

**DISSERTATION**  
**EPIDEMIOLOGY OF VESICULAR STOMATITIS VIRUSES IN THE**  
**SOUTHWESTERN UNITED STATES**

Submitted by

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Department of Clinical Sciences

In partial fulfillment of the requirements

for the degree of Doctor of Philosophy

Colorado State University

Fort Collins, Colorado

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UNDER OUR SUPERVISION BY BRIAN JAMES MCCLUSKEY TITLED  
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ABSTRACT OF DISSERTATION  
EPIDEMIOLOGY OF VESICULAR STOMATITIS VIRUSES IN THE  
SOUTHWESTERN UNITED STATES

The general objectives of these studies were to investigate the persistence of vesicular stomatitis viruses (VSV) in the southwestern United States, to identify management or environmental factors associated with exposure of horses to VSV in the southwest , and to investigate the applicability of sentinel herds for epidemiologic studies and for integration into animal disease surveillance systems.

A longitudinal study of sentinel equine operations (SEO) was conducted in Colorado and New Mexico from May 1998 to October 2001. Visits were made approximately every 100 days to each SEO in which between 2 and 20 horses were given examinations of the mouth, nasal cavity, coronary bands and external genitalia. In addition, blood samples were drawn by jugular venipuncture and swabs collected from the oral cavity. Information about operation management practices, animal movement history and other potential risk factors was collected by standardized questionnaire at each visit. All blood samples were tested by a competitive enzyme-linked immunosorbent assay (cELISA) for antibodies to each serotype of VSV. Samples positive by either cELISA were further tested by a serum neutralization test and IgM capture ELISA. There were 537 (27.1%) and 889 (44.8%) positive cELISA tests out of 1,984 total tests

for the New Jersey (VSV-NJ) and Indiana serotypes (VSV-IN) of VSV respectively. A total of 48 and 89 seroconversions occurred to VSV-NJ and VSV-IN respectively.

A mixed model was developed to examine the association of operation-level and animal-level factors and the change in serum neutralizing antibody titers between consecutive SEO visits. Questionnaire data and data collected through the use of geographic information systems were used in the model. The ecological section and plant hardiness zone were ecological variables that were associated with changes in antibody titer to VSV-IN. There were no factors associated with changes in antibody titer to VSV-NJ.

Climate data including daily maximum temperature, daily minimum temperature, daily mean temperature, daily mean relative humidity and daily total precipitation were collected at the Sevilleta Long Term Ecological Research site in central New Mexico. Discriminant analysis was used to identify the climatic variables best able to classify the months VS would occur between 1989 and 1999. The study found the amount of precipitation occurring 2, 10, 11, and 12 months previous to the month in which the cases were diagnosed were the climatic variables that best described the occurrence of VS cases. As an extension of this study, remote sensing data (normalized difference vegetation indices [NDVI]) along with climatic data were collected in relation to SEO in Colorado. The associations of the NDVI and climatic variables with the occurrence of cases of VS or seroconversions to either VSV-NJ or VSV-IN were investigated through discriminant analysis and logistic regression. A 10-period lag in total precipitation and 11-period lag in NDVI were variables that best predicted the occurrence of VS on Colorado sentinel equine operations.

The occurrence of VS was investigated in El Salvador through monthly visits to 12 sentinel cattle operations located in four different departments. Management, environmental and spatial data were collected. Heifers were enrolled on the operations and were examined and bled monthly for 3 years. Two cELISAs were used to detect antibodies on each sample for each serotype of VSV. Small terrestrial rodents were trapped on 8 of 12 operations. The seroprevalence of VSV-NJ was found to be higher than the seroprevalence for VSV-IN. It was confirmed that VSV is endemic in the four departments investigated in El Salvador.

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My Ph.D. program was born from my close involvement with three outbreaks of vesicular stomatitis that occurred in the southwestern United States in 1995, 1997 and 1998. As the USDA, Veterinary Services Area Epidemiology Officer for Colorado and Disease Reporting Officer for the Regional Emergency Animal Disease Eradication Organization (READEO) I was partially responsible for monitoring the spread of the disease and collating and analyzing data about the outbreaks. It quickly became clear to me that although extensive laboratory and field investigations on vesicular stomatitis had been conducted in the past, major epidemiologic questions remained unanswered. I wanted to answer a few of them.

I first must thank my advisor, Dr. Mo Salman for encouraging me to pursue not so much a degree, but answers to my myriad of questions. Dr. Jerry Diemer, Area Veterinarian in Charge in Colorado was instrumental in providing financial support but more importantly for giving me time away from my normal duties to conduct field studies. Dr. Nora Wineland also provided resources from the USDA for these studies.

Dr. Elizabeth Mumford was the co-coordinator of the sentinel studies in Colorado and New Mexico and without Liz's efforts the project could not have taken off or landed. The field studies described in this dissertation extended from northern Colorado to southern

New Mexico within the United States, and also include sentinel sites in El Salvador, Central America. They also extended over a four-year period. Studies of this geographic and temporal expanse inherently require assistance from many people. In New Mexico, Dr. Sherry Wainwright, Dr. Emma Adam and Dr. Dave White provided assistance with sentinel visits. In El Salvador, Dr. Adonias Serrano provided coordination of the sentinel dairy herd project. Sarah Tomlinson, Dr. Kevin McSweeney and Dr. Marco Hererro provided assistance with climate and remote sensing data collection. Statistical assistance with all phases of the project was provided by my colleagues at the Centers for Epidemiology and Animal Health, Dr. Bruce Wagner and Dr. Dave Dargatz.

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## **Chapter 1.**

### **Introduction and Overview**

Vesicular stomatitis (VS) must be considered one of the most enigmatic diseases of the western hemisphere. Throughout the second half of the last century, many bright minds have worked diligently to answer difficult questions surrounding this disease. What is the viral reservoir? How does disease appear to move over large geographic distances? Why did VS disappear from regions of the United States that were historically considered endemic for the disease? Although generally accepted to be an arthropod-borne disease, how do insects acquire the virus when a viremic host has yet to be identified? What are the immune mechanisms that protect most animals from developing clinical disease albeit a very short-lived protection? These and many other virologic, pathologic and epidemiologic questions remain unanswered about this disease whose effect on individual animals is generally innocuous but whose political effects are substantial.

