping of different types of forest and forest disturbances be improved to facilitate landscape epidemiology studies of hantavirus in the Paraguayan Atlantic Forest? (Chapters 4 and 6)

2. What landscape spatial patterns are associated with increased hantavirus presence? (Chapter 5)

3. What effect does small mammal community diversity have on hantavirus seroprevalence in *Akodon montensis*? (Chapter 7)

1.3. Research Hypotheses

1. Object-based classification of Landsat data of the study area produces a more accurate LULC classifications than pixel-based classifications because the different land use types in rural Paraguay are spectrally similar. (Chapter 4)

2. Landscape metrics calculated using landscape classifications from satellite imagery (Landsat) will have threshold values that distinguish a potentially high prevalence landscape from a low prevalence landscape for hantavirus in *Akodon montensis* in the Atlantic Forest in Paraguay because different landscapes affect the nature of population interactions in rodent communities. (Chapter 5)

3. The scale of measurement for landscape metrics (pattern) will affect how well that metric is associated with hantavirus because different types of population interactions (process) take place at different scales. (Chapter 5)

4. Forest understory density parameters (horizontal density and vertical density) that are associated with hantavirus in *Akodon montensis* will be detectable in Landsat imagery. (Chapter 6)

5. High diversity of the small mammal community will be associated with low seroprevalence of hantavirus due to a dilution effect from reduced amounts of intraspecies contacts. (Chapter 7)

6. Diversity of the small mammal community and seroprevalence of hantavirus will be associated with vegetation index measurements in Landsat imagery. (Chapter 7)

1.4. Outline of the Dissertation

In Chapter 2, I review the relevant literature and outline the broader scope of this research. The study area and data collection methods are discussed in Chapter 3. In Chapter 4 classification of land cover in the study area is presented, and the relative accuracies of per-pixel and object-based classifications is analyzed. This classification is then used in Chapter 5 to investigate the effects of landscape structure and composition on hantaviral seroprevalence. Other aspects of spectral information that can be used to measure forest understory variables important to serostatus of hantavirus in *Akodon montensis* are analyzed in Chapter 6. In Chapter 7 rodent community diversity and its association with hantavirus in *A. montensis* communities is explored, and the relationship between diversity and remotely sensed information is considered.

Chapter 2. Literature Review

2.1. Landscape Epidemiology

2.1.1. Introduction and History

Landscape epidemiology is the study of the effect that landscape has on pathogen or disease occurrence, transmission, and persistence (Ostfeld et al., 2005; Elliot and Wartenburg, 2004). It is, in part, a sub-discipline of landscape ecology/biogeography, especially in its consideration of the roles of land use/land cover (LULC) and landscape structure on the spatial aspects of disease or pathogens. Effects of landscape on disease can occur directly on the pathogens or indirectly, through the impact of landscape on behavior and health of pathogen vectors or reservoirs (Clements and Pfeiffer, 2009; Ostfeld et al., 2005). Landscape epidemiology is a subset of the more general field of spatial epidemiology. Spatial epidemiology involves any spatial aspect of studying disease or pathogens. Along with being concerned about the role of landscape and landscape patterns, spatial epidemiology is additionally used to analyze more general patterns of disease or pathogens and what those patterns can say about potential causes of disease and how disease moves through populations (Glass, 2000).

Use of the terms spatial epidemiology and landscape epidemiology have not been completely standardized. For the purposes of this dissertation the term landscape epidemiology will be used to refer specifically to study of disease related processes happening on the landscape and how they are affected by landscape patterns or landscape forming processes. This definition of landscape epidemiology extends beyond the distribution and processes of the disease itself and includes aspects of the entire environment-host-pathogen system (White et al., 1996). The term spatial epidemiology can also be used in more general situations where the spatial analysis might include elements that would not normally be considered 'on the landscape' or when the spatial aspects of the analysis do not concern landscape issues. For example, the study of disease spread through a feedlot might account for spatial factors in its analysis (Hessman et al,. 2009), but the only important factor might be pen proximity and not other landscape issues. Some of the literature discussing landscape epidemiology uses the more general term spatial epidemiology (Ostfeld et al., 2005). Other literature does not give it a special name or simply calls it epidemiology and applies it to aspects of the landscape (Eisenberg et al., 2007; Panah and Greene, 2005; Pavlovsky, 1966).

The concepts and ideas of landscape epidemiology were developed by Pavlovsky and his colleagues (see Pavlovsky, 1966 for a comprehensive summary of this work). Pavlovsky's work took place within a strong tradition of landscape studies taking place in Russian academia (Shaw and Oldfield, 2007). Pavlovsky recognized that some infectious diseases are associated with specific landscape factors. In his 1966 book, Pavlovsky states that many diseases have a specific focal geographic region or natural environment, which he called a natural nidality (i.e., hearth or locus). He also noted that spatial variation in the distribution of many diseases can be accounted for by variation in biophysical factors that affect vectors and reservoirs of pathogens. He further suggested that we can use the understanding of these factors to map and predict risk areas. For several diseases that were present in Russia at the time, Pavlovsky identified the types of landscapes that are associated with the hosts or vectors of their pathogens. He also discussed the role of other factors like climate, weather, terrain, biology and habitats. He then identified how these factors can be used to map the potential distribution of diseases and even showed a few maps for disease in the northern subcaspian region in Russia.

Despite its potential for understanding the distribution of disease, Pavlovsky's work remained little known outside of Russia, and little landscape epidemiology work was done by western scientists until the late 1990's. Since then, Pavlovsky's work has gained recognition by western epidemiologists and disease ecologists (Ostfeld et al., 2005). The recent growth in the importance of landscape epidemiology is due to several factors. One is improved technology, which allows more complicated analysis to be readily achieved (Panah and Greene, 2005). Another factor is improved access to various types of inherently spatial data, including remotely sensed data (Tran et al., 2007; Goetz et al., 2000; Hay, 2000) along with GIS tools (LaRoque, 2007; McLafferty, 2003; Hay et al., 2000) with which relevant landscape information can be derived across a wide spectrum of spatial scales (Elliot and Wartenburg, 2004; Langlois et al., 2001; Turner et al., 2001). In addition, there has been a concurrent development of landscape ecology which relies on many of the same resources as landscape epidemiology and does much to help inform landscape epidemiology (Kitron et al., 2006; Curran et al., 2000).

2.1.2. Landscape Controls on Pathogens

This research is primarily concerned with the effect of landscape on pathogen prevalence in a reservoir. For the purposes of this dissertation, landscape is defined as the natural and anthropogenic mesoscale arrangement of land use/land cover on the Earth's surface. Turner et al. (2001) define landscape as “area that is spatially heterogenous in at least one factor of interest”; this is a more inclusive definition that is also consistent with the way landscape is used here. In landscape epidemiology studies a variety of other vegetative, geologic, climatic, and other environmental conditions can be considered. Additionally, landscape epidemiology can be used to look at aspects of both disease and health (Ostfeld et al., 2005; Panah and Greene, 2005; Elliot and Wartenburg, 2004; Rushton, 2003). The ways that landscapes can have an effect pathogen prevalence are multifold and often includes its effects on inter-species interactions, (Keesing et al., 2006; LoGiudice et al., 2003) intra-species interactions, (Eisenberg et al., 2006; Rotureau, 2006) and individual organism health (LoGiudice et al., 2003; Ecke et al., 2002), through connectedness (Eisenberg et al., 2006; Goosem, 2001) and quality of the landscape (Lehmer et al., 2008; Ecke et al., 2002). Ways that changes to the landscape can influence disease processes and health are also often a subject of interest (Lehmer et al., 2008; Sallares, 2006; Patz et al., 2004). In addition to changing levels of active pathogens, changes made to landscapes can cause some pathogens to emerge or re-emerge (Eisenberg et al., 2007).

Landscape controls on the emergence, reemergence, or distribution of pathogens can occur directly or indirectly. Direct control generally occurs with pathogens that are not reservoir- or vector-borne. For example, coccidioidomycosis (Kolivras and Comrie, 2004) is a disease caused by a type of soil fungus. It occurs in parts of the Southwest US, Central America, and South America. The occurrence of coccidiodomycosis requires that a local environment have a period of time wet enough to allow the fungus *Coccidioides immitis* to complete a life-cycle and then dry enough that the soil and fungus becomes wind-borne, which is how humans are exposed to the fungus. Areas that stay too dry don't have the fungus and areas that stay too wet don't have airborne *C. immitis* and also may have less *C. immitis* due to competition from other soil fungi. There is also some speculation that certain types of anthropogenic landscape changes can increase the amount of *C. immitis* in the environment.

Indirect landscape controls on disease generally occur through the impact of landscape on pathogen reservoirs and vectors. Impact of landscape on the ability of pathogen reservoirs and vectors to carry pathogens occurs through its impact on reservoir and vector distribution, health, and interactions (Keesing et al., 2006; LoGiudice et al., 2003). These impacts on pathogen reservoirs and vectors are not just important to the maintenance of existing diseases, but also to the introduction of new diseases. About 75% of emerging and reemerging diseases are zoonotic (Taylor et al., 2001). The example of hantavirus will be more thoroughly discussed later, a few general examples that illustrate some important points regarding landscape epidemiology of reservoir- and vector-borne diseases are reviewed here.

Malaria is caused by a vector-borne pathogen that has a relatively clear link to the landscape and is probably the most widely studied disease in the context of landscape epidemiology. Humans contract the malaria parasite (*Plasmodium falciparum*) through the bite of infected female mosquitoes (Hay et al., 2000). The larval life stages of mosquitoes need water, so mosquitoes are found near water sources, a fact often used in malaria control and surveillance (Li et al., 2009; Wood et al., 1991). In many habitats the amount of water present influences the amount and vigor of vegetation. By measuring the amount of vegetation using remotely sensed imagery the potential mosquito population can be estimated (Hay et al., 2000; Wood et al., 1991). In other cases the water in a habitat can be estimated by monitoring rainfall through the use of remote sensing platforms (Savigny and Binka, 2004). Efforts to control mosquito populations operate through controlling the amount of water present or limiting the ability of mosquitoes to reproduce when water is present (Li et al., 2009). Additionally, understanding when mosquito numbers are apt to be high can help decisions regarding when and where to strengthen malaria prophylactic efforts. Other mosquito-borne pathogens and the various mosquito species that carry them have different specific water requirements, so different aspects of the environment are analyzed to control or monitor different mosquito species (Meade and Earickson, 2005).

Landscape controls on Lyme disease are more complicated, and appear to show some consequences of anthropogenic landscape change. Lyme disease is caused by *Borellia burgdorferi*, which is a tick-borne pathogen. Many different species of mammals are hosts for *B. burgdorferi*, but the vector through which it is spread between individual animals is the *Ixodes scapularis* tick. While there are many adequate mammal hosts for *B. burgdorferi*, *Peromyscus leucopus* (white-footed mouse) is a particularly good host. Many of the changes humans have made to the landscape, especially forest fragmentation, have been favorable for *P. leucopus* and detrimental to other mammal species. This means that an individual tick is more likely to encounter *P. leucopus* in a blood meal, increasing the likelihood that the pathogen will be transmitted. This in turn results in a situation where a higher proportion of the ticks present in the landscape carry *B. burgdorferi* and consequently human-tick encounters are more likely to end up in the human contracting Lyme disease (Brownstein et al., 2005; LoGiudice et al., 2003; Frank et al., 1998) regardless of the total tick population.

Rabies is an example of a non-vector-borne pathogen, or a pathogen transmitted directly from its animal reservoir to a human (Biek and Real, 2010). Landscape controls on the rabies virus include affecting how different strains of rabies virus spread through the landscape (Barton et al., 2010) and how different interactions between mammal species and mammals and humans occur (Clements and Pfeiffer, 2009; Biek and Real, 2010; Barton et al., 2010).

2.1.3. Landscape Ecology

As evidenced in the previous examples, both the spatial aspects of the landscape and the biology of the interactions are important to the various landscape epidemiology interactions (Kitron, 2006). Landscape ecology is an academic field that lies at the intersection of biology and geography (Turner et al., 2001), landscape ecological concepts link landscape effects and biological systems. Some of the important concepts in landscape ecology are the connections between pattern and process and the importance of scale in its application to biological studies (Turner et al., 2001).

Connections between pattern and process in ecology have been academically recognized since at least 1947 (Watt, 1947). Depending on the situation, active processes can leave behind specific patterns or patterns can constrain some processes (Turner et al., 2001). For disease processes (and in landscape epidemiology) patterns of disease provide clues about important processes in the maintenance and spread of that disease. The effect the pattern has on increasing or decreasing disease quantity can be analyzed. Patterns can suggest processes that were not previously recognized as important. Finally, patterns can also be used as proxies for measuring processes that are not directly measurable, are difficult to measure, or are not ethically directly measurable (Meade and Earickson, 2005; Saunders and Mohammed, 2009). Scale plays an important role in pattern analysis in part by allowing processes that operate at different scales than the pattern we are analyzing to be eliminated, scale will be discussed later.

An example of a disease process that has been elucidated using patterns is one involved in the spread of West Nile Virus (WNV) in the United States. West Nile Virus is a mosquito-borne virus that was introduced in the New York City area in 1999 (Nash et al., 2001). Because the virus is mosquito-vectored, it was assumed that the virus would spread at a more or less predictable rate (Peterson et al., 2003). Then, a second locus of WNV was found in the Southeastern U.S. (Peterson et al., 2003) and WNV quickly spread throughout the Southeastern U.S. This pattern of multinodal spread suggested that another factor influenced the spread of the virus, which was discovered to be migratory birds (Dusek et al., 2009; Peterson et al., 2003). In this case the pattern indicated that there were processes in addition to mosquitoes at work; the pattern also suggested that migratory birds could be involved and further studies confirmed that possibility (Dusek et al., 2009). Further, the pattern could indicate that there are even more unknown processes at work in the spread of WNV throughout the U.S. (Koch and Denike, 2007).

Landscape pattern can also affect disease processes. This generally happens through the effect of the landscape pattern on the way that individuals and populations interact. These interactions then affect how disease is spread through those communities and how vectors contact susceptible populations. An example of this is Lyme disease, mentioned above, where changes to the patterns of forests favors *P. leucopus* which ends up increasing the risk for humans contacting Lyme disease. Understanding the ways that landscape patterns affect various disease processes can help better define the potential impacts of human caused changes to landscapes.

2.1.4. Landscape Metrics

Landscape metrics are commonly used to quantify landscape patterns (Wu and Hobbs, 2002; Turner et al., 2001). There are many different metrics, but in general the various landscape metrics quantify things like size, shape, connectedness, and composition of landscape patches and habitats. By correlating the values obtained from landscape metrics with the presence of pathogens or reservoirs, the association of landscape patterns and diseases can be analyzed.

One important point about using patterns to analyze disease processes is that causality can not be absolutely determined through pattern analysis. However, many of the environmental causes of disease are very difficult to establish and many of the techniques used to analyze disease processes do not absolutely prove causality. What pattern analysis can do is help to reasonably determine likely causality and also add support to other types of analysis.

2.1.5. Scale

While not often discussed in epidemiology literature, scale is an important aspect of epidemiologic (and general scientific) understanding that is introduced via spatial analysis (Jacquez, 2000). Scale can be used to help fit pattern and process together; the scale of pattern and expected process need to match for them to be connected. However, analysis done at one scale is not always transferable to other scales (Kitron, 2006). The use of hierarchy theory can help determine the relationships among different scales; for example, how larger scale processes constrain smaller scale processes and how finer scale processes add up to larger scale processes.

Scale, as used here, refers to the characteristic spatial (or temporal) dimension of a phenomenon. Scale is generally measured in two different aspects, grain and extent. Extent refers to the overall size of the area in which a process or phenomenon occurs. The grain is the size of individual units of observation (Turner et al., 2001). When discussing issues of scale in reference to biological systems it is also important to define the level of community organization within which one is working.

Scales important to the analysis of landscape epidemiology can vary from global to individual organisms. At the global scale processes like global climate (Anyamba et al., 2006) and plate tectonics affect the patterns and distribution of disease transmission and maintenance. At the scale of an individual, how that individual changes its immediate environment can affect its ability to resist contracting disease or its potential for becoming exposed to pathogens (Pavlovsky, 1966). Of course, there are processes that act at many scales between those scales. Each process leaves a signature in the patterns that it leaves behind. For example, global climate places a limit on the areas that can support malaria because the mosquitoes that carry malaria are limited by temperature. Thus, malaria occurs in tropical and sub-tropical zones, can occur seasonally in warm temperate zones, but is relatively easy to control there (Meade and Earickson, 2005), and does not occur in polar zones. Within the large, global scale, tropical/sub-tropical zone there are regional limits placed on malaria by the amount of rainfall and altitudinal temperature variations, and other limitations and interactions can be associated on down in scales to individual habitat patches.

Different processes can operate at different scales in a single system. At times maybe even processes that are apparently diametrically opposed may be important at different scales. For example, schistosomiasis is a parasitic disease caused by exposure to *Schistosoma japonica*, which is carried by a species of snails (*Oncomelania hupensis*). Water is essential to the maintenance of schistosomiasis. Yang, et al. (2009) found that, when using remotely sensed imagery to map the potential schistosomiasis habitat it may be necessary to look for higher or lower values of NDVI, depending on the scale of the imagery (grain or spatial resolution). In coarse scale imagery higher values of NDVI are associated with higher levels of schistosomiasis, because at larger grains the pixels that represent water areas also contain a lot of vegetation and pixels with little vegetation represent dry land with no water. Conversely, in fine scale imagery lower NDVI values are more frequently associated with higher occurrence of schistosomiasis, because at smaller grains the pixels that represent vegetation are separate from the pixels that represent open water and open water is the habitat most associated with high schistosomiasis levels.

These issues of seeing different things at different scales helps highlight important aspects involved in applying data obtained at one scale to other scales. Various issues and techniques related to upscaling and downscaling information need to be recognized when considering the multiple scales of various processes and patterns (Kitron, 2006; Levin, 1992). Care must be exercised because while for some processes upscaling and downscaling can be done, for others one might get an incorrect answer.

2.2. Geospatial Technologies and Disease

2.2.1. Introduction

An improved understanding of the importance of spatial aspects of the physical environment on disease processes (Clements and Pfeiffer, 2009; Ostfeld et al., 2005) has played an important role in the current increased interest in landscape epidemiology (Ricketts, 2003). Also playing a role in the increased interest is an increase in the widespread availability of remotely sensed data (Hay, 2000) and GIS tools (Cromley, 2003), and the computing power needed to better analyze them to extract relevant landscape information from a wide spectrum of spatial scales (Reisen, 2010; Ricketts, 2003).

Remote sensing and Geographic Information Systems (GIS) are two geographic tools that can be used for analyzing the landscape ecology of disease and pathogen vectors (Clements and Pfeiffer, 2009). The role of remote sensing in researching and monitoring disease primarily involves the detection of secondary indicators that are associated with disease or pathogen vectors and also monitoring changes to those indicators (Ostfeld et al., 2005; Cline, 1970). GIS is a set of spatial analysis tools that are used to collect, store, analyze and display spatially referenced data (Panah and Greene, 2005; Ricketts, 2003) and is an essential part of most spatial and geographic analysis, including the analysis of spatial aspects of health and disease. I will review remote sensing and GIS separately, but these two aspects of geospatial technology are frequently used together as a cohesive unit.

2.2.2. Geographic Information Systems (GIS)

GIS is essentially a collection of software and hardware tools designed for acquiring, storing, and analyzing geospatial data (Panah and Greene, 2005; Ricketts, 2003). Nearly anything involving spatial data can be analyzed with the assistance of a GIS. GIS can be used to display spatial data, either for exploratory spatial data analysis (Arambulo and Astudillo, 1991) or for the presentation of final results (Clarke et al., 1996). Within a GIS, remote sensing data can be combined with other locational information important to disease and disease processes (Hay, 2000) and this combination can be analyzed or displayed. Appropriate spatial statistics, such as cluster analysis (Hay et al. 2000; Wakefield et al., 2000), kriging (Cressie, 2000), and spatial associations (Moore and Carpenter, 1999; Best et al., 2000; Langlois et al., 2001) can be used to analyze spatial data with the help of GIS programs. The ability to connect various sources and types of spatial data make GIS an appropriate platform to use in analyzing various aspects of spatial relationships relating to disease, including the dispersion of disease through communities (Morris and Wakefield, 2000) and access to health care (McLafferty, 2003). The biggest limitation in the use of GIS for analysis of spatial aspects of disease is data availability and data quality (Elliot et al., 2000; Stines and Jarup, 2000).

2.2.3. Remote Sensing

Remote sensing is the measuring of properties of the Earth's surface without being in contact with it, which is done through the detection of reflected or emitted electromagnetic radiation. The three major ways that remote sensing information is collected is by hand, by airplane, or by satellite. Data collected by hand (field data) are generally collected by portable or semi-portable spectrometers and are often used to characterize the electromagnetic spectra of particular land surface types. The biggest limitation to field data is that it is difficult to get full two dimensional coverage of any particular area, but the data tend to be detailed in terms of pixel size and/or spectra. Aircraft collected data generally give full two dimensional coverage of an area, but are limited in extent and are generally collected when commissioned, which means that there is typically no repetitive coverage of a particular area. Remotely sensed data collected by satellite usually has global coverage and has regularly repeating coverage, with the major limitation being grain size. The research in this document is based on satellite collected data.

There are four different resolutions important to remote sensing data: spatial, temporal, spectral, and radiometric. Radiometric is not generally a limiting factor, so I will not discuss it here. Spectral resolution refers to the electromagnetic radiation spectra measured by the detectors. The importance of spectral resolution is that through careful selection of spectra, classes can be effectively separated or important biophysical parameters can be determined. What kind of data satellites collect cannot be changed, but data can be selected from satellites that collect data closest to the needed type. With remotely sensed satellite data, spatial and temporal data are often inversely related. Satellites that have coarse spatial resolution generally collect data frequently and satellites that have fine resolution collect data less often.

Satellite systems that have very fine spatial resolution generally have pixel sizes of less than 5m and as small as 0.6m (Note: pixel resolution is expressed in terms of a single dimension, so that a resolution of 5m means that the pixel area is actually 5m x 5m or 25m^2). Data collection from these satellites is by user request, so there is not a complete global record at this resolution. In the next step up for spatial resolution are satellites that collect moderate resolution data, with pixel dimensions of about 15-100m. These satellites generally have a temporal resolution of about two weeks and there is a good global record of data within this set of resolutions. At coarse spatial resolution are satellites that collect data with pixel sizes from 250m to 5000m, these satellites usually obtain global coverage on a daily basis.

The potential for use of remote sensing data in the analysis of health has been recognized at least since 1970 (Cline, 1970). Satellite remote sensing data is the only major data source that provides a combination of global coverage and regular updates. The mixture of global scale coverage and repeated updates offers several advantages. The global nature of satellite data means that data can be collected regardless of political boundaries and the large scale two dimensional coverage in data means that patterns can be relatively easy to see, define, and measure. Repeated coverage means that changes to the landscape can be monitored as they happen and the importance of those changes to disease processes can be analyzed.

