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Seroprevalence of hantavirus in the Great Smoky Mountains National Park

Shawn L. Lewis
University of Tennessee

To the Graduate Council:

I am submitting herewith a thesis written by Shawn L. Lewis entitled "Seroprevalence of hantavirus in the Great Smoky Mountains National Park." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Comparative and Experimental Medicine.

John C. New Jr., Major Professor

We have read this thesis and recommend its acceptance:

Stephen A. Kania, Dorcas O'Rourke, Robert L. Donnell

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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Anne Mayhew, Vice Provost and
Dean of Graduate Studies

SEROPREVALENCE OF HANTAVIRUS IN THE GREAT SMOKY MOUNTAINS NATIONAL PARK

A Thesis
Presented for the
Master of Science
Degree
The University of Tennessee, Knoxville

Shawn L. Lewis
August 2002

Dedication

This thesis is dedicated to my husband, Leo whose support kept me from discharging my computer from a tall building. Also, my families, the Lewis' and Poorvins' who made life as a graduate student far more comfortable than perhaps, should have been. And my committee, Dr.'s New, Kania, O'Rourke, and Donnell who have inspired and encouraged me.

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M. Smith – Safety Officer, University of Tennessee, Institute of Agriculture

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J. Mills, T. Ksiazek – Viral and Rickettsial Zoonoses Branch, Centers for Disease Control and Prevention

M. Pelton, B. Minser, D. Buehler – Forestry, Wildlife and Fisheries, University of Tennessee

B. McNeil – Pharmacist, University of Tennessee College of Veterinary Medicine

College of Veterinary Medicine Center of Excellence for Livestock Diseases and Human Health

Abstract

In the fall of 2000 and 2001 we conducted a hantavirus survey in the Great Smoky Mountains National Park to gather preliminary information on the general distribution of hantavirus in this park. We tested 142 small mammals for antibodies against Sin Nombre Virus, a highly pathogenic strain of hantavirus. *Peromyscus spp.* were the only animals that were seropositive. Antibody-positive *Peromyscus spp.* were found in 6 of the 13 sites sampled. Of the 96 *Peromyscus spp.* tested, 16.7% of 42 *P. maniculatis* (deer mice) and 3.7% of 54 *P. leucopus* (white-footed mice) had antibodies reactive to Sin Nombre Virus. Although no human hantavirus cases have been reported in or originating from the Great Smoky Mountains National Park, reservoir populations in the park are infected with a pathogenic strain of hantavirus. The potential for human-rodent contact and subsequent human infection does exist in many areas of the park.

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Glossary Of Abbreviations

ARDS: Acute Respiratory Distress Syndrome

BCC: Black Creek Canal virus strain

DOB: Dobrava-Belgrade virus strain

EHF: Epidemic Hemorrhagic Fever

ENSO: El Nino Southern Oscillation

GMT: Geometric Mean Titre

GSMNP: Great Smoky Mountains National Park

HFRS: Hemorrhagic Fever with Renal Syndrome

HPS: Hemorrhagic Pulmonary Syndrome or Hantavirus Pulmonary Syndrome

HTN: Hantaan virus strain

IF: Immunofluorescent

KHF: Korean Hemorrhagic Fever

NE: Nephropathia Epidemica

PHV: Prospect Hill virus strain

PUU: Pumuula virus strain

SEO: Seoul virus strain

SNV: Sin Nombre virus strain

SR-11: virus strain known to cause HFRS in Asia

1.0 LITERATURE REVIEW

History of Hemorrhagic Fever with Renal Syndrome in Eurasia

An assortment of hemorrhagic fevers have been recognized and reported across Eurasia since the early 1900's. However clinically similar cases to a milder form of hemorrhagic fever, Nephropathia Epidemica (NE) were reported as "War Nephritis" as early as the American Civil War (1861-1865). During this time, approximately 14,000 cases were described among the Union army battalions occupying what were most likely Pennsylvania, Maryland and Delaware (Lee 1982).

Accounts describing Hemorrhagic Fever with Renal Syndrome (HFRS) have been found in a Chinese medicine text, *Whang Jae Nae Kyung*, which was written circa 960 A.D. in addition to other ancient writings (Lee 1982, Yanagihara and Gajdusek 1988).

HFRS or Hemorrhagic Nephroso-nephritis as it was known in Russia, has been recognized since 1944 although several thousand cases involving severe, moderately severe and mild forms of the disease have been described since 1913 (Smorodintsev et al. 1959, Lee 1982, Schmaljohn 1988). The severe and moderately severe forms of HFRS in Russia are now known to be caused by the *Hantaan* (HTN) and *Dobrava-Belgrade spp.* (DOB) of viruses. Their principal reservoir hosts are *Apodemus agrarius* (striped field mouse) and *Apodemus flavicollis* (yellow-neck mouse), respectively. Human outbreaks of HTN and DOB

range from southwestern Russia to the Urals; however, infected animals have been captured in far eastern Russia as well (Johnson 1986, Schmaljohn and Hjelle 1997).

The incubation period for HTN and DOB infections can be 4-42 days, 14-21 days on average. Clinical symptoms are fever, vomiting, prostration, shock and renal involvement. The clinical course may or may not include proteinuria, hemorrhagic manifestations and renal failure (Johnson 1986). The mortality rate is approximately 5-10% (Lähdevirta et al. 1971).

The mild form of HFRS, which has been reported since 1939 throughout Russia and Scandinavia, came to be known as Nephropathia Epidemica (NE) in 1945 (Lähdevirta 1971). Several thousand cases of "Trench Nephritis" or "War Nephritis", clinically similar to NE, had been described among British troops in Flanders (a region in northwest Europe) during World War I (1914-1918) (Lee 1982).

In the case of NE, the principal reservoir, *Clethrionomys glareolus* (wild bank vole) was identified in 1980 through antigen detection but the etiologic agent, *Pumuula* (PUU) virus was not identified until ca. 1984 (Brummer-Korvenkontio et al. 1980, Lähdevirta et al. 1984). The incubation period for NE is about 14-35 days. The clinical symptoms are acute febrile illness, abdominal pain and proteinuria. Patients rarely develop hemorrhagic manifestations (Johnson 1986). The mortality rate for NE is between 0-5% (Lähdevirta et al. 1984).

Epidemic Hemorrhagic Fever (EHF) is the form of HFRS that has been recognized in Asia and Eastern Europe. In China, about 20,000 cases had been reported annually from 1931 to ~1982 (Brummer-Korvenkontio et al. 1980, Lee 1982). EHF cases were described among Japanese troops in Manchuria in 1932 and again during World War II (1939-1945) in Japan but were not identified as EHF until 1964 (Lee et al. 1979, Lähdevirta et al. 1984). EHF had been reported in Eastern Europe in 1962 (Gajdusek 1962). A close antigenic relationship between EHF and HTN was demonstrated by Lee et al. (1978) and again by Gan et al. (1983). Lee also definitively identified that the principal reservoir for EHF was *Apodemus agrarius* (Black-striped field mouse) (Gan et al. 1983, Lee et al. 1979). The symptomology of EHF follows the same clinical courses as the three forms of HFRS described earlier (Lee et al. 1979).

From March to July of 1981, outbreaks of a mild hemorrhagic fever occurred in the Henan and Shaanxi provinces of northern China. This disease had a very short course (7-14 days), a very low mortality rate (<1%), and was characterized by acute febrile illness, proteinuria and slight hemorrhagic manifestations (Johnson 1986).

Thus far, HFRS cases had a sylvatic or rural association, primarily affecting agricultural workers, soldiers engaged in military operations and those involved with economic development (Johnson 1986). In the Chinese provincial cases, epidemiological evidence revealed that the patients had been in contact, sometimes frequently, with *Rattus norvegicus* (house rats) also known as Norway rats, in an urban setting. Samples of lung tissues from *R. norvegicus*

collected from patients' homes were found to be antigenically similar to HTN but clinical manifestations in patients were similar to NE found in Scandinavia and Russia (Gan et al. 1983). An earlier study in Japan reported that all cases of EHF during 1960-1972 had been among urban dwellers of Osaka City where there were *R. norvegicus* but no *Apodemus spp.* (Lee et al. 1979).

The most important epidemiological event to occur in the history of HFRS was surrounding the Korean Conflict (1950-1953). Approximately 3,200 cases of HFRS were reported among United Nations forces from 1951-1954 in Korea, with a mortality rate of 10-15%, and first attracted the attention of western physicians (Schmaljohn 1988, Schmaljohn and Hjelle 1997). The disease became known as Korean Hemorrhagic Fever (KHF) and the prototype viral agent was subsequently coined "*Hantaan*" virus, named for the Hantaan River in South Korea that flows through a KHF endemic area (Schmaljohn 1988).

A viral etiology had been suspected since the early 1940's when Russian investigators were able to successfully reproduce disease in human volunteers injected with sera and urine from HFRS patients (Lee 1982, Schmaljohn and Hjelle 1997). A rodent association with HFRS had been suspected for decades, but it was not until an HFRS outbreak in a Russian laboratory conducting research on tick-borne encephalitis in 1961 that a viral transmission was established. Two weeks after wild-caught rodents were brought into the laboratory, 113 workers, many of whom did not have any contact with the rodents or the animal rooms, were diagnosed with HFRS. This established a rodent

correlation with the disease and strongly suggested that the virus could be aerosolized (Lee 1982, Schmaljohn 1988).

Lee et al. (1978) identified the etiologic and reservoir host of KHF. An antigen, isolated from the lungs of wild-caught *A. agrarius*, produced a specific immunofluorescent (IF) reaction with sera from convalescing KHF patients (Lee et al. 1978, Lee 1982). With IF, combined with electron microscopy, other investigators were able to subsequently identify antigenically related, pathogenic viruses such as DOB, PUU, and Seoul (SEO) (Johnson 1986).

Given the primarily, sylvatic association with HFRS, and that the symptoms may be clinically mild, this disease is probably underreported. It is estimated that there are 150,000-200,000 cases of HFRS that require hospitalization annually in Asia and Europe. More than half of these cases are reported in China, with Russia and Korea reporting hundreds to thousands of cases annually. The mortality rate has decreased to 0.1%-10% depending upon the viral strain (Schmaljohn and Hjelle 1997).

Hantaan Agents

In 1975, the taxonomic family *Bunyaviridae* was established by the International Committee on Taxonomy of Viruses to encompass many morphologically and morphogenically similar arthropod-borne viruses. The *Bunyamwera* virus was the prototype for the *Bunyavirus* genus, the only genus within *Bunyaviridae* at the time. Several arboviruses were morphologically similar to *Bunyamwera* but antigenically unrelated to other viral species within the *Bunyavirus* genus. In 1982, 3 genera were added to the *Bunyaviridae* family:

Phlebovirus, Sandfly fever prototype; *Nairovirus*, Sheep or Crimean/Congo hemorrhagic fever virus prototype; and *Uukuvirus*, Uukuniemi virus prototype (Matthews 1982, Martin et al. 1985).

The viruses assigned to the *Bunyaviridae* family thus far were 80-120 nanometers (nm) in diameter with a primarily spherical shape, 5-10 nm surface projections anchored in a lipid bilayered envelope, and a tripartite RNA negative sense genome (Matthews 1982, Schmaljohn et al. 1983, Martin et al. 1985).

McCormick et al. (1982) purified the 76-118 Hantaan viral strain originally isolated by Lee et al. (1978) and later grown in tissue culture by French et al. (1981) in order to morphogenically characterize the virus. Viral strain 76-118 was inoculated into E-6 cells, a cloned line of Vero cells (a serially propagated heteroploid cell line used extensively for viral replication and plaque assays). Investigators found that the purified virus particles were morphologically similar to the viruses within the *Uukuvirus* genus and that the particles averaged 92.5 nm in diameter (McCormick et al. 1982, Schmaljohn et al. 1983).

Hung et al. (1983) attempted to further characterize Hantaan virus. HTN inoculated cells were harvested and fixed for indirect immuno-electron-microscopy. Investigators found both spherical and oval shaped particles with a larger average diameter than previously described, 122 nm and a variation of 110-160 nm (Hung et al. 1983). The virions possessed a membrane envelope comprised of granulofilamentous viroplasm arranged in a grid-like pattern and ~6 nm surface projections (Hung et al. 1983, Schmaljohn et al. 1983, Martin et al.1985).

Biochemical data were required to more completely characterize HTN. Schmaljohn (1988) demonstrated that HTN had the same sedimentation characteristics as Rift Valley fever in the *Phlebovirus* genus and consistent with *Bunyaviridae*. Disruption of the HTN sedimentation in nonionic detergent resulted in 2 distinct components that are characteristic of an enveloped virus with nucleocapsid and membranous components. The sedimented nucleocapsids were resolved into small, medium, and large components with similarity to the 3 nucleocapsids of LaCrosse virus in the *Bunyavirus* genus (Morita et al. 1985). HTN possessed characteristics very similar to the other genera of *Bunyaviridae*, a Large (L), Medium (M), and Small (S) segmented, single-stranded RNA genome enclosed in a lipid envelope with 2 virus-specified glycoproteins (Morita et al.1985, Schmaljohn 1988, Elliott 1991).

Attempts were made to antigenically relate the viruses throughout Eurasia and the Americas. Immunofluorescent (IF) antibody tests have demonstrated cross reactivity between the etiologic agents that cause KHF, EHF, NE, and HTN (Pyung-Woo et al. 1981). Antibody titres against the viruses that cause EHF and KHF, SR-11, and Hantaan are high regardless of the severity of the disease and the rodent reservoir (Lee et al. 1979, Pyung-Woo et al. 1981, Gan et al. 1983, Kitamura et al. 1983). Investigators were beginning to realize that the viral isolates from different rodent hosts and geographical areas were not identical but closely related. IF assays demonstrated that Hantaan viral isolates from the *Rattus* genus would rarely produce viral antigen in experimentally inoculated *Apodemus spp.* This indicated that there was no cross-infectivity of the viral

isolates. This suggested a unique viral strain in a respective host and that viral circulation amongst the *Rattus* hosts, at least, must be longstanding (Pyung-Woo et al. 1981, Lee et al. 1982, Kitamura et al. 1983).

Serological classification of HFRS viruses began in 1985. Three serotypes were identified at that time based on antigenic cross reactivity and blocking antibody titrations. Hantaan was designated serotype 1; Puumala, serotype 2; and Prospect Hill, a virus isolated from the *Microtus pennsylvanicus* (Meadow vole) in the United Stated but not associated with human illness, was serotype 3 (Goldgaber et al. 1985). Seoul and Dobrava/ Belgrade viruses have been designated fourth and fifth antigenically distinct groups respectively. As of 1987, Hantaan and related viruses had become a new genus of the family *Bunyaviridae* and recognized by the International Committee on the Taxonomy of Viruses as the genus *Hantavirus*. Thailand, Thottopalam and Muerto Canyon/Four Corners have also been added to the *Hantavirus* genus and several more serologically and genetically distinct viruses are being added continuously (Schmaljohn 1988, Elliott et al.1991).

Hantaviruses possess 3 segments of single-stranded RNA that comprise the major structural proteins and make up 1-2% of the virion particle. The large (L) segment, encoding a putative RNA dependent RNA viral polymerase, has a molecular weight of 2.2×10^6 Daltons and is 6500-8500 bases. The medium (M) segment encodes for G_1 and G_2 envelope glycoproteins and a non-structural protein (NSm), has a molecular weight of 1.2×10^6 Daltons and is 4200-5700 bases. The antigenic differences that occur among Hantaviruses are most likely

due to the less conserved and external amino half of the G_1 protein. The G_1 and G_2 coding sequences are contained within one continuous Open Reading Frame (ORF). This ORF is then transcribed as a single mRNA. The small (S) segment encodes for a nucleocapsid (N) core antigen and a non-structural protein (NSs), has a molecular weight of $0.6x10^6$ Daltons and is 1800-2300 bases (Morita et al. 1985, Yoo and Kang 1987, Schmaljohn 1988, Elliott et al. 1991, Rawlings et al. 1996). These 3 antisense RNA strands of Hantaviruses are highly conserved at the 3'- terminal nucleotide sequence (3' AUCAUCAUCUG) and are different than the other *Bunyaviridae* genera (Yanagihara and Gajdusek 1988).

Each hantaviral strain is associated with a specific rodent host and geographical region. There is a stronger association between the hantaviral phylogenetic relationship and rodent/insectivore host phylogenetic relationship than geographical distribution and thus, Hantaviruses may have coevolved with their specific hosts. There is strong evidence to support cospeciation among viral strains and rodent hosts as well as host switching or spillover. For example, HTN viruses are associated with *Apodemus sp.*; however an HTN-like strain was isolated from *Emberisa elegans* (Yellow-throated bunting). PUU viruses are associated with *Clethrionmys sp.* but some viral isolates from Russian *Clethrionmys sp.* were more closely antigenically related to HTN than to PUU. It has also been found that while two viral strains are monophyletic, their respective host species are much more divergent and distally related (Dzagurova et al. 1995, Ravkov et al. 1995, Hörling et al. 1996, Song et al. 1996, Monroe et al. 1999, Vapalahti et al. 1999, Rhodes III et al. 2000).

Pathogenesis of Human Disease

Hantaviruses throughout the Eurasian landmass are now referred to as "old world hantaviruses" and cause hemorrhagic fever with renal syndrome (HFRS). Hantaviruses found in North, Central and South America are referred to as "new world hantaviruses" and cause hemorrhagic pulmonary syndrome (HPS) with no renal involvement, however not all new world hantaviral strains identified thus far have been associated with human illness (Schmaljohn and Hjelle 1997, Mills et al. 1999*a*).

HFRS Clinical Features.-- HFRS may exhibit mild, moderate or severe disease symptomology depending upon the viral variant. The clinical course of classic Hantaan viral infection causing severe HFRS has been described by numerous investigators and is summarized in the following (Lee 1982, Lee and Johnson 1982, Ellis et al. 1995, Schmaljohn and Hjelle 1997).

HFRS occurs in five, often overlapping, clinical stages or phases; however these stages are arbitrarily categorized and not all stages of the disease are present or apparent in all cases (Gajdusek 1962, Lee 1982, Schmaljohn 1988, Schmaljohn and Hjelle 1997). The incubation period is usually 2-3 weeks but may vary from 4 days to 6 weeks (Lee 1982, Yanagihara and Gajdusek 1988).