The overall aim of this work was to investigate the epidemiology of VS in the southwestern United States in non-outbreak periods and specifically to investigate whether vesicular stomatitis viruses (VSV) are persistent in the environments of the southwest. This dissertation is presented in seven chapters. Chapters 3 through 6 have been written as separate manuscripts for publication and thus have been formatted to

meet the requirements of the journals to which they were or will be submitted, thus they will have different appearances. These chapters will contain some repetition in their introductions and materials and methods sections. To maintain consistency for this dissertation, figures and tables have been labeled by chapter and figure or table number throughout.

The aims of each chapter include:

Chapter 1 – An overview and introduction to the structure of the dissertation.

Chapter 2 – A literature review of vesicular stomatitis and the viruses which cause it with an emphasis on the epidemiology of the disease.

Chapter 3 – A description of the use of sentinel herds in animal disease monitoring and surveillance systems. The rationale for sentinel surveillance, as well as specific procedures to employ to establish sentinel surveillance, is provided. Specific examples of sentinel surveillance systems, including those used in the research for this dissertation are discussed.

Chapter 4 – In Part I of chapter 4, the methods used to establish sentinel equine operations for studying the epidemiology of vesicular stomatitis in the southwestern United States are given. Results of a three-year study of the sentinel equine operations are provided primarily as descriptive statistics. Discussion of the interpretation of results and the implications for regulatory action during vesicular stomatitis outbreaks are included. In Part II of chapter 4, a mixed model is developed to examine the association of animal-level and operation-level factors and changes in serum neutralization titers to VSV on the sentinel equine operations.

Chapter 5 – In Part I of chapter 5, the association of climatic factors and the occurrence of vesicular stomatitis cases in New Mexico is investigated through application of a discriminant model. In Part II of chapter 5, remote sensing data (normalized difference vegetation indices) in addition to climatic factors and their association with the occurrence of vesicular stomatitis cases or seroconversions to vesicular stomatitis viruses in Colorado are investigated. Both discriminant analysis and logistic regression models are employed to assess associations.

Chapter 6 – A description of the establishment of sentinel dairy herds in El Salvador to investigate the epidemiology of vesicular stomatitis in a reported endemic area is provided. Results are provided primarily as descriptive statistics. The value of the use of sentinel herds for surveillance is discussed.

Chapter 7 – This chapter provides bulleted conclusions from the research conducted for this dissertation.

Two extensive appendices with epidemiologic and geospatial data for sentinel equine operations in Colorado and New Mexico and for sentinel dairy operations in El Salvador are provided.

## Chapter 2.

### Vesicular Stomatitis

#### The Viruses

Vesicular stomatitis viruses (VSV) are members of the family Rhabdoviridae which include viruses that infect vertebrates, invertebrates and many plant species.<sup>96</sup> The viruses of this family known to infect mammals are classified into two genera, the *Lyssavirus* genus and *Vesiculovirus* genus. Rabies is the most well characterized and most devastating virus of the *Lyssavirus* genus while VSV are the prototype viruses of the *Vesiculovirus* genus. Vesicular stomatitis viruses are bullet-shaped and generally 180nm long and 75 nm wide.<sup>103</sup> The nucleocapsid or ribonucleoprotein core (RNP) and lipoprotein envelope surrounding the RNP are the two major structural components of VSV. Extending from the outer surface of the envelope are spike-like projections.<sup>96</sup> The genomic structure of VSV is a single strand of negative-sense RNA and is composed of 5 genes, N, P, M, G, and L representing the nucleocapsid protein, phosphoprotein (a component of the viral RNA polymerase), matrix protein, glycoprotein and the large protein (a component of the viral RNA polymerase), respectively.<sup>96</sup> A brief discussion of VSV transcription and replication is warranted to provide background for the epidemiologic and potential vaccine development implications to be discussed later.

The RNA genome is 11,161 nucleotides long and is transcribed by the RNA-dependent RNA polymerase composed of the L and P proteins. The polymerase generates 5 monocistronic, capped and polyadenylated mRNAs. Cellular translational mechanisms produce the 5 structural proteins of an intact virion.<sup>8</sup> Studies have shown that the synthesis of gene transcripts follows the order of the genes in the genome and there is a gradient in the amount of the transcripts which follows this same order (i.e. N>P>M>G>L).<sup>7</sup> The shift from transcription to replication is hypothesized to occur when large quantities of the N protein bind to the nascent leader RNA and prevents termination at the leader-N protein junction. Thus, continuous passage of the RNA polymerase down the genome occurs, resulting in production of a positive strand which in turn is replicated by the RNA dependent RNA polymerase to generate the negative sense RNA to be packaged in the virion. As is typical of RNA polymerases, their infidelity results in many nucleotide substitutions and researchers have suggested that with the established error rates of the polymerase one could expect every clone in a VSV population to differ from one another at a number of nucleotide positions.<sup>88</sup> It was further suggested that many of these base substitutions result in lethal mutations and subsequent production of non-infectious virions. Due to the potential for rapid evolution, VSV have been used to test evolutionary theory. Although RNA viruses, VSV do not appear to possess a molecular clock, that is stepwise evolution through time.<sup>71</sup> The evolutionary pattern of VSV suggest adaptations to geographical or ecological pressures and thus in some endemic areas virus genomes appear to remain relatively stable.<sup>78,94</sup>

Although there are many members of the *Vesiculovirus* genus, two are of particular interest in the United States, vesicular stomatitis virus - New Jersey serotype

(VSV-NJ) and vesicular stomatitis virus – Indiana serotype (VSV-IN). These two viruses are similar in size and morphology but generate distinct neutralizing antibodies in infected animals. Thus, although considered distinct viruses, they are often distinguished only by terming one serotype New Jersey and the other serotype Indiana.<sup>17</sup> Other members of the *Vesiculovirus* genus include Cocal, Jurona, Carajas, Maraba, Piry, Calchaqui, Yug Bogdanovac, Isfahan, Chandipura, Perinet, and Porton-S.<sup>96</sup> Cocal and Alagoas are subtypes of VSV-IN and have been associated with vesicular disease in animals in South America. Piry, Chandipura and Isfahan produced only mild lesions in experimentally infected animals.<sup>104</sup> The remaining Vesiculoviruses have been isolated from arthropods, mammals or both, but are not associated with disease.<sup>96</sup>