Remote sensing data can be processed to provide two types of information: biophysical and categorical. Both of these are useful in landscape epidemiology. Biophysical data includes information such as temperature, precipitation, or amount of vegetation. Currently, regularly updated global data sets of many biophysical parameters that can be used in landscape epidemiology work are available.

Remote sensing data can also be classified into discrete habitat types. The specific types that are useful depend on each specific vector/pathogen system, so there are fewer useful global data sets in this format and it is more common that individual studies will make their own classifications using available satellite data or modify other habitat classifications to fit the particular vector/pathogen system. Most habitat classifications use the spectral information available in each pixel and classify habitat types based solely on that spectral information. Recently, more techniques have been developed that use spatial information to help define habitat types. Usually, the spatial information methods group spectrally similar neighboring pixels together, and then analyze the spatial characteristics within each object or group of pixels. This spatial information can help separate otherwise spectrally similar habitats from each other.

An important feature of the satellite remotely sensed data is that it is inherently two dimensional data, which is a prerequisite for performing spatial analysis. Additionally, this inherent two dimensional property means that associations between disease, vector, or pathogen data and environmental conditions at a location can be made. Actual landscape patterns can be measured and the effects of those patterns can be measured. And a variety of scales can be analyzed, from as small as a few pixels up to global, and look for the scales important to a particular system.

2.2.4. Combining GIS and Remote Sensing

Remotely sensed data in conjunction with GIS have been applied to a wide variety of landscape epidemiological studies (Beck et al., 2000), and have been especially effective for analysis of a number of reservoir- and vector-borne and zoonotic pathogens with environmental co-factors. One of the principal advantages of using satellite remote sensing for analyzing disease-related environmental factors is the capability for rapid and repetitive collection of information, even from remote or inaccessible places (Curran et al., 2000). Some of the diseases analyzed using remotely sensed data include malaria (Beck et al., 1994; Mushinzimana et al., 2006), Lyme disease (Guerra et al., 2002; Allan et al., 2003; Brownstein et al., 2005), Chagas disease (Kitron, 2006), West Nile fever (Rogers et al., 2002; Ruiz et al., 2004), Ebola Hemorrhagic Fever

(Tucker et al., 2002; Pinzon et al., 2004), and Rift Valley Fever (Linthicum et al., 1987). Lately, good predictions have been made of Rift Valley Fever epidemics from remote sensing data (Anyamba, 2009; Linthicum et al. 1991). Hantavirus is also one of the diseases where remote sensing and GIS have been useful in understanding its dynamics (Glass et al., 2000; Langlois et al., 2001).

2.3. Hantavirus

2.3.1. Introduction

Hantaviruses are a genus of RNA viruses that belong to the Bunyaviridae family (Jonsson and Schmaljohn, 2001; Plyusnin and Morzunov, 2001). While most viruses in the Bunyaviridae family are arboviruses - that is, they are transmitted by arthropod vectors - hantaviruses are solely maintained in their rodent hosts (Plyusnin and Morzunov, 2001). The name hantavirus comes from the Hantaan River, which is located near the village of Songanaeri in Korea, where the prototype strain was obtained around 1982 (Johnson, 2001). The human diseases associated with hantavirus were described more than 1000 years ago in China (Klein and Calisher, 2007). However, identification of hantaviruses as the etiologic agent of those diseases occurred in the late 1970's in Eurasia and 1993 in the Americas (Johnson, 2001).

Hantaviruses can cause serious disease in humans. In Eurasia, hantaviruses can cause Nephropathia Epidemica (NE) or Hemorrhagic Fever with Renal Syndrome (HFRS) (Klein and Calisher, 2007). Throughout the Americas, the hantavirus-associated disease is Hantavirus Pulmonary Syndrome

(HPS) (Klein and Calisher, 2007; Raboni2005). Nephropathia Epidemica has a fatality risk of about 0.1%, HFRS has a case fatality risk of about 10%, and HPS is generally more serious, with fatality risk of about 35 to 50% depending on the strain (Klein and Calisher, 2007; Young et al., 2000; Schmaljohn and Hjelle, 1997; Williams et al., 1997). These diseases are thought to occur primarily through contact with the virus via the inhalation of rodent feces and urine, typically aerosolized while cleaning indoors or working outdoors (Young et al., 2000).

Hantaviruses are primarily hosted by rodents of the Muridae Family, with Subfamilies Murinae, Arvicolinae, and Sigmodontine (Chu et al., 2003; Schmaljohn and Hjelle, 1997). The exceptions are two shrew species, *Urotrichus talpoides* (Arai et al., 2008) and *Suncus murinus* (Johnson, 2001). The viruses are believed to have co-evolved with the rodents that carry them. Each of the individual species of rodent that carries hantavirus has a particular strain associated with it (Young et al., 2000, Williams et al., 1997). In general, presence of hantavirus does not cause disease-like symptoms in their reservoir species. The only known exception to this is Seoul virus infection, which produces symptoms in very young Norway rats (Klein and Calisher, 2007). There are, however, thought to be some minor effects of infection in some rodent species. These include changes in aggression (Klein and Calisher, 2007) and an interaction between ectoparasites and hantavirus (Deter et al., 2008) that may be protective for the rodent.

In rodent communities, hantavirus passes between adult animals, but not from a dam to her offspring. In other words, hantavirus is passed horizontally, not vertically in rodent communities (Klein and Calisher, 2007). This means that anything that affects the way rodents interact in their communities will affect the amount of hantavirus in that community, which makes hantavirus transmission in rodent communities a good candidate for study with Landscape epidemiology (Ostfeld et al., 2005; Langlois et al., 2001; Glass et al., 2000).

From a landscape perspective, the increased risk of human disease is determined by both the distribution of the host rodent population and the human population on the landscape. Both of these depend to some degree on land use and land cover, although the relationship between host, landscape, and human population varies (Yates, 2002). Variation in rodent hantavirus prevalence can occur seasonally, annually (Mills et al., 1999), with varying climactic conditions (Williams et al.,1997), and with varying habitat (Kuenzi et al., 1999, Root et al., 1999). While these factors play important roles in the prevalence of hantavirus in rodent communities, there are additional unidentified factors that play important roles (Calisher et al., 2007), likely these include factors like landscape patterns (Langlois et al., 2001) and possibly some anthropogenic land use/land cover changes.

2.3.2. Hantavirus in the Americas

Hantavirus is found in rodents throughout the Americas (Figure 2.1). The first New World strain of hantavirus identified and the one most often found in

North America is the Sin Nombre virus, which is carried by *Peromyscus maniculatus* and is found mostly in western North America (Mills et al., 1998). This strain of hantavirus is also responsible for the majority of HPS cases in North America (Schmaljohn and Hjelle, 1997). In South America there is a more diverse set of hantavirus strains generally spread throughout the southern portion of the continent (Figures 2.1 and 2.2). Several of these strains are responsible for HPS cases in South America (Klein and Calisher, 2007).

2.3.3. Hantavirus in Paraguay

At least four distinct strains of hantavirus are thought to be circulating within Paraguay, two of which have been identified in the study area (Chu et al., 2003). The strain of hantavirus that has most directly impacted the human population in Paraguay is called Laguna Negra and is endemic to *Calomys laucha*. This strain first emerged in 1995, (Johnson et al., 1997; Williams et al.,1997) and since then, there have been periodic occurrences of disease. Other Sigmodontine rodent species in Paraguay that were found to carry a hantavirus include *Akodon montensis*, *Holochilus chacarius*, *Oligoryzomys nigripes*, and *O. chacoensis* (Chu et al., 2003). Research suggests that the dynamics of hantavirus in South America is especially complex (Chu et al., 2003).

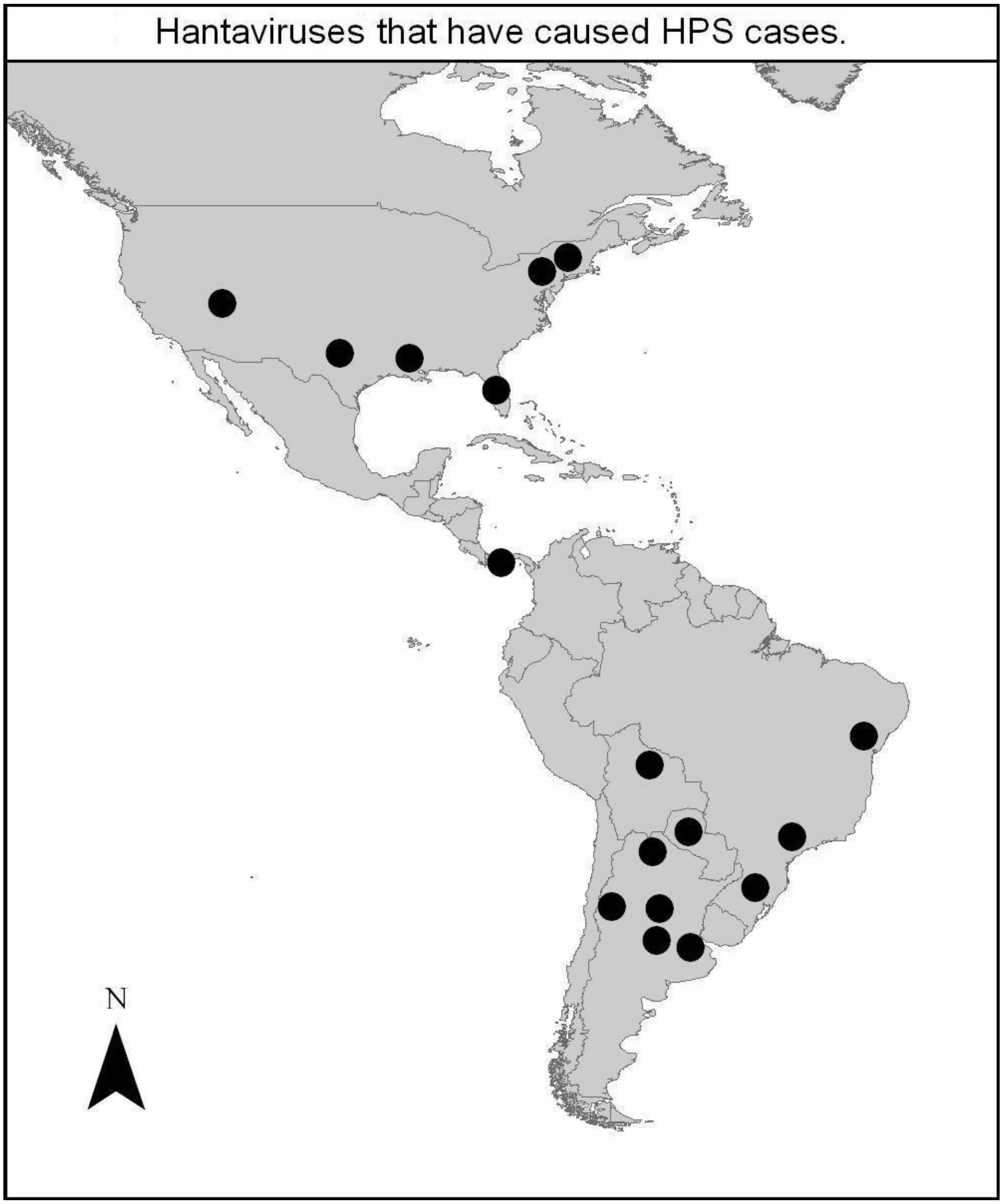

Figure 2.1. First identified locations for hantaviruses known to cause HPS (data from Klein and Calisher, 2007). Locations are approximate location of first confirmed case.

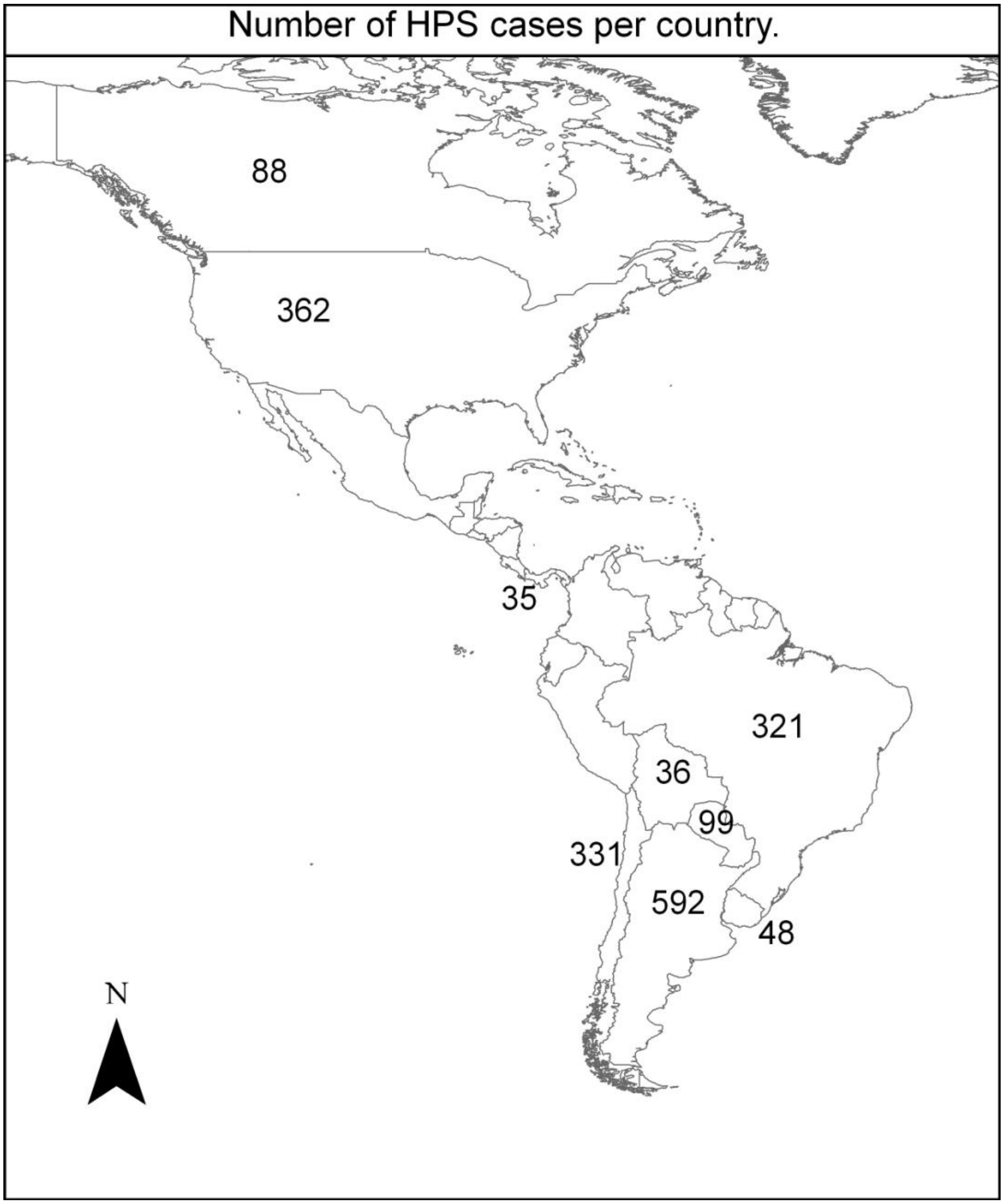

Figure 2.2. Number of HPS cases per country throughout the Americas. Most cases in North America are due to the Sin Nombre virus carried by P. maniculatus. Cases of HPS in South America are due to a broader variety of hantavirus strains carried by different rodent species (PAHO, 2010).

Chapter 3. Study Area and Data Collection

3.1. Paraguay

Paraguay is a small, landlocked country in the central part of South America. It has a nearly even ratio of rural to urban population, with 43% of the population living in rural areas (de la Mora, 2004) and the remainder in a few urbanized places. It is considered to be the most agrarian of South American countries (Carter et al., 1996). The major rural economic activity in Paraguay is farming, but forestry and hydroelectric generation also form significant portions of the economy. Agriculture and forestry sectors account for 26% of the total GDP of Paraguay and 90% of the exports (Glatzle and Stosiek, 2005), and 62% of the rural population is employed in farming (Robles, 2000). Paraguay a relatively poor country with the fourth lowest per capita gross national income out of 17 Latin American countries (Rowntree, 2008). Paraguay is made up of three distinct regions, which differ in several cultural and ecological aspects (Figure 3.1). One is east of the Paraguay River (Eastern Paraguay), another is west of the Paraguay River (the Chaco) (Glatzle and Stosiek, 2005), and the third is the urban area of Asuncion. Eastern Paraguay is primarily made up of the Interior Atlantic Forest ecoregion, but also contains some Cerrado, or natural grasslands, in the northeast, some Humid Chaco ecoregions near the Paraguay River, and small pieces of other ecoregions (Figure 3.1). In general, rainfall is not a limiting factor in Eastern Paraguay. The northern part of Eastern Paraguay is tropical and the southern part of this region is subtropical.

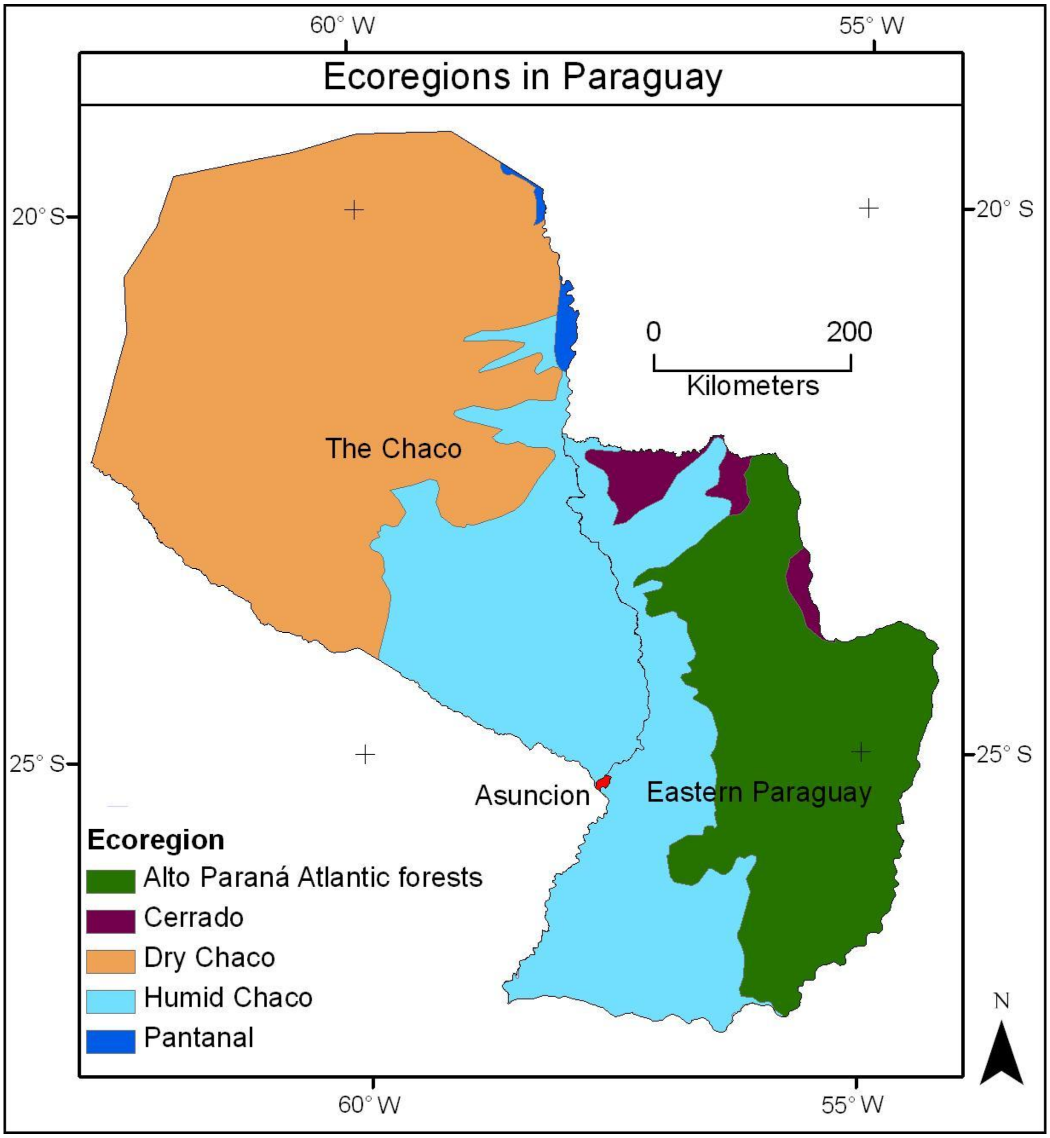

Figure 3.1. Map of Paraguay showing the three general regions and the Ecoregions (Olson et al., 2001).

In Paraguay, rural land cover changes are largely driven by two differing processes applied with a variety of intensities. These two processes correspond to the two major agricultural systems in Paraguay, termed the small-holder (or minifundia) and the commercial (latifundia) systems. The minifundia system is typically centered around subsistence agriculture with some cash crops. These small farms contain the majority of the rural population and are typically less than 20 hectares in size (Zoomers and Kleinpenning, 1996; Turner, 1993). The most common cash crop grown is cotton (Carter et al., 1996). Other typical cash crops for small farmers include tomatoes, sweet peppers, carrots, and cassava. Beef cattle and other animal products are also raised for income. Some cash crops that are a bit more specialized, but are either growing in importance, have an impact in some specific communities, or have strong future potential include: garlic (Douglas, 2003), pineapple, banana, sesame seed, nuts (pecan and macademia) (Rojas, 2001), citrus, and stevia (Douglas, 2003; Fuente, 2001). In the minifundia system land cover changes occur in small parcels, often times less than a hectare at a time.

Latifundias are typically from 1000 to 5000 hectares, but can be over 50000 hectares in area (Glatzle and Stosiek, 2005; Zoomers and Kleinpenning, 1996). The two predominant products produced on latifundias are beef and soybean. Other products that are produced on latifundias in Paraguay are yerba mate, sorghum, rice, and milk. In the latifundia system land cover changes occur in very large parcels of hundreds to thousands of hectares (Glatzle and Stosiek, 2005). These two types of land clearing impose very different patterns on the landscape. Large area land holders sometimes leave larger forest fragments on the landscape, but they are separated by much larger distances. Small area land holders leave smaller forest fragments on the landscape, but they are often closer to each other. Also, the levels of disturbance within the forest fragments can be different, with small area land holders often visiting their forest parcels more regularly to obtain firewood and building materials. Current laws require farmers to leave 25% of their land forested (Glatzle and Stosiek, 2005), but that law does not seem to be well enforced.