The first or febrile phase, lasting 3-8 days, has an abrupt onset of fever (102°F-104°F) accompanied by chills and intense frontal or retro orbital headache with occasional photophobia and ocular pain due to chemosis. This is followed by vomiting, and anorexia with lumbar and abdominal pain as a result of plasma extravasation causing retroperitoneal or peritoneal edema. A petechial

rash appears on the face, palate, pharynx, axillary folds, thorax and legs; erythema appears on the face and chest. There is a marked increase of albumin in the urine, urine specific gravity begins to decrease and proteinuria continues to increase. There is a decrease in blood platelets and an increase in hematocrit due to plasma extravasation (Myhrman 1951, Smadel 1953, Gajdusek 1962, Lee 1982, Yanagihara and Gajdusek 1988, Schmaljohn and Hjelle 1997).

The second or hypotensive phase lasts a few hours to 3 days and is marked by polyuria, continued febrile illness and onset of hypotension due to increased capillary permeability. Tachycardia due to increased blood potassium is present. Hematocrit levels are at their highest during the clinical course. Hematuria, hepatomegaly and splenomegaly may be present in some patients. Blood platelets continue to decrease and blood nonprotein nitrogen levels increase, delirium and confusion is observed. Acute hypotensive shock occurs resulting in a 30% mortality during this phase (Myhrman 1951, Gajdusek 1962, Lähdevirta 1971, Lee 1982, Yanagihara and Gajdusek 1988, Ellis et al. 1995, Schmaljohn and Hjelle 1997).

HFRS then progresses to the third or oliguric phase in which nearly half of all severe cases will result in death. The oliguric phase lasts from 3-13 days and at the beginning, blood pressure may normalize but in most cases, patients will become hypertensive due to hypervolemia. Erythema and petechia subside and hematocrit levels may return to normal. As the disease progresses, the clinical symptoms worsen; electrolyte imbalance occurs (hyperkalemia, hyponatremia and hypocalcemia), there is epistaxis with hemoptysis and purpura; cerebral,

conjuctival and gastrointestinal hemorrhage. Urinary output is decreased and there is a marked increase in blood urea nitrogen and azotemia is evident (Smaldel 1953, Gajdusek 1962, Lee 1982, Schmaljohn 1988,Yanagihara and Gajdusek 1988, Ellis et al. 1995, Schmaljohn and Hjelle 1997).

The fourth or diuretic phase lasts from days to weeks with an average of 10-28 days and during this time, clinical symptoms improve dramatically. Fluids or diuresis must be administered carefully to the dehydrated, electrolyte imbalanced patient. The small percentages of deaths that occur during this phase are due to fluid overload that cause hypertension and pulmonary edema. Urine output increases and there is significant renal improvement (Gajdusek 1962, Lee 1982, Schmaljohn 1988, Yanagihara and Gajdusek 1988, Ellis et al. 1995).

During the fifth or convalescent phase, which may last several months, proteinuria and azotemia continue to subside but full renal function, particularly urine concentration and glomular filtration rate may take more than 6 months to recover (Smadel 1953, Gajdusek 1962, Lee 1982, Yanagihara and Gajdusek 1988, Schmaljohn and Hjelle 1997).

HFRS Pathology.--The pathologic changes that occur with HFRS vary depending upon the severity and duration of the disease, also during which phase of the disease course the patient died in, particularly the histopathological changes in the kidneys that occur due to capillary damage (Smadel 1953, Lukes 1954). The primary lesion observed with HFRS found during postmortem examination is endothelial cell damage (Yanagihara and Gajdusek 1988).

Patients that die of shock during the hypotensive phase, usually by the eighth day of clinical onset, are found to have large quantities of protein-rich gelatinous fluid in the abdominal cavity (Yanagihara and Gajdusek 1988). The kidneys are swollen and pale in color, the subcortical medullary vessels are congested but without tubular damage, and hemorrhagic necrosis is present (Gajdusek 1962). Petechiae and ecchymoses are commonly found in the epicardium; congestion with fresh erythrocytes causing severe hemorrhage in the right atrium is almost always present in patients dying during the hypertensive phase. Hemorrhagic necrosis and intense congestion of the anterior lobe of the pituitary is apparent. The adrenal glands have focal to diffuse hemorrhagic necrosis. The lungs are only slightly congested and are of normal size in most cases although mild pulmonary edema or bronchopneumonia may be observed. Congestion of the spleen and bone marrow, and thrombocytopenia are observed. There is massive enlargement of the lymph nodes and distention of small intestine submucosa lymphatics (Lukes 1954, Gajdusek 1962, van Ypersele de Strihou 1979, Yanagihara and Gajdusek 1988).

The pathologic changes that occur during the oliguric and diuretic phases, after the ninth day of clinical onset, are much more severe with primarily renal involvement. The most distinctive characteristic of the renal lesion is intense congestion and hemorrhage at the corticomedullary junction with extensive tubular epithelial necrosis (Smadel 1953). Vascular congestion in the renal intertubular spaces increases and the tubular lumens become filled with desquamated cells, eosinophilic casts and hyaline material indicative of

progressive tubular damage and medullary hemorrhage (Lähdevirta 1971). Severe necrosis of the pituitary is common in the diuretic group and in some cases, collapsed connective tissue stroma and absence of parenchymal cells may be observed. There is a significant increase of pulmonary edema and/or abscess formation, both of which cause bronchopneumonia in many of the fatal cases during the diuretic phase (Lukes 1954, Gajdusek 1962, Yanagihara and Gajdusek 1988).

HPS Clinical Features and Pathology.--The occurrence of hantavirus pulmonary syndrome (HPS) has thus far been limited to the Americas and there have been no viral HPS strains associated with renal involvement identified in North America (Bradshaw 1994, CDC 2000). The reported mortality rate of untreated HPS is 43%-66% and about 50% for treated cases, 5 times more fatal than the most severe forms of old world Hantaviruses (Eidson and Ettestad 1995, Kitsutani et al. 1999).

Serosurveys of cricetid and microtine rodents conducted in the United States 11 years prior to the first, highly publicized, and diagnosed cases of HPS revealed antibody titres to a genetically distinct hantavirus. Prospect Hill virus (PHV) was isolated from *Microtus pennsylvanicus* in 1982 and antibody titres in *Peromyscus maniculatis* were higher against PHV than to HTN (Nerurkar et al. 1994). In 1984, several *Rattus norvegicus* were antibody positive for a hantavirus that was antigenically distinct from the prototype HTN and subsequently named Girard Point virus (GPV) after the granary in Philadelphia, PA where the rats were found (LeDuc et al. 1984).

Since there were no cases of archetypal hemorrhagic fever with renal syndrome diagnosed in the United States, it was unknown if these new hantaviruses were pathogenic to humans (LeDuc et al. 1984, Nerurkar et al. 1994). It was discovered that in a 1985 serosurvey of patients with idiopathic febrile illness, along with healthy blood donors, that 13 subjects (out of 1699 serum samples) had antibody titres against hantavirus. None of the donors had traveled to geographical areas that were endemic with HFRS nor had a history of HFRS illness. The absence of neutralizing antibody titres in 12 of the 13 donors with low titres of reactive antibodies may be suggestive of a cross reaction to an antigenically related Hantaan virus (Yanagihara et al. 1985).

In May of 1993, a highly publicized outbreak of HPS in the Four Corners area (New Mexico, Utah, Colorado and Arizona) of the United States was reported although hantavirus was not the suspected culprit at the time. On May 14, 1993, the Indian Health Service reported to the New Mexico Department of Health that 2 young, previously healthy, adult Navajo Indians had died within 5 days of each other of acute respiratory failure. Several more adult Navajo Indians succumbed to the same illness in the Four Corners area. Those and the index cases were negative for tests to all suspected pathogens. The public health departments of Arizona, New Mexico and Utah were asked by the Centers for Disease Control and Prevention (CDC) to provide blood and tissue samples from suspected cases. Again, all test results were negative for known pathogens in the region except for positive reactions with Puumala virus. The CDC, Army and National Institutes of Health performed extensive immunologic and molecular

tests, and on June 9, 1993 the CDC confirmed a previously unidentified genotype of hantavirus. As of November 5, 1993, there were 42 confirmed cases of hantavirus infection with a 62% mortality rate (Rand 1994, Weigler 1995). This novel hantavirus, originally called Muerto Canyon or Four Corners virus after the area HPS appeared was subsequently coined Sin Nombre virus (SNV). It is the prototype new world hantavirus (Quarles 1995, Weigler 1995, Kitsutani et al. 1999).

The most distinguishing feature of severe or fatal HPS is acute respiratory distress syndrome (ARDS) caused by noncardiogenic pulmonary edema (Eidson and Ettestad 1995, Kitsutani et al. 1999, Leslie et al. 1999). HPS occurs in 3 arbitrarily assigned phases; prodromic, pulmonary or cardiopulmonary, and convalescent. The syndrome is characterized by a rapid clinical progression and high case fatality rate (Eidson and Ettestad 1995, Kitsutani et al. 1999, CDC 2000),

The incubation period for HPS is on average 2-3 weeks but can be up to 45 days as was the case for the only HPS fatality in Rhode Island thus far (Kitsutani et al. 1999). The prodromic or febrile phase lasts 1-6 days and is a non-specific presentation. This phase begins with febrile illness with a fever between 101°F-104°F, generalized myalgia, prostration, nausea and diarrhea. Tachypnea and tachycardia may be present in the late prodromal phase but usually occur during the abrupt onset of the cardiopulmonary phase (Weigler 1995, Kitsutani et al. 1999, CDC 2000). During the late prodromal phase, there is a marked increase in large atypical lymphocytes and thrombocytopenia. A

characteristic of the differential white blood cell count that distinguishes HPS from other viral infections is an elevated left shift of neutrophils with circulating myelocytes (CDC 2000).

Six or 7 days after clinical onset, there is an abrupt progression into the cardiopulmonary phase marked by dyspnea, hypotension, hypoxemia and noncardiogenic pulmonary edema leading to respiratory distress, which can occur within 24 hours. Clinical pathologic findings at this time are a rise in hematocrit and a fall in serum albumin, which reflects a fluid shift from the circulatory system to the lungs. Radiographically, there is a progression of interstitial or alveolar infiltrates to severe bilateral pleural effusions. Immunohistochemically, there is a distribution of viral antigens within the capillary endothelium of various tissues particularly within the pulmonary microvasculature, spleen and lymph nodes. The resulting lesion is the functional impairment of the vascular endothelium that may be attributed to the cellular effect of viral inclusions and/or virally induced, immune-mediated response within the pulmonary endothelium. The severe pulmonary edema, prolonged prothrombin time, proteinuria and high lactate dehydrogenase activity mark a poor prognosis. The convalescent phase is somewhat unremarkable except that it is quite rapid and polyuria is frequently present in most survivors (Rand 1994, Eidson and Ettestad 1995, Weigler 1995, Kitsutani et al. 1999, CDC 2000).

The varied clinical courses observed with new world HPS infections might depend upon 2 factors. The first is the genetic diversity of the hantavirus, for instance, the previously described pathology is of infection due to SNV or closely

related hantavirus. Bayou, Black Creek Canal and Andes virus infections have been associated with renal insufficiencies and elevated serum creatnine kinase. PHV infection has thus far not been associated with any apparent clinical course in humans. Clinical descriptions of some human cases have not progressed past the prodromal phase and are thought to represent unique hantaviral strains. The second determining factor for different clinical manifestations may be within the human patient; it has been hypothesized that some patients may have a weaker immune response to infection thus causing a less intense virally induced immune response. Integrins expressed on platelets and endothelial cells may serve as receptors for HPS viral cellular entry and variations in these receptor molecules may alter viral pathogenicity (Schmaljohn and Hjelle 1997, Kitsutani et al. 1999, CDC 2000).

The answer to disease severity may be solved with the recent discovery of an animal model for hantavirus pathogenicity. Thus far, infected rodents have remained asymptomatic but now virologists at the U.S. Army Medical Research Institute of Infectious Diseases have reported that Syrian hamsters exhibit HPS symptoms when inoculated. Although the hamsters are susceptible only to Andes virus, theories about cellular entry and subsequent immune response to the virus can now be tested (Enserink 2001).

Rodent-Human Hantavirus Transmission

The primary route of human hantavirus infection is the inhalation of virus present in the urine, feces and saliva shed by infected murid rodents. After several proposed routes of infection, the aerosol route was established in 1961

after an outbreak in a Moscow laboratory that resulted in the infection of 113 of 186 employees and visitors. Several sylvatic rodents captured from endemic areas were brought to the laboratory during hantavirus field studies and many of the infected people had no contact with the rodents. Investigators had once believed that the other possible routes of infection, in addition to respiratory, were via rodent ectoparasites, mucous membrane penetration, and percutaneous since all of these potential confounders could occur with close contact with rodent carriers. The 1961 Moscow outbreak where several infected people had no contact as well as a 1954 laboratory outbreak that occurred in another Russian laboratory studying ectoparasite-free rodents in which visitors that had no contact with rodents became infected convinced investigators that infection must occur via aerosolization (Lee 1982, Lee and Johnson 1982, Johnson 1986, Tsai 1987, Hjelle and Glass 2000).

Since then, laboratory associated outbreaks have occurred throughout the world; imported Louvain (Lou/WSL/M) rats were responsible for an outbreak in a United Kingdom laboratory, as were Wistar rats in medical research laboratories in Japan and Belgium. Wild-caught rodents brought into laboratories have been associated with Korean and Chinese laboratory infections (Lee and Johnson 1982, Destmyter et al. 1983, Lloyd et al. 1984).

The majority of hantavirus infections are associated with temporally sporadic outbreaks and sylvatic settings, occurring in localized foci, eliciting the concept of a "place" disease. Historically, the highest risk group for contracting a hantaviral infection had been those conducting military operations due to the

disruption of the rodents' habitats (Gajdusek 1962, Johnson 1986, Yanagihara and Gajdusek 1988). Now, at-risk groups or individuals are persons that engage in activities that bring them into contact with infected rodents. Agricultural activities such as threshing and working in grain silos along with forestry related activities bring humans in contact with potentially infected rodent excreta. Several rural incidents are results of working and/or sleeping in closed structures such as cabins, barns and outbuildings that are or have been infested with rodents. Field investigators are, of course, at a high risk (Yanagihara and Gajdusek 1988, Bradshaw 1994, Ellis et al. 1995).

Urban hantavirus cases are more prevalent in Asia than in North America and outbreaks are of more epidemic proportions due to poorer housing conditions that would bring humans in contact with infected rodents in densely human populated dwellings. Infestations of house rats are responsible for outbreaks in dormitories, army barracks and apartment buildings (Lähdevirta 1971, Gan et al. 1983, Schmaljohn and Hjelle 1997). There have been isolated urban cases in the United States such as the ones that occurred in New York and Rhode Island (Ellis et al. 1995).

Secondary routes of hantavirus infection occur as a result of rodent bites as was the case in Haute-Sovoie in rural France (Dournon et al.1984). This scenario is plausible since infected rodents excrete virus in their saliva and horizontal transfer of hantavirus between animals is often a result of fighting. While infection due to contact with contaminated fomites or food is possible, it is far less common than aerosol or even percutaneous transmission (Dournon et al.

1984, Ellis et al. 1995, Otteson et al. 1996, Schmaljohn and Hjelle 1997). The possibility and perhaps occurrence of nosocomial infection has been documented (Enria et al. 1996). A Buenos Aires physician with no other risk factors such as visits to endemic areas, contracted HPS 27 days after direct contact with an HPS infected patient's blood. Early work by Russian investigators, from 1940-1941, produced HFRS in human volunteers by intramuscular and intravenous injections of serum and urine from naturally infected HFRS human patients (Smorodintsev et al.1959 and Gajdusek 1962). Japanese investigators, from 1941-1943 claimed to have produced HFRS infection with injected filtrates of *Apodemus agrarius* tissues along with ectoparasites collected from HFRS endemic areas (Gajdusek 1962). The possibility of nosocomial transmission of hantaviruses remains unclear although plausible since viral isolates can be obtained from blood and urine of infected patients (Gajdusek 1962, Lee et al. 1978, Tsai 1987, Enria et al. 1996, Schmaljohn and Hjelle 1997).

Hantavirus Epidemiology

Seasonal incidences of HFRS have been noted in Korea since 1951 and appear to be the highest during late spring and late fall. These seasonal peaks of incidence may be exclusively a result of an increase in rodent populations or confounded by activities that would bring humans into more contact with infected rodents and their excreta (Lee 1982, Lee and Johnson 1982, Ellis et al. 1995).

Since the 1993 HPS outbreak in the Four Corners area, Investigators have tried to find a correlation between rodent populations and HPS cases (Mills

et al. 1995*b*, Jay et al. 1997). Preceding the 1993 outbreak there was an increase in precipitation and a mild winter attributed to the El Nino Southern Oscillation (ENSO) of 1991-1992. This, in turn, resulted in an increase in abundant food sources such as insects and vegetation, primarily rich crops of pinyon nuts. Sevilleta National Wildlife Refuge in New Mexico reported a 20-fold increase in the rodent population over 1992 estimates. This scenario makes it likely that a rodent population explosion and subsequent increase of viral transmission via more rodent-rodent contact would also increase the likelihood of humans coming into contact with HPS infected rodents (Bradshaw 1994, Engelthaler et al. 1999, Mills et al. 1999*a*). A similar pattern of increased precipitation followed by an HPS outbreak was observed in Paraguay from 1995-1996. A second ENSO event began in mid-1997 and prior to that time there were approximately 4 cases/year that had been reported throughout CO, AZ, NM and UT, the region of the original outbreak. HPS cases in the four states increased to 33 between January 1998-July 1999 from an incidence of 6 cases that was predicted for that time frame (Engelthaler et al. 1999, Mills et al. 1999*a*, Hjelle and Glass 2000).

Attempts to construct a model that could predict epidemiological patterns of HPS in humans based upon rodent population dynamics alone have led to more questions than answers. The association between rodent populations and prevalence is complex (Lloyd et al. 1984, Childs et al. 1987, Boone et al. 1998, Engelthaler 1999).

Each viral strain known to cause HPS is associated with a single primary Sigmodontine host species. Although evidence suggests that the same hantavirus may be maintained by 2 closely related species, a secondary host species may represent a spillover from the primary reservoir. There is also cospeciation of virus-host, which will eventually lead to a unique viral strain maintained in that host. There have been no cyclical or seasonal fluctuations observed in Sigmodontine rodent populations but rather dramatic unpredictable increases or decreases in densities based on factors such as climatic changes, changes in biotic communities, inter- and intraspecific competition and predation (Schmaljohn and Hjelle 1997, Mills et al. 1999*a*).

The *Peromyscus maniculatis* (deer mouse) is widely distributed throughout the contiguous United States and is the primary reservoir for SNV and SNV related viruses. Other Peromyscus species such as *P. boyii* (brush mouse) and *P. truei* (pinyon mouse) have a southwestern geographical focus and are also reservoir hosts for SNV and related viruses. Most of the host-habitat association work has been conducted in the Southwest United States because it remains the most HPS-endemic area and has one of the richest assemblages of biotic and rodent communities in North America (Mills et al. 1997, Abbott et al. 1999, Mills et al. 1999*a*).