### **Pathology and Clinical Picture**

The molecular basis of VSV pathogenesis is typical of viral infections with a series of events terminating in the release of progeny virions and cell death. Adsorption of virus to cell surface receptors through the G protein spike, penetration and uncoating of viral particles leads to transcription and replication. Ultimately new viral particles are assembled at the cellular plasma membrane. The matrix protein (M protein) plays a specific role in the attachment of condensed nucleocapsids to the plasma membrane and to subsequent budding of the new virions.<sup>12</sup> The rounding of infected cells is caused by the M protein due to a disruption in the cytoskeleton. Inhibition of the cellular RNA, DNA and protein synthesis occurs even prior to cellular rounding.<sup>101</sup>

Infection of epithelium with VSV induces intercellular edema in the malphigian layer and the epithelial cells become separated by vacuolar cavities.<sup>77,82</sup> Cellular necrosis

is concomitant with edema with cells shrinking in size but not undergoing lysis. The infiltration of inflammatory cells, including granulocytes and monocytes, eventually results in cellular lysis. Vesicles develop when the necrotic, edematous mucosa breaks free from underlying tissue forming a cavity filled with cellular exudates. The separation occurs at the basal layer. Vesicle formation through intercellular edema, cellular necrosis and inflammatory cell infiltration generally occurs within 48 hours following experimental inoculation and vesicles may disappear through seepage of edematous fluid soon after this time.

The clinical signs of vesicular stomatitis (VS) infection occur in cattle, horses, swine and rarely in llamas (Figures 2.1 and 2.2). Signs follow a typical viral incubation period of 3 to 7 days with an initial febrile period followed by ptyalism in cattle and horses.<sup>10,56,75</sup> Cattle often can be heard smacking their lips or seen immersing their mouths in water troughs without drinking. Lesions of the oral mucosa include raised, blanched and rarely fluid filled vesicles. The dorsal lingual surface often is affected but the gingival surfaces, palate and mucocutaneous junctions may also exhibit lesions.<sup>75</sup> Vesicles are very short-lived and rupture leaving ulcerations and erosions. Lesions often coalesce to form large denuded areas of oral mucosa with the presence of epithelial tags. Vesicular and/or ulcerative lesions outside of the oral mucosa occur on the snout of pigs, teats of cattle and coronary bands of pigs, cattle and horses. Teat lesions are not as common as oral lesions but in cattle may be associated with severe mastitis. Milk production may drop dramatically due to both the inability of the animals to eat and mastitis. An atypical outbreak of VS occurred in Peru in which lesions occurred almost exclusively on teats of cattle with oral lesions being exceptional.<sup>89</sup> Lesions of the feet

typically manifest as a coronitis with edema and inflammation extending from the coronary band proximally up the lower leg. Hoof lesions in swine often result in sloughing of the hoof wall. Again, coronary band lesions are much less common than oral lesions during outbreaks in the southwestern United States.

A very common lesion observed by diagnosticians during VS outbreaks in the 1990's, yet rarely described in the literature are crusting or scabbing lesions of the muzzle, ventral abdominal wall, prepuce and udder of affected horses. These lesions typically start as discrete, small (approximately 1cm) erosions that quickly coalesce so that large crusted or scabbed areas are observed. These lesions are often associated with feeding sites of hematophagous arthropods. Vesicular stomatitis viruses have been isolated from samples collected from these crusting or scabbing lesions.

Stomatitis and other ulcerative, erosive or vesicular diseases not caused by VSV also occur in horses, cattle and swine. An extensive review of the differential diagnosis of VS in horses has been published by this author.<sup>64</sup> In cattle and swine, foot and mouth disease (FMD) must be considered the most important differential diagnosis. Swine vesicular disease and vesicular exanthema of swine are other viral agents that may create lesions similar to VS in swine.

Subclinical infections are common in livestock during outbreaks of VS. One study reported disease prevalence of 44.7% in horses on 17 premises but a seroprevalence of 61.0%. Only 4.5% of cattle on the 17 premises investigated showed clinical signs while 67.6% were seropositive to VSV-NJ.<sup>100</sup> In another study, also conducted during the 1982 outbreak in the southwestern United States, disease prevalence ranged from 0 to 30% but the percent seropositive ranged from 14 to 100%.<sup>97</sup> Extensive whole herd testing

conducted on 4 livestock operations in 1995 by the author confirmed the extensive nature of subclinical infections. Operation A was a production dairy (n=250) located in northwestern New Mexico, 3 miles south of the Colorado state line. The cattle on this dairy were first identified as infected on August 13, 1995. Blood samples were collected on August 30 (n=210) and September 12 (n=244). At the time of blood collection, each animal was observed for clinical signs of VS (ptyalism and/or vesicles or ulcers on labial or nasal mucosa). Those exhibiting ptyalism were given an oral examination. Prevalence of clinical disease was estimated from clinical examinations and seroprevalence was calculated for both collection dates. Operation B was a dude ranch located in southcentral Colorado. Ranch census indicated the presence of 98 horses at the time VS was first diagnosed on August 22. Blood samples were collected as previously described from 98 horses on September 20. All animals were given an oral examination following blood collection. Horses that were clinically negative were re-bled on October 24. Operation C was a cow/calf (n=594) operation located in southcentral Colorado. Summer pasture is part of a grazing cooperative on public lands in the Rio Grande National Forest. Cows and their calves are removed from the National Forest in September or October. Animals were first diagnosed as infected with VS after being moved from the National Forest allotment on October 18. Blood samples were obtained from cattle (n=593) on November 2 and 3 and from horses (n=8) on November 11. At the time of cattle blood collections, observation of each animal for clinical signs of VS (ptyalism and/or vesicles or ulcers on labial or nasal mucosa) was conducted. Each horse was given an oral examination at the time of blood collection. Operation D was a cow/calf operation also located in southcentral Colorado. The cattle herd consisted of 36 animals of mixed ages and breeds.

Vesicular stomatitis was diagnosed in this herd on August 14 at which time blood samples were obtained from all animals in the herd. All animals (n=37) were re-bled on September 11. All animals were given a physical examination at the time of the first blood sample collection. No additional clinical cases were seen on the second visit.