3.2. Atlantic Forest

The site chosen for this research lies within the Atlantic Forest region of Eastern Paraguay. Although not as well known as the Amazon rain forest, the Atlantic Forest (Figure 3.2) is among the most threatened ecosystems on Earth. Its rate of land cover conversion is among the fastest in the world and it is thought that only about 8% of the original 1.5 million km2 of the ecosystem remains today (Hansen and DeFries, 2004; Galindo-Leal and Gusmao-Camara, 2003). The rapid anthropogenic Atlantic Forest conversion includes Paraguay (Huang et al., 2007) and the dynamics of that change are evolving fast (Cartes and Yanosky, 2003). As of 2000 it was estimated that about 25% of the Paraguayan Atlantic Forest remained, with nearly 15% of that deforestation happening since 1990 (Huang, 2009). The entire Atlantic Forest consists of eight biogeographic subdivisions, the Atlantic Forest in Paraguay lies entirely within the Interior Atlantic Forest subdivision (Silva and Casteleti, 2003).

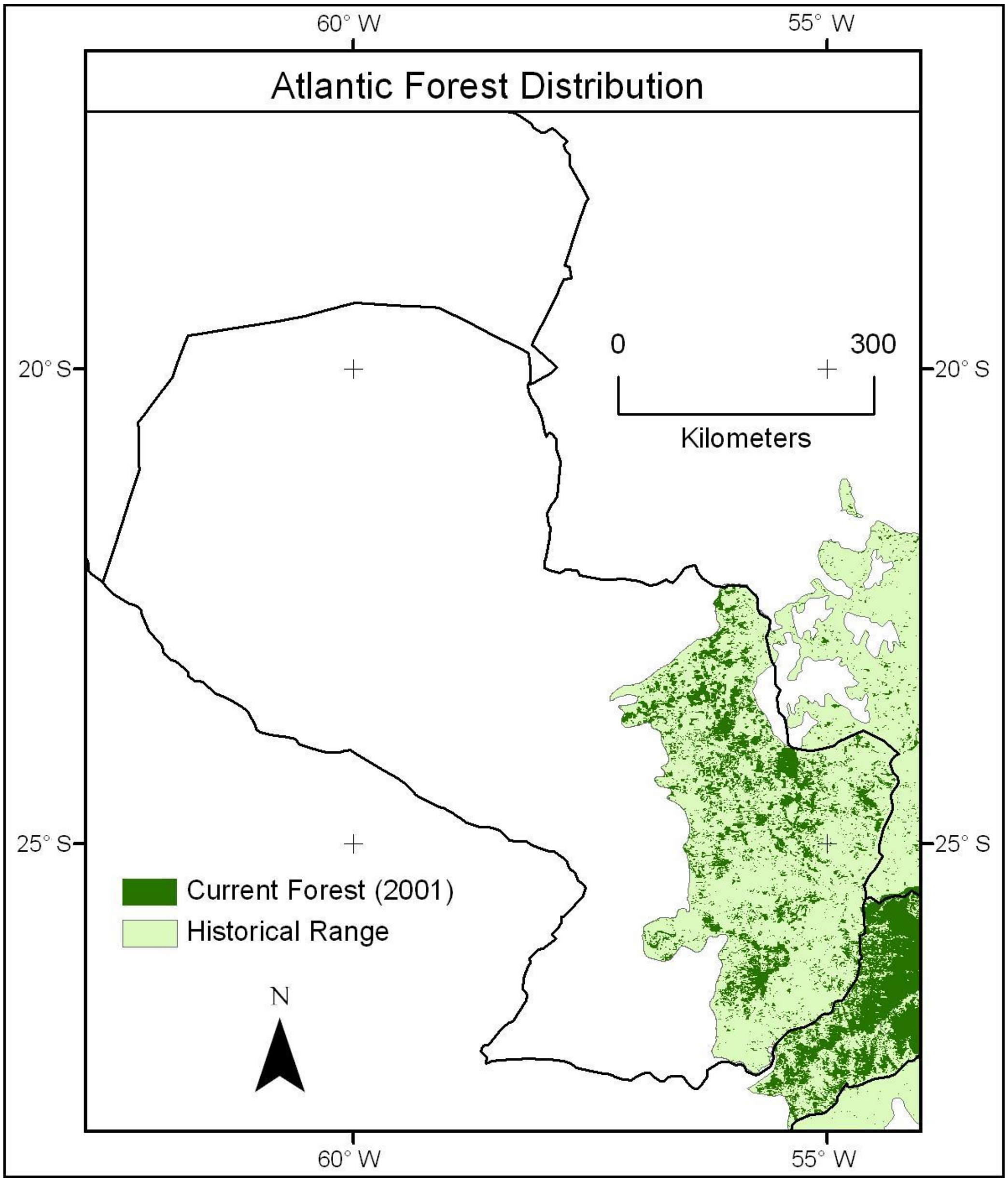

Figure 3.2. The Atlantic Forest ecoregion in Paraguay showing both historical distribution (Olson et al., 2001) and remaining forest as of 2001 (Eva et al., 2003).

3.3. Mbaracayú (Biosphere and Reserve)

The research for this dissertation took place within the Mbaracayú Biosphere (RBM, Reserva de Biosfera del Bosque Mbaracayú) in the

Department of Canindeyu in Eastern Paraguay (Figure 3.3). The Biosphere is a protected multi-use zone which contains the Mbaracayú Forest Reserve (RNBM, Reserva Natural del Bosque Mbaracayú) and various types of farm land and some small urban communities (FMB/BM, 2005). The Reserve and Biosphere were established in 1991 (FMB/BM, 2005).

The Biosphere consists of the entire watersheds of the Rio Jejui'mi and Rio Jejui Guazu upstream from their confluence, approximately 15 km West of Villa Ygatimi (Figure 3.4) and is 280,000 ha in area (FMB/BM, 2005). The main land use/land cover types in the biosphere include undisturbed forest in the Reserve, natural cerrado areas, and various farming communities that are comprised of forest fragments, crop fields, and pasture lands. As of 2003 the Biosphere was approximately 50% forest and 33% agricultural cover (FMB/BM, 2005) with ongoing relatively rapid conversion of some forest land to agriculture (Huang et al., 2007).

The Mbaracayú Forest Reserve (hereafter referred to as RNBM) is 64,400 ha, most of which is in the Rio Jejui'mi watershed (Figure 3.4). Less than 1% of the Reserve land area is actively occupied by humans, the rest is protected natural area, with about 85% forest cover and the remainder as cerrado (savannah) and other grasslands (FMB/BM, 2005). The Reserve contains the largest remaining fragment of Atlantic forest in Paraguay (Galindo-Leal and Gusmao-Camara, 2003).

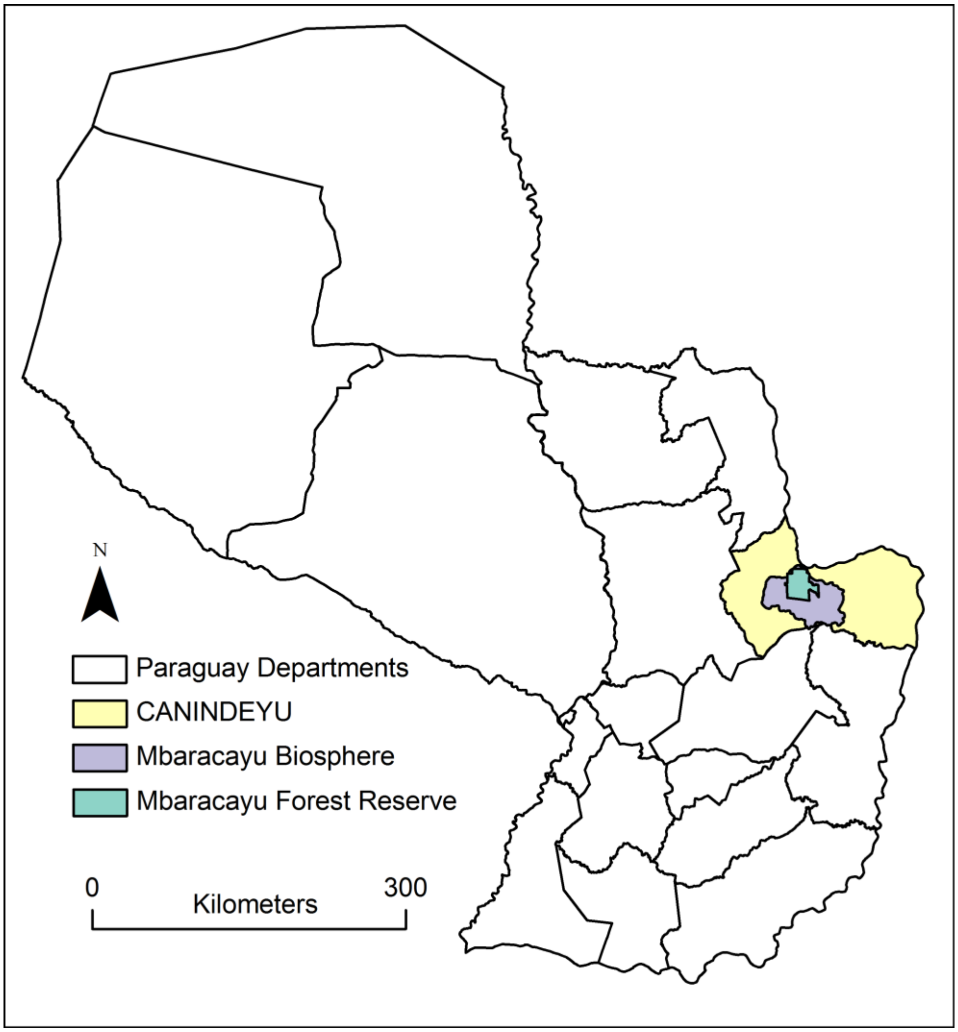

Figure 3.3. Map of Paraguay showing the location of the Mbaracayú Biosphere and Forest Reserves.

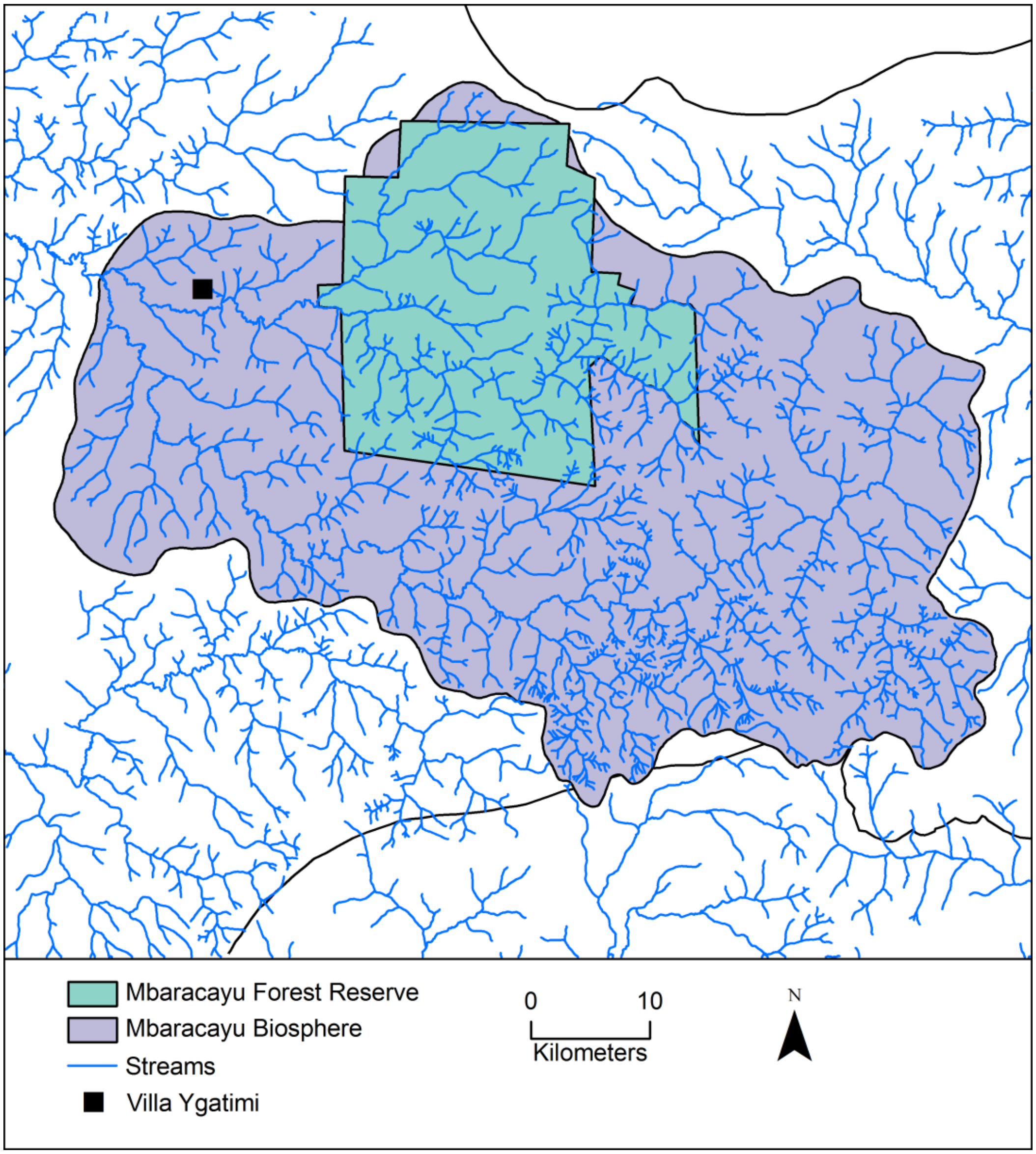

Figure 3.4. The Mbaracayú Biosphere and Reserve showing their relationship to the local watersheds.

3.4. Data Collection

A combination of remotely sensed and field-collected data was used in this research. With a minor exception, all of the field data were collected within the boundaries of the Biosphere. These data consisted of rodent seroprevalence data, vegetation data, and georectification data. Some of the georecitification data (used to correct locational accuracy in satellite imagery) was collected outside of the Biosphere. All rodent data was collected following field protocols in the ASM guidelines for the use of wild mammals in research (Gannon et al., 2007) and the CDC guidelines for handling potentially infected rodents (Mills et al., 1995) and were approved by the Texas Tech University Animal Care and Use Committee (Note: trapping campaigns were organized and conducted by personnel from Texas Tech).

3.4.1. Mark/Recapture Rodent Trapping Data

3.4.1.1. Mark/Recapture Rodent Trapping Sites

Rodent trapping to obtain hantavirus seroprevalence data was done using two main types of trap sites. One set of trap sites were a series of Mark/Recapture trapping grids. These were located within the Reserve and in the Biosphere near the East and West sides of the Reserve (Figures 3.5 and 3.8). The other type is described in section 3.4.2. The Mark/Recapture grids were established to collect demographic and ecological information and to collect physical samples from captured rodents within a single land cover/disturbance type.

There were ten Mark/Recapture sites, selected to represent a range of human disturbance. Two of the sites (Jejui-mi A and Jejui-mi B) were selected to represent forested areas with minimal levels of human disturbance. Both of these sites had some evidence of prior selective logging disturbance, more on Jejui-mi A than on Jejui-mi B, but those disturbances were more than 20 years in the past. Two more sites (Rama III A and Rama III B) were selected to represent high levels of human disturbance of the latifundia (see Section 3.1), or large agriculture, type. Rama III B was a forested site adjacent to the RNBM, which showed signs of recent and ongoing selective logging. Rama III A was a deforested grassland/pasture site that had some scattered woody shrubs and trees on it. The remaining 6 sites were selected to represent disturbance associated with the minifundia (see Section 3.1), or small agriculture, type. Originally, the minifundia trap sites were at the Maria Auxiliadora sites. This site had been a small farmer community that had been abandoned within a few years prior to trapping. It was chosen because it had all the landscape characteristics of a minifundia community and was accessible. Shortly after trapping commenced, the sites were claimed by a group of small farmers who did not wish to allow the trapping to continue there. The minifundia sites were then moved to Horqueta-mi, which was also an abandoned minifundia site. This site was abandoned 15 years prior to commencement of trapping and was more secure against resettlement. These sites were eventually determined to be unsuitable for this study due to low rodent capture numbers. The final move of the minifundia sites was to an active small farmer community, Britez Kue. Permission to trap was obtained from two of the residents in this community and trapping occurred here until the end of the trapping portion of the study.

3.4.1.2. Mark/Rapture Rodent Trapping Method

The Mark/Recapture sites were monitored on a rotating basis between February, 2005 and December, 2007 (Table 3.1). Each trapping session lasted for eight consecutive nights. Each of the grids consisted of an 11 x 11 array of trap stations placed 10 m from each other, covering an area of 1 ha. Each trap station had one standard Sherman live-trap (7.5 × 9.0 × 23.0 cm; H. B. Sherman Traps, Tallahassee, Florida) placed on the ground and, where vegetation structure permitted, also had one placed about 1-2m above-ground to capture arboreal small mammal species.

During the trapping sessions, each trap was checked each morning. All rodent data were collected following field protocols in the ASM guidelines for the use of wild mammals in research (Gannon et al., 2007) the CDC guidelines for handling potentially infected rodents (Mills et al., 1995) and were approved by the Texas Tech University Animal Care and Use Committee (Note: trapping campaigns were organized and conducted by personnel from Texas Tech). Trapped animals were processed and released at the site of capture. For the first capture, each animal had a Passive Integrated Transponder (PIT) tag (Biomark Inc., Boise, ID, USA) implanted subdermally and a 1-2 mm snip taken from its tail. The PIT tag allowed identification of each individual during subsequent captures. The tail snip provided material for DNA confirmation of specific identification, when needed. If the capture was the first for an animal during an 8-day trapping session, demographic information, such as the animals specific identity, sex, age class, reproductive condition, weight, general appearance, and presence of lesions or scars were noted. Additionally, a blood sample was obtained by retro-orbital bleeding. The blood sample was stored in a 2ml cryotube in liquid nitrogen and was tested for hantaviral antibodies using Immunofluorescence Assay (IFA) (Chu et al., 2003). A positive IFA test result, indicating that the animal was seropositive (S+), was used as an indication that the animal had been exposed to hantavirus in its past. Any subsequent captures of individual animals during a trapping session was confirmed by reading their identification from the PIT tag using a hand-held electronic reader held within 10 cm of the animal.

3.4.2. Megagrid Rodent Trapping Data

3.4.2.1. Megagrid Rodent Trapping Site

The second type of sampling strategy used to obtain hantavirus seroprevalence data was a large grid of trap transects (hereafter referred to as the Megagrid) that were located in the Eastern side of the reserve and in the Britez Cue minifundia community along the Eastern side of the reserve (Figures 3.6 and 3.8).

The Megagrid trap sites totaled 71 sites. The sites were selected to represent a range of human disturbance. Each site consisted of two 500m long transects (Figure 3.6), with traps set every 10m along each transect. The two transects were perpendicular to each other and the center point of the two transects intersected.

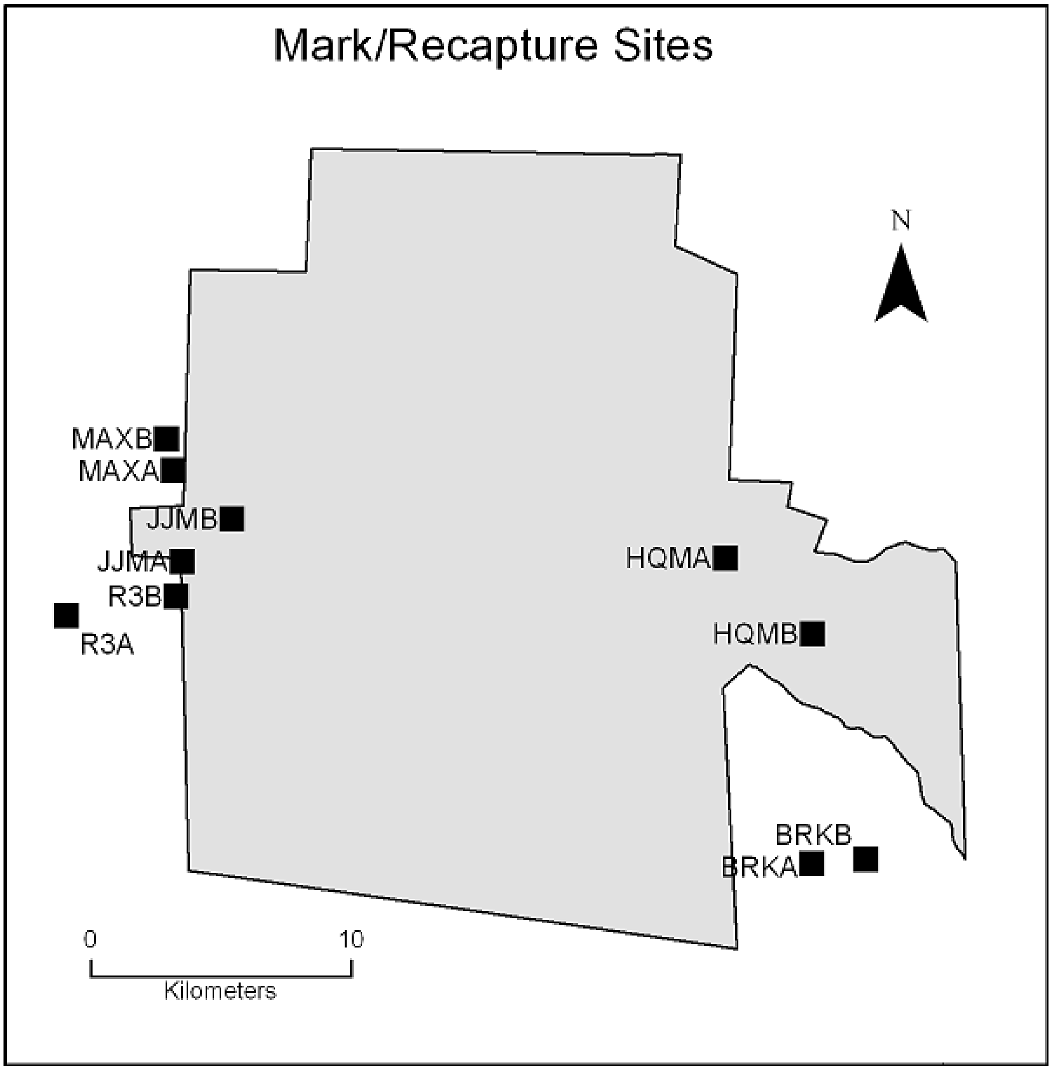

Figure 3.5. Locations of the Mark/Recapture trap sites. Labels match abbreviations given with the site names in Table 3.1.

Table 3.1. Trap dates and site description for all Mark/Recapture trapping grids.