A temporal pattern of fluctuating rodent densities and a linear correlation of hantavirus incidence is better documented in Korea, China and Scandinavia. The population density of the reservoir host of KHF in Korea and EHF in rural China, *Apodemus agrarius* experiences an increase in the late spring and more

so in the fall; this corresponds to the increased incidences of human disease. In Sweden, the reservoir host of NE, *Clethrionomys glareolus* (an Arvicoline rodent) experiences a fairly regular population cycle every 3-4 years and this also corresponds to the incidence of NE in humans (Lee and Johnson 1982, Schmaljohn and Hjelle 1997, Mills et al. 1999*a,b*).

The establishment of a temporal pattern of HPS cases in the United States is not so clear-cut. In studies of Sigmodontine rodents in the Southwest, population densities of *P. boyii* experience a slight spring-fall bimodal peak but HPS cases were unevenly distributed displaying a higher incidence in spring-summer. While this is an oversimplification, in actuality rodent population dynamics experience year-year and seasonal trends varied by biotic communities. In order to establish a temporal pattern of HPS cases, longevity of a study that also factored in environmental variables and increased statistical power would need to be conducted (Engelthaler et al. 1999, Kuenzi et al. 1999, Mills et al. 1999*a*, Boone et al. 2000).

Community types and altitudinal data are important variables for monitoring rodent densities. The lowest SNV prevalence in rodents is found at altitudinal and climatic extremes such as alpine tundra and salt desert scrub, ≥3,384m and ≤873m respectively. This corresponds to the majority of HPS cases found at mid altitudes (1,800m-2,500m) consisting of chaparral, pinyon-juniper and grassland. This sometimes coincides with increased densities of rodent reservoirs but is not consistently found from study to study due to factors such as longevity of the study and biases introduced as results of survivability

and seroconversion of rodents during mark-release-recapture monitoring (Mills et al. 1997, Abbott et al. 1999, Engelthaler et al. 1999, Parmenter 1999, Boone et al. 2000).

There is a positive correlation between favorable climatic events initiating abundant mast and increased reproduction of *Peromyscus spp.* and visa versa. While this may lead to an increase in rodent densities and seroprevalence, data collected during the spring after a mild winter for instance, will be biased due to maternal antibodies conferred to the offspring. The same scenario could also lead to an increase in rodent densities but a decrease in seroprevalence if data were collected after juveniles have cleared maternal antibodies (the juvenile dilution effect). This may represent an increase in seroprevalence for the following year due to subsequent infection of older juveniles, even though population densities may decline. This has been observed as a result of unfavorable environmental conditions causing a decline in newborn mice. The subsequent population will consist of older mice that are commonly infected with hantavirus (Graham and Chomel 1997, Mills et al. 1997, Abbott et al. 1999, Kuenzi et al. 1999, Mills et al. 1999*a*).

Another difficulty with predicting epidemiological patterns is the focality of HPS cases and viral infection in host reservoir populations. Long term monitoring in endemic areas has demonstrated that HPS outbreaks in the U.S. are temporally and spatially sporadic as are infections in host reservoir populations. Hantaviral infection appears as distinct "islands" associated with the preferred microhabitat of the reservoir host during periods of population stasis.

These islands expand and contract temporally and during periods of irruptions (extreme population explosions). These foci become difficult to identify for the purpose of assessing human risk. The same phenomenon has been observed in urban and suburban areas in the U.S (Korch et al. 1989, Mills et al. 1997, Boone et al. 1998, Abbott et al. 1999, Engelthaler 1999, Kuenzi et al. 1999, Mills et al. 1999*a*, Glass et al. 2000).

In order to develop a model that could predict the frequency or even timing of HPS outbreaks continual monitoring of endemic areas needs to be implemented. Long-term prospective studies need to include the following variables; temporal patterns of rodent host population dynamics and infection; altitudinal and climatic data, community types and reservoir host ecology all as a function of a geographical site's microenvironment, given the spatially incongruous nature of HPS (Graham and Chomel 1997, Mills et al. 1999*b*, Parmenter et al. 1999, Boone et al. 2000).

As mentioned earlier, each hantaviral strain is associated with a primary reservoir host, most notably the Murid and Arvicoline families; *Peromyscus maniculatis* (deer mouse) and *Sigmodon hispidus* (Cotton rat, sub-family *Sigmodontinae*) and *Clethrionomys glareolus* (Bank vole) and *Microtus pennsylvanicus* (meadow vole, family *Arvicolidae*) (Yanagihara and Gajdusek 1988, Nerurkar 1994, Quarles 1995) (Table 1). Other less frequently infected reservoir hosts include *Lemmus sibiricus*, *Arvicolidae* (Black-footed lemming), *Blarina brevicauda* (Northern short-tailed shrew), *Suncus murinus* (house shrew, *Soricidae*) and *Talpa europea* (European mole, *Talpidae*) -

Table 1. Distribution of Hantaviruses in the Continental U.S.

Viral Strain	Primary Rodent Reservoir	U.S. Reservoir Distribution	Human Illness
Sin Nombre	*Peromyscus maniculatis* (grassland form) (Deer Mouse)	All of U.S. except the SE seaboard and southernmost CA	HPS
El Moro Canyon	*Reithrodontomys megalotis* (Western Harvest Mouse)	West and Central U.S. S to N Baja, CA	None documented
Monongahela	*Peromyscus maniculatis nubiterrae* (Deer Mouse)	Eastern U.S.	HPS
Blue River	*Peromyscus leucopus* (SW/NW haplotype) (White-footed Mouse)	Central U.S.	HPS
New York	*Peromyscus leucopus* (eastern haplotype) (White-footed Mouse)	Eastern U.S.	HPS
Bayou	*Oryzomys palustris* (Rice Rat)	SE Kansas to E Texas to S New Jersey and Florida	HPS
Black Creek Canal	*Sigmodon hispidus* (eastern haplotype) (Cotton Rat)	SE U.S. from S Nebraska to C Virginia to SE Arizona and Florida	HPS
Muleshoe	*Sigmodon hispidus* (western haplotype) (Cotton Rat)	Southern U.S.	HPS
Prospect Hill	*Microtus pennsylvanicus* (Meadow Vole) *montanus/ ochrogaster* (Prairie Vole)	Throughout U.S.	None documented*
Isla Vista	*Microtus californicus* (California Vole)	California	None documented
Seoul	*Rattus norvegicus* (Black Rat)	Throughout U.S.	HFRS

*Human antibody reactive to Prospect Hill virus has been documented (Monroe et al. 1999)

(Childs et al. 1987, Childs et al. 1994*a*, Mills et al. 1995*b*, Rowe et al. 1995, Schmaljohn and Hjelle 1997, Bennett et al. 1999, Calisher et al. 1999, Ksiazek et al. 1997, Monroe et al. 1999)

and are responsible for the maintenance and transmission of their respective viruses (Tang et al. 1985, Yanagihara and Gajdusek 1988, Nerurkar 1994, Vapalahti 1999).

Two species of bats, *Rhinolophus ferrum-equinum* (horse-shoe bat, *Rhinolophidae*) and *Eptesicus serotinus* (serotine bat, *Vespertilionidae*) were identified in 1994 as maintenance hosts for Hantaan or related viruses. Both species exhibited no differences in seropositivity in both summer and winter, which may be an explanation for year-round HFRS occurrences in non-endemic areas. Large amounts of antigen were detected in lung and kidney sections of both species. Genetic sequencing of the PCR products that were amplified with primer pairs for the S segment of Hantaan strain was not performed so exact homology to the prototyped virus in unknown. *E. serotinus* often inhabits human dwellings as do rats which makes it plausible for the bats to have contact with infectious rodent excreta and visa versa. More genetic analysis is required to determine the relatedness of the viral strains and if bats represent a spillover host or an example of cospeciation (Kim et al. 1994).

Other unusual non-rodent species that were seropositive to hantavirus during serosurveys are: *Mustela frenata* (long-tailed weasel), *Tamias spp.* (chipmunks), *Sylvilagus auduboni* (desert cottontail), domestic dogs, chickens,

and pigs. Russian investigators claimed to have found hantaviral antigen in 13 species of birds in Eastern Russia. It is unlikely that these species are involved in virus maintenance and shedding, rather are end-stage hosts like humans (Yanagihara and Gajdusek 1988, Nerurkar 1994, Rand 1994, Quarles 1995, Malecki et al. 1998).

Carnivores, particularly *Felis catus* may play a role in hantavirus epidemiology or be end-stage hosts themselves. Chinese investigators have reported that cats have been responsible for human HFRS cases in China and in a case-control study conducted there, cat ownership carried a significant relative risk (3.73; 95% CI=1.24-11.20), even when controlling for other variables. This evidence may be biased since households that have rodent infestations are more likely to have cats and/or cats may be bringing infected rodents into contact with humans as a result of hunting (Yanagihara and Gajdusek 1988, Eidson and Ettestad 1995, Weigler 1995). Serologically positive cats have been found in residential areas of Baltimore, MD although hantavirus antigen was not detected (Yanagihara and Gajdusek 1988, Weigler 1995). Serum samples from 200 domestic cats in Austria that were allowed outside had a seroprevalence of 5% and titred higher against PUU than HTN (Notwotny 1994). A serosurvey in Great Britain of domestic cats resulted in a prevalence of 23% in some regions with a mean of 9.6%. Small foci of antigen were detected in the lungs on 2 (out of 100) cats that died of unknown illness and had histories of hunting; this is in contrast to rodent hosts where antigen is often found throughout the lungs (Notwotny et al. 1994). Another study conducted in Great Britain found a 9.6%

seroprevalence in domestic and feral cats. In addition, 23% of chronically ill cats were antibody positive and 8 (out of 19) were both FeLV and FIV negative (Bennett et al. 1990).

More extensive studies need to be performed such as genetic detection and characterization of viral isolates as well as serosurveys and accurate health data collected (in the cases of domestic cats). It remains to be determined if cats can shed infective virus and remain chronically infected, asymptomatic hosts as are rodents and insectivores or if they represent a susceptible dead-end host (Yanagihara and Gajdusek 1988, Bennett et al. 1990, Weigler 1995).

Viral Maintenance in Rodent Reservoirs

Hantaviruses are associated with a primary reservoir host and in the United States *Peromyscus maniculatis* (deer mouse) has been identified as the primary rodent reservoir of SNV (Childs et al. 1994*b*). Several other murid rodents have been identified, along with their respective hantaviral strains however, those will be discussed later. This section will focus on the population dynamics of the deer mouse and how that relates to viral maintenance and intra- and interspecific transmission; concepts that are applicable to other host species.

Deer Mouse Biology.-- *Peromyscus maniculatis* (deer mice) have been responsible for most of the human HPS cases in the United States thus far. Deer mice are the most abundant small mammals in North America and are comprised of more than 60 subspecies. *Peromyscus spp.* are thought to be "chaparral" dwelling species although there is a tremendous variation of habitats that they occupy. Deer mice are found at elevations ranging from sea level to more than

4,200 meters and occupy terrains consisting of forest, prairie, and desert (McCabe and Blanchard 1950, Childs et al. 1994*b*, Joyner et al. 1998, Mills et al. 1999*a*).

It is probably no mistake that *Peromyscus spp.* have been the primary maintenance hosts in the United States. It is a genera of antiquity and great adaptability that is able to thrive in diverse climates and terrains (McCabe and Blanchard 1950). The deer mouse is very much a terrestrial creature exhibiting a limited ability to climb, having a preference for burrows and rotted tree trunks instead. Deer mice will often use tunnels and burrows abandoned by moles, gophers and even spiders' holes under the chaparral. The mice themselves will dig their own burrows if the ground is soft. In an Indiana study, these burrows averaged 16 feet in length and were up to 12 inches below the surface (McCabe and Blanchard 1950, Hoffmeister 1989, Quarles 1995).

Deer mice are extraordinary opportunists, invading and exploiting areas altered by natural disasters and human disturbances such as flood areas, fires, landslides, strip mining, over grazing and land development. Most importantly, deer mice will often build nests inside human dwellings and storage buildings such as grain silos, barns, and sheds. Evidence of migration into buildings is observed primarily in the fall of colder weather climates but frequently occurs at anytime the deer mice find the opportunity to do so. This is in contrast to other *Peromyscus spp.*, which are rarely, if ever found in human dwellings (Hoffmeister 1989, Quarles 1995, Calisher et al. 1999).

Subspecies of *P. maniculatis* are most often separated both geographically and ecologically, being found in almost any habitat. In the Four Corners area alone, subspecies are found in montane, mixed coniferous, and spruce-fir forests; Northern Great Basin pinyon-juniper and sage-grassland; woodlands and desert scrub. This region is of particular interest due to the variation of biomes and enzoonotic status of hantaviruses. What has been observed in this region and other areas in the United States with biome diversity spanning a relatively moderate geographical area are the unique population dynamics of *Peromyscus spp.* For instance, in Northern New Mexico subspecies of *P. maniculatis* populations (*P.m.rufinus*) are continuous throughout montane, woodland, and lowland zones whereas in Central and Southern New Mexico, *P. maniculatis* are the dominant species in montane zones and sympatric with *P. leucopus* in desert scrub but virtually non-existent in woodland zones which are dominated by other *Peromyscus spp.* (Sevilleta 1998, Calisher et al. 1999, Engelthaler et al. 1999).

The Great Smoky Mountains National Park (GSMNP) in Eastern Tennessee and Western North Carolina is another area with overlapping *Peromyscus spp.* home ranges. The deer mouse is generally found throughout the park but is more abundant at higher altitudes (≥1986m) and the white-footed mouse is readily found at lower elevations (<900m). There is extensive overlapping of the 2 species at approximately 900m and they may be found in close proximity to one another despite the different habitat preferences (Linzey 1995). In Cades Cove along Abram's Creek (519m), *P. leucopus* is the dominant

species and this area is virtually devoid of *P. maniculatis* (Pavorun, personal communication).

In other regions of the United States, *P. maniculatis* remains more disjunct and such overlapping amongst subspecies and species is not readily observed (Hoffmeister 1989, Sevilleta 1998, Engelthaler et al. 1999). Such extensive habitat diversity of the deer mouse creates a particular risk factor for rodent-human contact and HPS cases as they will inhabit structures and are quite prolific (Calisher et al. 1999).

Deer mice are omnivorous and they are opportunistic feeders as well as dwellers. The deer mouse diet is highly variable depending upon regional biomes and seasonality; from spring until fall mice will consume herbaceous matter, insects, and insect larvae, the latter primarily in the spring. During the fall, diets are mostly berries near cultivated fields, and in prairies deer mice will consume seeds of crops and regional plant seeds. Montane and woodland species consume a vast array of material such as acorns, nuts, carrion, insects, plant matter, leaves, bark, tubers, and roots. Deer mice will sometimes engage in copraphagy (McCabe and Blanchard 1950, Hoffmeister 1989, Linzey 1995, Quarles 1995, Bunker 1997, Joyner et al. 1998, Sevilleta 1998, Calisher et al. 1999).

Both *P. maniculatis* and *P. leucopus* will go into a state of torpor for several days or weeks at a time during cold temperatures. It is the *P. maniculatis* however, that will câche berries, seeds, and seedlings (often poached from birds and squirrels) more so than any other *Peromyscus spp.* The deer mouse habitat

is often subject to washing, gullying and flooding and the mice will build elaborate drainage foundations of stones, twigs and hard leaves for food caches. This is supported by the fact that when the proper materials are provided, deer mice will construct the same agglomerations in traps and cages (McCabe and Blanchard 1950, Hoffmeister 1989, Linzey 1995, Quarles 1995, Bunker 1997, Joyner et al. 1998, Sevilleta 1998, Calisher et al. 1999). *Peromyscus spp.* are extremely important to the ecologic hierachy, they consume insects and other invertebrates and are important for the dispersal of seeds and mycorrhizal fungi spores. They are a food source for reptiles and several small carnivores such as owls, fox and weasels (Quarles 1995, Bunker 1997, Joyner et al. 1998).

Peromyscus females are reproductively prolific and can produce litters every month, year-round in both captivity and in sylvatic settings in more temperate climates (Hoffmeister 1989). Typically, females are seasonally polyestrous and breeding extends from early spring into late fall with an average of 4 litters produced each year. On average, the estrous cycle is 5 days and usually commences at around 49 days of age although female sexual maturity can begin as early as 35 days old. Females born in the spring will come into estrous as soon as sexual maturity occurs and those born in fall will first breed the following spring (Quarles 1995, Bunker 1997, Joyner et al. 1998, Sevilleta 1998).

Gestation is usually 23-25 days but variation occurs depending upon lactation status; non-lactating females exhibit gestation periods of 21-25 days and lactating females, 24-30 days. Females will come into estrous again shortly

after parturition and the longer gestation period may be attributed to an embryonic implantation delay due to lactation. The life expectancy of wild *Peromyscus spp.* is short and lifetime litter production for females is rarely more than 2-3 (Quarles 1995, Bunker 1997, Joyner et al. 1998, Sevilleta 1998).

Litter sizes are highly variable and are correlated with the mother's size, age, and weight. The range is 1-10 young per litter and can be as high as 13 but average 3-5 per litter. Subsequent litter sizes will increase until the fifth or sixth litter and then declines. Post-natal *P. maniculatis* are altricial except for suckling instincts and develop rapidly with eyes opening around day 15. They are weaned between 25 and 35 days although weaning can be as early as days 18 or 19. Weaned juveniles will move to new nesting sites prior to sexual maturity and by 6 weeks old, both males and females appear sexually mature (Hoffmeister 1989, Quarles 1995, Bunker 1997, Joyner et al. 1998, Sevilleta 1998). Depending upon the time of year females give birth, it is common for the parents and 1 or 2 litters to overwinter together; often, unrelated groups will occupy the same nest for the winter.

The male will assist in preparing the nesting site, grooming and protecting young and teaching juveniles to find food outside of the nesting site. The females will also aggressively defend her young against intruders; in fact, reproductive females are more territorially aggressive than males (Bunker 1997, Sevilleta 1998).

When young mice leave their nests, they will disperse 100 yards or more from the original nest. This distributive instinct increases the survivability of

young mice as competition and intolerance of mature animals established at the original nesting site would undoubtedly result in the demise of the smaller animals. There is also long term ecologic importance of *P. maniculatis* dispersal; the burden on vegetation and food sources of the microenvironment grows as does the population and therefore, it is more beneficial for young mice to establish elsewhere (McCabe and Blanchard 1950).