On operation A prevalence of clinical VS was estimated to be 10% on both the initial and follow-up visits. Cows were observed with ptyalism and active vesicles and ulcers on the oral and nasal mucosal surfaces. Of the 210 cows tested on the initial visit, 94 were positive by the cELISA (45%) and 117 were positive by the serum neutralization test (SNT) (56%). Of the 244 animals tested on the follow-up visit, 27 were calves not tested previously. The calves were pastured approximately 8 miles from the infected dairy and were considered non-exposed. None of the 27 calves tested was positive on either the cELISA or SNT. Of the 217 exposed animals tested on the follow-up visit, 153 were positive by the cELISA (71%) and 167 were positive by the SNT (76%). Overall on operation B, 25 of 98 (26%) horses examined were considered clinically positive for VS. Horses were found to have active and healing ulcers on the oral mucosa and one was considered to have flu-like signs consistent with the initial stages of VS. Of the 95 horses tested, 64 (67%) were positive by the cELISA and 73 (77%) were positive by the SNT. Horses negative on the first test were re-bled 34 days later on October 24. Of the horses retested due to a negative first test, 3 of 25 were positive (12%) by the SNT. The retested animals, combined with the original horses tested, provided an overall seroprevalence of 81% by the SNT. Overall on operation C, 59 of 593 (10%) cows examined were considered clinically positive. Seroprevalence was determined by cELISA testing only. Of the 593 cows tested, 484 were positive (82%). None of the 8 horses examined

exhibited clinical signs consistent with VS. However, one horse (13%) was positive by both the cELISA and the SNT. On operation D, examination of the 36 cattle on the initial visit revealed 5 that were clinically positive (14%). Testing by the complement fixation and SNT indicated 6 of 36 (17%) and 7 of 36 (19%) positives, respectively. Retesting 28 days later revealed 5 of 37 clinically positive (13.5%), 12 of 37 (33%) positive by complement fixation testing and 27 of 37 (73%) positive by SNT. The results of this study indicate that from 0 to 26% of animals on any one premises can exhibit clinical signs consistent with VS. Seroprevalences observed in this study were from 3 to 9 times higher than clinical prevalences.

### **Natural History**

The first report of VS in the United States was from 1916. However, anecdotal reports from the Civil War period leave little doubt that VS was occurring in horses during that time.<sup>43</sup> In 1904 a disease with a clinical picture indistinguishable from VS, although it was called mycotic stomatitis, reportedly occurred in the summer and fall in numerous eastern and mid-western states.<sup>69</sup> Cases of a similar disease were reported to have occurred in western Colorado as early as 1906 and in the Chicago stockyards in 1907.<sup>46</sup> However, accounts of VS were first reported in the literature in 1916 when horses in the Denver stockyards showed the clinical signs typical of VSV infection. Later that summer, an outbreak of VS in cattle and horses in the San Luis Valley of Colorado occurred. In this same year cases occurred in horses in Illinois, Iowa, Kansas, Missouri Montana, Nebraska, South Dakota, Utah and Wyoming.<sup>43</sup> In 1925 a train load of healthy cattle arrived in Indiana from Kansas City. These cattle were dispersed to

individual farms and soon after developed lesions of the tongue and oral mucosa. The disease spread to horses in the area. The infectious agent was isolated and was termed the vesicular stomatitis Indiana strain.<sup>26</sup> In 1926, an extensive outbreak of VS occurred in New Jersey where approximately 750 cattle on 33 farms were affected. The disease appeared only in very few horses. The agent again was isolated and found to be distinct from the Indiana strain isolated in the previous year. This new strain of VSV was termed vesicular stomatitis New Jersey strain.<sup>26</sup> Over the next 6 decades VS occurred sporadically throughout the United States (Table 2.1). Only states in New England appear to have been spared incursions of VS.

In the 1990's three outbreaks of VS occurred, but these were limited to the southwestern United States. On May 9, 1995 the first case of VS was confirmed in Las Cruces, New Mexico. A case was defined as an animal with positive results on virus isolation or positive results on a serologic test in combination with clinical signs consistent with VS. An extensive review of this outbreak has been previously published by this author.<sup>13</sup> Briefly, 1,162 investigations were conducted in 42 states during the outbreak. Vesicular stomatitis was confirmed in 6 states including Arizona (1 premises), Colorado (165 premises), New Mexico (186 premises), Texas (1 premises), Utah (6 premises), , and Wyoming (8 premises). Overall 78% of the positive premises housed horses that were positive for VS, 22% of positive premises housed cattle that were positive for VS and there was one VS-positive llama. All cases where virus isolation was successful were due to the VSV-NJ serotype.

A detailed review of the outbreak occurring in 1997 has been previously published by this author.<sup>63</sup> In 1997 the index case for the outbreak in the United States

was investigated on May 27 in Yavapai County, Arizona following a report of suspicious vesicular lesions in a horse by a private veterinary practitioner. One of 9 horses on this farm had clinical signs of VS and was confirmed to be infected with VSV-NJ by a four-fold increase in titer on the complement fixation test. During the 1997 outbreak, 689 total investigations for suspected VS occurred in 40 states. There were a total of 380 (55%) premises identified as housing animals positive for VS in four states; Arizona, Colorado, New Mexico and Utah. Similarly to the 1995 outbreak, clusters of cases occurred in the Albuquerque, New Mexico and Grand Junction, Colorado areas. However, unlike the 1995 outbreak, a large number of cases were identified in the counties east of the Continental Divide in Colorado extending from Pueblo, Colorado as far east as Brush, Colorado. Nationwide, horses comprised 704 of 802 (88%) of the examinations conducted for suspect VS and 362 of 374 (97%) positive premises had horses diagnosed as positive for VS. Cattle comprised 78 of 802 (10%) of the examinations conducted and 12 of 374 (3%) positive premises had cattle diagnosed with VS. There were no premises that had both cattle and horses diagnosed with VS. There were 6 positive premises that did not report a primary species where VS was diagnosed. The remaining 2% of examinations were conducted in sheep, goats, swine, llamas, elk and one dog. None of these species were positive for VS. Both VSV-NJ and VSV-IN were isolated from clinical cases.

In 1998 the index case of VS occurred in a horse in Tularosa, New Mexico. Virus isolation was confirmed on May 18, 1998 and was identified as VSV-IN. Overall, 130 of 232 (6%) investigations nationwide were positive for VS. A total of four states were affected including Arizona (15 positive premises), Colorado (102 positive premises),

New Mexico (12 positive premises) and Texas (1 positive premises). Premises where an equid was the species positive for VS represented 99% of all positive premises. Only 1 premises was identified as housing cattle with VS and this occurred in only one cow. All of the VSV isolated during this outbreak were VSV-IN.