Site name	Trap dates	Site description
Jejui-mi (JJA and JJB)	12 FEB-18 FEB, 2005 14 JUN-21 JUN, 2005 12 SEP-19 SEP, 2005 10 NOV-17 NOV, 2005 27 FEB-06 MAR, 2006 19 MAY-26 MAY, 2006 27JUL-03AUG, 2006 03DEC-10DEC, 2006 21MAY-28MAY, 2007 18NOV-25NOV, 2007	Native forest with some evidence of prior selective logging history. Least disturbed sites of all sites trapped for small mammals.
Rama III (R3A and R3B)	15MAR-22MAR, 2005 07AUG-14AUG, 2005 01OCT-09OCT, 2005 15FEB-22FEB, 2006 09JUL-16JUL, 2006 22NOV-29NOV, 2006 05MAY-12MAY, 2007 27NOV-04DEC, 2007	A – Heavily managed pasture land. Seasonal burning, some woody shrubs on site. B – Native forest cover with recent and occasional logging activity.
Maria Auxiliadora (MAA and MAB)	18MAY-25MAY, 2005 13JUL-20JUL, 2005	Heavily disturbed minifundia site. Heterogenous land cover.
Horqueta-mi (HMA and HMB)	23AUG-30AUG, 2005 20OCT-27OCT, 2005 03FEB-10FEB, 2006 17JUN-24JUN, 2006 22SEP-29SEP, 2006	Forest area growing from minifundia activity 10 years prior to trapping.
Britez Kue (BKA and BKB)	22FEB-01MAR, 2007 27JUN-04JUL, 2007	Heavily disturbed minifundia site. Heterogenous land cover.

3.4.2.2. Megagrid Rodent Trapping Method

The Megagrid sites were each trapped for one two night session sometime between February, 2006 and August, 2006. Each trap station within the transects had one standard Sherman live-trap (7.5 × 9.0 × 23.0 cm; H. B. Sherman Traps, Tallahassee, Florida) placed on the ground and, where vegetation structure permitted, also had one placed about 1-2m above-ground.

During the trapping sessions, each trap was checked each morning. All rodent data was collected following field protocols in the ASM guidelines for the use of wild mammals in research (Gannon et al., 2007) the CDC guidelines for handling potentially infected rodents (Mills et al., 1995) and were approved by the Texas Tech University Animal Care and Use Committee (Note: trapping campaigns were organized and conducted by personnel from Texas Tech). Trapped animals were euthanized via methoxyflurane inhalation, and the animals specific identity, sex, age class, reproductive condition, weight, general appearance, and presence of lesions or scars was noted. In addition, each animal had lung, liver, kidney, heart, and muscle tissues and blood samples collected. All samples were frozen in liquid nitrogen and transported to the Museum of Texas Tech University where they were stored in a -80°C freezer. Blood samples were tested for hantaviral antibodies using Immunofluorescence Assay (IFA) (Chu et al., 2003). A positive IFA test result means that the animal is seropositive (S+), which was used as an indication that the animal had been exposed to hantavirus at some point in its life cycle (Note: a positive IFA does not necessarily mean that the individual rodent is currently hosting live virus).

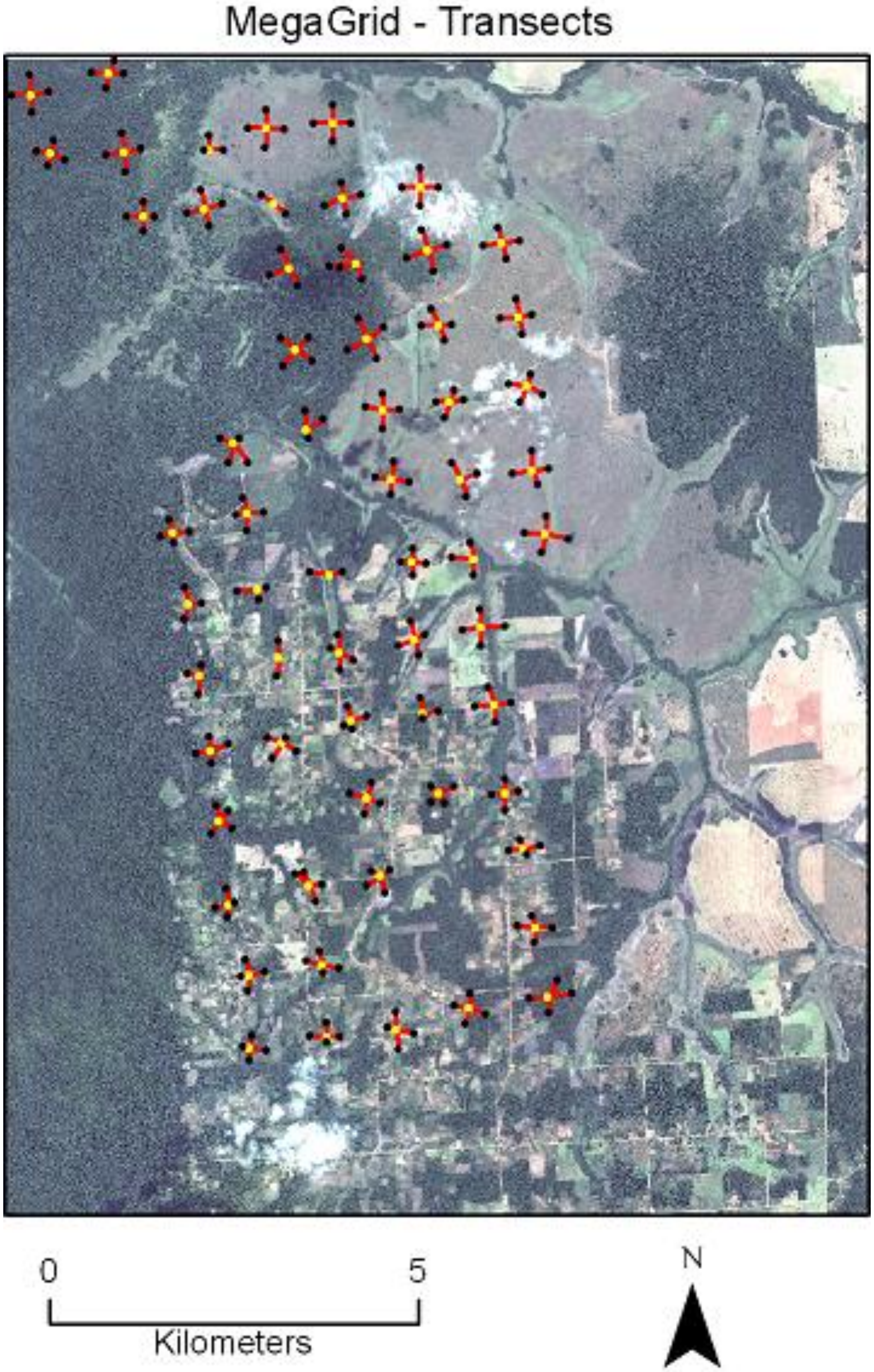

Figure 3.6. Map of Megagrid sites showing transect patterns and landscape.

3.4.3. Vegetation Data

Data about vegetation characteristics (Table 3.2) were collected from a variety of sites in and around the Reserve (Figure 3.8). The sites were somewhat limited due to accessibility, but examples of all the major land cover types on and off the reserve were represented. In addition to the displayed vegetation sites, vegetation data was collected for all of the Mark-Recapture sites and several of the Megagrid sites.

Each sample plot had a nested sub-plot design (Figure 3.7) based in part on the design described in Lu et al. (2004). This type of plot design is common in vegetation structure analysis and accommodated vegetation data that was previously collected at the rodent trapping sites. The larger plot was a 100m2 plot, in this plot all trees were counted and each tree had its diameter at breast height (DBH), tree height and stem height recorded. Trees were defined as woody stems with a DBH > 10cm. The middle sized plot was a 9m2 plot with its center in the center of the larger plot, this plot had all saplings counted and each sapling had its height and DBH recorded. Saplings were defined as woody vegetation with a DBH < 10cm and >2.5cm. The smallest plot had nine locations, the center of the larger plots, and the corners of the larger plots. These plots were 0.1m2, from each of these plots ground cover information following the Daubenmire method (1959) and vertical density information was collected in each plot. In addition, horizontal density information (Kelt et al., 1994) was collected from the central point. The ground cover, vertical density, and horizontal density data collected was the same as had been previously collected by me for the hantavirus project.

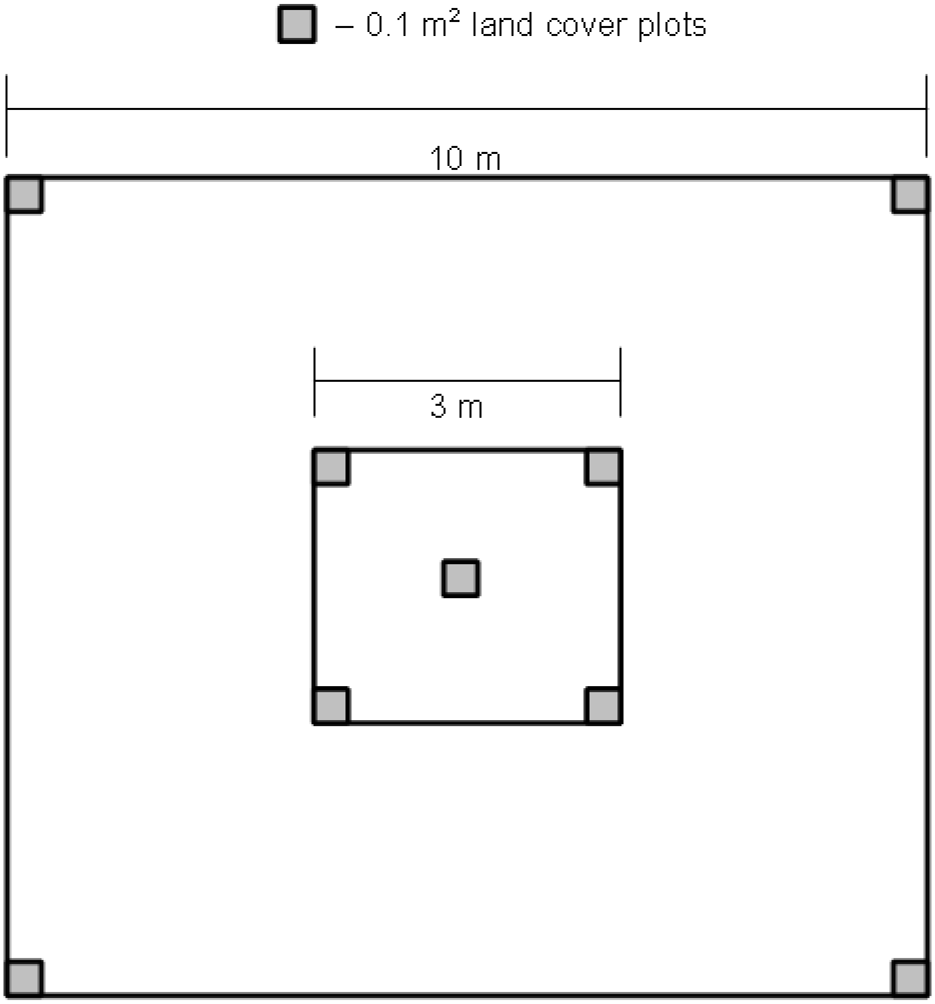

Figure 3.7. Nested sub-plot design used to collect forest vegetation data in the study area.

Table 3.2. Summary of vegetation data.

Name	Variables	Notes
Horizontal Density	Ground level, 0.25m, 0.5m, and 1m.	Measure density of vegetation using a white board. A 10 x 20cm white board placed at sample site, the distance at which half of the board is visible is measured.
Vertical Canopy Density	Primary (top), secondary, and tertiary canopies.	Measured presence or absence of vegetation immediately above sample site. Tertiary canopy is from 2-5m above site, secondary canopy is from 5-15m above site, primary canopy is >15m above site.
Ground Cover	Soil, Litter, Grass, Bromeliad, Bamboo, Overall vegetation, Herbacious vegetation, Fern, Woody debris.	Ground cover percentage estimated into one of seven categories within a 20cm x 50cm sampling frame. 0 – not present, 1 – 0 to 5% cover, 2 – 5 to 25% cover, 3 – 25 to 50% cover, 4 – 50 to 75% cover, 5 – 75 to 95% cover, 6 – >95% cover.
Tree Variables	Count, DBH, height, distance from center.	Count of trees within plot. Height, distance from center, and Diameter at Breast Height measured for each tree.
Sapling Variables	Count, DBH, height, distance from center.	Count of saplings within plot. Height, distance from center, and Diameter at Breast Height (if over 1cm) measured for each sapling.

3.4.4. Georectification Data

Data for georectifying satellite imagery (i.e., ground control points) were collected throughout the Biosphere and in some locations outside of the Biosphere. These data primarily consisted of road intersections and were collected while traveling in and out of the region and during data collection episodes. The regions outside of the Biosphere were primarily to the West and South of the Biosphere.

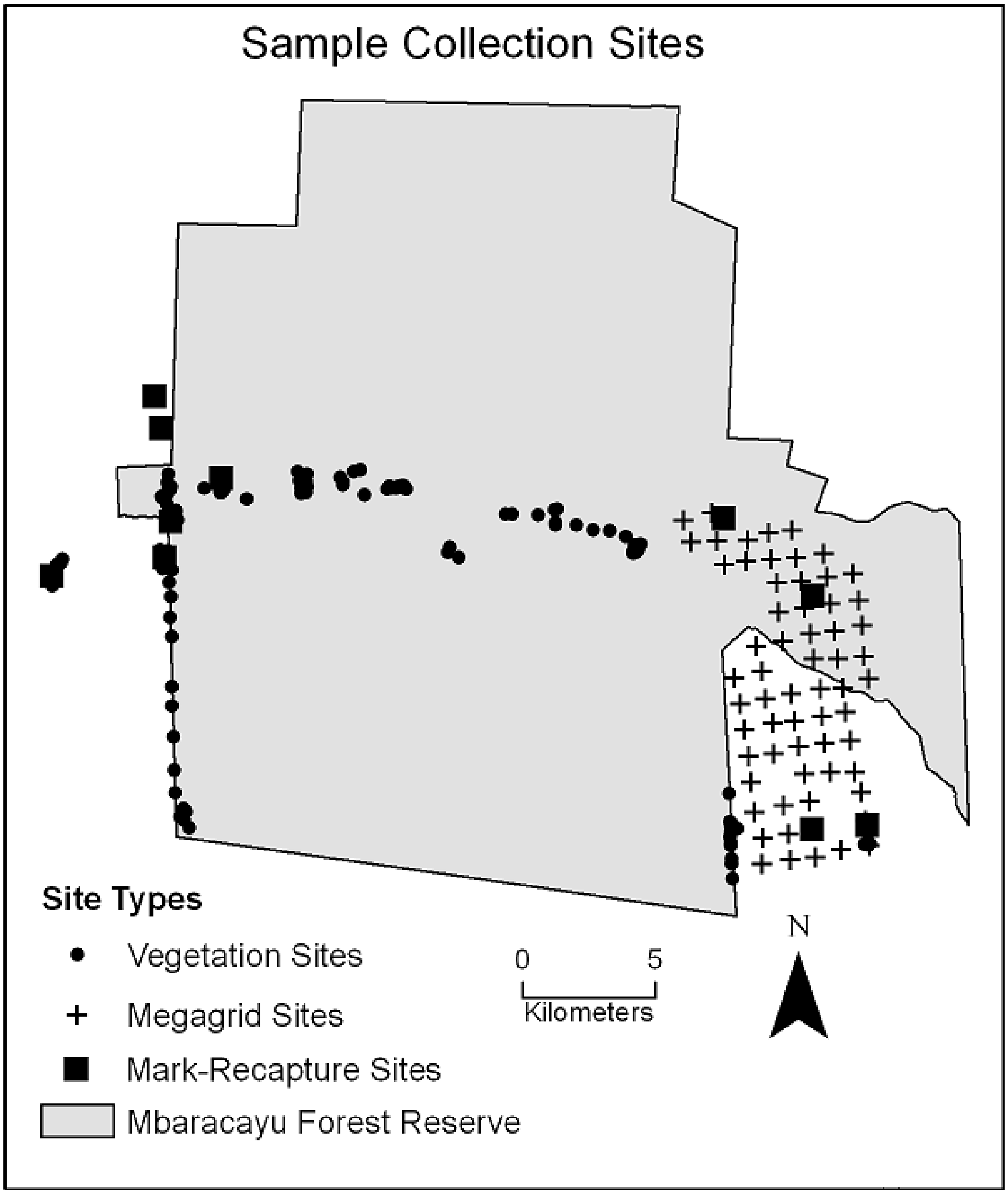

Figure 3.8. Map showing all sample collection sites and their spatial relationship to each other.

Chapter 4. Landscape Classification

4.1. Introduction

4.1.1. Review

Geospatial analysis technologies such as remote sensing and GIS provide tools for gathering and analyzing spatial data over a range of spatial scales. An additional advantage of using classifications from remotely sensed imagery for analyzing disease-related environmental factors is the capability for rapid and repetitive collection of information, even from remote or inaccessible places (Curran et al., 2000). This capability for generating a responsive spatio-temporal set of data has led to an increase in the interest in using satellite imagery as a data source in epidemiological work (Beck et al., 2000), especially when dealing with zoonotic diseases (Glass et al., 2006).

A common use of remote sensing images is to classify them into land use/land cover information. In landscape epidemiology, that information is generally in the form of vector or host habitat maps (Kazmi and Usery, 2001). Most classifications are based on using spectral information associated with individual pixels. However, this per-pixel classification approach does not use the spatial information that also exists in the imagery, which can be used to help correctly identify habitat types. Object-based classifications (Jansen and van Amsterdam, 1991) are an alternative way to map land use/land cover and habitat types, including ones that can be useful for studying the influence of landscape on disease processes. Object-based classifications work by partitioning images into sets of homogenous regions, or objects. The objects are then analyzed for the spectral properties and geometric patterns that can help distinguish the habitats of interest (Jansen and van Amsterdam, 1991; Lobo et al., 1996). In this chapter an object-based classification of the study area is presented, which will then be used for landscape epidemiological analysis of rodent hantavirus seroprevalence in a subsequent chapter.

In the study area, the different land use/land cover types that are expected to play a role in hantavirus prevalence are spectrally very similar. Anthropogenic land cover disturbance is one of the factors that has been shown to be associated with prevalence of hantavirus in rodent communities (Goodin et al., 2006). Different land uses like minifundia agriculture and latifundia agriculture (see Section 3.4) have nearly identical spectral signatures, but vary greatly in the size of their patterns (Figure 4.1). Specifically, latifundia agriculture typically consists of very large cleared fields (100s to 1000s of ha) that are rectangular in shape with distinct edges, whereas minifundia agriculture consists of land holdings of 10-20 ha with individual fields of 1ha or less being common. An additional pattern imposed by minifundia agriculture is that fields cleared for farming are typically nearer a road and the part of the farm farther from the road remains forested, this results in a 'fishbone' type pattern imposed on the larger landscape. The minifundia landscape is also much more densely populated by humans than the latifundia landscape (Robles, 2000).

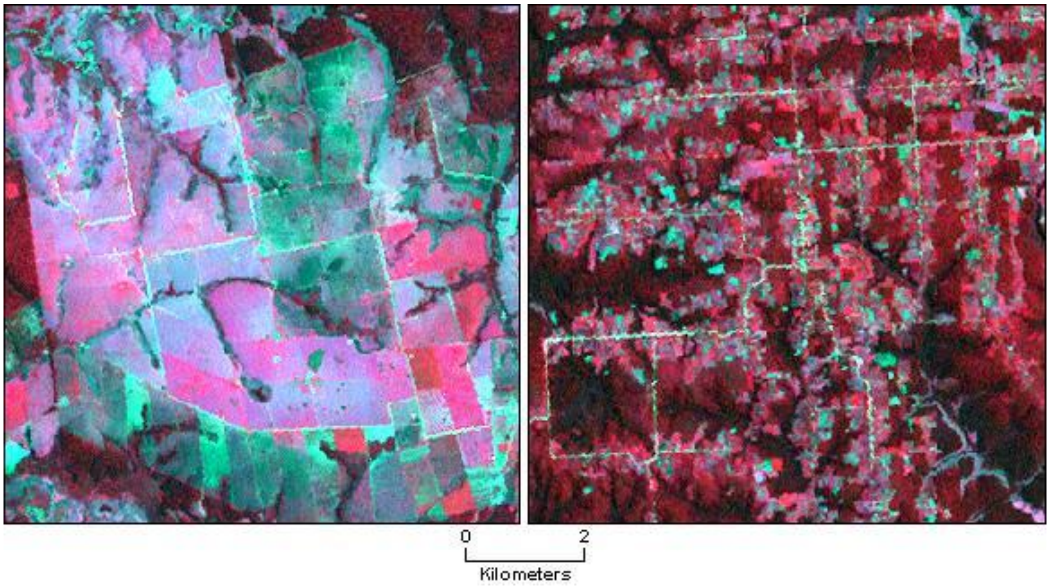

Figure 4.1. False color satellite image showing the spectral similarities and spatial size differences between latifundia and minifundia agriculture. Latifundia agriculture is on the left, minifundia is on the right, both images are the same scale. Image is from Koch et al. (2007).

4.1.2. Objectives

The objective of this chapter is to classify habitat types. This method is an object-based technique, which is an improvement over traditional per-pixel classifications in this site. One of the reasons for this improvement is that many of the habitat types are spectrally very similar, but vary in spatial properties. These spatial properties can be measured using the object-based classification. This chapter is largely based on the data from Koch et al. (2007).

4.2. Materials and Methods

4.2.1. Study Area

The region of this classification is centered on the Reserva Natural del Bosque de Mbaracayú (RNBM) in Eastern Paraguay. The RNBM is a protected forest area that is surrounded by the Mbaracayú biosphere: much of the biosphere is included in the image. The forest fragment that constitutes the core of the classified region has had minimal human impacts for more than 20 years. Conversion of forest to agriculture in the surrounding region is ongoing, with most of the agriculture having been established within the last couple of decades. Along with the forest, minifundia, and latifundia land use/land cover, there are savanna and grassland areas, some of which have also been converted to agriculture and pasture use (Sarmiento, 1983).

4.2.2. Satellite Image

The satellite image used for classifications was a Landsat ETM+ image from path 225/row 77 that was acquired on 28 February 2003. This was the latest cloud-free image available in this scene before the failure of the ETM+ scan line corrector (Howard and Lacasse, 2004). The image was prepared for analysis by subsetting the study area from the full scene and georectifying it using the Universal Transverse Mercator grid (zone 21S), WGS84 datum, and using a 1st order polynomial with nearest neighbor resampling (Richards and Jia, 1999). Georectification data consisted of in-situ surveys, a minimum of ten points were used for each georectified image. Total root mean square error for the georectification was less than 1.0. Raw pixel digital numbers were corrected for atmospheric backscatter (Song et al., 2001) using the IDOS model (Chavez, 1988).

4.2.3. Classification

The classification was designed to obtain information about both rodent habitat and anthropogenic aspects of that habitat, so it was important to distinguish different land uses from the same land cover habitats. The landscape classes used here are: Forest, Wet Cerrado, Dry Cerrado, Minifundia, Latifundia, Pasture, and Unclassified. These seven classes were chosen based on a variety of sources including previous classifications (Naidoo and Hill, 2006) from the region, and expert opinions from others familiar with the study area. These habitat categories matched habitat preferences of various rodents from the region, but especially *Akodon montensis* (Owen, et al., 2010). In this classification I originally attempted to separate forest into different classes (Koch et al., 2007), the two forest classes were the least effectively separated classes, so they were collapsed into one forest class for the landscape analysis conducted in Chapter 5. Chapter 6 discusses ways to improve the separation of different forest classes.