Home ranges of *P. maniculatis* can vary greatly; one study estimated home ranges of 242 square meters – 3,000 square meters. These estimates will vary both temporally and spatially, study to study due to environmental changes of an area and recapture success that can be altered by die-offs and trap responsiveness. Male deer mice have greater home ranges than do females and there is greater intra- and interspecific territory overlap for males than females. This leads to greater territory defense and more conspecific aggressive encounters as well as with other species and genera thus creating another route of horizontal transmission of hantaviruses (i.e. fighting) (Korch et al. 1989, Bunker 1997, Calisher et al. 1999).

Rodent-Rodent Hantavirus Transmission.--It is well documented that hantaviral transmission between rodents and insectivores is responsible for viral maintenance in its respective host as well as spillover into closely related species. Despite attempts to prove otherwise, vertical transmission has not been documented and is unlikely (Lee et al. 1981, Mills et al. 1999*a*).

Viral RNA in infected rodents can be detected in the lungs, kidneys, submaxillary glands and rectal tissue; subsequently, large quantities of virus are

shed persistently or sporadically in urine, saliva and feces for extended periods of time, even up to the lifetime of the host. Despite high circulating antibody titres in the serum, virus can still be isolated from organ tissue (Lee et al. 1981, Kariwa et al. 1998, Mills et al. 1999*a*).

Several studies have confirmed that rodent-rodent transmission of hantaviruses occurs much in the same way that rodent-human transmission occurs, that is aerosolization of virus infected excreta. Urine from Seoul virus infected rats, inoculated intranasally into susceptible rats resulted in viral infection and shedding. Horizontal intracage transmission has been demonstrated by simply placing susceptible animals in cages with infected animals (*Apodemus agrarius*). This study also revealed that transmission is not sex – related as infection rates for inter and intra-sex pairings were not significantly different. It is unknown if the virus may be transmitted venerally in a sylvatic setting (Lee et al. 1981, Kariwa et al. 1998).

Aerosolization, grooming, and fighting are the modes of transmission amongst wild population of rodent host. Infected excreta in a shared burrow system are thought to serve as a source for new infection of susceptible animals. Infant and juvenile mice have become infected by their mothers as a result of grooming and exposure to infected excreta present in nests. There has been no demonstration that has shown that hantaviruses can cross the placental barrier or are present in the milk of lactating females (Lee et al. 1981, Kariwa et al. 1998, Mills et al. 1999*a*).

Several studies have noted significant correlations between hantavirus prevalence amongst male rodents and fight wounds. In late summer and early fall, during the period of population peaks, male fighting is increased due to declining availability of food, territorial aggression, and competition for breeding partners. Data regarding a gender correlation with hantavirus prevalence are inconsistent and vary amongst species tested, but most support a higher male to female ratio of hantavirus prevalence. Fighting is the primary mode of interspecific transmission amongst closely related, sympatric species and may be important for viral maintenance (Korch et al. 1989, Boone et al. 1998, Calisher et al. 1999, Mills et al. 1999*a*).

The average lifespan of rodents is brief, perhaps a few months, but mark-recapture studies have shown the some rodents can live 1-2 years or more. This is important for transseasonal persistence of hantaviruses, particularly during periods of low population densities. Infected overwintering adults sharing nests are an important source of infection for susceptible young animals, thus even a small percentage of persistently infected animals serve as reservoirs during low population densities (Lee et al. 1981, Korch et al. 1989, Rand 1994, Schmaljohn and Hjelle 1997, Boone et al. 1998, Kariwa et al. 1998, Calisher 1999, Mills et al. 1999*a*).

There are 3 intrinsic factors associated with hantavirus prevalence: age, weight, and gender. Most long-term mark and recapture monitoring programs have demonstrated that male mice have a higher seroprevalence than females (Mills et al. 1999*a*). One study conducted in Arizona concluded that antibody

positive male Brush mice (*Peromyscus boylii*) had longer survivability than antibody negative *P. boyii* and in disjunct optimal habitats; resident mice were predominantly dominant, antibody positive males. More data will need to be collected in order to determine if this phenomenon truly exists or if it is merely a function of population density and/or recapture success. Another Arizona study found no difference in survivability of seropositive male and females but had much smaller sample sizes and a shorter trapping period (7 months versus 35 months) (Kuenzi et al. 1999). If seropositive male mice do have a longer survivability, they could serve as important maintenance reservoirs for both transseasonal infection and during low population densities (Abbott et al. 1999, Kuenzi et al. 1999)

The other 2 intrinsic factors associated with hantavirus seroprevalence are age and weight and are discussed together because one is a function of the other and cannot easily be stratified. In most field studies, animal weight is used to determine age and thus, the 2 factors must be considered together. Even in studies where eye lens weight was used to determine age, there were no significant differences in hantavirus prevalence when controlling for age or animal weight (Childs et al. 1985, Mills et al. 1997, Glass et al. 1998, Abbott et al. 1999).

Most studies of *Peromyscus spp.* and rat species have demonstrated that antibody prevalences were highest in the highest weight classes. Body weight classes for *Peromyscus spp.* can be assigned I (juvenile), II (young adult), and III (adult) and are 6.0-19.0g, 19.1-22.0g, and 22.1-30.0g respectively. The weight

classes for rats vary from study to study, most likely due to higher weight variability amongst the different species. The trend of increasing seroprevalence as weight class increases is very consistent for *Peromyscus spp.* in the Four Corners region. Additionally, antibody positive *Peromyscus spp.* in the heaviest weight class were also males and presumably the oldest. Since most of these were not mark-recapture studies, it is unknown whether the higher sero-prevalence in class III males represents newly acquired or long-standing infections. In a Walker River Basin study (NV, CA), Boone and others (1998) found no significant difference in seroprevalence between juveniles and adult *Peromyscus spp.* They did however find that antibody positive males were heavier than antibody negative males. While 2 independent studies yielded heavier male biases and hantavirus prevalence correlations, they were not on mark-recapture sites (Tsai 1987, Childs et al. 1995, Mills et al. 1997, Glass et al. 1998, Mills et al. 1999*a*).

While the seroprevalence in weight class I is the lowest, it is interesting to note that the youngest mice in this weight class have the highest seroprevalence. Mills and others (1999*a*) conducted a survey of small mammals in the Southwestern United States and divided the deer mice weight class I into 3 subclasses. Antibody prevalence in the heaviest subclass was 1%; 4% in the middle subclass; and 14% in the lightest subclass. The reason for this is that maternal antibodies are conferred to infants and that passive antibody is lost as young adults, making them susceptible to subsequent hantavirus infection. This

phenomenon could potentially skew seroprevalences and should be considered when conducting field studies (Boone et al. 1998, Mills et al. 1999*a*).

The seroprevalence amongst Norway rats (*Rattus norvegicus*) in Baltimore, MD demonstrated a weight, and thus age bias but not a gender bias. Heavier rats (>300g) had the highest prevalence but no significant differences among male and female rats. Glass and others conducted a study of Cotton rats (*Sigmodon hispidus*) in Florida; *S. hispidus* is the primary rodent reservoir of Black Creek Canal virus (BCC) in the Southeastern U.S. Their findings were more consistent with deer mice than with studies of rats. There were both male and weight / age biases and a higher seroprevalence in the lightest (youngest) weight class than in intermediate weight class which again reflects maternal antibody present, the loss of passive immunity and then subsequent infection (Childs et al. 1985, Childs et al. 1987, Mills et al. 1997, Boone et al. 1998, Glass et al. 1998, Abbott et al. 1999, Kuenzi 1999, Mills et al. 1999*a*).

Rodent Hantavirus Pathology.--In general, the rodent host remains asymptomatically, systemically infected upon experimental inoculation or naturally acquired hantaviruses. Full characterization of the viral pathogenesis; immune response; viral persistency; and hantavirus-host biological relationship is incomplete. Further investigation of viral pathogenicity in the natural rodent host needs to be examined (Kim and McKee 1985, Kariwa et al. 1996, Netski et al. 1999).

Inferences regarding viral shedding, humoral and cell mediated immune responses; and to some degree, pathogenesis in wild populations can only be

made from controlled laboratory experiments. Many of these studies have demonstrated that viral persistence and exhibition of clinical symptoms are age dependent (Morita et al. 1985, Yanagihara and Gajdusek 1988, Kariwa et al. 1996, Hutchinson et al. 1998).

Experimentally infected young adult (6 weeks old) *Sigmodon hispidus* with BCC will have systemic viral complimentary RNA (vcRNA) between 7 and 14 days post-inoculation (p.i.). Infectious virus was present in all tissues, including salivary glands at this time and blood virus titres peaked on 14 days p.i. while titres in all other tissues peaked on day 21 p.i Virus titres declined rapidly after peaking, between 21 and 50 days p.i. but could still be detected on day 150 even though vcRNA could only be found in brain tissue. IgG was detected 14 days p.i., peaked by day 50 p.i. and remained at high levels throughout the duration of the experiment (150 days). Shedding of infectious virus in the urine began on day 7 p.i. Viral titres peaked between 21 and 50 days p.i. and declined after that although infectious virus was present in the urine and feces on day 150 p.i. It is interesting to note that vcRNA was present in the testicles between days 7 and 50, which suggests the possibility of venereal transmission although that has yet to be documented. No clinical symptoms such as weight loss, meningoencephalitis or mortality were observed in any of the animals (Yanagihara and Gajdusek 1988, Hutchinson et al. 1998).

A similar experiment, inoculating adult *A. agrarius* mice with both 76/118 and Lee strains yielded similar results. Viremia was present on day 7 p.i., subsequently transient, and infectious virus was present in all other tissues and

excreta by day 12 p.i. Infectious virus isolated from the lungs, kidneys, and urine persisted for up to 180, 260, and >360 days respectively. High titres of neutralizing and low titres of immunofluorescent antibodies were present for at least 360 days p.i. (Lee et al. 1981). Both of these experiments demonstrated a semichronic pattern of tissue infection with an initial acute phase and viral shedding via rodent excreta persisting for longer periods (Lee et al. 1981, Hutchinson et al. 1998).

Two studies using Wistar rats inoculated with either the KI-262 (Seoul virus) or SR-11 (laboratory derived) strains differed from studies previously mentioned but supported an age dependent factor involved in viral persistence and the ability to act as a source of secondary infection. Three or seven week old rats inoculated with Hantaan strains had either no viral antigen in any tissue (3 week olds) or viral genome in the lungs only (7 week olds). The 7-week-old rats had no virus in the lungs after 50 days p.i. IgG avidity (bond strength between an antibody and an antigen) increased linearly with infection whereas neutralizing antibodies peaked on day 50 p.i. and were maintained after that. Antibody responses persisted throughout the course of the study (Morita et al. 1985, Kariwa et al. 1996).

Newborn rats, 24-48 hours old, when inoculated with the same strains had a much different response. Viral genome was detected in all tissues examined between 1 and 5 days p.i. viral antigen was detected by 3 weeks p.i. and persisted for at least 6 weeks in the lungs serving as a secondary source of infection for cagemates previously uninfected. These data indicate that viral

persistency is age dependent and that newborn rats may be a factor in viral maintenance in a natural setting (Morita et al. 1985, Kariwa 1996).

Early attempts to discover an animal model for hantaviral infections and to understand the biological relationship of the host and hantavirus infection revealed that some pathology does occur in the reservoir host (Kim and McKee Jr. 1985, Netski et al. 1999). The pathological changes that occur in experimentally infected animals differs greatly from naturally infected animals due to the amount of viral inoculums and use of serially diluted, passaged strains in experimental infections (Yamanouchi et al. 1984, Netski et al. 1999).

Inoculation of newborn (<24 hours old) mice and rats with *Apodemus* or *Rattus* derived hantavirus isolates exhibit clinical symptoms that do not resemble human hantaviral infection. Animals will first exhibit hyperactivity and hyperexcitability, which lasts approximately 2-3 days. Initially, animals will not gain weight and over the disease course will lose weight. They will often have a ruffled coat, hunched posture and hind limb paralysis. Convulsions, coma and death usually occur shortly after inoculation. On postmortem examination, animals are runted, dehydrated, and ~50% the weight of normal controls. Animals that do survive will have growth retardation and significant atrophy and paralysis of the hindquarters. Histologically, small foci of necrotic neurons in brain tissue; mononuclear infiltration of the meninges, myocardium, epicardium, and liver; congestion, edema, and interstitial pneumonitis in the lungs; and medullary interstial congestion were observed (Kurata et al. 1983, Yamanouchi et al. 1984, Kim and McKee Jr. 1985).

This is an age dependent phenomenon. With increasing age, the mortality rate decreases, clinical symptoms diminish and by young adulthood, clinical manifestations are inapparent and resemble the naturally infected state. The differences in immune responses in an age-dependent fashion may dictate clinical manifestations and viral clearing in the rodent host (Kurata et al. 1983, Yamanouchi et al. 1984, Kim and McKee Jr. 1985, Yanagihira and Gajdusek 1988).

Naturally infected, wild-caught deer mice exhibited no outward clinical symptoms although pathological changes would be observed histologically. The viral antigen load was positively correlated with the pathology observed. Septal edema in lung tissue with mononuclear infiltrates; immune infiltrates in liver portal zones; and infected kidney glomeruli endothelium were observed. These data are consistent with the pathological changes that occur in *P. leucopus* infected with New York virus and humans infected with SNV. The deer mice spleen, which contains numerous immune cell types, had viral antigen present, which suggests that SNV may infect immune cells (Netski et al. 1999).

The reservoir hosts' humoral immunity is important for neonatal protection from hantaviral infection. Rodent dams confer protective antibodies to their young in utero and via mammary transfer. Maternal immunoglobins G (IgG) and A (IgA) give neonatal resistance to infection when challenged with hantaviral isolates. Cross fostering experiments have shown that both IgG and IgA antibody titres in neonates peak 2-3 weeks after fostering and when challenged with low virus titres (lethal titres) infants developed no clinical symptoms or had

signs of viral infection as compared to nonimmune controls. Neonates challenged with higher virus titres (also lethal dose) exhibited one of two antibody titre changes. One-half of the group had decreasing daily titres of IgG and IgA with transient IgM, followed by an increase of IgG and IgA. This group became persistently infected as evidenced by recovered virus from organs. The other half of this group had a decrease in IgG and IgA, no virus recovered, and demonstrated complete dissipation of antibodies. Neither groups had clinical symptoms. In the group that was challenged with the lower virus titres, it is believed that complete protection was acquired by the dams' immunity since IgM was not detected (Dohmae et al. 1993, Gavrilovskaya et al. 1993, Dohmae and Nishimune 1995, Dohmae and Nishimune 1998).

There are little data addressing specific IgM circulation in infected animals other than one report of Wistar rats challenged with a B1 strain of hantavirus had detectable IgM for over 6 months (Dohmae et al. 1993, Gavrilovskaya et al. 1993, Dohmae and Nishimune 1995, Dohmae and Nishimune 1998).

Neonates born to and/or suckling from immune dams have no detectable antibody after they are 3 months old and thus are susceptible to infection or reinfection in natural settings or maintained with infected cagemates (Dohmae et al. 1993).

A humoral response may be insufficient to clear virus after systemic infection. T-lymphocytes are thought to protect against and assist in recovery from hantavirus infection in mice. T-cell deficient nude rats are more susceptible to Seoul virus than immunocompetant rats. Infant BALB/c mice inoculated with

serial dilutions of immune spleen cells 24 hours after challenge with Hantaan virus were protected 30%-100%, which correlated directly with the dilution of the immune spleen cells. Additionally, IgG, IgM and neutralizing antibody titres appeared earlier and higher titres in immune spleen cell protected mice compared to unprotected controls (Nakamura et al. 1985 Kariwa et al. 1996).

Hantavirus and Tennessee

Between December 1994 and April 1995, the Centers for Disease Control and Prevention (CDC) conducted a hantavirus serosurvey of 39 natural, Central and Eastern U.S. National Park Service sites. The 1993 Four Corners hantavirus outbreak prompted Mills and others to determine the extent of hantavirus activity outside the Southwestern endemic area of the U.S. (Mills et al. 1998).

Of particular interest are the results from GSMNP. As mentioned earlier, *P. maniculatis* and *P. leucopus* are sympatric in many areas of the park. Although both species were captured and tested, 2 *P. maniculatis* out of 27 (or 7.6%) tested were antibody positive for a hantavirus that cross-reacted with Sin Nombre antigen (Mills et al. 1998).

The characteristics of seropositive deer mice in this study were consistent with previously and subsequently published data, that is hantavirus prevalence was higher among males than females and a trend of increasing seroprevalence with increasing weight class (Mills et al. 1998).

Genetic analysis of hantavirus positive *P. maniculatis* in Tennessee reveals a distinct lineage that has been associated with a 1995 HPS case fatality in Eastern North Carolina. Within this distinct lineage is the Sevier County, TN

strain that differs from the Tuckasegee, NC strain by 7.9% at the nucleotide level. The Tennessee lineage is 12.2%, 14.4%, and 15.8% different from the New York, Monogahela, and Sin Nombre lineages respectively (Monroe et al. 1999). Although antibody positive deer mice have been captured in Eastern Tennessee, there have been no documented human cases thus far (Mills et al. 1998).

2.0 MATERIALS AND METHODS

Trapping

From September 13, 2000 to December 1, 2000 and again from November 28, 2001 to November 30, 2001 thirteen areas in GSMNP, Tennessee and North Carolina were selected for sampling (Table 2). These sites included three sites previously surveyed by the Centers for Disease Control and Prevention (CDC) in 1994 and 1995 (Mills et al. 1998). According to park staff that were on hand during the CDC survey, the 3 sites selected by the CDC and also sampled by our team were: Clingman's Dome Road at the Mount Collins Shelter trail head, Campbell Lead, and Cades Cove along Abrams Creek, intersecting Hyatt Lane.

The other 10 sites were selected on the basis of varying risks of human-rodent contact at high and low elevations. These 10 sites were: Mount Collins Shelter, Indian Gap, Clingman's Dome observation area, Newfound Gap observation area, Greenbriar picnic area, Metcalf Bottoms picnic area, Sugarlands' Visitor Center, Cades Cove, the intersection of Loop Road and Abrams Creek, Elkmont Campgrounds and Ranger Station, and Elkmont Clubhouse and adjacent cabin (Table 2).

Most areas were visited for 2 nights, 4 areas: Mount Collins Shelter, Clingman's Dome observation area, and both Elkmont Trapsites were visited for 1 night each. GSMNP wildlife biologists and Student Conservation Assistants (SCA's) provided assistance.