Although only occurring sporadically in the southwestern United States since the 1980's there is an endemic focus of VS on Ossabaw Island, Georgia. Since 1964, VS in Georgia has been restricted to positive serologic test results from Ossabaw Island.<sup>87</sup> Antibodies to VSV-NJ have been found in many animal species on Ossabaw Island including cattle, raccoons, white-tailed deer, horses, and in the highest prevalence among the feral swine population.<sup>36</sup> Serologic evidence indicates that only VSV-NJ is present on the Island and that transmission occurs annually, is seasonal and is generally associated with one habitat type on the Island, maritime forest.<sup>85</sup> Clinical disease has been observed only in feral swine and is rare.

As mentioned previously, VS is a disease of the western hemisphere and although only one endemic focus of disease occurs in the United States, areas throughout South America, Central America and Mexico are considered endemic for VS. Although limited, there are reports of the endemic nature of VSV and other vesiculoviruses from Argentina,<sup>16</sup> Brazil,<sup>5,33</sup> Columbia,<sup>90</sup> and other South American countries.<sup>5</sup> Extensive research conducted in Costa Rica has shown the endemic nature of the virus and the disease.<sup>6,78,80,93</sup> Work conducted by the author in El Salvador also indicated that the virus and disease were endemic in that country (see Chapter 6). A review of VS in Mexico showed that cases occurred in every year between 1981 and 1995 with both serotypes identified in most years (Dr. Cristobal Zepeda, unpublished data, 1995). This review also

indicated that VS has a national distribution in Mexico although most cases occur in the southern states of Chiapas, Tabasco and Veracruz. The central area of Mexico also is considered endemic although at a lower level and the northern area of Mexico is more similar to the southwestern United States with sporadic cases occurring.

## **Diagnostics**

Three approaches to diagnosis of VSV infection are available; antibody detection through a variety of serologic tests, virus detection through isolation and detection of viral genetic material by molecular techniques.

Detection of antibodies to VSV can be accomplished through application of the serum neutralization test (SNT), complement fixation test (CFT), and enzyme-linked immunosorbent assays (ELISA). The SNT has been considered the standard serologic test for VS antibodies for many years. The Office International des Epizooties (OIE) recognizes the neutralization test as a prescribed test for international trade.<sup>81</sup> Samples with detectable antibody greater than 1:40 are considered positive for international trade purposes. Detailed descriptions of SNT procedures have been previously reported.<sup>4,19,80</sup>

The CFT also is recognized as a prescribed test for international trade purposes and samples with titers greater than 1:5 are considered positive. A detailed description of CFT procedures is provided in the OIE Manual of Standards.<sup>81</sup>

Numerous ELISAs have been developed for detection of VSV antibodies<sup>1,47,55</sup> but most recently the competitive enzyme linked immunosorbent assay (cELISA) has become the serologic test of choice for screening purposes during outbreaks of VS in the United States. The cELISA is considered a prescribed test for international trade by the

OIE with a sample considered positive if the absorbance is greater than or equal to 50% of the absorbance of the diluent control. Specific protocols for conducting the cELISA have been published.<sup>4,81</sup>

An ELISA (mcELISA) capable of detecting the IgM class of antibody to VSV was developed following the 1982 outbreak of VS in the southwestern United States.<sup>95</sup> The assay was shown to be capable of identifying recent exposure to VSV. This assay is not a prescribed test for international trade as determined by the OIE.

A comparison of these four tests (SNT, CFT, cELISA, mcELISA) by examination of experimentally inoculated animals indicated that the cELISA performed comparably to the SNT. The relative sensitivity and specificity of the cELISA to the SNT was 88% and 99% respectively.<sup>31</sup> Positive mcELISA and cELISA responses consistently appeared 1-2 days prior to CFT seroconversion but all animals reverted to mcELISA negative status by 49 days post-exposure. In a recent report, comparison of the SNT and cELISA on 1,106 samples collected from cattle on sentinel farms in Costa Rica indicated very good agreement between the two tests.<sup>4</sup> Kappa values of 0.8871 for detection of antibodies to VSV-NJ and 0.6912 for antibodies to VSV-IN when comparing the two tests were reported. The current serologic diagnostic testing scheme employed during outbreaks in the United States is to screen samples with the cELISA for antibodies to either serotype of virus with those considered positive on these assays further tested by the SNT and CFT.

For virus isolation vesicular fluid, epithelial tags or swabs from fresh lesions are the ideal diagnostic sample. Vesicular stomatitis viruses are propagated easily in cell culture as evident by their use in a variety of basic virologic studies. Samples can be

inoculated onto an assortment of cell types including VERO, BHK-1 and IB-RS-2 cell cultures. Vesicular stomatitis viruses will cause cytopathic effects in all three cell types.<sup>81</sup> Fluorescent antibody staining using conjugates specific for VSV-NJ and VSV-IN can be employed for serotype differentiation.<sup>19</sup>

The detection of genomic sequences of VSV also may be used to identify the presence of virus in tissue or swab samples. A polymerase chain reaction (PCR) assay was developed to detect the phosphoprotein gene of VSV-NJ, but not VSV-IN, and was shown to be more sensitive in detecting positive samples than the isolation of virus from tissue.<sup>79</sup> A hemi-nested PCR was developed later using the genomic sequence of the L protein as the primer. This assay detected the presence of both VSV-NJ and VSV-IN even when viable virus was not present as shown by positive PCR assays run on blood samples which were non-infectious in cell culture.<sup>49</sup> A reverse transcriptase PCR (RT-PCR) was developed that allowed for simultaneous detection of three important diseases of swine including FMD, swine vesicular disease and VSV.<sup>72</sup> This assay also employed the L gene sequence for VSV and was able to differentiate between VSV-NJ and VSV-IN. Very recently, a single-tube multiplex PCR for detection of VSV-NJ and VSV-IN in insect pools was developed.<sup>61</sup> This assay could detect either VSV-IN or VSV-NJ, or both VSV serotypes, with as little as 20 femptograms of total RNA present. Viral RNA also could be detected in macerates containing two infected mosquitoes in pools including 10 to 30 non-infected mosquitoes.<sup>61</sup>

## **Epidemiology**

### *Movement of disease through time and space*

Outbreaks of VS in the southwestern United States typically begin in the late spring or early summer. The index cases for the 1982-1983, 1995, 1997 and 1998 outbreaks were diagnosed in the first week of June, May 9, May 27 and May 14, respectively (McCluskey, B., USDA, APHIS, Veterinary Services, 1998, unpublished data).<sup>13,63,97</sup> Subsequently, premises with livestock positive for VS then are diagnosed throughout the summer with the last cases usually identified in the late fall. Also typical is the northward progression of recognized VS cases over time. Index cases for outbreaks in the United States usually are identified in southern New Mexico or Arizona. The index cases for the 1982, 1995, 1997 and 1998 outbreaks were found in Camp Verde, Arizona; Las Cruces, New Mexico; Taylor, Arizona; and Tularosa, New Mexico respectively. Figure 2.4 provides an example of the northward progression of cases through time for the 1995 VS outbreak. It has been suggested that both the temporal and spatial characteristics of VS outbreaks are suggested to be associated with arthropod abundance with identification of new cases occurring as warmer weather induces insect hatches and ceasing when cold weather predominates, inhibiting vector hatches.