Non-forest classes were defined by a combination of vegetation cover and disturbance history. Cerrado (savanna) regions were divided into two classes, wet and dry. Dry cerrado is found in areas where fire reduces tree cover; the vegetation in this area consists of grasses, forbs, palm trees, and some firetolerant tree species. Wet cerrado occurs in areas that are too persistently wet to support tree growth; vegetation in these areas consists of grasses, forbs, and some shrubs. Pasture areas are dominated by both native and introduced grasses; they typically were converted from both cerrado types and from previously forested areas. Agricultural areas (both latifundia and minifundia) are generally converted from previously forested areas and they are dominated by a variety of cultivated crops. Crops in the latifundia system primarily consist of soybean and corn. Minifundia system crops are much more diverse and typically include cotton, sesame, corn, mandioca, and various vegetable and fruit crops.

4.2.4. Classification Method

Along with producing a classification to use with a landscape study, this classification served to evaluate the potential improvement of object-based classifications over per-pixel classifications. To do this I produced a classification using each of the two strategies.

The per-pixel classification used a supervised, maximum likelihood classification algorithm (Richards and Jia, 1999) implemented in the ENVI software package (v. 4.2, ITTVIS). Training site selection was guided by ground truth data collected during two visits to the study area (October 2002 and June 2005), along with inspection of high-resolution satellite data of the study area. Mixed training pixels were reduced by avoiding transitional and edge areas for training sample selection. A minimum of 800 training pixels were used per land use/land cover category. Prior to classification, I used the transformed divergence index (Jensen, 1996) to optimize the spectral features for classification. Based on the transformed divergence index, ETM+ bands 1, 3, 4, and 6 were used to classify the image. A 3 x 3 majority filter was also applied to reduce random pixel classification errors and noise (Gurney and Townshend, 1983). The per-pixel classification is shown in Figure 4.2.

The object-based classification was conducted in two steps. The first step was segmentation, where the spatial objects were formed. Segmentation was based on the fractal net evolution approach implemented in the eCognition software, v.4.2 (Baatz et al., 2004). Objects are formed by merging adjacent pixels based on similarity in pixel values until a set of heterogeneity factors are exceeded. The heterogeneity factors are defined by both spectral and geometric properties (Benz et al., 2004) and are user-defined, the factors are: scale parameter, shape factor, and compactness. The values that yielded an optimal segmentation for this image were: scale parameter, 16; shape factor, 0.1; and compactness, 0.5.

The second step in the object-based classification is to sort the objects into classes. Each object from the segmentation is described by a number of spectral, geometric, and textural features. Spectral information used in this classification were mean spectral values and standard deviations for ETM+ bands 1-5 and 7. Geometric information used in this classification were area and assymetry metrics (Baatz et al., 2004). Textural information used in this classification were dissimilarity and standard deviation for each band derived from the grey-level co-occurrence matrix (Haralick and Shanmugam, 1974). The classes were then determined using a minimum distance decision algorithm with fuzzy class boundaries. A proximity algorithm using contextual information separated objects with nearly equal probabilities of belonging to two or more classes. The object-based classification is shown in Figure 4.3.

4.2.5. Accuracy Assessment

Accuracy was assessed similarly for both of the classifications. In both classifications, validation sites were selected by proportional stratified random sampling (Beyer, 2004). For the per-pixel classification, pixels were selected randomly from within the area of objects selected for assessing the object-based classification. Sites in which the cover type could not be identified through either field data or high resolution imagery were eliminated. The final sample sizes were 632 validation pixels for the per-pixel classification and 225 objects for the object-based classification.

Objects and pixels were assessed for correctness and the results were tabulated into error matrices (Table 4.1 and 4.2). The error matrices were used to quantify producer and user accuracies for each cover class and the total accuracy for all classes (Jensen, 1996). Additionally, Cohen's Kappa statistic (Cohen, 1960; Congalton and Mead, 1983), a measure of improvement of the classification over random, was calculated as was the significance of the difference between the two Kappa statistics.

This accuracy assessment method is commonly used for comparing per-pixel and object-based classifications (Wang et al., 2004, Matinfar et al., 2007, Perea et al., 2009, Ouattara et al., 2010, Mas et al., 2010). However, there are potential issues involved with comparing pixels against objects. The pixels and objects are different sizes, and thus present potential problems related to having different scales. Whether a per-pixel or object-based classification might be more poorly represented by the traditional error matrix would depend on how the accuracy model is used and relationships between pixel size and real (field) object size (Dungan, 2006, Mas et al., 2010). Additionally, objects that cover two different basal LULC types present problems in determining whether or not they should be considered accurate (Congalton, 2008). Potential solutions to the problems of comparing pixels and objects have been presented in map feature comparison research. For example, Hargrove et al. (2006) have presented a quantitative goodness-of-fit model that is designed for comparing categorical maps. The goodness-of-fit model compares the percentage of spatial matching between features, but appears to be potentially adaptable to a pixel v. object type of system.

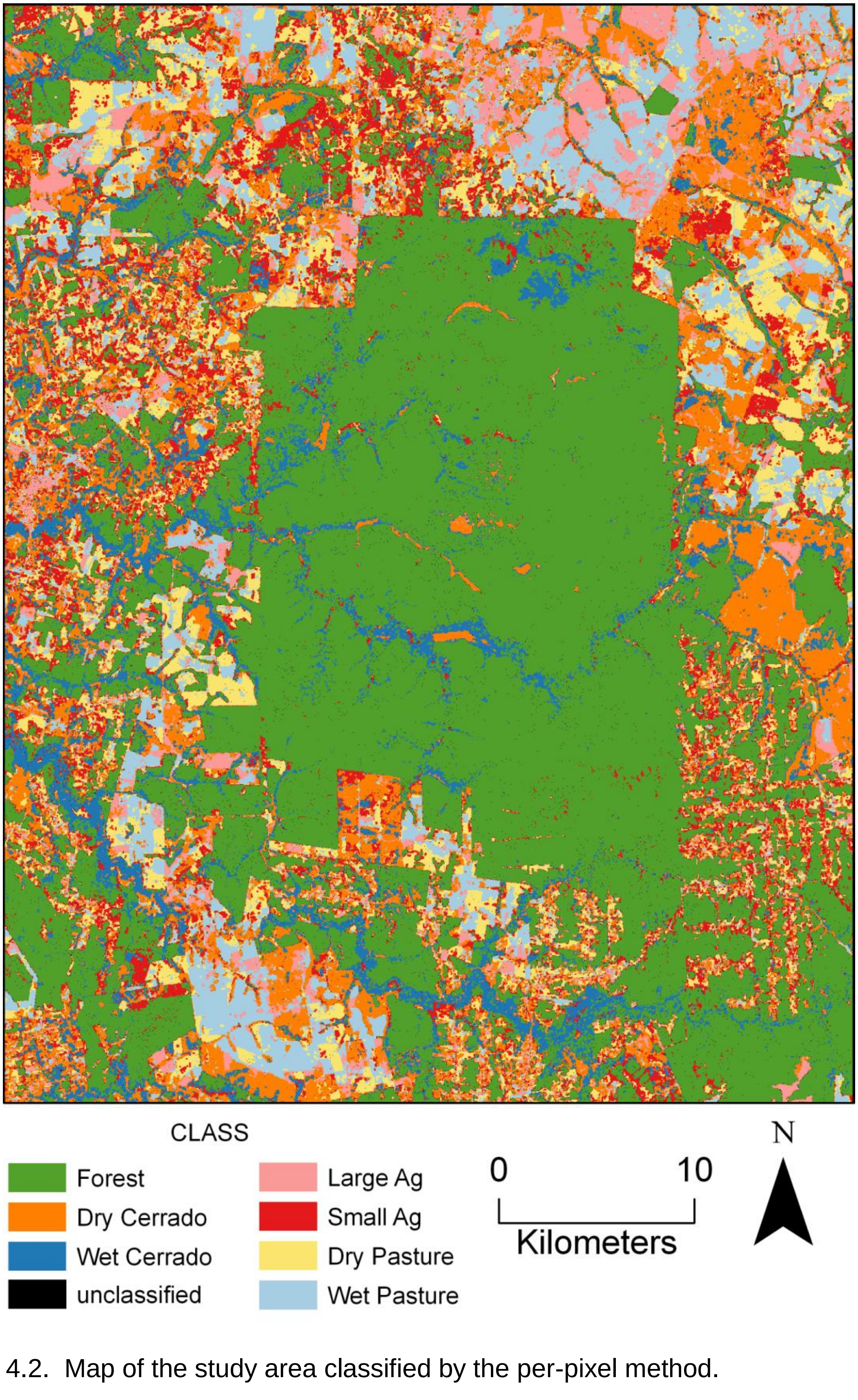

Figure 4.2. Map of the study area classified by the per-pixel method.

Figure 4.3. Map of the study area classified by the object-based method.

4.3. Results and Discussion

4.3.1. Comparison of Object-based and Per-pixel Classifications

Qualitatively, the object-based classification appears to be superior to the per-pixel classification (Figures 4.2 and 4.3). Both classifications show the same overall pattern, but the map from the per-pixel classification is much noisier and there is poor definition between the class regions. Quantitatively, that observation is confirmed (Tables 4.1 and 4.2). The object-based classification correctly classified 92% of scene objects, whereas only 43% of the pixels in the per-pixel classification were correct. In addition, the Kappa statistic for the object-based classification is significantly higher than that of the per-pixel classification ($z= 11.5$, $p < 0.001$).

Both classifications showed that their best ability was in separating the forest class from other classes. The most accurately classified class in the object-based classification was the forest, which was over 95% accurate in both user's and producer's accuracy. The most accurate classification in the per-pixel classification was the forest class at 78% accurate. Only two out of seven classifications fared worse than that in the object-based user's and object-based producer's accuracies, the worst of those coming in at 74% accurate. The majority of the classes in the pixel-based classifications were less than 50% accurate.

Table 4.1. Error matrix for the per-pixel based classification.

Map Class	Reference Class								
	Forest	Wet Cerrado	Dry Cerrado	Latifundia	Minifundia	Wet Pasture	Dry Pasture	Total	User's Accuracy
Forest	118	9	0	47	12	0	0	186	0.63
Wet Cerrado	4	15	0	20	2	20	0	61	0.25
Dry Cerrado	0	5	16	27	7	0	14	69	0.23
Latifundia	16	4	4	64	19	2	1	110	0.58
Minifundia	12	4	9	40	18	3	1	87	0.21
Wet Pasture	1	5	0	21	25	3	1	56	0.05
Dry Pasture	0	4	15	11	10	1	22	63	0.35
Total	151	46	44	230	93	29	39	632	
Producer's Accuracy	0.78	0.33	0.36	0.28	0.19	0.10	0.56		
	Overall Accuracy = 0.43					Kappa = 0.30			

Table 4.2. Error matrix for the object-based classification.

Map Class	Reference Class								
	Forest	Wet Cerrado	Dry Cerrado	Latifundia	Minifundia	Wet Pasture	Dry Pasture	Total	User's Accuracy
Forest	65	0	0	0	0	0	0	65	1.00
Wet Cerrado	0	11	0	0	1	2	0	14	0.79
Dry Cerrado	0	1	20	2	0	0	4	27	0.74
Latifundia	0	0	2	42	3	1	1	49	0.86
Minifundia	0	0	0	0	17	0	1	18	0.94
Wet Pasture	1	0	0	0	1	20	1	23	0.87
Dry Pasture	0	0	4	1	1	1	22	29	0.76
Total	66	12	26	45	23	24	29	225	
Producer's Accuracy	0.98	0.92	0.77	0.93	0.74	0.83	0.76		
	Overall Accuracy = 0.92					Kappa = 0.91			

User's accuracy (equivalent to Type I statistical error) is the type of accuracy that describes how the map performs in the field; that is, from the perspective of a map user, would observed cover type agree with the map. Both classifications struggled with cerrado classes and the dry pasture classification. In general, confusing dry cerrado and dry pasture was a common mistake for both classifications, which seems reasonable as the land cover is nearly identical, and spatial clues are not strong distinguishers between these classes. A user who saw wet cerrado on a map might not be overly surprised to find wet pasture or a large farm at the site when they are in the field. In terms of user's accuracy, the per-pixel classification struggled the most with wet pasture classifications.

Producer's accuracy (equivalent to Type II statistical error) is the type of accuracy that describes how well the field is represented on the map; that is, from the perspective of an observer in the field, would the map agree with the observed cover type. Both classifications showed considerable confusion between the dry cerrado and minifundia classes. In both, the minifundia was frequently mistaken for latifundia, however, the per-pixel classification mistakenly classified minifundia sites as every other available class. This is not surprising, as the minifundia agricultural system is a heterogenous mixture of many land cover types in a much smaller spatial scale. The object-based classification was able to use the spatial factors to help distinguish this class from the other classes.

4.4. Conclusion

Object-based classification was clearly superior to the per-pixel based classification. The least accurate classes in the object-based classification were about as accurate as the best classes in the per-pixel classification. In the object-based classification, the forest class was the most accurate, which is important because the forest class is the habitat class preferred by *Akodon montensis*. The overall accuracy of 92% is an improvement over other classification's from the region (Huang et al., 2007), even though we used a single image, whereas others have used multi-image classifications. Some experimentation was needed to establish the proper parameters for the object-based classification, but successive classifications should be able to use these same parameters in the future.

Using this technique with multi-date imagery might improve the ability of the classification to distinguish many of the classes. Another potential improvement, which is addressed in Chapter 6, would be distinguishing between different forest classes. In Koch et al. (2007) this classification distinguished two forest types, which was the weakest part of that classification scheme.

Use of satellite-derived land use/land cover maps are an important facet of landscape epidemiological studies of infectious disease. Adding geometric and textural components to the spectral information that is commonly used is one way to improve the usefulness of habitat classifications for landscape epidemiology studies.

Chapter 5. Landscape Ecology of Hantavirus in *Akodon montensis* in the Atlantic Forest in Eastern Paraguay

5.1. Introduction

5.1.1. Literature Review

Composition and configuration of landscapes can play an important role in the maintenance and spread of pathogens, especially for reservoir-borne pathogens like hantavirus (Langlois et al., 2001) or other reservoir- or vector-borne pathogens and diseases. Composition and configuration of landscapes tend to exert significant control over disease processes when habitats favorable to vectors or reservoir species are one of the mosaic of cover types present on the landscape (i.e. composition, Pavlovsky, 1966) and/or when the juxtaposition of differing cover types favors the movement or interaction of individuals or communities of disease reservoir species (i.e. composition, McCallum, 2008).

In rodent communities, hantavirus is passed between reservoir species (transmission) or between a competent reservoir and another host (spillover). Transmission does not occur between a dam and her offspring. In other words, hantavirus is transmitted horizontally, but not vertically in rodent communities (Klein and Calisher, 2007). Horizontal transmission implies that factors that affect the way individuals and communities interact will affect the quality and quantity of pathogen transmission on those communities. Habitat type and habitat patterns are landscape factors that can affect animal interactions and thus may impact pathogen transmission within reservoir communities (McCallum, 2008). Additionally, anthropogenic changes to landscapes often leave specific patterns on the landscape; if these patterns are associated with increased hantavirus presence in reservoir communities, then there can be increased potential for human contact with the virus.

Whenever landscape effects are considered it is also important to recognize the spatial scales at which those interactions take place (Turner, 2001). As different landscape spatial scales are analyzed, different potential levels of interactions occur within small mammal populations. Each species has a different range of interactions, so the scales at which different populations interact need to be determined empirically for each species (Holland, 2009). With hantavirus in rodent populations in other regions, scale of observation and landscape configurations have been found to important in the analysis of the rodent communities (Langlois et al., 2001; Yahnke et al., 2001; Goodin et al., 2006; Suzan et al., 2006).

5.1.2. Objectives

The objectives of this chapter are to analyze what types of landscape compositions and configurations are the most important for hantavirus seroprevalence on the landscape. In particular, I look at what types of anthropogenic disturbances are important, at what scales are they important, and what type of effect they have on hantavirus seroprevalence. Anthropogenic disturbance in Paraguay and other parts of North and South America have been shown to affect the presence of hantavirus within the rodent communities (Langlois et al., 2001; Yahnke et al., 2001; Goodin et al., 2006; Suzan et al., 2006). Once the types of anthropogenic changes that affect hantavirus presence on the landscape are elucidated, it becomes possible to make recommendations regarding the way anthropogenic changes are conducted in order to reduce the risk of hantavirus exposure to the human population. An additional objective of this study is to investigate the size of some important mesoscale interactions in *Akodon montensis*, both in terms of the individual rodents and community dynamics and how they affect hantavirus seroprevalence, to help guide future research efforts.

5.2. Methods

5.2.1. Study Area

The study area was located within the Mbaracayú biosphere in the Department of Canindeyu in Eastern Paraguay, which is described in Section 3.3. The trapping for this study was conducted in the Megagrid (Section 3.4.2), which was a grid of transects located along the eastern side of the reserve and in the neighboring small farmer community. Transect sites were chosen by placing a regular grid through a diverse set of LULC types typical for this area. Sites within the RNBM Reserve included forest and dry cerrado habitats. The rest of the grid was located within the mostly minifundia community of Britez Kue (there are some latifundia/pasture sites).

5.2.2. Rodent Trapping

Trapping was done on a grid consisting of a central point and two transects, which were 500m long and intersected perpendicularly at their midpoints (Figure 3.5). Traps were placed at the center point and then every 10m along each transect. Trapping was conducted between February and August 2006. Each site was trapped for two nights, traps were checked each morning during the trapping session, captured rodents were kept, euthanized via methoxyflurane inhalation, and their tissues and blood were harvested and frozen. These samples were used to determine hantavirus seropositive status of the captured rodents. This site was originally set up to measure some rodent demographic parameters, due to the variety in the landscape it was later recognized that this setup would also allow for a comparison of the effect of different landscape patterns on rodent communities. All procedures involving animals followed American Society of Mammalogists guidelines (Gannon et al., 2007) and the CDC guidelines for handling potentially infected rodents (Mills et al., 1995) and were approved by the Texas Tech University Animal Care and Use Committee (Note: trapping campaigns were organized and conducted by personnel from Texas Tech).

5.2.3. Landscape Classification

The land use/land cover (habitat) map used for this analysis is from the classification described in Chapter 4, which is a minor modification from Koch et al. (2007). The two forest classes from Koch et al. (2007) were collapsed to give one forest class and a total of seven classes of LULC, all of which are included in the analysis here. This was done because the two forest classes were not well separated, potential solutions for this issue are further analyzed in Chapter 6. The landscape classes used here are: Forest, Wet Cerrado, Dry Cerrado, Minifundia, Latifundia, Pasture, and Unclassified.

5.2.4. Landscape Structure Metrics

Landscape metrics can be calculated at two different levels: class, and landscape (McGarigal et al., 2002). Class level metrics are calculated for each class type in the mosaic (all patches for each class type). With these results, if there is more than one patch in an analysis area, averages and distributions for the metrics regarding each class type can be obtained. Class level metrics are used primarily with the Forest and Minifundia class in this analysis. Landscape metrics are calculated for the entire landscape mosaic (all patches for all class types). Here, too, averages and distributions of the group of patches can be obtained from each analysis site. I calculated and analyzed metrics associated with hantavirus seroprevalence for the landscape mosaic as a whole. I then calculated and analyzed metrics associated with seroprevalence for the two most numerous classes in the mosaics, Forest and Minifundia.

The landscape structure metrics for the area surrounding each trap site were extracted using a series of different sized windows, with the center of the trapping transect at the center of each window. These windows were squares with side length of 250m (6.25 ha), 500m (25 ha), 1000m (100 ha), and 2000m

(400 ha) (Figure 5.1). Fragstats 3.3 (McGarigal et al., 2002) was used to calculate the spatial pattern metrics for each window. These metrics (Table 5.1) were then tested for associations with seroprevalence and used to determine what aspects of landscape patterns were the most important for hantavirus prevalence in this landscape. The output from Fragstats included metrics for the overall landscape as well as for each classification type present in each window.

Fragstats is a program developed for the spatial pattern analysis of categorical maps in a raster data format. The program calculates a variety of areal and shape configuration metrics at patch, class, and landscape scale. Selected areal and shape configuration metrics are calculated for each patch in an landscape input. For class level calculations, the metric calculations for all patches of one class are combined and their statistical distributions are reported (mean, standard deviation, etc.). For landscape level calculations, the metric calculations for all patches are combined and their statistical distributions are reported. Adjacency metrics can also be reported for class and landscape level calculations. Shape configuration metrics that Fragstats can calculate include metrics that measure patch shape compactness and metrics that measure patch shape edge complexity (McGarigal et al., 2002), shape configuration metrics used in this study are described in Table 5.1.

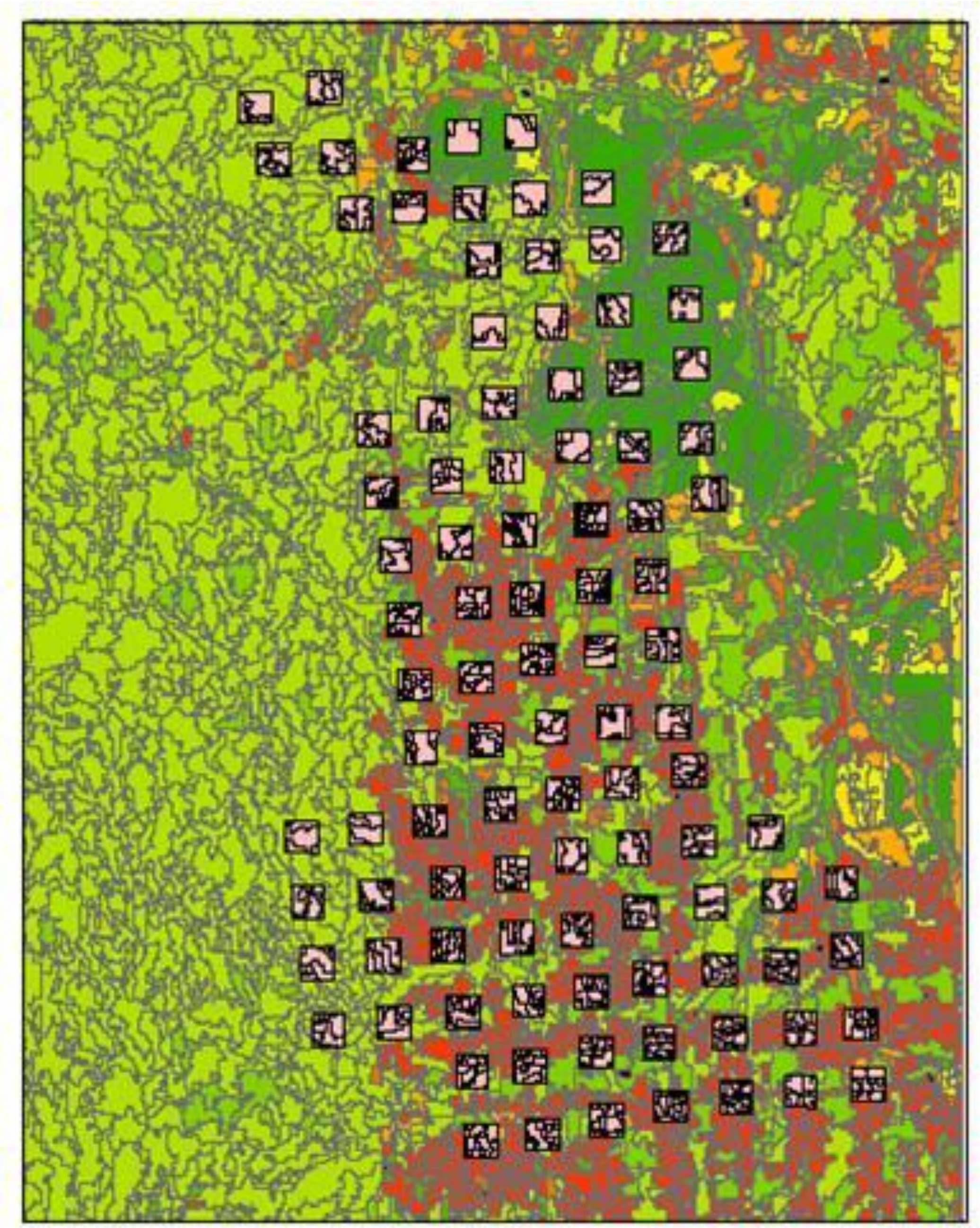

Figure 5.1. Example of the windows used to extract landscape metrics. This example shows the 500m (25ha) windows. The center of each square is the center of one trap transect.