Table 2. Vegetation Types and Elevation (m) of Each Trapsite

Trapsite / Elevation (m)	Vegetation Type[a]
Mount Collins Shelter 1759m	Thelypteris noveboracensis Forest: Northern Red Oak, Red Maple, Carolina Allspice and Buffalo Nut
Clingmans Dome Road / Trail Junction 1778m	Red Spruce Forest: Northern Hardwoods, Yellow Birch and Yellow Buckeye
Indian Gap 1576m	Red Spruce Forest: Northern Hardwoods, Yellow Birch and Yellow Buckeye
Clingmans Dome 1893m	Human Influence[b] and Blue-eyed Grass, Red Spruce Forest: Northern Hardwoods, Yellow Birch and Yellow Buckeye
Newfound Gap 1534m	Red Spruce Forest: Dead Trees and Exposed Northern Hardwood Forest with Mountain Ash and Fire Cherry
Campbell Lead 515m	Virginia Pine Successional Forest: Virginia Pine, Tulip Poplar, Red Maple and Black Locust
Greenbriar 504m	Chestnut Oak, Scarlet Oak, Mountain Laurel and Wandflower
Metcalf Bottoms 498m	Sub Mesic to Mesic Oak Hardwood Forest and Thelypteris noveboracensis Forest: Red Oak, Red Maple, Carolina Allspice and Buffalo Nut
Sugarlands 448m	Human Influence[b] and Montane Alluvial Forest: American Sycamore, Tulip Poplar and White Pine
Cades Cove: Hyatt Lane x Abrams Creek 519m	Fescue Herbaceous Vegetation
Cades Cove: Loop Road x Abrams Creek 515m	Red Maple Seasonally Flooded Forest
Elkmont Campgrounds/ Ranger Station 647m	Human Influence[b] and Yellow Poplar, Sub Mesic to Mesic Oak Hardwood Forest: Northern Red Oak, Red Maple, Carolina Allspice, Buffalo Nut and Thelypteris noveboracensis Forest
Elkmont Clubhouse/Cabin 652m	Human Influence[b] and Yellow Poplar, Sub Mesic to Mesic Oak Hardwood Forest: Northern Red Oak, Red Maple, Carolina Allspice, Buffalo Nut and Thelypteris noveboracensis Forest

[a]Vegetation types are listed by dominance. Vegetation descriptions have been compiled by the University of Georgia (Madden, personal communication).

[b]Areas that have been altered by human activity and land use change such as heavy deforestation and paving.

Each trapnight, an average of 35 (range=16-50) Sherman™ (3x3.5x9", Sherman live traps, Tallahassee, FL) traps were placed in traplines of 6-22 traps at approximately 30-foot intervals. Traps were marked with stickers identifying them as property of the University of Tennessee College of Veterinary Medicine as part of a research study. Trapline and number were marked on the sticker prior to placement. Surveyors' tape was tied to a branch above the trap to mark location. Traps were baited with a mixture of peanut butter and rolled oats and an apple slice. During the colder months, cotton balls were also placed in traps for nesting material to reduce trap related deaths (Mills et al. 1999). Traps were set out in late afternoon and collected early the following morning. Investigators donned latex gloves before partially opening sprung traps for confirmation of captured animals. Traps containing animals were placed in biohazard bags, double bagged in plastic garbage bags, and marked on a trap tally form. Empty traps were snapped shut to prevent animals from entering during the day and left in place if a second night of trapping was planned. The double-bagged traps were transported to a central processing area.

The processing area was opened on 1 side. A stainless steel table was used for processing animals and a gurney with a fan was placed in back of the processing table, approximately 5 feet, to create a laminar flow to the outside and away from the investigators. Investigators donned disposable Tyvek® (E.I. du Pont de Nemours and Company, Wilmington, DE) coveralls, boot covers, hair bonnets, latex gloves, and face shields with powered air purifying respirators

equipped with HEPA filters (3M™ Air-Mate, St. Paul, MN). Investigators conducting data collection remained behind the laminar flow. Processing was performed according to standardized protocols (Mills et al. 1995*a, b*) with the following exceptions: Isofluorane™ (Rhodia Asia Pacific, Singapore) was used as the anesthetic agent. Animals were first placed in a Ziploc™ bag with an Isofluorane™ soaked cottonball to induce anesthesia. A Falcon™ (BD-Becton, Dickinson and Company, Franklin Lakes, NJ) 15 ml conical tube containing an Isofluorane™ soaked cottonball placed over the animal's nose and used to maintain anesthesia during cardiac puncture (Mills et al. 1995*a*). Blood was then transferred into a serum separator microtainer® (BD-Becton, Dickinson and Company, Franklin Lakes, NJ). After euthanasia by cervical dislocation (University of Tennessee Animal Care and Use Committee Protocol #1040), tissue samples were collected for RT-PCR analysis and placed in an appropriately marked 2.0 mL cryovials (Fisherbrand®, Suwanee, GA) containing Trizol™ reagent (GibcoBRL Life Technologies, Gaithersburg, MD) (150µl for kidneys and 75µl for all other tissues). Specimen identification was made using morphological characteristics as described by Laerm and Boone (1997). Traps were cleaned, disinfected, re-baited, and returned to the trapline, all other traps were re-set for a second night. On the last morning of trapping at each trapsite, all traps were collected and cleaned according to standardized protocols (Mills et al. 1995*a*, *b*).

North American Datum of 1927 (NAD27) was the basis used for Global Positioning System (GPS) coordinates identifying trapsites in GSMNP (Figure 1

and Table 6). GPS coordinates were obtained using TOPO! ® Interactive Maps (version 2.0, Wildflower Productions San Francisco, CA).

Serology

Blood collected by cardiac puncture was centrifugated at 12,000 rpm, 35°C for 6 minutes in a Biosafety Level 3 (BSL-3) facility at the University of Tennessee College of Veterinary Medicine. The serum portion was drawn off under a laminar flow biological safety cabinet with an Eppendorf® 100µl pipette (Brinkmann Instruments, Inc. Hamburg, Germany) with aerosol barrier tips (Fisherbrand®, Suwanee, GA) (tips discarded after each serum sample) and expelled into a cryovial. Serum samples were kept frozen at -40°C in a locked freezer until analyzed.

Through the Tennessee Department of Health and Environment, we acquired SNV Control Antigens and Positive Control; Positive Control Antigen SPR568 (Cat. No. VA2272, Lot No. 98-0044L), Negative Control Antigen SPR569 (Cat. No. VA2273, Lot No. 98-0042L) and Positive Control Sera (Cat. No. VS2365, Lot No. 99-0005L), produced by the CDC. This assay was performed as per Feldmann et al. (1993) and the CDC Sin Nombre Virus IgG ELISA (1994) with modifications. The positive control antigen consisted of affinity purified, inactivated SNV recombinant nucleocapsid (N) protein produced in either baculo virus/SF-9 cells or *E. coli* (CDC 1994). The negative control antigen was prepared in a similar manner (CDC 1994). The positive control serum was produced in mice injected with recombinant, nucleocapsid antigen. The negative control for this assay was pooled sera from hantavirus negative,

laboratory mice with no known exposure to hantavirus, provided by the University of Tennessee Office of Laboratory Animal Care.

The positive control antigen, negative control antigen, and positive control sera were reconstituted with 0.20 mL each of ultra pure water. The positive and negative control antigens were used at a 1:4000 dilution, diluted with phosphate buffered saline (PBS) (pH 7.2, Invitrogen Corp., Grand Island, N.Y). Wash buffer was made with PBS (Fisherbrand®, Suwanee, GA) and 0.1% Tween-20 with thimersol (Sigma®, St. Louis, MO). Serum diluent (SerDil) was made with wash buffer and 5% powdered skim milk.

Falcon®, flexible, 96-well polyvinyl chloride (PVC), flat-bottom microtitre plates (BD-Becton Dickinson and Company, Franklin Lakes, NY) were coated overnight at 4ºC with diluted antigens. Positive control antigen coated the top half of the plate and negative control antigen coated the bottom half of the plate. Unbound antigen was removed by washing plates three times with wash buffer.

The affinity purified, goat anti-mouse IgG (γ-chain specific) horseradish peroxidase, conjugate (Kirkegaard and Perry Laboratories, Gaithersburg, MD) dilution was determined by checkerboard titration with reference sera; optimal dilution was found to be 1:4000. Test sera were initially diluted 1:100 and then serially diluted two fold with 750 µl SerDil through 1:1600. The negative control sera were initially diluted 1:100 and then serially diluted two fold to 1:1600. The positive control sera were initially diluted to 1:4000 and then serially diluted two fold to 1:64,000. 100µl each of test sera, positive control sera, and negative control sera were plated in duplicate on microtitre plates. Plates were incubated

for 90 minutes at room temperature (25°C). Unbound antibodies were removed by washing plates three times with wash buffer. Bound IgG was detected with conjugated antibody at a 1:4000 dilution (previously described). 100µl was added to each microtitre plate well. Plates were incubated for 60 minutes at room temperature (25°C). Unbound conjugate was removed by washing plates three times with wash buffer. ABTS (2,2' azino-di [3-ethyl-benzothiazolnin (6)-sulfonic acid]) (Kirkegaard and Perry Laboratories, Gaithersburg, MD) substrate used to detect bound IgG labeled with conjugate; 100µl was added to each well. Microtitre plates were incubated at room temperature for 30 minutes. Optical densities were read at a wavelength of 405 nanometers (OD_{405}), and recorded using KC junior™ software. The values of the 1:100 test sera dilutions of each specimen were averaged to yield the adjusted OD_{405} values used for titre determination.

Antibody titres were measured by ELISA and converted to Geometric Mean Titres (GMT) by taking the reciprocal of the mean OD values where they transected the halfway point of the highest OD value (0.8) (Figure 6, page 76). Samples were considered positive if their log titre was greater than 1:100 or if their sum adjusted OD_{405} value was greater than or equal to 0.65 (Figures 7 and 8, pages 77 and 78).

Statistical Analysis

Data were analyzed using SPSS software (version 10.0, SPSS Inc., Chicago, IL). Trapnights were calculated by multiplying the number of nights traps were set by the number of traps set each night. The weight classes for

deer mice and white-footed mice used were those calculated by Mills et al. (1998) and were: I (4.0-17.7g), II (17.8-20.9g), and III (21.0-37.0g) for deer mice and I (6.0-16.9g), II (17.0-20.0g), and III (20.1-37.0g) for white-footed mice. Overall trap success was calculated as the number of captures per trapnights at each location. For *Peromyscus spp.*, a corrected trap success rate was calculated similarly but by subtracting half of the non-*Peromyscus spp.* captured from the overall number of trapnights to account for trap unavailability (Mills et al. 1998).

3.0 RESULTS

Trapping Results

Between September 13, 2000 and December 1, 2000; and on November 28 and 29, 2001, a total of 761 trapnights (trapnight = 1 trap for 1 night) were conducted. A total of 171 rodents and insectivores representing 7 genera and 12 species were captured (146 rodents and 25 insectivores) (Table 3).

Sample Collection

Blood samples were obtained from 142 (83.0%) captured animals and tested for the presence of IgG antibodies against hantavirus. Tissue samples (spleen, liver, lung, and kidneys) were collected from 169 animals (embryos were collected from 15 of these animals). Two animals, *Ochrotomys nuttali* (Golden mice) were released from the trapsite as they were thought to be a threatened species at the time of the survey; they were included in calculations for trapping success but excluded from all other data analysis.

Peromyscus leucopus was the most frequently captured species (32.7%), followed by *P. maniculatis* (25.1%), and *C. gapperi* (24.0%). All other species accounted for 18.2% of the total captures. The average number of captures per trapsite was 14.3 (range=2 – 35) and the overall trapping success was 23.4% (171/731).

Table 3. Captured Species by Gender, Age Category, Weight, and Elevation

Species	Total[a] (%)	Gender		Age[c,d] (%)	Weight Range (g)	Mean ±SD	Elevation Range (m)	Mean ±SD
		Male(%)[b]	Female(%)[b]					
Peromyscus leucopus	56 (32.7)	29 (51.8)	27 (48.2)	29 (51.8) Juvenile; 10 (17.9) Sub-adult 17 (30.4) Adult	9.0 – 35.0	17.5 ±6.2	1345 - 5680	2958.5 ±1596.1
Peromyscus maniculatis	43 (25.1)	19 (44.2)	24 (55.8)	22 (51.2) Juvenile; 17 (39.5) Sub-adult 4 (9.3) Adult	10.0 – 23.0	16.7 ±3.1	1494 - 5680	3994.2 ±1726.2
Clethrionomys gapperi	41(24.0)	15 (36.6)	26 (63.4)	N/A	14.0 – 39.0	25.0 ±6.1	4603 - 5680	5344.6 ±359.8
Sorex longirostris	3 (1.8)	0 (0.0)	3 (100.0)	N/A	3.0 – 6.5	4.8 ±1.8	5726 - 5680	5410.1 ±233.3
Blarina brevicauda	7 (4.1)	5 (71.4)	2 (28.6)	N/A	10.0 – 18.0	13.7 ±2.4	1345 – 5680	3012.6 ±2058.2
Sorex fumeus	5 (2.9)	1 (20.0)	4 (80.0)	N/A	6.0 – 8.0	7.0 ±1.0	4603 - 5680	5274.4 ±557.2
Microtus ochrogaster	2 (1.2)	1 (50.0)	1 (50.0)	N/A	14.0 – 37.5	25.8 ±16.6	5680 – 5680	5680.0 ±0.0
Sorex cinerus	1 (0.6)	0 (0.0)	1 (100.0)	N/A	8.0 –8.0	8.0 ±0.0	4603 – 4603	4603.0 ±0.0
Blarina carolinensis	9 (5.3)	7 (77.8)	2 (22.2)	N/A	11.0 – 19.0	14.0 ±2.6	1345 – 1557	1498.4 ±65.1
Sigmodon hispidus	1 (0.6)	1 (100.0)	0 (0.0)	N/A	65.0 – 65.0	65.0 ±0.0	1557 – 1557	1557.0 ±0.0
Synaptomys cooperi	1 (0.6)	1 (100.0)	0 (0.0)	N/A	26.0 – 26.0	26.0 ±0.0	1544 - 1544	1544.0 ±0.0

[a] Two *O. nuttali* were not included in this table. They were released at the trapsites where they were captured and no other data were collected for this species.

[b] Gender number and frequencies were calculated for within species

[c] Approximate ages for *P. maniculatis* (deer mice) and *P. leucopus* (white-footed mice) were based on weight categories: I (4.0-17.7g), II (17.8-20.9g), and III (21.0-37.0g) for deer mice and I (6.0-16.9g), II (17.0-20.0g), and III (20.1-37.0g) for white-footed mice. Weight category I = Juvenile; weight category II = Sub-Adult; and weight category III = Adult. (Mills et al. 1998).

[d] Approximate ages for all other species were not calculated. Only *P. maniculatis* and *P. leucopus* were seropositive for hantavirus and only these species were used for analysis.

The specific trap success indicates relative population number of *Peromyscus spp.* at each trapsite (Table 4). The specific trapsite success varied greatly throughout the park (range= 1.7% - 42.1%) with an average of 16.3%.

Table 4. Percentage of Trap Success for All Animals and *Peromyscus spp.* by Trapsite

Trapsite	All Species Trapped (%)[a]	*Peromyscus spp.* Trap Success (%)[b]
Mount Collins Shelter	29/50 (58.0)	14/42.5 (32.9)
Clingmans Dome Road / Mt. Collins Trail Junction	7/60 (11.7)	3/58 (5.2)
Indian Gap	11/60 (18.3)	3/56 (5.4)
Clingmans Dome Observation Area	35/50 (70.0)	11/38 (28.9)
Newfound Gap	20/40 (50.0)	16/38 (42.1)
Campbell Lead x Gatlinburg by-pass	2/60 (3.3)	1/59 (1.7)
Greenbriar	5/59 (8.5)	5/59 (8.5)
Metcalf Bottoms	17/70 (24.3)	11/67 (16.4)
Sugarlands	11/60 (18.3)	7/58 (12.1)
Cades Cove: Hyatt Lane x Abrams Creek	15/96 (15.6)	10/93.5 (10.7)
Cades Cove: Loop Road x Abrams Creek	4/96 (4.2)	4/96 (4.2)
Elkmont Campgrounds / Ranger Station	3/30 (10.0)	3/30(10.0)
Elkmont Clubhouse / Cabin	12/30 (40.0)	12/30 (40.0)

[a] All species Trap Success was calculated by: Animals trapped / Number of traps set.

[b] *Peromyscus spp.* Trap Success was calculated by subtracting half of the non-*Peromyscus spp.* captured from the denominator of All Species trapped and dividing the number of *Peromyscus spp.* by the adjusted denominator.

An average of 8.3 (range=1 – 15) *Peromyscus spp.* were captured at all trapsites. There was a significant difference in the number of *P. leucopus* versus *P. maniculatis* captured at 2 elevation ranges (P<0.001). *P. leucopus* accounted for 73.6% (39/53) of *Peromyscus spp.* captured below 750m. *P. maniculatis* accounted for 63.0% (29/46) of *Peromyscus spp.* captured above 1500m (Figure 1).

Seroprevalence of Antibody Positive Rodents

Of the 142 animals that were tested for the presence of hantavirus IgG antibodies, 9 were found to be seropositive. The only seropositive animals were 4.9% (7/142) *P. maniculatis* and 1.4% (2/142) *P. leucopus* with an overall seroprevalence of 6.3% (9/142).

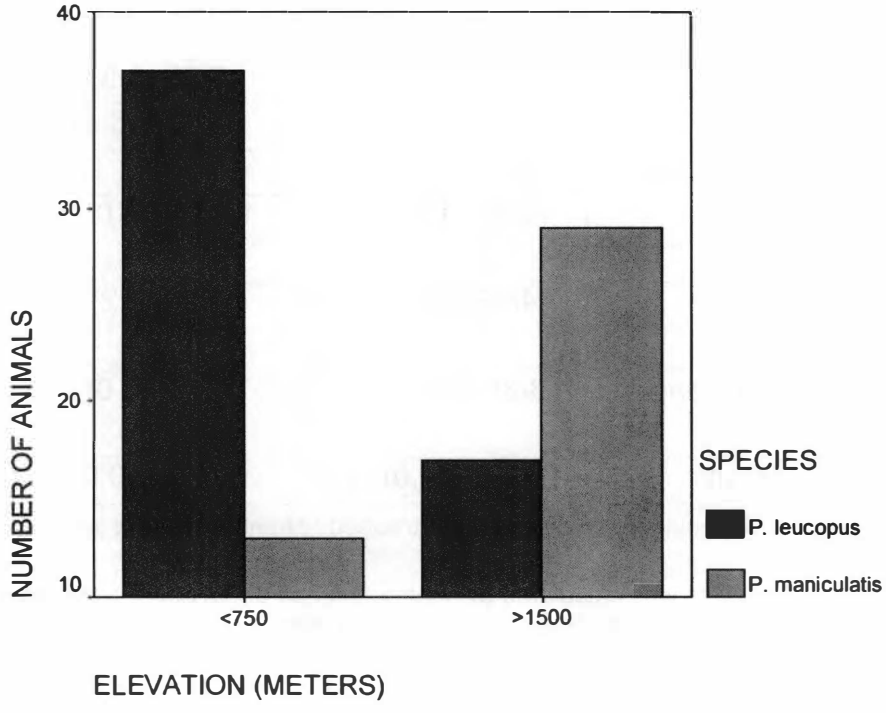


Figure 1. Comparison of *Peromyscus spp.* Trapped by Elevation

P. leucopus accounted for 56.3% (54/96) of the *Peromyscus spp.* that were serologically tested. *P. maniculatis* accounted for 43.8% (42/96) of the *Peromyscus spp.* that were serologically tested (Table 5).