#### *Virus Trafficking*

During the VS outbreak in 1982-1983, disease entered California through transport of infected cattle purchased in Idaho.<sup>42</sup> Additional evidence from investigative reports suggests that VSV can be moved to new locations through infected animals. For example, the only case of VS infection identified in Texas during the 1995 outbreak was due to movement of a horse from an area in New Mexico that was experiencing increased VSV activity into Texas. It was apparent that this horse had become infected in New Mexico and then subsequently exhibited clinical signs of VS infection after being moved

back to Texas (McCluskey, B., USDA, APHIS, Veterinary Services, 1995, unpublished data). In a study conducted by the United States Department of Agriculture, Veterinary Services in 1995, the movement of livestock from states with positive premises to other states and the association with the number of VS cases was examined.<sup>28</sup> The survey did not reveal a correlation between the number of horses and dairy cattle imported from an infected state or their proportion in relation to other states and the number of VS cases in importing states.

Direct contact transmission was observed when pigs were experimentally inoculated with VSV-NJ.<sup>84</sup> Serologically naive pigs were housed in direct contact with pigs that were experimentally inoculated with VSV-NJ by routes that would simulate contact or mechanical transmission.<sup>84</sup> In a second experiment, pigs infected with VSV-NJ by contact were housed with additional naive pigs. Pigs were monitored and sampled daily for clinical disease and virus isolation and serologic testing was performed before and after infection or contact. Contact transmission developed only when vesicular lesions were evident and contact pigs shed virus as early as 1 day after contact. Transmission was lesion-dependent, that is transmission only occurred when the infected pigs had visible lesions. Contact transmission was efficient, with infections ranging from subclinical to clinical with development of vesicular lesions.

#### *Arthropods*

Vesicular stomatitis viruses are classified as arboviruses, that is, they use insects as biological vectors. There is irrefutable evidence of insect transmission of VSV in endemic areas including Ossabaw Island. Experimental evidence exists to support insect transmission in the southwestern United States but the mechanism of virus acquisition

and potential amplification and spread of disease by arthropods in this region remains an enigma.

Trapped wild sandflies (*Lutzomyia trapidoi*; Diptera: Psychodidae) were infected experimentally with VSV-IN by feeding on viremic infant hamsters. The virus titers increased in the flies and bite transmission occurred within 3 days of the infective blood meal.<sup>91</sup> Later experiments showed that VSV-IN could be transmitted transovarially in two species of sandflies.<sup>91</sup> Virus titers in first generation female sandflies were comparable to those found in their parents and the first generation flies were capable of transmitting virus to their offspring.<sup>91</sup> Replication of VSV-NJ in sandflies also was shown to occur experimentally.<sup>98</sup> Eight species of sandflies were collected on dairy farms in a VS endemic region of Costa Rica.<sup>48</sup> Some of the species collected are known to be mammophilic. Isolation of VSV from collected flies was not attempted. On Ossabaw Island, VSV-NJ has been isolated from trapped sandflies (*Lutzomyia shannoni*) and virus activity as measured by seroconversions in feral swine populations which corresponded to the seasonal appearance of sandflies.<sup>14,22</sup> The hosts of the sandflies on Ossabaw Island were determined through ELISA testing of blood-fed flies trapped in the wild.<sup>21</sup> It was shown that sandflies were feeding on feral swine, white-tailed deer and to a lesser extent on horses and raccoons. All of these species have been shown to carry antibodies to VSV-NJ on Ossabaw Island. However, experiments indicated that neither pigs nor white-tailed deer were competent amplifying hosts of VSV-NJ for the sandflies.<sup>23,24</sup>

Black flies (Diptera: Simuliidae) were shown experimentally to be competent vectors for VSV-NJ.<sup>27</sup> *Simulium vittatum* (black fly) females intrathoracically infected with VSV-NJ transmitted infectious virus in their saliva after 10 days.<sup>27</sup> In other

experiments, mice exposed to experimentally infected black flies seroconverted to VSV-NJ by 21 days after infection indicating that virus transmission had occurred.<sup>65</sup> In a recent report, efficient transmission of VSV-NJ was demonstrated between infected and non-infected black flies co-feeding on non-viremic deer mice and pigs.<sup>66,67</sup> This suggests that black flies could act as a transfer vector between non-viremic vertebrate hosts and domestic livestock. VSV-NJ has been isolated from Simuliidae trapped in the wild during outbreaks of VS.<sup>38</sup>

Other species of insects have either been shown to be competent biological or mechanical vectors of VSV or have had the viruses isolated from them following trapping. Table 2.2 lists arthropods from which VSV have been isolated.

Flight ranges of insect vectors previously mentioned vary but none would be adequate to explain the often large distances observed between either individual or clusters of infected premises. Backward trajectories of winds were examined for VS outbreaks in 1982 and 1985.<sup>83</sup> Findings from the trajectory analysis suggest the feasibility of infected insects being transported for long distances on wind currents and subsequently landing on non-infected premises many miles from the infected premises or cluster of premises.