Table 5.1. Description of metrics discussed in Chapter 5 (McGarigal et al., 2002).

Metric	Metric Type	Description
Area	Area	Patch area.
Circle	Shape	Measure of the smallest circle able to contain entire patch.
Core Area	Area	Area in patch that is 50m or more from the edge of that patch.
Core Area Index	Area	Percentage of patch area that in the core area.
Fractal Dimension	Shape	A measure of the complexity of the shape edge, very little effect from patch size.
Gyration	Density	A measure of how disperse the patch is, or how much of the patch is far away from the patch center.
Nearest Neighbor	Proximity	A measure of the shortest Euclidean distance between two patches.
Number Patches	Count	Number of patches of a class present in a landscape mosaic.
Perimeter-area Ratio	Shape	A simple measure of shape complexity, value affected by patch size.
Proximity	Proximity	A measure of number of patches within a certain radius and how close those patches are.
Shape	Shape	A ratio of the patch perimeter and the smallest possible perimeter for a patch of the same area. Similar to Perimeter-area ratio, but corrects for patch size.

5.2.5. Statistical Analysis

Seropositivity is a binary variable. That is, an individual rodent is either S+ or S-, as determined by IFA (see Section 3.4.2). Odds ratios and logistic regression are appropriate statistical tests for these types of data (Dohoo et al., 2003), and were used to measure association between presence of seropositive rodents and the various landscape metrics. Logistic regression was used in a bivariable comparison of seroprevalence against all landscape metrics and in a multivariable comparison of seroprevalence with select landscape metrics. All tests were run at each of the four landscape window scales. The transects are assumed to be spatially independent, as home range sizes for *A. montensis* are on the order of 1600 m^2 (40 x 40 m) or less (Owen et al., 2010). For clarity, selected examples from the bivariable and multivariable tests will be shown in Section 5.3, the full set of results significant at a p-value < 0.1 for the bivariable tests and all of the final multivariable models can be seen in Appendix A.

5.2.5.1. Odds Ratio

Odds ratios were used in cases where the predictor and response variables are both discrete. Specifically, odds ratios were used to evaluate the presence/absence of seropositive rodents in comparison to the presence/absence of the different LULC classes within a window. Additionally, odds ratios were used to test presence/absence of rodents and presence/absence of *A. montensis* against the presence/absence of the seven LULC classes.

5.2.5.2. Logistic Regression (Bivariable)

Logistic regression was used in cases where the response variable (S+) was discrete, but the predictor variables (landscape metrics) were continuous. For the bivariable tests, each individual predictor variable (i.e. each of the landscape metrics listed in Table 5.1) was tested against the presence of seropositive *A. montensis*.

5.2.5.3. Logistic Regression (Multivariable)

For the multivariable tests, backward stepwise regression was used to find the set of landscape metrics that best explained the presence of seropositive *A. montensis*. The initial set of potential predictor variables tested were those that had some association with seroprevalence based on the results from the bivariable test, but were not too closely correlated with each other. Potential predictors that were correlated with each other with an r > 0.95 had one representative selected to include in the initial multivariable model. Screening for the initial set of predictor variables involved using all potential predictor variables that correlated with seroprevalence with a bivariable p-value <0.2 (Tables A.1-A.4). Backward stepwise regression consisted of starting with the initial full model, then dropping one term from the model, putting it back in and dropping another until all the terms have been tested. Goodness of fit for the model was determined using the Akaike Information Criteria (AIC). The model that had the lowest AIC that was at least 2 units less than the original model was kept (i.e. that predictor variable was dropped from further consideration). This process was then repeated starting with the new model until there were no more improvements from the previous model, that is when testing stopped and the multivariable model was established. Statistical results were calculated using R version 2.8.0 (R Development Core Team, 2009) and the R Commander package (version 1.4-10).

5.3. Results and Discussion

5.3.1. Rodent Trapping

Trap success for the 71 trapped transects used in this analysis is summarized in Table 5.2. The majority of sites had some rodents captured. Just under half of the transects registered a capture of at least one *A. montensis*. About a tenth of all transects, and about a quarter of transects that had *A. montensis* had some seropositive rodents trapped in them. Two analyses were conducted, one with the entire data set including *A. montensis* and one with only *A. montensis*, as *A. montensis* is the primary hantavirus reservoir in the study area.

Table 5.2. Summary of trap success and serostatus for *Akodon montensis* (AKMO) and other rodent species. (S+ indicates seroposive).

	All Species	AKMO	All Species S+	AKMO S+	Other Species S+
Total Number	268	165	10	9	1
Number of Trapsites (of 71)	54	32	7	7	1

5.3.2. Site Composition (Odds Ratio)

The 71 trapped sites contained a mixture of forest, dry savanna, wet savanna, latifundia, minifundia, pasture, and other land use/land cover types. The number of sites containing these land use/land cover types varied with scale of observation (varying window size), the number of sites containing a particular land use/land cover type are listed in Table 5.3. As expected, there are fewer LULC types represented in the smaller window. Forest and Minifundia were the only classification types to be represented more than 30 times at all four of the observational scales, so these two classes (along with overall landscape) were used for additional site composition analysis.

Results from the odds ratio evaluation of the strength of association between presence or absence of a particular landscape type and the presence of seropositive rodents (Table 5.4) show that there are no associations between the presence (or absence) of any particular LULC type and presence of seropositive rodents. There was no association at any of the four scales of observation. There were associations between the presence of LULC types and presence of *A. montensis* and between the presence of LULC types and the presence of other rodent species (Table 5.4). The presence of forest was strongly associated with the presence of *A. montensis*. This is not surprising, as the species is recognized as a forest generalist species (Owen et al., 2010). This association did not show up at the 1000m and 2000m diameter observational windows. In the case of the 2000m windows, every site had some forest present, so that particular scale could not make a prediction based on presence/absence, at the 1000m window size, only 5 windows did not have any forest, so this also had little chance to make a prediction. While the presence of forest does predict the presence of *A. montensis*, but not the presence of seropositivity, I will show that the amount of forest present will be associated with seroprevalence at some scales of observation (Section 5.3.5).

Presence of a few landscape types was associated with the absence of *A. montensis*. In particular, the presence of the two types of savanna (wet cerrado and dry cerrado) were associated with the absence of *A. montensis* at all observational scales, with the exception of wet cerrado observed through 2000m windows, which was another landscape type that was present in all windows at that scale of observation. There is something about the savanna landscape types that is repellent to *A. montensis*, or perhaps the forest types bordering the cerrado landscape types are not particularly attractive to *A. montensis*. Another association that involves presence of a particular LULC type and absence of *A. montensis*, is the presence of latifundia or pasture in the larger window sizes.

Finally, there were some associations between presence of wet and dry cerrado and the absence of all rodent species in general. These results were less consistent than the results for *A. montensis*, but still the cerrado environment, or otherwise suitable habitat near cerrado LULC types, seems to be generally unfavorable for rodents.

Table 5.3. The number of sites that contain a particular LULC type for each window size.

	2000 m	1000 m	500 m	250 m
Forest	71	66	59	50
Wet Cerrado	71	58	38	22
Dry Cerrado	27	22	16	14
Minifundia	69	59	52	40
Latifundia	31	13	7	3
Pasture	65	41	20	11
Unclassified	51	24	12	5

Table 5.4. Association of land use class presence and rodents, *A. montensis*, and presence of seropositive rodents. Odds Ratios and p-values reported only for those with p-value <0.05. Odds ratios higher than 1 indicate presence of that land use class has a positive impact on the factor and odds ratios lower than 1 indicate that presence of that land use class has a negative impact on the factor.

	250 m		500 m		1000 m		2000 m	
	Odds Ratio	p-value	Odds Ratio	p-value	Odds Ratio	p-value	Odds Ratio	p-value
All rodents								
Forest								
Dry Cerrado					0.282	0.0248		
Wet Cerrado	0.200	0.0044			0.088	0.0417		
Lati-fundia								
Mini-fundia								
Pasture								
Akodon montensis								
Forest	5.409	0.0043	12.179	0.0050				
Dry Cerrado	0.027	0.0003	0.052	0.0004	0.109	0.0004	0.099	<0.01
Wet Cerrado	0.109	0.0004	0.300	0.0142	0.183	0.0107		
Lati-fundia					0.170	0.0173		
Mini-fundia								
Pasture					0.346	0.0306	0.142	0.049
Seropositive rodents								
Forest								
Dry Cerrado								
Wet Cerrado								
Lati-fundia								
Mini-fundia								
Pasture								

5.3.3. Scale of Influence

Results show a stronger association between seroprevalence and landscape metrics in the 250m and 2000m diameter window sizes, where the 500 and 1000m windows were less strongly associated (Table 5.5). The number of bivariable associations is the total number (sum) for the three different types of landscapes measured (whole landscape, forest class, and minifundia class). There were fewer associations significant with a p-value < 0.1 at the 500m and 1000m diameter window scale. Additionally, at the 500m window scale, there were no bivariable associations at a p-value < 0.1 in the landscape mosaic tests and at the 1000m window scale, there were no bivariable associations at a p-value < 0.1 in the forest mosaic tests. Higher AIC values indicate a poorer overall model fit. With the multivariable models, at the 1000m diameter window scale, the model had consistently higher AIC values and usually needed more variables to achieve this poorer explanation.

The 250m windows are most likely capturing the effects of landscape on one population interaction scale of rodents, whereas the 2000m windows may be capturing the effects of landscape on another scale of rodent population interactions. The 500m and 1000m windows would then be at or near the transition scale between these two population scales and in that case would be expected to be less meaningful. While the 250m and 2000m windows are at the extremes of the scales measured here, they are by no means the extremes of potential rodent interaction, but rather are two important scales of community organizational levels. Finding both scales of interaction and transition zones is important, so that future research efforts do not try to measure activity at a transition scale, which often yields unreliable results (Turner et al., 2000). Further study would need to be done to know with certainty what population levels are being captured at the 250m and 2000m scales. This further study would need to look specifically at movement within the *A. montensis* community and also include genetic tests to see if the 2000m scale is a metapopulation scale or simply a larger population interaction scale. The remainder of the results that will be shown and discussed will focus on the 250m and 2000m windows.

Table 5.5. Some of the factors that show the relatively improved association of landscape metrics and presence of seropositive rodents at the 250m and 2000m window sizes.

	250 m	500 m	1000 m	2000 m
Number of bivariable associations	36	14	8	27
Average AIC	38	35	46	36

5.3.4. Metrics From the Entire Landscape Mosaic (LULC Independent)

With the overall landscape mosaic patterns metrics (Table 5.6), there is an association between the nearest neighbor metric and seroprevalence. In the landscape mosaic calculations, nearest neighbor measures the separation between patches of the same type. In the 250m windows, increasing nearest neighbor values are associated with seroprevalence. This suggests that rodents can move between patches that are 100-200m from each other well enough to maintain enough of a population to have seropositive *A. montensis*. In the 2000m window, there is also an association with nearest neighbor (range), in this case indicating that diversity in distances between patches is associated with seroprevalence. Additionally, two shape metrics, perimeter-area ratio and fractal dimension, are added to the associations with seroprevalence. Both the perimeter-area ratio and fractal dimensions are measures of shape complexity. Both are negatively associated with seroprevalence, which means that less complex shapes in the overall landscape are associated with increased seroprevalence. In the minifundia system at the 2000m windows a higher number of patches with less complex shapes are expected. This indicates that this land use system may have more potential for seroprevalent small mammal communities. The perimeter-area ratio is significantly associated in both the bivariable and multivariable tests (Table 5.6), with a stronger association in the multivariable test. The fractal dimension is only significantly associated in the multivariable test. These three metrics (nearest neighbor, perimeter-area ratio, and fractal dimension) were the only components for the multivariable model for the 2000m window landscape mosaic test, the significance of all associations was improved in the multivariable model over the bivariable test, which suggests there are some issues with confounding that appear to be accounted for in the multivariable model.

Table 5.6. Selected metrics from the Landscape mosain tests.

		Bivariable		Multivariable	
Window size	Metric	Odds Ratio	p-value	Odds Ratio	p-value
250 m	Nearest neighbor (median)	1.0264	0.0060	1.0754	0.0300
2000 m	Perimeter-area ratio (SD)	0.9925	0.0476	0.9853	0.0136
	Nearest neighbor (range)	1.0019	0.0522	1.0034	0.0108
	Fractal dimension (mean)	<0.0001	0.1940	<0.0001	0.0896

5.3.5. Metrics from the Forest Mosaic

Metrics from the forest mosaic give the clearest links between LULC patterns and seroprevalence. Presence of forest was associated with the presence of *A. montensis*, but not seroprevalence (Section 5.3.2). In the results presented here, (Table 5.7) the amount of forest present, as measured by area, was associated with seroprevalence.

There were some associations between the amount of forest and the presence of seropositive rodents within some of the smaller window sizes. In the 250m windows, landscapes with at least 1 hectare of forest were positively associated with seroprevalence. With the 500m windows landscapes with at least 6 hectares and at 1000m at least 35 hectares of forest were positively associated with seroprevalence. At coarser scales, the area of forest present become less significantly associated with seroprevalence. In the 250m windows the number of patches of forest was also significantly positively associated with seroprevalence. Combining the forest area and number of forest patches says that, within 250m windows, *A. montensis* populations are mixed and can move between forest patches enough to have the type of community connectivity needed to be positively associated with seroprevalence. In other words, rodents travel between patches that are closer than 100-200m from each other, and when there is enough forest they can sustain a population that is large enough to maintain the presence of the virus, even when that forest is highly fragmented.

The multivariable model for forest (Table 5.8) in the 250m window that best explained the presence of seropositive rodents contained just two metrics as independent variables. Those two metrics were the total area of forest and the coefficient of variation (CV) of the forest patch areas, both were positively associated with seroprevalence. This supports the basic composition discussed in the previous paragraph, increasing total forest area in a 250m window was associated with the presence of seropositive rodents. The inclusion of CV of patch area in the multivariable models indicates that one or some of those patches should be relatively large. However, just having a large patch does not appear to be enough, because the average size is not associated with seroprevalence, there appears to be some interaction between a diversity of patch sizes that plays a role. This modifies the picture from the last paragraph by adding the stipulation that there should be one relatively large fragment, probably to give a suitable large habitat patch for the rodent population and the maintenance of hantavirus in that large fragment would then allow for seropositive rodents to exist in all nearby fragments.

The AIC score for the multivariable model for forest in the 2000m window was the lowest (strongest association) among all multivariable models tested. This model result shows that, while the number of patches is associated bivariably with presence of seropositive *A. montensis*, when several other variables (core area index, core area, proximity, circle, and gyration) were accounted for, the number of patches is significant. More patches associated with presence of seropositive rodents at this scale indicates that *A. montensis* populations are connected at this scale.

Table 5.7. Forest composition metrics that were tested using logistic regression. The columns labeled +/- indicate the direction of the relationship, positive indicates an increase in the metric is associated with the presence of seropositive *A. montensis*.

	250 m		500 m		1000 m		2000 m	
Metric	Odds Ratio	p-value	Odds Ratio	p-value	Odds Ratio	p-value	Odds Ratio	p-value
Area	1.40	0.0588	1.09	0.0594	1.02	0.1450	1.00	0.2826
Mean Patch Area	1.27	0.1370	1.07	0.1080	1.01	0.3890	0.99	0.5270
Number Patches	12.14	0.0045	1.49	0.3774	1.32	0.2751	1.28	0.0437
Core Area	1.02	0.0874	1.02	0.0769	1.02	0.1680	1.01	0.3762

Table 5.8. Selected metrics from the Forest mosaic tests.

		Bivariable		Multivariable	
Window size	Metric type	Odds Ratio	p-value	Odds Ratio	p-value
250 m	% landscape	1.0231	0.0874	1.0416	0.0455
	Area (CV)	1.0398	0.0103	1.0551	0.0065
2000 m	Number patches	1.2755	0.0437	13.451	0.0098
	Core Area Index (mean)	0.9962	0.8620	1.5715	0.0190
	Core Area (SD)	1.0079	0.3520	1.1635	0.0147
	Proximity (median)	1.0007	0.4380	0.9945	0.1007
	Circle (median)	0.4272	0.8310	>1000	0.0429
	Gyration (range)	1.0027	0.1929	1.0251	0.0200

5.3.6. Metrics from the Minifundia Mosaic

With the minifundia LULC type, the distribution of metrics (rather than the mean) was associated with seroprevalence (Table 5.9). Distributions (coefficient of variation and standard deviation) were positively associated with seroprevalence, which means that more diverse arrangements of minifundia LULC types was associated with a higher rodent seroprevalence.

The coefficient of variation of shape and core area were positively associated with seroprevalence in *A. montensis* in both the 250m windows and the 2000m windows. Individual farm sizes are around 10-20ha, which is between the 250m and 500m window sizes. A diversity in size and shape of minifundia

LULC type at the scale of an individual farm is thus associated with seroprevalence in *A. montensis*. The 2000m window size would include several farms within it, the association with these two metrics at this scale says that the way a minifundia community is arranged can play a role seroprevalence in those minifundia communities. Combined with relatively high human population levels in these communities, there is likely a high risk for human exposure to hantavirus in the minifundia communities. Further study should be able to elucidate whether changing the way that forest fragments are arranged could alleviate this potential problem.

Table 5.9. Selected metrics from the Minifundia mosaic tests.

		Bivariable		Multivariable	
Window size	Metric	Odds Ratio	p-value	Odds Ratio	p-value
250 m	Shape (CV)	1.0907	0.1020		
	Core Area (CV)	1.0240	0.0444	1.1172	0.0507
	Fractal Dimension (SD)	>1000	0.0518	>1000	0.0815
2000 m	Shape (CV)	1.0390	0.0852		
	Core Area (CV)	1.0076	0.0555		
	Nearest Neighbor (CV)	1.0211	0.0762	1.1060	0.0548

5.4. Conclusion

Many aspects of landscape patterns are important to hantavirus seroprevalence in small mammal communities in Eastern Paraguay. Patterns of

LULC patches are associated with seroprevalence, especially patterns associated with anthropogenic disturbance, like highly fragmented forest patches, and smooth edged patch shapes. These patterns of LULC patches are most apparent when viewed using 250m windows and 2000m windows, which likely correspond to different *A. montensis* population interaction scales. In the overall landscape, patches of the same type that are within 100-200m of each other are sufficient to have seropositive rodents in the landscape. At the larger (2000m window) scale nearness of patches is still associated, but shape metrics are also associated. These shape metrics (perimeter-area ratio and fractal dimension) show that some anthropogenic changes, rectangular patches with relatively straight edges, are important to seroprevalence in *A. montensis*. Metrics from the forest mosaic give the clearest links between LULC patterns and seroprevalence. The presence of forest is associated with *A. montensis* presence, but not hantavirus seroprevalence. However, forest area in the 250m window size is positively associated with seroprevalence in *A. montensis*, especially when it is highly fragmented. Highly fragmented forest patch landscapes were also associated at the 2000m window size, with area being less important. When analyzing the minifundia LULC type, both 250m and 2000m windows show high coefficient of variation of shape and core area was associated with high seroprevalence. In the smaller window size, an additional shape metric (fractal dimension, a measure of shape complexity) is important, highlighting the importance of shape at this scale. In the larger window size, closeness of minifundia patches is an additional important factor in high seroprevalence.

Chapter 6. Forest Disturbance

6.1. Introduction

6.1.1. Review

Some of the indicators associated with human disturbance of the Atlantic Forest ecoregion in Eastern Paraguay are associated with higher *Akodon montensis* populations and with increased seroprevalence levels in rodents in the Atlantic Forest (Goodin et al., 2009, Chapter 5). In this analysis, ways to use spectral information in satellite imagery to better classify some of the types of forest and landscape changes associated with changes in hantavirus prevalence in rodent communities were examined.

The types of forest canopy structure associated with higher *A. montensis* populations include less upper and middle canopy and more dense low vegetation (especially if that vegetation is bamboo). Hantavirus seroprevalence in *A. montensis* was associated with denser upper canopy (Goodin, 2007). On a landscape scale, human disturbed forests are susceptible to high seroprevalence levels in *A. montensis* populations.

Forest classifications tend to be presence/absence type of classifications. In the classification scheme used in Chapter 4, the research attempted to separate undisturbed from disturbed forests. Unfortunately, the different forest types present in the ecosystem are rather complex and separating the forest types was the weakest part of the classification. The object-oriented classification used in Chapter 4 was an improvement over traditional per-pixel classifications. Subsequent analysis has shown that vegetation density is the canopy variable most associated with the presence of hantavirus reservoir species, particularly *A. montensis* (Owen et al., 2010). Additionally, successional stages in forest regeneration (Lu, 2004; Viera, 2003) and forest disturbance from logging (Asner, 2004; Souza et al., 2005) in the Amazon can be detected. Better elucidation of the spectral behavior of detailed aspects of vegetation densities in the Atlantic Forest, especially when that is combined with object-oriented classification, should result in improved classification of forest sub-types in the study region.

6.1.2. Objectives

The purpose of this chapter is to examine ways to classify forest understory densities in satellite imagery. Of particular interest are the types of disturbances that are associated with high *A. montensis* populations or hantavirus seroprevalence in *A. montensis* populations.