Seropositive animals were captured during each month of the survey except December (trapping was conducted on only 1 day during December). An equal number of seropositive *Peromyscus spp.* were captured during the months of September and November (n=4), and one seropositive animal was captured during the month of October. The fewest number of *Peromyscus spp.* were captured during September (Figure 2). Considering *Peromyscus spp.* that were tested for SNV antibodies (n=96), 2.1% (2/96) of *P. leucopus* were positive and 7.3% (7/96) of *P. maniculatis* were positive. The proportion of seropositive deer mice was more than 4 times the proportion of seropositive white-footed mice and was statistically significant (P=0.03) (Tables 5 and 6, page 65 and Figure 3, page 66).

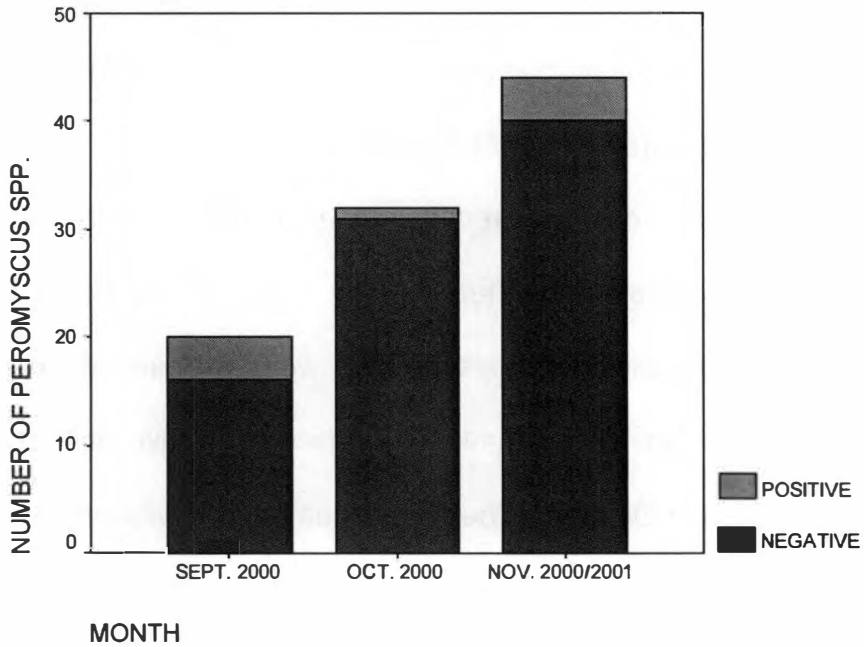


Figure 2. Seropositive and Seronegative *Peromyscus spp.* by Month

Table 5. Proportions of Seropositive and Seronegative *Peromyscus spp.*

SPECIES by POSITIVE and NEGATIVE Crosstabulation

			NEGATIVE and POSITIVE		
			negative	positive	Total
SPECIES	*P. leucopus*	Count	52	2	54
		% within SPECIES	96.3%	3.7%	100.0%
		% within POS_NEG	59.8%	22.2%	56.3%
		% of Total	54.2%	2.1%	56.3%
	P. maniculatis	Count	35	7	42
		% within SPECIES	83.3%	16.7%	100.0%
		% within POS_NEG	40.2%	77.8%	43.8%
		% of Total	36.5%	7.3%	43.8%
Total		Count	87	9	96
		% within SPECIES	90.6%	9.4%	100.0%
		% within POS_NEG	100.0%	100.0%	100.0%
		% of Total	90.6%	9.4%	100.0%

The POS_NEG rows are the seropositives of 1 *Peromyscus sp.* divided by the total number of seropositive animals, and seronegatives of 1 *Peromyscus sp.* divided by the total number of seronegatives.

Table 6. Profile of Hantavirus Seropositive Animals

Trap Date	Trap site	Elevation (m) /GPS coordinates	Species	Gender	Age[a]	Weight (g)	*Peromyscus* Specific Seroprevalence[b]	% Seropositive *Peromyscus*[c]
09/21/00	Clingmans Dome Road / Trail Junction	1778 X:276,146 Y:3,941,241	*P. maniculatis*	Female	Sub-adult	20.0	100% 3/3	60.0% 3/5
09/21/00	Clingmans Dome Road / Trail Junction	1778 X:276,146 Y:3,941,241	*P. maniculatis*	Male	Juvenile	17.0	100% 3/3	60.0% 3/5
09/22/00	Clingmans Dome Road / Trail Junction	1778 X:276,146 Y:3,941,241	*P. maniculatis*	Female	Juvenile	13.0	100% 3/3	60.0% 3/5
09/28/00	Indian Gap	1576 X:278,379 Y:3,943,198	*P. maniculatis*	Female	Sub-adult	18.0	33.3% 1/3	30.0% 3/10
10/05/00	Clingmans Dome Observation Area	1893 X:273,809 Y:3,937,662	*P. maniculatis*	Female	Sub-adult	18.0	10.0% 1/10	34.5% 10/29
11/02/00	Metcalf Bottoms	498 X:260,251 Y:3,951,745	*P. maniculatis*	Male	Adult	23.0	18.2% 2/11	91.7% 11/12
11/02/00	Metcalf Bottoms	498 X:260,251 Y:3,951,745	*P. maniculatis*	Male	Sub-adult	19.0	18.2% 2/11	91.7% 11/12
11/10/00	Sugarlands	448 X:270,371 Y:3,951,897	*P. leucopus*	Male	Adult	21.0	14.3% 1/7	77.8% 7/9
11/29/01	Elkmont Clubhouse / Cabin	652 X:266,295 Y:3,948,630	*P. leucopus*	Male	Sub-adult	17.0	6.7% 1/15	100% 15/15

Table 6 continued

[a] Approximate ages for *P. maniculatis* (deer mice) and *P. leucopus* (white-footed mice) were based on weight categories: I (4.0-17.7g), II (17.8-20.9g), and III (21.0-37.0g) for deer mice and I (6.0-16.9g), II (17.0-20.0g), and III (20.1-37.0g) for white-footed mice. Weight category I = Juvenile; weight category II = Sub-Adult; and weight category III = Adult (Mills et al. 1998).

[b]*Peromyscus* Specific Prevalence was calculated by dividing the number of positive *Peromyscus* by the total number of *Peromyscus* captured at that trapsite.

[c]% *Peromyscus* was calculated by dividing the number of *Peromyscus* by the total number of animals captured and serologically tested at that trapsite.

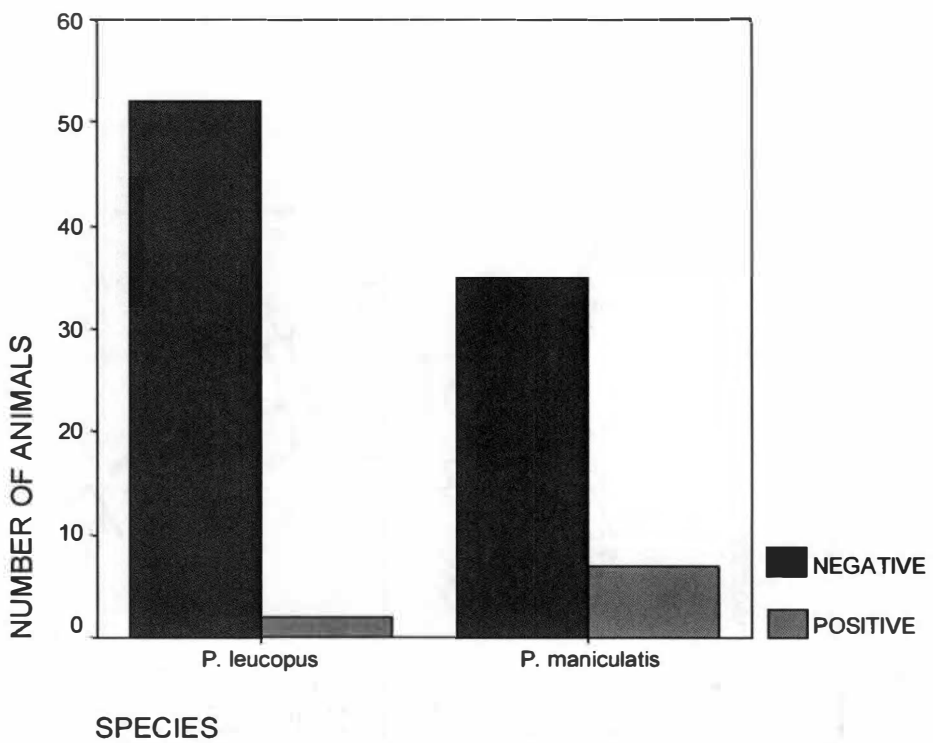


Figure 3. Comparison of Seropositive and Seronegative *Peromyscus spp*.

Geographic Variation in Seroprevalence

Seropositive *Peromyscus spp.* were found at 6 trapsites out of 13 in GSMNP and *Peromyscus* specific trapsite seroprevalence ranged from 6.7% - 100% (Table 6 and Figure 4).

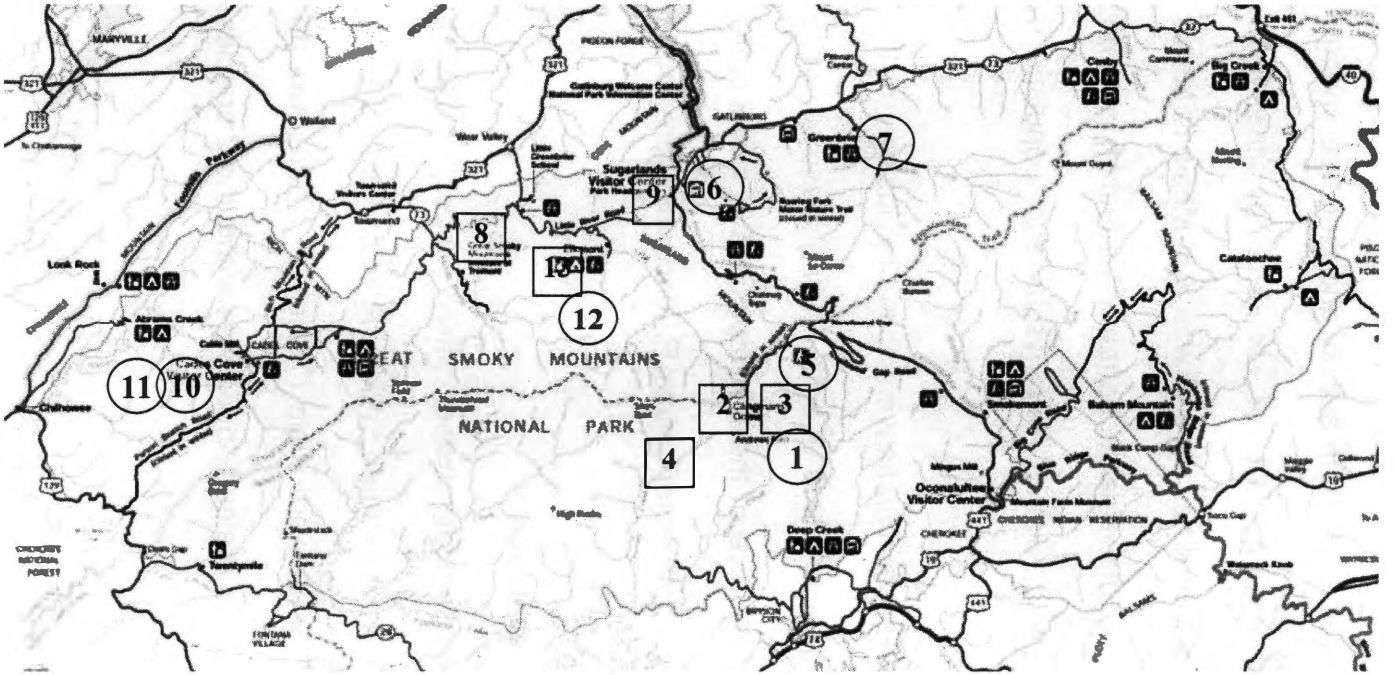


Figure 4. Trapsites in GSMNP where at least one Seropositive (□) and only Seronegative (○) Animals were Trapped

1= Mount Collins Shelter
2= Clingmans Dome Road / Trail Junction; 3 seropositive *P. maniculatis*
3= Indian Gap; 1 seropositive *P. maniculatis*
4= Clinmans Dome Observation Area; 1 seropositive P. maniculatis
5= Newfound Gap
6= Campbell Lead x Gatlinburg by-pass
7= Greenbriar
8= Metcalf Bottoms; 2 seropositive *P. maniculatis*
9= Sugarlands; 1 seropositive *P. leucopus*
10= Cades Cove: Hyatt Lane x Abrams Creek
11= Cades Cove: Loop Road x Abrams Creek
12= Elkmont Campgrounds / Ranger Station
13= Elkmont Clubhouse / Cabin; 1 seropositive P. leucopus

Fifty percent (3/6) of the trapsites where seropositive animals were found were dominated by human influence (Table 2). Human influence dominated is defined as areas that have been altered by human activity and land use change such as heavy deforestation and paving. Two of six (33.3%) sites were predominantly Red Spruce forest and the remaining site was Sub-Mesic to Mesic Oak hardwood forest. There was no discernable pattern of infection and no correlation between seroprevalence and relative *Peromyscus spp*. populations (Tables 4 and 6).

Among seropositive animals, *P. maniculatis* were found exclusively at the higher elevations (>1500m) while equal numbers of both *Peromyscus spp.* were found at the lower elevations (<750m). Five of the 7 seropositive *P. maniculatis* were found above 1500 meters while both seropositive *P. leucopus* were found below 750 meters.

Association of Gender and Seroprevalence

Of the 96 *Peromyscus spp.* serologically tested, within *P. leucopus*, 50.0% (27/54) were female and 50.0% (27/54) were male. Within *P. maniculatis*, 57.1% (24/42) were fe4male and 42.9% (18/42) were male (Table 7). There was no significant difference in the proportions of males and females within the 2 species (P=0.49)

Table 7. Comparison of Serologically Tested *Peromyscus spp.* by Gender

SPECIES by GENDER Crosstabulation

			GENDER		
			female	male	Total
SPECIES	*P. leucopus*	Count	27	27	54
		% within SPECIES	50.0%	50.0%	100.0%
		% within GENDER	52.9%	60.0%	56.3%
	P. maniculatis	Count	24	18	42
		% within SPECIES	57.1%	42.9%	100.0%
		% within GENDER	47.1%	40.0%	43.8%
Total		Count	51	45	96
		% within SPECIES	53.1%	46.9%	100.0%
		% within GENDER	100.0%	100.0%	100.0%

The percentages within GENDER rows are the percentage of species within that gender

Within *P. leucopus*, the only seropositive animals were male. There was no significant correlation between gender and species specific seropositivity (P=0.15). Within *P. maniculatis*, the proportion of males and females that were positive was equal (16.7%). There was no correlation between gender and species specific seropositivity (P=1.00) (Table 8). Even when considering the males and females of both *Peromyscus spp.*, there was still no significant correlation between gender and seropositivity (P=0.58) (Table 9).

Among seropositive animals (n=9), significantly more positive females (44.4%; 4/9) were captured at the higher elevations (>1500m) than males (11.1%; 1/9). Significantly more positive males (44.4%; 4/9) were captured at the lower elevations (<750m) than females (0.0%; 0/9) (P=0.02) (Table 10).

Table 8. Species Comparison by Gender and Seropositivity

GENDER by POSITIVE/NEGATIVE for SPECIES crosstabulation

SPECIES				NEGATIVE and POSITIVE		Total
				negative	positive	
P. leucopus	GENDER	female	Count	27		27
			% within GENDER	100.0%		100.0%
			% within POS_NEG	51.9%		50.0%
			% of Total	50.0%		50.0%
		male	Count	25	2	27
			% within GENDER	92.6%	7.4%	100.0%
			% within POS_NEG	48.1%	100.0%	50.0%
			% of Total	46.3%	3.7%	50.0%
	Total		Count	52	2	54
			% within GENDER	96.3%	3.7%	100.0%
			% within POS_NEG	100.0%	100.0%	100.0%
			% of Total	96.3%	3.7%	100.0%
P. maniculatis	GENDER	female	Count	20	4	24
			% within GENDER	83.3%	16.7%	100.0%
			% within POS_NEG	57.1%	57.1%	57.1%
			% of Total	47.6%	9.5%	57.1%
		male	Count	15	3	18
			% within GENDER	83.3%	16.7%	100.0%
			% within POS_NEG	42.9%	42.9%	42.9%
			% of Total	35.7%	7.1%	42.9%
	Total		Count	35	7	42
			% within GENDER	83.3%	16.7%	100.0%
			% within POS_NEG	100.0%	100.0%	100.0%
			% of Total	83.3%	16.7%	100.0%

Table 9. *Peromyscus spp.* Specific Comparison of Seronegatives and Seropositives by Gender

POSITIVE and NEGATIVE by GENDER Crosstabulation

			GENDER		
			female	male	Total
POSITIVE and NEGATIVE	negative	Count	47	40	87
		% within POS_NEG	54.0%	46.0%	100.0%
		% within GENDER	92.2%	88.9%	90.6%
		% of Total	49.0%	41.7%	90.6%
	positive	Count	4	5	9
		% within POS_NEG	44.4%	55.6%	100.0%
		% within GENDER	7.8%	11.1%	9.4%
		% of Total	4.2%	5.2%	9.4%
Total		Count	51	45	96
		% within POS_NEG	53.1%	46.9%	100.0%
		% within GENDER	100.0%	100.0%	100.0%
		% of Total	53.1%	46.9%	100.0%

Table 10. Comparison of Seropositive Animals by Gender and Elevation

GENDER by ELEVATION Crosstabulation

			ELEVATION (METERS)		
			<750	>1500	Total
GENDER	female	Count		4	4
		% within GENDER		100.0%	100.0%
		% within ELEVATION		80.0%	44.4%
		% of Total		44.4%	44.4%
	male	Count	4	1	5
		% within GENDER	80.0%	20.0%	100.0%
		% within ELEVATION	100.0%	20.0%	55.6%
		% of Total	44.4%	11.1%	55.6%
Total		Count	4	5	9
		% within GENDER	44.4%	55.6%	100.0%
		% within ELEVATION	100.0%	100.0%	100.0%
		% of Total	44.4%	55.6%	100.0%

Association of Weight (Age) and Seroprevalence

The relative ages of the *Peromyscus spp.* were based on weight (Table 6). Within *P. leucopus* tested, 53.7% (29/54) were juveniles (weight category 1); 16.7% (9/54) were sub-adults (weight category 2); and 29.6% (16/54) were adults (weight category 3). There were no seropositive juveniles; 1.9% (1/54) of sub-adults were seropositive; and 1.9% (1/54) of adults were seropositive.