### *Reservoirs*

Arboviruses generally use vertebrates as reservoirs for transmission via arthropods.<sup>32</sup> A vertebrate reservoir normally would experience a viremia during which time they are infective for hematophagous insects. Viremia has not been observed in livestock species that exhibit clinical signs. Bats (*Myotis lucifugus lucifugus*) were inoculated subcutaneously with Cocal vesicular stomatitis virus to determine their

potential as maintenance hosts of VSV. Inoculated bats were shown to remain viremic for 10 days when housed at 22 C and those kept in hibernation conditions were viremic for 16 days.<sup>29</sup> It was not determined if these periods of viremia were due to virus replication or merely persistence. Persistent infections of VSV-IN were established in immunocompetent Syrian hamsters with virus recoverable up to 8 months after infection.<sup>39</sup> Virus was isolated from brain, spleen and liver homogenates in these experiments.<sup>39</sup> Other experimental work with hamsters revealed viral RNA in the brain, cerebellum, spleen, liver, kidney and lung 2 months after infection and in central nervous system tissues at 10 and 12 months post infection.<sup>9</sup> However, infectious virus was not recovered from any experimental animals in this study. The pathogenesis of VSV-NJ was investigated in deer mice (*Peromyscus maniculatus*), a potential reservoir species in the southwestern United States. Virus was inoculated experimentally into mice and virus was identified by immunohistochemistry in central nervous system tissues and the heart for up to 5 days post-inoculation.<sup>25</sup> Blood clearance experiments in wild mammals, chickens, pigeons, and other birds from Panama, indicated that virus was cleared in all animal species by 120 minutes post-inoculation.<sup>92</sup>

Serologic evidence of exposure to VSV has been shown in many vertebrate species. Table 2.3 lists select species from which antibodies to one or both VSV serotypes have been detected.

### *Risk Factors*

There are few published epidemiologic studies that were designed specifically to investigate risk factors for VS. A cross-sectional study of 348 farms conducted in Costa Rica found that cattle residing in areas between 500 and 1500 meters in premontane or

lower montane moist forest, had a higher risk of seropositivity to VSV-NJ as compared with cattle at lower elevations (O.R.  $\geq 3.6$ ).<sup>6</sup> Cattle residing at 0 to 500 meters and less than 2 meters of annual rainfall (tropical dry forest) were also at higher risk of seropositivity to VSV-NJ.<sup>6</sup> No factors were associated with increased risk of seropositivity to VSV-IN. A prospective case-control study conducted on 22 Costa Rican dairy farms evaluated cow, farm and ecological risk factors of clinical VS.<sup>93</sup> Affected cattle were generally older, with 7-year old cows having the highest age-specific incidence rate. Clinical disease also was associated with cows in lactation, and with higher acute antibody titers to VSV-IN. Farm factors associated with clinical disease included the presence of poultry and a longer calving interval on the farm. Two ecological factors were forced into the multivariate models, the reported presence of sandflies and the farms location in forest land. The two forced ecological variables were the only ones found to be associated significantly with clinical disease.<sup>93</sup>

A case control study was designed to identify management factors affecting the risk of animals developing VS in the southwestern United States.<sup>50</sup> Horses, cattle and sheep with suspected VS on 395 premises in Arizona, Colorado, New Mexico and Utah were included in the study. Data were collected during the VS outbreak in 1997 with cases defined as those premises completing a questionnaire and that had at least one animal confirmed positive for VS. Control premises were those investigated and completing a questionnaire but on which animals were tested for VS and then determined to be negative for VS. Results indicated that animals with access to a shelter or barn had a reduced risk of developing VS (O.R. 0.6, 95% C.I. 0.35 to 0.99). This effect was more pronounced for equine premises (O.R. 0.5, 95% C.I. 0.3 to 0.9). Risk of developing

disease was increased where animals had access to pasture (O.R. 2.01, 95% C.I. 1.1 to 3.7). On all premises where owners reported increased insect populations and where animals were housed less than 0.25 miles from a source of running water odds of developing VS were increased (insects O.R. 2.5, 95% C.I. 1.47 to 4.47; running water O.R. 2.6, 95% C.I. 1.32 to 5.0).

A retrospective case control study was designed to determine potential risk factors for VS in Colorado in 1995.<sup>70</sup> Data were collected on 52 premises that had VS-positive animals and 33 that did not have VS positive animals during the 1995 VS outbreak and 8 premises that were in the vicinity of premises with VS positive animals during the 1995 outbreak. Premises level and animal level data were collected including management practices and ecological variables. Premises that had at least 1 seropositive animal in 1996 were significantly more likely to be case premises than control premises. For case premises, there was an association between serologic status of the animals in 1996 and their clinical disease status in 1995. There were no significant premises level or animal level risk factors identified in this study.

As previously mentioned, some studies have shown that animals located in specific ecologic zones were more likely to be seropositive for VSV than animals in other zones. It was suggested from these studies that habitat requirements of either reservoirs or arthropod vectors were the factors associated with zone preference. An extensive study of human inhabitants of rural Central America found that antibodies to VSV-NJ were associated with persons living at elevations between 350 and 649 meters, with relatively dry climate, low density vegetation and seasonal alterations in ground moisture.<sup>20</sup> Similar risks were found for VSV-IN with the addition of increased risk in

moist, high density tree cover habitats. Chapter 4 of this dissertation describes studies conducted in New Mexico and Colorado that examine climatic variables potentially associated with VS in the southwestern United States.

### *Economic Impact*

Financial impacts of VS on livestock producers have been reported during a number of outbreaks in the United States. An outbreak on an Alabama dairy in 1962 resulted in net losses of \$13,889 on sale of cows, \$15,000 to \$20,000 in milk production, and \$10,000 to \$14,000 in milk quota.<sup>30</sup> A cost of \$253.31 per clinical case was calculated for 13 Colorado dairies affected by VS in 1982.<sup>3</sup> The greatest loss was due to cows culled (46.6% of the total loss). Two California dairies affected by VS in 1982 sustained losses of \$202/cow for one dairy and \$97/cow for the other dairy for total losses of \$225,000 for both dairies over a two month period.<sup>41</sup> Again culling accounted for the highest percentage (56%) of the loss. Another dairy affected by VS in 1982, located in Idaho, sustained total losses of approximately \$50,000.<sup>59</sup> Losses were attributed to involuntary culling, secondary bacterial infections and death, lost milk production and losses due to early dry-off of cows. Information concerning financial effects of VS on 16 Colorado beef ranches was investigated following the 1995 outbreak.<sup>45</sup> Median financial loss was \$7,818/ranch and mean financial loss was \$15,565/ ranch for total losses for the 16 ranches approaching \$250,000. Financial losses in these beef herds primarily were attributed to increased culling rates, death of pregnant cows, loss of income from calves, and costs of additional labor during the outbreaks.