6.2. Methods

6.2.1. Data Collection

Vegetation data were collected during July of 2008 and 2009. Sample plots were randomly selected from forested sites in the Mbaracayú Biosphere. Samples were also taken from the trap sites (Sections 3.4.1 and 3.4.2) to help correlate with other existing data) and samples were taken from disturbed and undisturbed forest sites that were misclassified in Koch et al. (2007), as well as from other disturbed and undisturbed forest sites that were correctly classified in Koch et al. (2007). Disturbed sites are forested sites known to have had anthropogenic activity in the last 20 years. Sample plots for disturbed forest were primarily on private property, so permission to enter those sites was obtained from the landowners. When access was denied, an appropriate replacement site was selected, either nearby or one that has similar spectral properties. Sites were primarily within 1km of road or trail access. Data were collected from 148 sites. Data collection is described in Section 3.4.3.

6.2.2. Satellite Data

Cloud free March and June Landsat images from both field years were analyzed, results from the 2008 images are shown below as both years gave very similar results. June images were used because they were near the time of data collection and are a relatively consistently cloud free time of the year. March images were used because March was also relatively cloud free. Additionally, June corresponds with winter, which is a season where some leaf loss from trees has occurred, but the vegetation phenology is relatively stable and March is near the end of the summer and for vegetation is another vegetatively stable time of year.

Landsat imagery was georectified to a root mean square error of less than one pixel with nearest neighbor resampling, using ground control points collected in situ. NDVI and the Tasseled Cap transformations were calculated using ENVI 4.5 (ITT Visual Information Solutions). Responses of vegetation data were compared with the original data bands, NDVI, and Kaufman-Thomas transformation bands.

6.2.3. Statistics

Statistical results were calculated using R version 2.8.0 and the R Commander package (version 1.4-10) (R Development Core Team, 2009). For the Disturbed/Undisturbed and On/Off Reserve data, significance of band response was determined with logistic regression. Continuous data, vertical and horizontal densities, was tested against spectral response using linear regression.

6.3. Results and Discussion

6.3.1. Disturbance and Location On/Off Reserve

Results from the logistic regression tests are shown in Table 6.1. The Disturbed/Undisturbed site classifications led to strong associations with Band 4, NDVI, Brightness, and Greenness in both the March and June images. The On/Off RNBM site classifications were strongly associated with several satellite bands and band transformations, but with few June image bands. None of the sites off of the RNBM were classified as undisturbed, some sites on RNBM were classified as disturbed by those familiar with the past history of the area, these were primarily sites that had been cleared for agriculture or logging prior to the time that RNBM was established and since then were allowed to revert to forest. This suggests that, in March imagery, sites that have had 20 years or more of undisturbed forest growth can be distinguished from sites that have recent disturbance activity.

Table 6.1. Satellite band imagery response to disturbed sites and on/off reserve sites.

	Undisturbed 2008-03-29	Undisturbed 2008-06-17	On Reserve 2008-03-29	On Reserve 2008-06-17
L7-Band 1			*	
L7-Band 2	*		**	
L7-Band 3			**	
L7-Band 4	***	**		.
L7-Band 5	*	.	*	
L7-Band 6a			***	
L7-Band 6b			***	
L7-Band 7			**	
NDVI	*	**	.	
Brightness	***	**		
Greenness	**	**	.	.
Wetness	*		**	
TC4			*	.
TC5				
TC6			.	
For the significance codes: '.' = $p < 0.1$, '*' = $p < 0.05$, '**' = $p < 0.01$, and '***' = $p < 0.001$.				

6.3.2. Vertical Density

In Tables 6.2 and 6.3, are results from logistic regression of vertical density measurements with spectral information. The March image does not show many associations with vertical density, but it does suggest that further study might be able to show middle canopy density using Tasseled Cap transformations. In June, however, there seems to be good potential to distinguish density of the highest canopy. The association was especially strong in the Fourth category of the Tasseled Cap transformation. The existence of this canopy is one of the factors associated with rodent seroprevalence in this forest area. In the images from the region, high values in the Tasseled Cap Fourth (TC4) band are associated with areas that have exposed soil (especially tilled latifundia fields) or areas that are composed of high amounts dead plant matter (grassland areas within the reserve). The TC4 was associated with areas that contain the highest canopy in the June imagery because some of the high canopy trees lose their leaves during this time of year, thus exposing the soil and leaf litter below at this time of year.

6.3.3. Horizontal Density

Horizontal density measurement associations with imagery are shown in Tables 6.4 and 6.5. Here, March imagery shows the stronger relationship and the June imagery does not show many strong relationships. Most of the horizontal density measurements were somewhat associated with Band 4, NDVI, and Greenness. There is one interesting and relatively strong association with the June imagery. The Fourth band in the Tasseled Cap transformation is associated with horizontal density at ground level. This is the same imagery information that is associated with the upper canopy density.

Table 6.2. Satellite band imagery (image date 2008-03-29) response to vertical canopy density.

	Highest Canopy	Middle Canopy	Lower Canopy	Average of all canopies
L7-Band 1		*		
L7-Band 2				
L7-Band 3				
L7-Band 4				
L7-Band 5		*		.
L7-Band 6a				
L7-Band 6b				
L7-Band 7				
NDVI			.	
Brightness				.
Greenness				
Wetness		.		
TC4		*		
TC5		*		*
TC6				

For the significance codes: '.' = $p < 0.1$, '*' = $p < 0.05$, '**' = $p < 0.01$, and '***' = $p < 0.001$.

Table 6.3. Satellite band imagery (image date 2008-06-17) response to vertical canopy density.

	Highest Canopy	Middle Canopy	Lower Canopy	Average of all canopies
L7-Band 1	.			
L7-Band 2	.			*
L7-Band 3	**		*	
L7-Band 4				
L7-Band 5	*			*
L7-Band 6a	*			
L7-Band 6b	*			
L7-Band 7				
NDVI				
Brightness				
Greenness				
Wetness				
TC4	***			***
TC5				
TC6				.
For the significance codes: '.' = $p < 0.1$, '*' = $p < 0.05$, '**' = $p < 0.01$, and '***' = $p < 0.001$.				

The heavy dependence of NDVI and Tasseled Cap Greenness on Band 4 can be seen in the apparently close associations between Band 4, NDVI, and Greenness in Table 6.4. In this image from 2008-03-29, Band 4 and NDVI had a correlation of 0.838, Band 4 and Greenness had a correlation of 0.941, and NDVI and Greenness had a correlation of 0.952. While these correlations are close, they are not perfect and these different bands can pick up subtle, but important, differences in habitat.

Table 6.4. Satellite band imagery (image date 2008-03-29) response to horizontal vegetation density.

	Density at ground level	Density at 0.5 m height	Density at 1.0 m height	Density at 2.0 m height	Average of all densities
L7-Band 1					
L7-Band 2					
L7-Band 3				.	
L7-Band 4	*	.	.		.
L7-Band 5					
L7-Band 6a					
L7-Band 6b					
L7-Band 7					
NDVI	*	.	.	.	*
Brightness					
Greenness	*	.	.		*
Wetness					
TC4					
TC5					
TC6					
For the significance codes: '.' = p < 0.1, '*' = p < 0.05, '**' = p < 0.01, and '***' = p < 0.001.					

Table 6.5. Satellite band imagery (image date 2008-06-17) response to horizontal vegetation density.

	Density at ground level	Density at 0.5 m height	Density at 1.0 m height	Density at 2.0 m height	Average of all densities
L7-Band 1					
L7-Band 2	*				
L7-Band 3	.				
L7-Band 4					
L7-Band 5					
L7-Band 6a					
L7-Band 6b					
L7-Band 7					
NDVI					
Brightness	.				
Greenness					
Wetness					
TC4	**				
TC5					
TC6					
For the significance codes: '.' = $p < 0.1$, '*' = $p < 0.05$, '**' = $p < 0.01$, and '***' = $p < 0.001$.					

6.4. Conclusion

These data show that Landsat imagery can be used to determine some aspects of forest disturbance and understory vegetation density in the Atlantic Forest in the Mbaracayú Biosphere. Specifically, forest areas that have been recently impacted by humans are detectable using the Tasseled Cap Brightness and Greenness bands, NDVI, or ETM+ Band 4. A dense upper canopy, which is a vegetative variable associated with seroprevalence can be mapped using June imagery to deriving the Tasseled Cap Fourth Band. March imagery can be used to detect horizontal density, another factor associated with seroprevalence, using ETM+ Band 4, NDVI, or Tasseled Cap Greenness Band.

Typically, methods for distinguishing forest disturbances focus on comparing deforested areas with forested areas. However, ability to use remotely sensed data to determine understory vegetative factors is an important aspect of the current development of RS data. Studies have shown that some aspects of understory (Eriksson et al., 2006), successional stages in forest regeneration (Lu, 2004; Viera, 2003) and forest disturbance from logging (Asner, 2004; Souza et al., 2005) can be detected in the Amazon. Because understory density (Goodin et al., 2009) and some of the medium levels of anthropogenic change in forests (Chapter 5) are associated with seroprevalence in *A. montensis*, improving forest mapping techniques will be an important tool in better understanding the processes involved with seroprevalence.

Chapter 7. Linkages Between Small Mammal Diversity and Hantavirus Seroprevalence and their Association with Remotely Sensed Vegetation Indices.

7.1. Introduction

7.1.1. Review

The diversity of pathogen hosts and vectors can have an affect on the presence and prevalence of a pathogen on a landscape. Depending on the way the pathogen interacts with various hosts and vectors, diversity on the landscape can reduce or increase the amount of pathogen present (Begon, 2008). Decreases in the prevalence of pathogens in diverse communities can happen through species competition or through a dilution effect (Begon, 2008). Increases in pathogen prevalence can occur through combinatorial effects via multi-species interactions (Begon, 2008).

Diversity of pathogen hosts on a landscape can be measured using a few metrics for species richness, evenness, and diversity (Turner, 2001; Keylock, 2005; Legendre and Legendre, 1998). Richness is a measure of the number of different species present (Turner, 2001). Evenness is used to measure the equality in numbers between the species present (Legendre and Legendre, 1998). Simpson's Index of Species Diversity is a metric that measures the probability of interspecific contact, it is essentially a way of combining richness and evenness into a single metric (Legendre and Legendre, 1998). In some habitats, vegetative diversity or vegetative productivity as measured with NDVI is closely tied to diversity of animal species (St. Louis et al., 2009; Lassau et al., 2005).

The dilution effect is a model that explains how changes in species diversity may affect disease transmission (Ostfeld and Keesing, 2000; LoGiudice et al., 2003; Keesing et al., 2006; Clay et al., 2009). Generally the change explained by the dilution effect is that communities with high biodiversity will have lower levels of a particular pathogen, but the effect may also increase pathogens (Keesing et al., 2006). The dilution effect reduces pathogen prevalence by reducing the amount of intraspecific contacts, when those reduced intraspecific contacts occur among the most competent hosts, then pathogen loads are reduced (LoGiudice et al., 2003). One of the clearest examples is with Lyme disease. The white-footed mouse (Peromyscus leucopus) is an especially good host for the pathogen that causes Lyme disease, when habitat is fragmented and the number of other mammals is reduced, the ticks that can carry the pathogen are more likely to have the pathogen, and tick encounters with humans are more likely to result in Lyme disease. Regarding hantavirus, a study conducted with *Peromyscus maniculatus*, the host for SNV, showed that a more diverse small mammal system reduced the number of intraspecific contacts, which reduced the prevalence of seropostivie rodents within a natural host-pathogen system (Clay et al., 2009).

7.1.2. Objectives

The objectives of this chapter are to analyze the effects of small mammal community diversity on *Akodon montensis* and the presence of hantavirus in *A. montensis* communities, and to evaluate the use of satellite-derived data (see Chapter 6) to help evaluate small mammal community biodiversity and the potential presence of hantavirus on the landscape. Presence of hantavirus in *A. montensis* communities is represented by testing for individuals that are seropositive from prior hantavirus exposure. Biodiversity is measured by calculating diversity metrics on the rodent species caught in trapping sessions and rodent species caught at trap sites.

7.2. Materials and Methods

7.2.1. Trapping Data

The trapping data used here is from the Mark/Recapture trapping data (see 3.2.1), which consists of information from 53 different trapping sessions conducted over a course of two and a half years (February, 2005 to November, 2007) in 10 different locations (see Figure 3.4 and Table 3.1). This analysis herein looks at the relationship between diversity metrics and serostatus in two different ways. In one, the trapping sessions are used as the sample sets, these results are shown in Tables 7.1 and 7.2. Two of the available sessions were eliminated because there were no rodents trapped during the session (and thus have no diversity and no chance of presence of hantavirus seropositive rodents). The remaining 51 sessions were used in this analysis.

In the second type of analysis, the data for each Mark/Recapture grid is pooled together and diversity metrics from those 10 sites are compared to serostatus. This arrangement also allowed for a comparison of remotely sensed data to the diversity metrics and in extension allows for a test of seroprevalence against remotely sensed data. These data were arranged by trap site (instead of trap session). The total counts of each species by trap site can be seen in Appendix B, this trap site data is a sum of the species caught during a number of trapping sessions at each Mark/Recapture site (R3A & B – 10 sessions, JJA & B – 8 sessions, MAA & B – 2 sessions, HMA & B – 5 sessions, BKA & B – 2 sessions).

7.2.2. Diversity Indices

Species diversity was measured using three indices: species richness (Turner, 2001), species evenness (Keylock, 2005), and Simpson's Index of Species Diversity (Keylock, 2005). Species richness is a count of the number of species present in a certain landscape, sometimes expressed as a percentage or as a raw count (both yield identical relationships). Here, evenness is calculated as a count of the number of different species caught during an individual trapping session. Evenness was calculated using the 'asbio' package (version 0.3-24) in R version 2.11.1 (R Development Core Team, 2010). The 'asbio' package calculates evenness using the Pielou's measure of species evenness

$$J = H'/\ln(S),$$

where H' is Shannon Weiner diversity and S is the total number of species in a sample). Simpson's Index of Species Diversity (1-D) was calculated using the 'vegan' package (version 1.17-4) in R version 2.11.1 (R Development Core Team, 2010). In the 'vegan' package

$$D = \Sigma \text{ pi2},$$

where pi is the proportional abundance of species i. Once 1-D is calculated, Simpson's Index of Species Diversity results in a number that is between 0 and 1, where lower numbers mean lower diversity and higher numbers mean higher diversity.

7.2.3. Statistical Analysis

A combination of linear and logistic regressions were used to test the relationship between rodent diversity and the *A. montensis* community and the seroprevalence in that community. Logistic regression was used in cases where the response variable (seroprevalence or presence *A. montensis*) was binary and the predictor variable (i.e. diversity metric) was continuous. Linear regression was used in cases where both the response variable (count or prevalence of rodents or seroprevalence) and the predictor variable (diversity metric) were continuous. The trapping grids are assumed to be spatially independent, as home range sizes for *A. montensis* are on the order of 1600 m^2 (40 x 40 m) or less (Owen et al., 2010), there was a small amount of temporal overlap at the same site for subsequent trapping sessions. Relating spectral information about the trap sites with diversity and with seroprevalence was done using linear regression. All regressions were run using R version 2.11.1 (R Development Core Team, 2010) and the R Commander package (version 1.6-0).

7.2.4. NDVI Calculation

NDVI was calculated from a Landsat ETM+ image from path 225/row 77 that was acquired on 2008-03-29, using the ENVI software package (v. 4.2, ITTVIS). Means and standard deviations of NDVI were obtained from a 5 pixel x 5 pixel window centered over the trap sites used in this analysis.

7.3 Results and Discussion

7.3.1. Logistic Regression on Trap Session Presence/Absence Data.

This test included 51 trapping sessions and compares the presence of seropositive *A. montensis* and the presence of *A. montensis* in a trapping session to diversity metrics. The presence of seropositive rodents in a particular trap session was related to a small mammal community that has a higher count of species, but displayed less diversity (Table 7.1). The positive association of richness and presence of *A. montensis* seroprevalence is likely due to the high population of *A. montensis* that occurs in the same type of trap session (see Table 7.2). The association between low diversity and higher likelihood of presence of seropositive rodents is the same as is seen in the rest of the results herein.

Table 7.1. Results from comparison of presence of *A. montensis* and seropositive *A. montensis* against small mammal diversity. Estimates are shown for associations with a p-value < 0.1.

	Richness		Evenness		Simpson's Index of Diversity	
	Estimate	p-value	Estimate	p-value	Estimate	p-value
Presence of S+ AKMO	0.329	0.063	--	0.241	-2.277	0.067
Presence of AKMO	--	0.134	--	0.813	--	0.441
(AKMO – *Akodon montensis*; S+ – seropositive)						

7.3.2. Linear Regression on Trap Session Presence/Absence Data.

This test included 51 trapping sessions and compares the seroprevalence of the entire rodent community, the seroprevalence of *A. montensis*, and the *A. montensis* population size in a trapping session to diversity metrics. Results from the analysis of seroprevalence and number of seropositive *A. montensis* (Table 7.2) show that the seroprevalence in a particular trap session is related to low small mammal community diversity. The number of *A. montensis* has the opposite relationship, there is a high population where there is also a rich small mammal community.

These results together show that *A. montensis* sustains better populations in situations where many other species of rodents also find favorable conditions. When the rodent community is less even in population or less diverse, then the population of *A. montensis* shows higher seroprevalence. Environments that may be somewhat less favorable for rodents and small mammals in general (i.e. support fewer other rodent species, and in lower numbers) are also associated with greater hantavirus seroprevalence in *A. montensis* communities. Combined with the results from presence data in Table 7.1, there is a higher potential for seeing at least the presence of seropositive *A. montensis* in rodent species rich communities, but the population is high and the number of seropositive *A. montensis* are low leading to a very low seroprevalence in rich communities. Imbalance in rodent communities is associated with high numbers of seropositive in *A. montensis* communities.

Table 7.2. Results from comparison of seroprevalence, number of seropositive *A. montensis* and number of *A. montensis* against small mammal diversity. Estimates are shown for correlations with a p-value < 0.1.

	Richness		Evenness		Simpson's Index of Diversity	
	Estimate	p-value	Estimate	p-value	Estimate	p-value
S+ prevalence	--	0.692	--	0.210	-0.151	0.083
Number of S+ AKMO	--	0.822	-9.821	0.038	-7.380	0.012
Number of AKMO	6.367	0.019	--	0.368	--	0.160
(AKMO – *Akodon montensis*; S+ – seropositive)						

7.3.3. Comparison by trap site (diversity metrics v. seroprevalence)

This test included only the 10 Mark/Recapture grid locations as samples, the total counts of small mammals were pooled over all trapping sessions for each trap site. Comparison of sites (as opposed to individual trap sessions), to seroprevalence (Table 7.3 and Figure 7.1) was significantly associated with evenness and Simpson's Index of Species Diversity. Both of these relationships were negative, indicating again that low small mammal community diversity is correlated with seroprevalence.

7.3.4. Comparison by trap site (NDVI v. diversity metrics and seroprevalence)

This arrangement of trap information by trap site allowed me to investigate the association between vegetative indices (NDVI) and rodent diversity and seroprevalence. Mean NDVI signal was somewhat negatively associated with evenness and Simpsons Index of Diversity. This says that less vegetation, as represented with lower NDVI, is associated with the types of small mammal community diversity that is associated with high seroprevalence. Seroprevalence was also somewhat associated with mean NDVI, although not significantly in this analysis.

The standard deviation of NDVI for these trap sites was significantly negatively associated with richness in rodent communities. Diverse vegetative communities (as measured with NDVI) are associated with low richness in rodent communities. The sites that showed the highest NDVI standard deviation here are sites that were situated at habitat ecotones, these sites also had low richness in their small mammal species numbers. It has been shown in other tests (Table 7.1) that diverse rodent communities tend to have low seroprevalence. The data also suggest that more thorough (more trap sites) tests of mean NDVI against small mammal communities would show that mean NDVI (weaker vegetative signal) is negatively associated with evenness and small mammal diversity. These are characteristics that were associated with more seroprevalence.

Table 7.3. Comparisons between NDVI, *A. montensis* seroprevalence, and rodent biodiversity. Estimates are shown for associations with a p-value < 0.26.

	Richness		Evenness		Simpsons Index of Diversity		NDVI (mean		NDVI (SD)	
	Est.	p-value	Est.	p-value	Est.	p-value	Est.	p-value	Est.	p-value
S+	--	0.90	-0.27	0.06	-0.18	0.08	0.60	0.16	--	0.51
NDVI mean	--	0.54	-0.15	0.24	-0.10	0.25				0.59
NDVI SD	-0.01	0.07	--	0.95	--	0.86				
(S+ – seropositive; Est. – Estimate)										

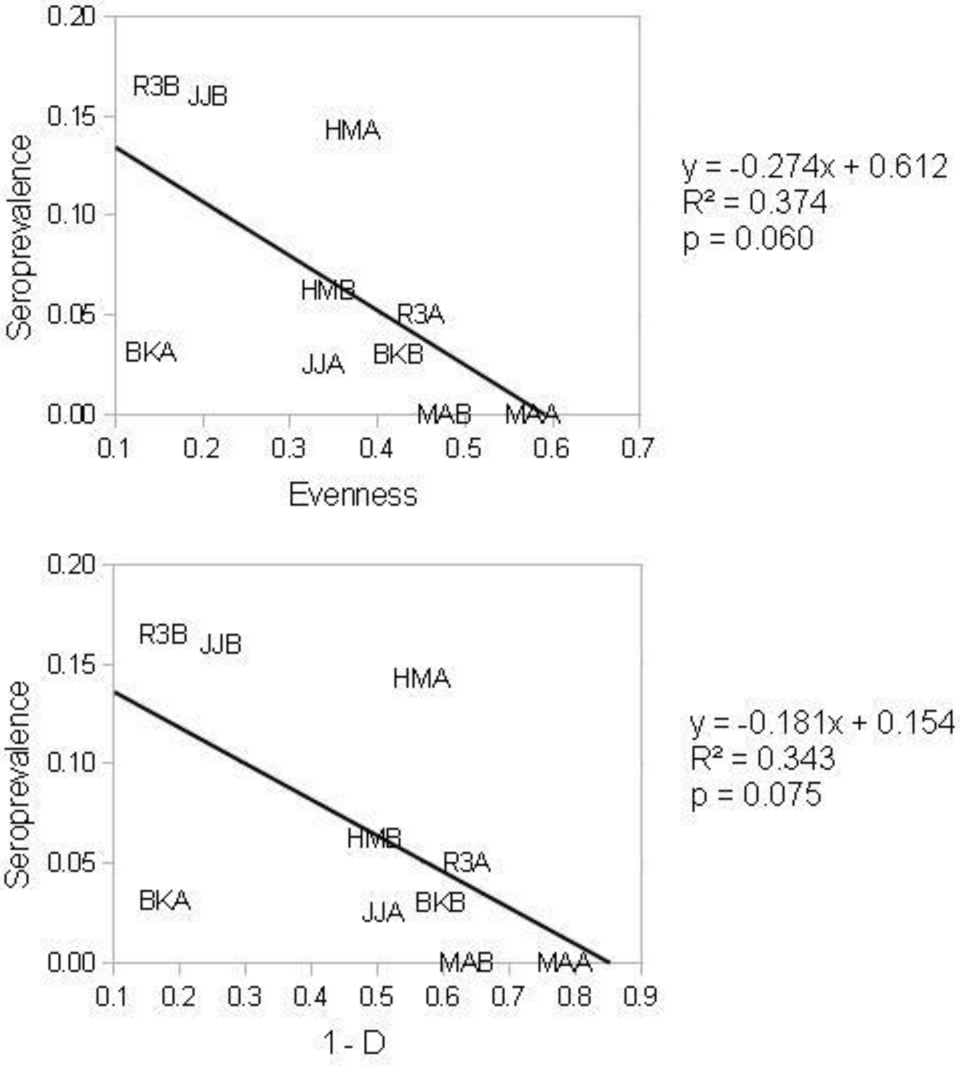

Figure 7.1. Plots of ten trap sites showing comparisons between *A. montensis* seroprevalence, and rodent biodiversity metrics.