Within *P. maniculatis* tested, 50% (21/42) were juveniles (weight category 1); 40.5% (17/42) were sub-adults (weight category 2); and 9.5% (4/42) were adults (weight category 3). Two of Forty-two (4.8%) juveniles were seropositive; 9.5% (4/42) of sub-adults were seropositive; and 2.4% (1/42) adults were seropositive (Table 11 and Figure 5, page 72).

Table 11. Species Comparison Between Seropositive / Seronegative by Weight (Age) Categories

POSITIVE/NEGATIVE by WEIGHT CATEGORY for SPECIES Crosstabulation

SPECIES				WEIGHT (AGE) CATEGORY 1	2	3	Total
P. leucopus	POSITIVE / NEGATIVE	negative	Count	29	8	15	52
			% within POS_NEG	55.8%	15.4%	28.8%	100.0%
			% within Weight Cat	100.0%	88.9%	93.8%	96.3%
			% of Total	53.7%	14.8%	27.8%	96.3%
		positive	Count		1	1	2
			% within POS_NEG		50.0%	50.0%	100.0%
			% within Weight Cat		11.1%	6.3%	3.7%
			% of Total		1.9%	1.9%	3.7%
	Total		Count	29	9	16	54
			% within POS_NEG	53.7%	16.7%	29.6%	100.0%
			% within Weight Cat	100.0%	100.0%	100.0%	100.0%
			% of Total	53.7%	16.7%	29.6%	100.0%
P. maniculatis	POSITIVE / NEGATIVE	negative	Count	19	13	3	35
			% within POS_NEG	54.3%	37.1%	8.6%	100.0%
			% within Weight Cat	90.5%	76.5%	75.0%	83.3%
			% of Total	45.2%	31.0%	7.1%	83.3%
		positive	Count	2	4	1	7
			% within POS_NEG	28.6%	57.1%	14.3%	100.0%
			% within Weight Cat	9.5%	23.5%	25.0%	16.7%
			% of Total	4.8%	9.5%	2.4%	16.7%
	Total		Count	21	17	4	42
			% within POS_NEG	50.0%	40.5%	9.5%	100.0%
			% within Weight Cat	100.0%	100.0%	100.0%	100.0%
			% of Total	50.0%	40.5%	9.5%	100.0%

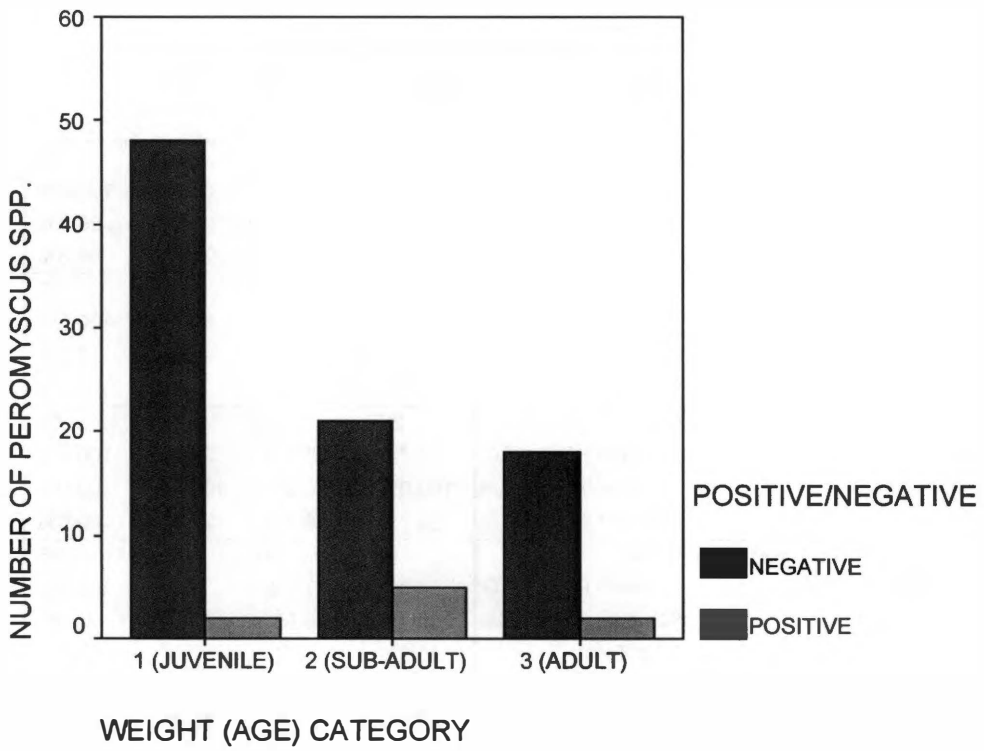


Figure 5. Weight / Age Comparison of Seropositive and Seronegative *Peromyscus spp.*

Antibody Titres

Geometric Mean Titres (GMTs) were converted from ELISA OD_{405} values (Figure 6). GMTs from most seropositive animals ranged from 233 – 7143, however one sample with a GMT of 42 was considered antibody positive because its sum-adjusted OD value exceeded the negative mean plus 4 standard deviations (0.328) (Figure 7, page 75).

A random sample of seronegative animals was paired with the seropositive animals to demonstrate the differences in GMT's at the cut-off point of 100 (for positive antibody titre determination) (Figure 8, page 76).

There were no discernable patterns between GMT's and gender, species or age.

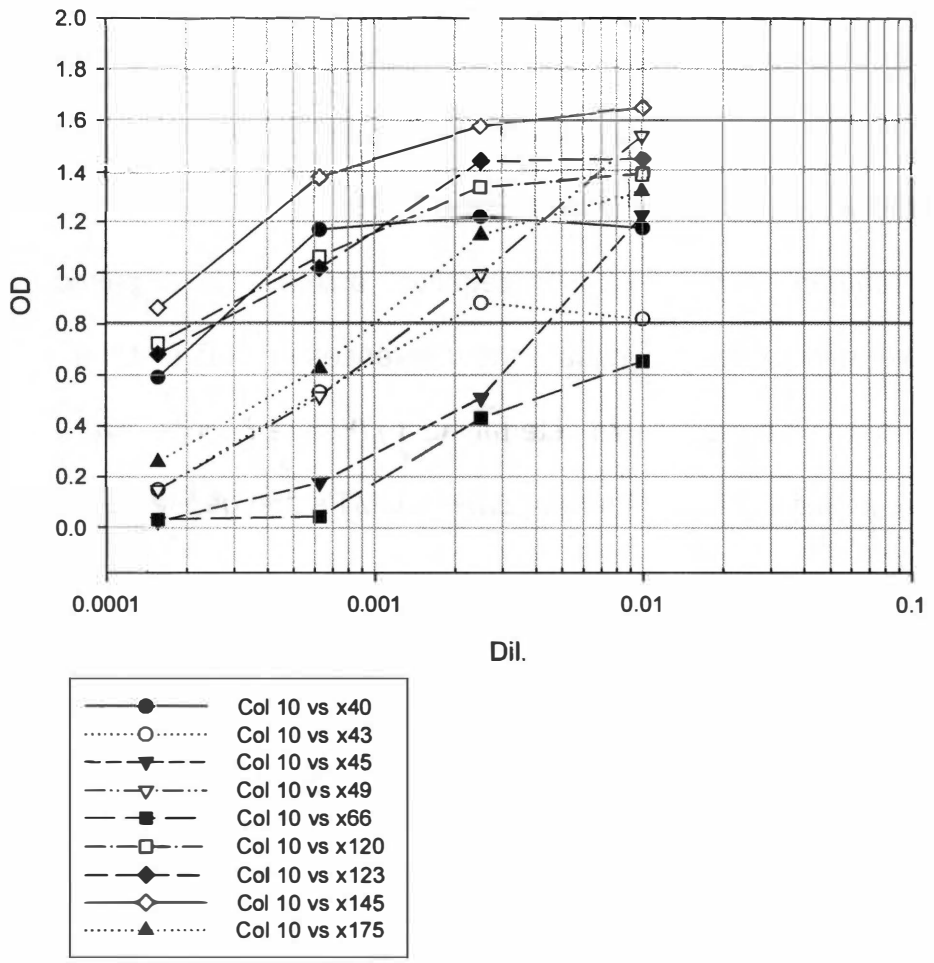


Figure 6. Log of ELISA OD_{405} Values for Determination of GMT's

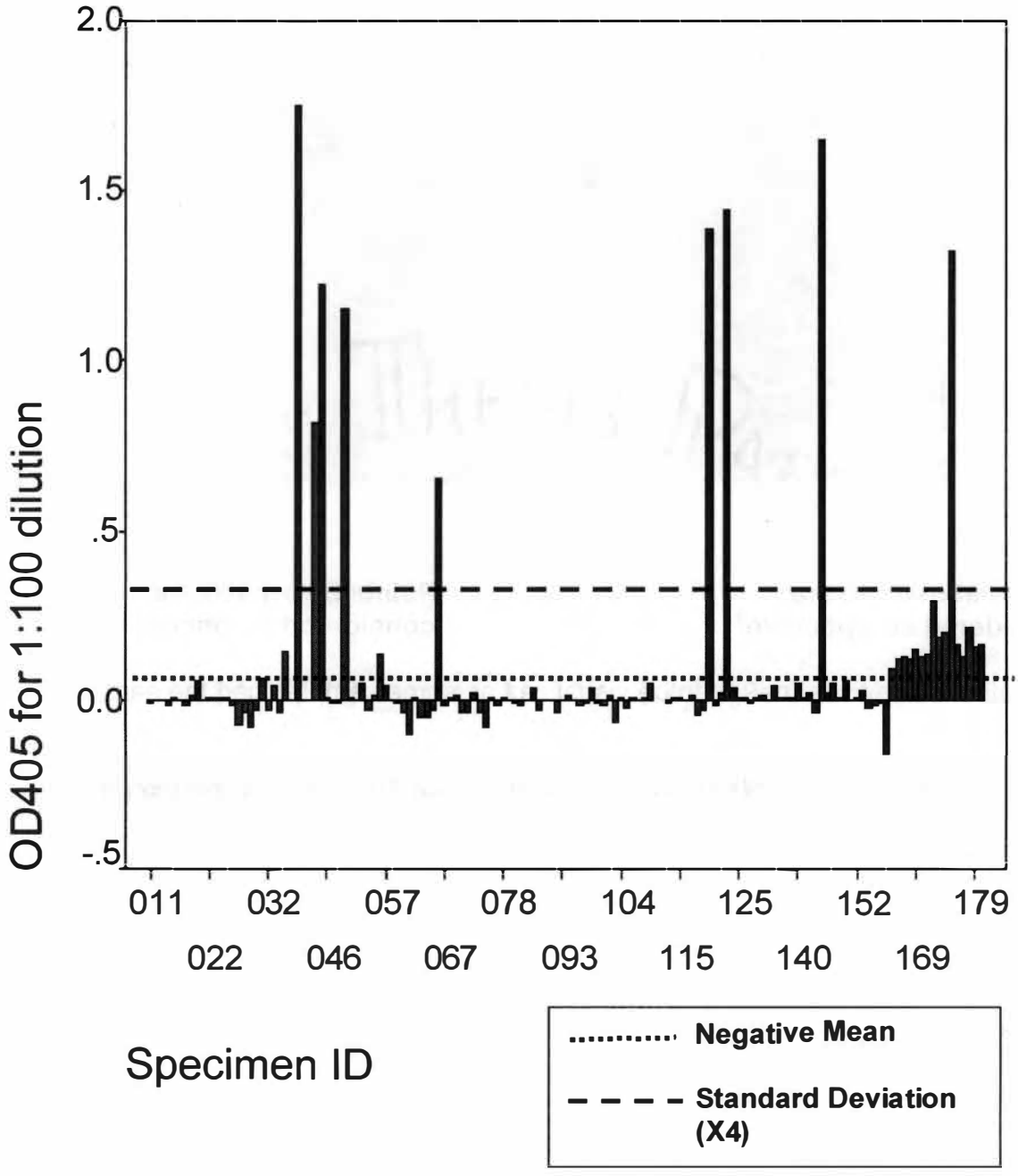
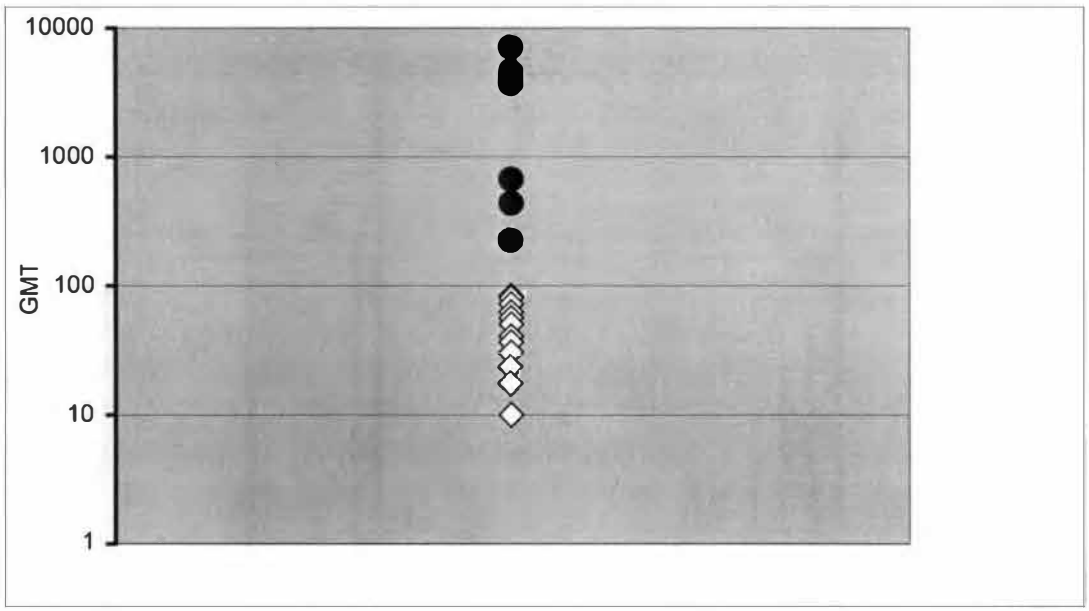


Figure 7. ELISA OD_{405} Values for all Animals Tested with the Negative Mean and 4X Standard Deviation



Points above 100 are considered seropositive[a]

Points below 100 are considered seronegative

[a]Not all seropositives (n=9) can be visualized as some samples had the same GMT's.

Figure 8. Positive and Negative Geometric Mean Titres for *Peromyscus spp.*

4.0 DISCUSSION

The overall hantavirus antibody prevalence in the Great Smoky Mountains National Park among *Peromyscus spp.* (9.4%) is higher than the previously reported seroprevalence of 6.1% among *Peromyscus spp.* (Mills et al. 1998). The 1995 study conducted by Mills et al. (1998), which was limited in the number of sites sampled (n=3) reported a *P. maniculatis* specific seroprevalence of 7.4% and no seropositive *P. leucopus*. Our study has shown a *P. maniculatis* specific prevalence of 16.7% and a *P. leucopus* specific prevalence of 3.7%. Our study is the first to find evidence of hantavirus infection in *P. leucopus* in the park.

The results of the 3 trapsites surveyed by Mills et al. (1998) and revisited by us in 2000 are worthy of comparison. According to park staff, the 2 seropositive deer mice that Mills et al. (1998) found in GSMNP were located at the Clingmans Dome Road / Mt. Collins Trail Junction. Our investigators found 3 seropositive deer mice in the same general location. The specific trap success of the Mills survey was 9.4%. The specific trap success found by our investigators was 5.2% for the same 3 sites used in the original GSMNP survey. One interpretation is that there was a decrease in the *Peromyscus spp.* population, as relative populations may be inferred from specific trap success. The differences in seroprevalences at the same 3 trapsites were not significantly different (P=0.12) but suggestive that the 21.4% seroprevalence found in 2000 was different than the 6.1% seroprevalence reported by Mills et al. (1998). The possible decrease in *Peromyscus spp.* population and increase in seroprevalence may be just that, at least in this biotic community. This is

supported by the differences in respective specific trap success rates and seropositive animals found. It is difficult however, to make a meaningful comparison due to 1) the small sample sizes of both surveys and 2) variables such as month of trapping and weather that were not reported in the initial survey.

We found no seropositive animals in the other 2 trapsites that Mills et al. (1998) visited in the 1995 survey (Cades Cove: the intersection of Hyatt Lane and Abrams Creek, and Campbell Lead). There are no data to compare to the remaining 8 trapsites initially surveyed by our team.

The 16.7% (7/42) seroprevalence of *P. maniculatis* is close to that reported in a Walker River Basin (NV and CA) study of 18.5% (Boone et al. 1998). It is higher than those reported in California in 1999 and the Four Corners Region in 1997 (9.1% and 11.0% respectively) (Mills et al. 1997 and Bennett et al. 1999); but lower than the Four Corners Region during the 1993 hantavirus outbreak of 30.4% (Childs et al. 1994*b*). There were no significant differences of seroprevalences found in GSMNP and these previous studies ($P>0.05$). There were however, significant differences when comparing the 1998 Walker River Basin study to the 1999 California and 1997 Four Corners studies ($P<0.001$). When comparing the 1999 California and 1997 Four Corners studies, there was no significant difference ($P=0.25$). The differences may be attributed to temporal, spatial, and environmental variables that would cause fluctuations in rodent populations.