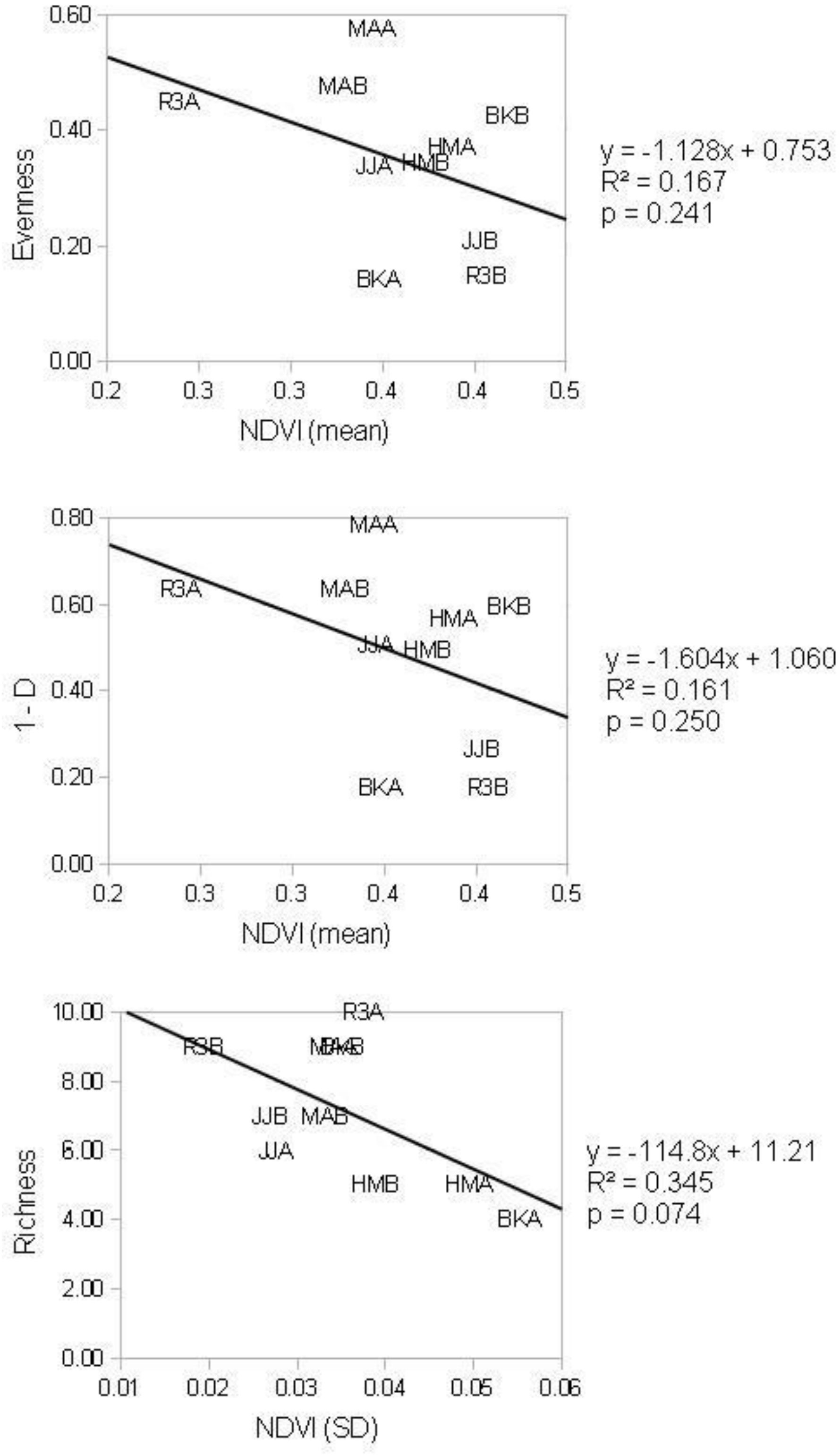

Figure 7.2. Plots of ten trap sites showing comparisons between NDVI and rodent biodiversity metrics.

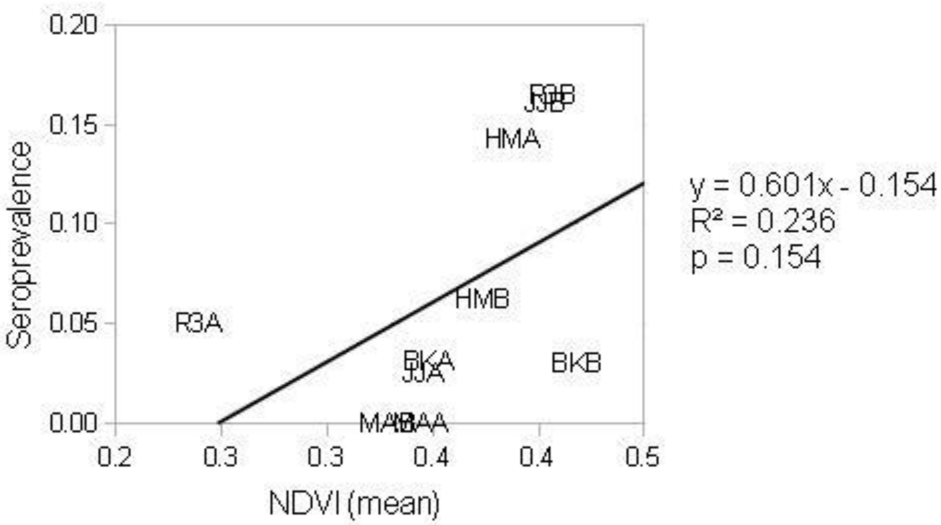

Figure 7.3. Plot of ten trap sites showing comparisons between NDVI and rodent seroprevalence.

7.4. Conclusion

Hantavirus seroprevalence in *A. montensis* is associated with low diversity in small mammal communities. This trend is apparent both when measured for trap sites and when including temporal changes in those trap sites. In addition, *A. montensis* communities have their highest populations when embedded in rich small mammal communities. The high population *A. montensis* communities are not associated with high seroprevalence, although they are associated with presence of seropositive *A. montensis*, likely at low seroprevalence.

These data suggest that *A. montensis* thrives in a type of habitat where many other rodents also do well. *A. montensis* is a habitat generalist (Owen et al., 2010; Umetsu and Pardini, 2007), so it follows that habitat that is good for more rodent species would also support larger populations of *A. montensis*. On the other hand, when the overall rodent community was less even or less diverse, seroprevalence in the *A. montensis* community was greater. This further suggests that habitat type plays a role in hantavirus seroprevalence in the *A. montensis* community.

Relationships between habitat and seroprevalence in general (McIntyre et al., 2005; Mackelprang et al., 2001; Lehmer et al., 2008; Suzan et al., 2006) and habitat suitability and increased hantaviral seroprevalence specifically (Goodin et al., 2009) have been noted previously. Dilution effects (Begon, 2008; LoGiudice et al., 2003) are one possible causal mechanism for lower seroprevalence in habitats that support more diversity. In habitats with diverse and high small mammal populations, encounters between a potential viral shedder (that is, an individual of *A. montensis* that is actively infected and capable of infecting others) would be less frequent compared to less diverse habitats, simply because in a diverse habitat, encounters between competent reservoir hosts would be less probable. In other words, 'dilution' of the potential host community reduces intra-specific encounters among *A. montensis*. Habitat quality might also affect seroprevalence indirectly, via the host physiology. Ecosystem stress might lead to physiological stress, which would increase susceptibility to pathogen exposure. In this case, differences in seroprevalence appear in different habitats, but the differences could also be temporal in nature, such as a population explosion during exceptionally good conditions, followed by too high of a population competing for limited resources later (Yahnke et al., 2001; Engelthaler, 1999) or by typical seasonal fluctuations (Calisher et al., 2007), or by changes to habitat (Mackelprang et al., 2001, Lehmer et al., 2008; Suzan et al., 2006). Regardless of the cause, understanding that changes to habitat (temporal and spatial) play a role in seroprevalence in *A. montensis*, and more generally can play a role in pathogen maintenance in various animal communities can allow humans and human communities to make more informed land use decisions.

Chapter 8. Summary and Conclusions

Many aspects of landscape patterns are important to hantavirus seroprevalence in small mammal communities in Eastern Paraguay. Patterns of LULC patches are associated with seroprevalence, especially some patterns associated with anthropogenic disturbance. These patterns of LULC patches are most apparent when viewed using 250m windows and 2000m windows, which likely correspond to different *A. montensis* population interaction scales. In the overall landscape, patches of the same type that are within 100-200m of each other are sufficient to have seropositive rodents in the landscape. At the larger (2000m window) scale, proximity of patches is still associated with seroprevalence, but shape metrics are also associated with seroprevalence. These shape metrics (perimeter-area ratio and fractal dimension) show that some anthropogenic changes, rectangular patches with relatively straight edges, are important to seroprevalence in *A. montensis*. Metrics from the forest mosaic give the clearest links between LULC patterns and seroprevalence. The presence of forest is associated with *A. montensis* presence, but not seroprevalence. However, forest area in the 250m window size is positively associated with seroprevalence in *A. montensis*, especially when it is highly fragmented. Highly fragmented forest patch landscapes were also associated at the 2000m window size, with area being less important. Some smaller scale patterns are also associated with seroprevalence in *A. montensis*. Vegetative diversity (shown using NDVI) is associated with rodent richness, which is consequently associated with high populations of *A. montensis* and low seroprevalence.

Several of the aspects of landscape patterns important to hantavirus seroprevalence can be studied using satellite-derived data. The LULC classification map used to analyze associations between LULC patterns and seroprevalence is an obvious way that satellite data can be used to analyze association between patterns and seroprevalence. Remotely sensed data can also be used to determine understory density variables through the use of satellite imagery. Horizontal and vertical density in the understory has been found to be associated with the presence of hantavirus in *A. montensis* (Goodin et al., 2009). Vertical and horizontal density measurements were correlated with NDVI and the Fourth band in the Tasseled Cap transformation. Seroprevalence in *A. montensis* is associated with low diversity in small mammal communities. Low small mammal community diversity is somewhat associated with mean NDVI at trap sites. High standard deviations in NDVI data is associated with areas that have high rodent richness, which is associated with high *A. montensis* populations, but low seroprevalence.

The next step in the landscape pattern research might be to divide the forest areas into different forest types to further analyze the importance of forest pattern metrics on seroprevalence and *A. montensis* populations. Additionally, habitat classification of the Megagrid site could be done using finer scale (QuickBird. 0.6m) and coarser scale (MODIS, 250m) imagery. These classifications can then be used to look at different scales of pattern associations with seroprevalence. Differences in the results from different seasons in the forest density research suggest that phenological signals could be an important variable to include when classifying forest types in the Interior Atlantic Forest in Eastern Paraguay. Many of the variables used to test against seroprevalence had variance as an important part of the relationship; this indicates potential utility for more use of spatial aspects in remotely sensed data analysis. These spatial aspects include refining the use of object-oriented classification techniques and use of texture metrics. Better understanding of individual animal movements is another potential avenue of research and could be accomplished through further Mark/Recapture studies or through telemetry studies. This line of research would help determine what population organizational levels are being measured at the 250m and 2000m window landscape grains.   Finally, more detailed vegetative community and pattern classifications should help clarify the link between small mammal diversity and *A. montensis*; these detailed vegetative community and pattern classifications can be extended to include all other species present at the RNBM.

This research mostly relied on Landsat for its remotely sensed data source. Other satellites that are in a similar data grain scale that could also be used for this research include CBERS (China-Brazil remote sensing satellite), SPOT, and ASTER. At a finer grain size, QuickBird could be used, these data would allow for finer details of the patterns to be measured and would also allow for an extension of the pattern metrics research into smaller scales, possibly even individual *A. montensis* habitats. The research herein is the beginning of the pieces needed to create risk maps of hantavirus based on aspects of the landscape. At this point, the mapped risk zones would only be useful to guide further research, but some fine tuning of these results with more details would make a more broadly useful risk map.

The results of this dissertation contribute to landscape epidemiology through its application of landscape ecology and remotely sensed data analysis techniques to seroprevalence of hantavirus in *A. montensis* in the Interior Atlantic Forest region in Eastern Paraguay. Landscape pattern analysis has been previously applied to hantavirus research (Langlois et al., 2001; Suzan et al., 2006), this research extends this previous research through application of a broader selection of landscape metrics and consideration of a wider range of habitat types. This dissertation also adds to remotely sensed data analysis through the applications of remotely sensed data to landscape analysis, through improving detection of subtle habitat differences within a forest, and through better understanding of LULC dynamics in Paraguay. Better understanding of the spectral reflectance properties of Atlantic Forest and how those properties may be used to map vegetation density beneath the forest canopy, combined with object-oriented classification, will result in improved classification of forest sub-types in the study region.

The interactions between hantavirus and its rodent reservoir are complex in Paraguay. This research adds to the understanding of this system, which will lead to better understanding of broader hantavirus dynamics. These types of improvements in understanding will eventually allow for improved ability to predict risks of hantavirus exposure to human populations.

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Appendix A. Bivariable and Multivariable associations

A.1 Bivariable landscape metric associations

All bivariable associations between presence of seropositive rodents and landscape metrics that are significant with a $p < 0.1$ are shown in Tables A.1-A.3.

Table A.1. Results from bivariable tests on association between hantavirus serostatus and landscape mosaic metrics. All metrics with $p < 0.1$ were selected.

Landscape mosaic window size	Metric	p-value
250 m	Proximity (CV) Nearest neighbor (mean) Nearest neighbor (median)	0.0379 0.0072 0.0060
500 m	none	--
1000 m	Gyrate (SD) Proximity (CV) Nearest neighbor (range) Nearest neighbor (SD) PR	0.0928 0.0708 0.0697 0.0920 0.0870
2000 m	Perimeter-area ratio (mean) Perimeter-area ratio (range) Perimeter-area ratio (SD) Perimeter-area ratio (CV) Contiguity (mean) Contiguity (range) Contiguity (SD) Contiguity (CV) Nearest neighbor (range) Nearest neighbor (SD)	0.0908 0.0944 0.0476 0.0477 0.0814 0.0935 0.0492 0.0470 0.0522 0.0779

Table A.2. Results from bivariable tests on association between hantavirus serostatus and forest patch mosaic metrics. All metrics with $p < 0.1$ were selected.

Forest patch window size	Metric	p-value
250 m[1]	CA PLAND NP LPI LSI Area (median) Gyrate (median) Fractal dimension (SD) Fractal dimension (CV) CPLAND NDCA Core area index (median) Proximity (mean) Proximity (median)	0.0587 0.0588 0.0045 0.0864 0.0798 0.0971 0.0792 0.0535 0.0718 0.0874 0.0326 0.0751 0.0757 0.0757
500 m	CA PLAND Area (median) Gyrate (mean) Gyrate (median) CPLAND Core area index (mean) Core area index (median) Proximity (median)	0.0594 0.0594 0.0886 0.0770 0.0622 0.0796 0.0442 0.0385 0.0968
1000 m	none	
2000 m	NP Area (CV) Circle (range) NDCA Core (range) Core (CV) Proximity (range) Proximity (SD)	0.0437 0.0621 0.0822 0.0784 0.0995 0.0592 0.0474 0.0649

[1] Metrics confounded by number of patches were eliminated from the forest 250m window, these included metrics where standard deviation, range, and coefficient of variation were all associated. The metrics deleted were: Area, Gyrate, Shape, Perimeter-area ratio, Circle, Contiguity, Core, and Core Area Index.

Table A.3. Results from bivariable tests on association between hantavirus serostatus and minifundia patch mosaic metrics. All metrics with $p < 0.1$ were selected.

Minifundia patch window size	Metric	p-value
250 m	Shape (SD)	0.0969
	Shape (CV)	0.1020
	Fractal dimension (range)	0.0823
	Fractal dimension (SD)	0.0518
	Fractal dimension (CV)	0.0583
	Circle (range)	0.0729
	Circle (SD)	0.0466
	Circle (CV)	0.0455
	DCORE (CV)	0.0444
500 m	Core area index (CV)	0.0900
1000 m	Gyrate (mean)	0.0746
2000 m	Gyrate (median)	0.1030
	Gyrate (range)	0.0965
	Shape (CV)	0.0852
	Circle (CV)	0.1027
	DCORE (range)	0.0903
	DCORE (CV)	0.0555
	Nearest neighbor (CV)	0.0762

A.2 Multivariable landscape metric associations

Full models for multivariable associations between presence of seropositive rodents and landscape metrics are shown in Tables A.4-A.6.

Table A.4. Results from the multivariable test on association between hantavirus serostatus and landscape mosaic metrics. The combination of variables that gave the lowest AIC scores as determined using a backward stepwise logistic regression.

Landscape mosaic window size	Metric	Relationship direction	p-value
250 m	Area (range)	-	0.0795
	Nearest neighbor (median)	+	0.0333
	Shape (CV)	+	0.0898
	Split	-	0.0903
500 m	DCORE (range)	+	0.0138
	DCORE (mean)	+	0.0135
	Shape (CV)	+	0.0217
	Shape (range)	+	0.0172
	Shape (Standard Deviation)	-	0.0340
	Core (CV)	-	0.0148
1000 m	Area (CV)	+	0.0411
	Area (SD)	-	0.0565
	Core area index (SD)	+	0.0582
	Connect	+	0.0399
	Contiguity (CV)	-	0.0589
	DCORE (SD)	-	0.2323
	Nearest neighbor (mean)	-	0.1583
	Perimeter-area ratio (mean)	+	0.0611
	PR	-	0.0347
	Proximity (mean)	-	0.0736
	Proximity (CV)	-	0.0488
	Proximity (SD)	+	0.0882
	Shape (median)	+	0.0464
2000 m	Nearest neighbor (range)	+	0.0108
	Fractal dimension (mean)	-	0.0896
	Perimeter-area ratio (SD)	-	0.0136

Table A.5. Results from the multivariable test on association between hantavirus serostatus and forest patch mosaic metrics. The combination of variables that gave the lowest AIC scores as determined using a backward stepwise logistic regression.

Forest patch window size	Metric	Relationship direction	p-value
250 m	Area (CV)	+	0.0065
	CPLAND	+	0.0455
500 m	Perimeter-area ratio (median)	-	0.0396
	Proximity (SD)	-	0.0374
	Proximity (CV)	+	0.0394
	CA	+	0.0378
	Contiguity (mean)	+	0.0524
	Core area (median)	-	0.0336
1000 m	Area (mean)	+	0.1197
	Area (median)	+	0.0460
	Core area index (mean)	+	0.0485
	Gyrate (mean)	-	0.0330
	Mesh	-	0.0990
	NLSI	+	0.0901
	Proximity (median)	-	0.0847
	Proximity (mean)	+	0.0429
	TE	+	0.0221
2000 m	Core area index (mean)	+	0.0190
	Circle (median)	+	0.0429
	DCORE (SD)	+	0.0147
	Gyrate (range)	+	0.0230
	NP	+	0.0098
	Proximity (median)	-	0.1007

Table A.6. Results from the multivariable test on association between hantavirus serostatus and minifundia patch mosaic metrics. The combination of variables that gave the lowest AIC scores as determined using a backward stepwise logistic regression.

Minifundia patch window size	Metric	Relationship direction	p-value
250 m	DCORE (CV)	+	0.0507
	Fractal dimension (SD)	+	0.0485
	CPLAND	-	0.0808
	Division	-	0.3608
500 m	Area (median)	-	0.2191
	Core area index (range)	+	0.0752
	Fractal dimension (SD)	+	0.0815
	Gyrate (SD)	-	0.0811
	Gyrate (CV)	-	0.1134
	LSI	+	0.0804
	NP	-	0.0817
	Perimeter-area ratio (median)	-	0.0850
	TE	-	0.0836
1000 m	Contiguity (mean)	+	0.1855
	Core (median)	-	0.0788
	Fractal dimension (median)	-	0.1495
	Gyrate (median)	+	0.0354
	IJI	-	0.0667
2000 m	Circle (mean)	+	0.0977
	DCORE (median)	+	0.0321
	Nearest neighbor (CV)	+	0.0548
	Gyrate (mean)	-	0.0453
	Gyrate (range)	+	0.0415
	IJI	-	0.0549
	Perimeter-area ratio (mean)	-	0.0260
	Proximity (SD)	-	0.0788

Appendix B. Mark/Recapture species counts

The total counts of each species by trap site. These counts are the totals collected over all trapping sessions conducted at each site. Each table contains a different set of species.

Table B.1. Trapping data for each Mark/Recapture trap site and seven species of nineteen species captured.

Trap Site	AKMO	AKSP	CACA	CSPP	MDOM	NELA	OXDE
R3A	30	0	14	7	0	209	115
R3B	611	1	7	0	0	0	0
JMA	162	0	9	0	0	0	0
JMB	176	0	1	0	0	0	0
MAA	30	0	0	0	0	0	0
MAB	33	0	0	0	0	0	0
HMA	29	1	25	0	0	0	0
HMB	2	0	16	2	1	0	0
BKA	29	0	1	0	0	0	0
BKB	192	0	17	0	0	0	0

Table B.2. Trapping data for each Mark/Recapture trap site and six species of nineteen species captured.

Trap Site	OLFO	ORME	OLNI	OSPP	LUCR	DASE
R3A	0	0	14	2	1	1
R3B	33	16	2	1	0	0
JMA	20	48	3	0	0	0
JMB	12	11	0	1	0	0
MAA	2	10	0	1	0	0
MAB	2	7	2	0	0	0
HMA	11	6	25	0	0	0
HMB	1	0	4	0	0	0
BKA	0	0	0	0	0	0
BKB	85	5	1	6	0	0

Table B.3. Trapping data for each Mark/Recapture trap site and six species of nineteen species captured.

Trap Site	CTEN	ORAN	THSP	GAGI	OXMI	MIPA
R3A	7	0	0	0	0	0
R3B	0	2	1	0	0	0
JMA	0	1	0	0	0	0
JMB	0	4	0	1	0	0
MAA	0	0	0	0	0	0
MAB	0	4	0	0	0	0
HMA	2	1	0	2	0	0
HMB	0	2	0	0	0	0
BKA	0	1	1	0	0	0
BKB	0	14	0	0	11	2