The 3.7% seroprevalence of *P. leucopus* is lower than that reported in Montana, Indiana, and New York (8.0%, 4.6%, and 12.0% respectively) (Douglass et al. 1996, White et al. 1996, Dietrich et al. 1997). Again, there were no significant differences in *P. leucopus* specific seroprevalence found in GSMNP and these previous studies (P=0.05). There was also no significant difference between the Montana and Indiana studies (P=0.18). The differences between seroprevalences in New York and Montana, and New York and Indiana were significant (P=0.02 and P=0.01, respectively). These differences may also be attributed to temporal, spatial, and environmental variables that would cause fluctuations in rodent populations. It is worth noting that while the seroprevalences for New York and Montana were only 4.0% different, New York had a much larger sample size. In National Parks east of the Rocky Mountains surveyed by Mills et al. (1998) where *P. leucopus* were found, seroprevalences ranged from 0.0% - 33.3% (overall prevalence=2.0%) (Mills et al. 1998). The overall seroprevalence we found in GSMNP was higher than the overall prevalence in all the other eastern parks. The distribution of *P. leucopus* has an eastern to mid-west distribution and *P. maniculatis* is distributed throughout the U.S. with the exception of the eastern seaboard. The 2 species will often have overlapping home ranges; such as in GSMNP. It is the abundance and opportunistic nature of the deer mouse that puts them in contact with humans, more so than white-footed mice (Calisher et al. 1999 and Linzey 1995).

No other rodent or insectivore hosts were found to be antibody positive by serologic testing. Since we used anti-mouse IgG conjugate and Sin Nombre

Virus antigens to conduct our serologic testing, these animals may have fallen outside of our test's ability to detect antibodies against hantavirus. *C. gapperi* (SNV), *M. ochrogaster* (PHV), *S. hispidus* (BCC), and Insectivores (Thottapalayam virus) have been demonstrated to be either primary hosts or spillover hosts for hantaviruses (Ksiazek et al. 1997; Schmaljohn and Hjelle 1997; Mills et al. 1998; and Monroe et al. 1999). It is also possible that our sample sizes were too small for infection to be detected (e.g. *S. hispidus* n=1, *M. ochrogaster* n=2, *Sorex spp.* n=4, and *Blarina spp.* n=16). It should be noted that none of the insectivores captured in this survey have been documented to have hantavirus infections elsewhere in North America based on antibody assays or viral RNA detection assays.

Based on our results, *P. maniculatis* is the primary reservoir host for this strain of hantavirus and *P. leucopus* is either a co-reservoir host or represents a spillover host. Based on previous studies conducted in Tennessee and North Carolina, the former is the most likely scenario. Both *Peromyscus spp.* have overlapping distribution in the Southeast and both species have been captured at the same sites. Since both species are so similar morphologically, they were considered together when determining seroprevalences in North Carolina. Mills et al. (1998) found that where the 2 species were sympatric, they were captured at the same sites, and found seropositive *P. leucopus* both with and without seropositive *P. maniculatis*. This implies that *P. leucopus* may be acting as a maintenance host for hantavirus (Weigler et al. 1996 and Mills et al. 1998).

A future goal of this study is to conduct RT-PCR analysis on tissue collected from all animals to determine the presence of viral RNA. We will purify and sequence RNA fragments in order to determine viral lineage.

Additionally, mitochondrial DNA will be isolated and sequenced from *Peromyscus spp.* to provide a comparison between morphological and genetic identifications of the 2 species since they are very similar phenotypically. This type of phylogenetic analysis will provide us with information regarding any virus-host differences that may be present to determine if *P. leucopus* is a spillover host or if co-speciation may be occurring. Phylogenetic analysis of both reservoir host and respective virus will allow us to determine if genetic variants exist in different hosts, which would represent co-speciation or if *P. leucopus* represents a spill-over host (Monroe et al. 1999).

Despite the low rate of seroprevalence in *P. leucopus*, they are still important epidemiologically. Both *Peromyscus spp.* are sympatric in GSMNP and hantavirus infections in *P. leucopus* may represent transmission from *P. maniculatis* rather than from other *P. leucopus* (Jay et al. 1997). Infected *P. leucopus* have been found in areas that have a high potential for human-rodent contact and subsequent human infection. Although no human cases have been reported in or originating from GSMNP, this may be a function of missed diagnoses. Health care professionals need to be aware that the virus is present in East Tennessee and aware of clinical manifestations of human infection.

We could not ascertain any correlation between rodent host populations and antibody prevalence (Tables 4 and 6). This is consistent with the results of

previous studies that had much larger sample sizes. As in previous studies, a complex relationship between rodent host densities and prevalence likely exists, albeit, not linearly. Rodent population dynamics are highly variable based on biotic community, weather, season, and predation. Thus far, no studies have been conducted that are temporally adequate and take into consideration enough environmental variables to establish any linear correlation between rodent population densities and hantavirus prevalence (Engelthaler et al. 1999, Kuenzi 1999, Mills et al. 1999*a*, Boone et al. 2000). A long-term, mark-recapture survey would aid in establishing such a relationship (Mills et al. 1997 and Boone et al. 1998).

There appears to be a geographical clustering of seropositive mice in GSMNP even though the distribution of *Peromyscus spp.* is widespread (Figure 5). The areas in which we trapped support earlier findings in Tennessee and North Carolina that suggest an overlap between the 2 species. Our study also confirms earlier findings of elevational preferences in *Peromyscus spp. P. leucopus* is more abundant at lower elevations and *P. maniculatis* is more abundant at higher elevations (Figure 1) (Linzey 1995 and Weigler et al. 1996). This factor could have introduced bias into our study by not sampling the elevation range where the most overlap between the 2 species may occur. Our sites were primarily selected based on varying risks of human-rodent contact. Subsequently, we did not sample any sites between 750m and 1500m. This is a limitation of this study that will need to be addressed by future surveys in GSMNP.

The disjunct distribution in prevalence in this study may be explained by insufficient sample size or, if there is a newly emerging infection within a particular microhabitat, our sample power was too limited to detect a low frequency of infection (Dearing et al. 1998 and Glass et al. 1998). For example, we surveyed 3 sites where reasonable numbers of *Peromyscus spp.* were trapped and found that our sample power was indeed too low to detect even the overall prevalence we found of 6.3%. We trapped 11 *Peromyscus spp.* in Cades Cove. There would have had to be ≥25% seroprevalence to detect at least one infected animal within a 95% confidence interval (Cannon and Roe 1982). Fifteen *Peromyscus spp.* were trapped in Newfound Gap, a 20% seroprevalence would have had to exist in order to detect at least 1 seropositive within a 95% confidence level. Finally, 5 *Peromyscus spp.* were trapped in Greenbriar. This sample size could have detected a ≥ 50% seroprevalence within a 95% confidence interval.

Previous studies incorporating more extensive spatial and temporal surveys have also reported focality of rodent reservoir infection. They too have attributed their results to inadequate sample sizes, influences of home range sizes, and trap responsiveness (Korch et al. 1989 and Mills et al. 1997). Additionally, there may be the lack of connectivity between populations as a result of topographic features resulting in a disruption of viral ecology (Boone et al. 1998). In other words, viral maintenance requires the immigration of antibody-negative animals and/or newborn animals into an area that has infected animals, resulting in subsequent infection of new animals. Conversely, infected animals

need to emigrate to areas with antibody negative animals in order for the virus-host cycle to continue, particularly if the infected animal population becomes extinct in its geographical area. If connectivity between maintenance hosts is somehow disrupted, so is viral ecology.

This was a preliminary study and thus the duration was limited to September 2000 – November 2000 (December 1, 2000 inclusive) and again for 2 nights in November 2001. Any temporal distribution of infected animals could not be established, as this was a terminal study. Nonetheless, there are some interesting observations that may be inferred from our results. An equal number of seropositive *Peromyscus spp.* were captured during the months of September (n=4) and November (n=4). However, the total number of *Peromyscus spp.* tested for those months was 20 and 44 respectively (Figure 2). So, the seroprevalence for September was 20.0% and declined to 9.1% yet the relative *Peromyscus spp.* population for November was more than double that of September's relative *Peromyscus spp.* A direct comparison cannot be made as trapsites differed in their respective months. However, it is interesting to note that 6/7 females captured November 2001 were either lactating or pregnant most likely due to unusually warm weather. This lends further support to previous studies' findings that rodent populations cannot be linearly correlated to seroprevalences.

All of the seropositive mice from September were juveniles or sub-adults. This may represent the presence of maternal antibodies in juveniles and recent infections in the sub-adults (Glass et al. 1998 and Mills et al. 1998). When

considering only seropositive animals, the data suggest that a positive animal was more likely to be a sub-adult and that a negative animal was more likely to be a juvenile (P=0.10) (Figure 5). This comparison and most others are limited due to the small number of seropositive animals detected.

There appears to be a trend towards increased prevalence with increasing body weight class (Figure 6). Of the seropositive animals found, the heaviest (oldest) weight class was exclusively male.

ELISA was useful for detecting hantavirus IgG antibodies in the *Peromyscus spp.* tested. The N protein is expressed by the S segment of the hantaviral genome and is more sensitive for anti-hantavirus antibody detection than glycoproteins (Feldmann et al. 1993). This assay would detect antibodies against Sin Nombre virus but could not distinguish infections that are cross-reactive with Sin Nombre Virus (Mills et al. 1999). As mentioned previously, we will be conducting RT-PCR analysis on tissue samples from all the animals captured. This will allow us to distinguish between animals with active infections and those that have either cleared the virus (as would be the case with older animals) or have passive immunity conferred by their mothers.

The objectives of this survey were to confirm the CDC's previous findings. Secondly, we wanted to sample more sites that had high human visitation and could potentially put visitors and park staff at risk of coming into contact with hantavirus infected rodents. And lastly, we wanted to gather preliminary information on the general distribution of hantavirus in GSMNP.

The results of this preliminary study demonstrate the need for a more comprehensive and extensive survey of GSMNP. Ideally, we would like to expand both spatial and temporal aspects to gain a broader understanding of the rodent-virus dynamics. Some future goals would be long-term monitoring of different habitats to determine the extent and frequency of infection in rodent hosts. This would allow us to assess human risk by identifying environmental factors that could contribute to increased rodent populations and subsequent increase in hantavirus prevalence.

Since voles accounted for 25% (43/171) of the total capture and are also widely distributed in GSMNP, ELISA panels and RT-PCR primers should include Prospect Hill Virus (PHV). While no human cases have been attributed to infection by PHV, antibodies against PHV have been found in humans (Monroe et al. 1999). The possibility exists to identify human illness from PHV with increased surveillance by field investigators and health care professionals. Molecular characterization of any additional hantavirus variants that may exist would contribute to the understanding of virus-host ecology. As mentioned previously, phylogenetic analysis of both rodent host and virus will allow researchers to identify a host-switching event or spillover infection. In other words, we wish to distinguish a separate viral strain emerging from a simple spillover from a primary host species. This information would be epidemiological relevant in the event of a human case originating from the park. If a viral isolate from a human case could be obtained, this could elucidate information on the

rodent host responsible. The unique opportunity exists to further define virus-host dynamics prior to a diagnosed human infection.

We most recently found a seropositive *P. leucopus* in 1 unoccupied structure out of 2 surveyed. Both of these structures were infested with mice and excreta were abundant, which pose a health risk to park staff and visitors who may enter these structures. There is a need, based on our preliminary results to educate park staff and visitors who may be at increased risk, in addition to health care professionals of the potential for human hantavirus infection in GSMNP.

5.0 RECOMMENDATIONS

Precautions for Workers in Affected Areas Who are Regularly Exposed to Rodents

The Centers for Disease Control and Prevention (CDC 2000) has issued recommendations for people engaged in activities that may place them in contact with infected rodents and their excreta. Persons who frequently handle or are exposed to rodents (e.g., mammalogists, pest-control workers) in the affected area are probably at higher risk for hantavirus infection than the general public because of their frequency of exposure. Therefore, enhanced precautions are warranted to protect them from hantavirus infection.

Precautions to be Used:

- Anyone engaged in activities that bring them in contact with rodents should be informed about the symptoms of the disease and be provided with detailed guidance on prevention measures.
- Anyone developing febrile or respiratory illness within 45 days of potential exposure to rodents or rodent infested structures should immediately seek medical attention and inform the attending physician of the potential occupational risk of hantavirus infection. The physician should contact local health authorities promptly if hantavirus-associated illness is suspected. A blood sample should be obtained and forwarded through the state health department to CDC for hantavirus antibody testing.

- Workers should wear Positive Atmosphere Pressure Respirators (PAPR) equipped with High Efficiency Particulate Air (HEPA) filters when removing rodents from traps or handling rodents in the affected area. (Please note: the HEPA classification recently has been discontinued. Under the new classification system, the N-100 filter type is recommended. Read the Federal Occupational Safety and Health Administration (OSHA) directive online, at http://www.osha-slc.gov/OshDoc/Directive_data/CPL_2-0_120.html.
- Respirators (including positive-pressure types) are not considered protective if facial hair interferes with the face seal, since proper fit cannot be assured. Respirator use practices should be in accord with a comprehensive user program and should be supervised by a knowledgeable person.
- Workers should wear rubber or disposable latex gloves when handling rodents or handling traps containing rodents. Rubber gloves should be washed and disinfected before removing them, latex gloves should be disposed of in accordance with biohazardous waste disposal protocols.
- Traps contaminated by rodent urine or feces or in which a rodent was captured should be disinfected with a commercial disinfectant (an approved disinfectant with virucidal properties) or bleach solution (≥10% concentration). Dispose of dead rodents as described in the section on Eliminating Rodents inside the Home (available online at: http://www.cdc.gov/ncidod/diseases/hanta/hps/noframes/prevent5.htm)

- Persons removing organs or obtaining blood from rodents in affected areas should contact the Special Pathogens Branch, Division of Viral and Rickettsial Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, to check for detailed safety precautions and updates to the published 1995 Methods for Trapping and Sampling Small Mammals for Virologic Testing (Mills 1995*a*).

Precautions for Park Staff and Research Workers

- All park staff and anyone conducting research in GSMNP should be notified of the potential for hantavirus infection and follow the precautionary measures previously mentioned.
- Structures that are used by workers should be rodent-proofed, cleaned, and disinfected on a regular basis. Seasonal structures frequented by visitors should also be rodent-proofed. Structures should be cleaned and properly winterized for seasonal closure. For seasonal openings, workers may enter buildings with full respiratory protective equipment and clothing; open all windows and doors; and thoroughly clean and disinfect with commercial disinfectant or bleach solution previously described.

- Workers should keep track of incidents resulting in exposure to rodents and their excreta in the event of subsequent hantaviral infection. Such events should also be reported to appropriate park staff.

- The Department of the Interior should initiate a long-term hantavirus monitoring program of national parks in conjunction with local universities to identify areas that may be at high risk to park staff and visitors.
- Health care workers in the surrounding GSMNP communities need to be given detailed diagnostic information on hantavirus infection and follow the procedures mentioned previously.

Precautions for Other Occupational Groups Who Have Potential Rodent Contact

Insufficient information is available at this time to allow general recommendations regarding risks or precautions for persons in the affected areas who work in occupations with unpredictable or incidental contact with rodents or their habitations. Examples of such occupations include telephone installers, maintenance workers, plumbers, electricians, and certain construction workers. Workers in these jobs may have to enter various buildings, crawl spaces, or other sites that may be rodent infested. Recommendations for such circumstances must be made on a case-by-case basis after the specific working environment has been assessed and state or local health departments have been consulted.

Precautions for Campers and Hikers in the Affected Areas

There is no evidence to suggest that travel into areas where HPS has been reported should be restricted. Most usual tourist activities pose little or no risk that travelers will be exposed to rodents or their urine and/or droppings. However, persons who participate in outdoor activities such as camping or hiking in areas where the disease agent has been reported should take precautions to reduce the likelihood of their exposure to potentially infectious materials.

Useful Precautions:

- Avoid coming into contact with rodents and rodent burrows or disturbing dens (such as pack rat nests).
- Air out, and then disinfect cabins or shelters before using them. These places often shelter rodents.
- Do not pitch tents or place sleeping bags in areas in proximity to rodent droppings or burrows or near areas that may shelter rodents or provide food for them (e.g., garbage dumps or woodpiles).
- If possible, do not sleep on the bare ground. In shelters, use a cot with the sleeping surface at least 12 inches above the ground. Use tents with floors or a ground cloth if sleeping in the open air.
- Keep food in rodent-proof containers!

- Promptly bury (or--preferably--burn followed by burying ashes, when in accordance with local regulations) all garbage and trash, or discard in covered trash containers.
- Use only bottled water or water that has been rendered safe for consumption by filtration, boiling, chlorination, or iodination for drinking, cooking, washing dishes, and brushing teeth.
- And lastly, do not play with or handle any rodents that show up at the camping or hiking site, even if they appear friendly.

6.0 SUMMARY

In summary, we collected 171 rodents and insectivores from GSMNP in 2000 and 2001. Two animals were released; tissue samples were collected from 169 animals; and blood samples were collected from 142 animals (96 of these were *Peromyscus spp.*). Nine animals, all *Peromyscus spp.* were found to have antibodies against SNV for a total seroprevalence of 6.3%. Seven of 42 (16.7%) *P. maniculatis* (deer mice) and 2 of 54 (3.7%) *P. leucopus* (white-footed mice) accounted for the only seropositive animals. The deer mouse is most likely the primary reservoir host in GSMNP.

These preliminary findings warrant more extensive molecular testing of tissue samples to determine if more than 1 viral strain exists in GSMNP in order to define their relationship to known pathogenic strains of hantavirus that are present elsewhere in the United States. Reverse Transcription-Polymerase Chain Reaction (RT-PCR) and genetic sequencing performed on one of our antibody positive samples has revealed a unique viral strain that is closely related to Monongahela and responsible for a human HPS fatality in North Carolina. We have named this strain "Newfound Gap" and registered it in GenBank (accession number AF406788, available by September 1, 2003).

Seropositive animals were collected from areas in the park that places visitors, park staff, and researchers at risk for contact with infected rodents. More sampling sites need to be added in addition to permanent trapping webs to identify areas that may experience an increase in hantavirus infected animals.

This would allow park staff to take precautions such as closing structures or modifying shelters and bunkhouses, and using protective respirator equipment where needed in order to prevent human hantavirus cases. At present, there have been no known human hantavirus cases associated with GSMNP.

Healthcare workers can now be informed that at least 1 pathogenic strain of hantavirus exists in GSMNP and be provided with information on disease symptomology for efficacious diagnosis and treatment. Again, a unique opportunity exists to identify where hantavirus infected animals may be present and to characterize viral strains prior to a human case occurring.

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VITA

Shawn L. Lewis was born and raised in Connecticut. She graduated from the University of Connecticut, Storrs College of Agriculture and Natural Resources with a B.S. in Animal Science in 1997.

Shawn is currently pursuing her doctorate in Comparative and Experimental Medicine at the University of Tennessee, Knoxville.