A survey of horse, beef cattle and dairy cattle owners was conducted to assess the financial impact of the 1995 VS outbreak in New Mexico (Ott, S., USDA,

APHIS, Veterinary Services, unpublished data, 1996). Financial impact was comprised of extra expenses and lost revenues. Table 2.4 provides a summary of these impacts.

### *Zoonotic potential*

Three people were infected with VSV through exposure to experimentally infected animals while working in laboratories.<sup>44</sup> Fever, general malaise and muscle pain were signs similar in all three individuals. Mild stomatitis was observed in two of the three individuals. In all three recovery was complete and rapid. Although no virus was isolated from any of these individuals, high neutralizing antibody titers to VSV-NJ were present in all three. Two of the individuals handled experimentally infected cattle and the third was splashed with virus containing material while harvesting infected allantoic fluids.

In the 1950's, laboratory workers at the Agricultural Research Laboratory in Beltsville, Maryland were tested routinely for VSV complement fixing antibodies.<sup>74</sup> A summary of this work indicated that VS in humans appears as an acute, self-limiting infection with signs similar to influenza. Overall, 96% of laboratory workers and animal handlers tested had positive titers to VSV while only 57% of the seropositive individuals could recall having clinical signs.

An investigation of owners and handlers of infected cattle was conducted during an outbreak of VS in 1965.<sup>35</sup> A total of 41 persons was interviewed and had specimens collected for virus isolation and serologic testing. Eight persons had serologic evidence of exposure to VSV and all 8 had lived or worked on ranches where cattle were confirmed to have been infected with VSV-IN. Fever, general malaise, myalgia, nausea and pharyngitis were observed. Vesicular lesions of the gums occurred in 2 people. Similarly,

a study was conducted of veterinarians, research workers and regulatory personnel who were exposed to VSV during an outbreak in Colorado in 1982.<sup>76</sup> Neutralizing antibody prevalence was higher in exposed persons with clinical signs than in those without a history of clinical illness. Higher risk of seropositivity was found for individuals who examined the oral cavity of infected animals, had open wounds on hands or arms and for those examining horses rather than cattle. Overall, infectivity of humans was low.

A survey of approximately 20,000 Central Americans, living in areas endemic for VSV revealed an overall prevalence of neutralizing antibodies to VSV-NJ and VSV-IN of 48% and 18% respectively.<sup>20</sup>

### *Vaccine*

The concept of using an unmodified viable VSV as a vaccine was proposed first in the 1920's.<sup>73</sup> Cattle inoculated intramuscularly with VSV did not develop lesions and when challenged with virus locally, were resistant. A field trial of attenuated virus vaccine was conducted in a large dairy herd in Panama. Cattle were vaccinated over a three year period, 1962-1964, and then serum antibody titers as well as prevalence of disease was followed until 1966.<sup>59</sup> Protection for up to 4 years after vaccination was reported.

A special license was obtained from the United States Department of Agriculture in 1967 to produce and sell an attenuated VSV-NJ lyophilized vaccine, although sale of this vaccine in the U.S was discontinued in 1972.<sup>58</sup> However, this same vaccine was used in Guatemala for many years with reported success.<sup>58</sup>

A commercially available inactivated VSV-NJ vaccine was used in Colorado during the 1985 outbreak but serologic data regarding its immunogenicity and efficacy

were not available. A field trial to examine the humoral responses to this vaccine was conducted in a 350 cow dairy.<sup>40</sup> Two doses of this formalin inactivated cell culture-derived vaccine were administered intramuscularly 30 days apart to lactating and non-lactating adult cattle in this herd. Geometric mean titers peaked at 1:530 by 21 days after the second vaccination and declined to a geometric mean titer of 1:65 by 175 days post-vaccination. The lack of detectable antibody in the control group of cattle indicated that exposure to wild-type virus did not occur and therefore the efficacy of the vaccine could not be ascertained. A similar study was conducted by the author during the 1995 VSV outbreak. Three commercial dairies approved to use an autogenous inactivated virus vaccine produced from a 1995 isolate of virus were enlisted in a field trial.<sup>62</sup> Serum samples were collected from all cattle in the study prior to vaccination and all were determined to be free of antibodies to both VSV-NJ or VSV-IN. Two doses of vaccine were administered 14 days apart and all cattle in the study subsequently were bled three times. On all operations, all vaccinated cattle generated serum neutralizing antibodies to VSV-NJ but antibody titers waned quickly to low levels by 250 days post vaccination. There was no indication that wild type virus infected livestock on these operations or on operations near them so that determining whether cattle were protected with the vaccine was not accomplished.

A DNA vaccine that expressed the glycoprotein gene of VSV-NJ was evaluated for neutralizing antibody responses in mice, calves and horses.<sup>18</sup> The vaccine elicited antibody titers in individuals from all species, but the level of antibody required to afford protection to challenge was not determined.

Recently, gene expression of VSV-NJ was altered through rearrangement of the viral genome. Translocation of the N gene attenuated the virus to increasing extents and reduced lethality in mice without reductions in the ability to generate protective immunity.<sup>102</sup> This may provide a novel approach to the development of an attenuated VSV vaccine that will not elicit clinical disease but may afford adequate protection.

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Table 2.1. Occurrence of Vesicular Stomatitis in the United States, 1930-1990.

Year of Occurrence	States Affected	Serotype
1937	Wisconsin, Minnesota, South Dakota, Montana, West Virginia	New Jersey
1941	Texas, Louisiana, Kansas, Alabama	New Jersey
1942	Colorado	Indiana
1943	Colorado, Missouri	New Jersey
1944	Colorado, Texas	New Jersey
1945	California	New Jersey
1946	Idaho	Not reported
1947	Arizona	Not reported
1949	Arizona, Alabama, Mississippi, Georgia, Tennessee, Florida, Minnesota, Wisconsin, Colorado, Utah, Montana, Wyoming	New Jersey
1950	Texas, New Mexico	Not reported
1952	Georgia	New Jersey
1953	Georgia, North Carolina, Virginia, West Virginia, Maryland, Oklahoma, New Jersey	New Jersey
1954	Florida, Georgia, South Carolina	New Jersey
1955	Georgia, South Carolina, North Carolina, Louisiana	New Jersey
1956	Georgia, South Carolina, North Carolina, Louisiana, Colorado*, New Mexico*	New Jersey Indiana
1957	Florida, Georgia, South Carolina, North Carolina, Virginia, West Virginia, Louisiana, Arkansas, Oklahoma, Texas	New Jersey
1958	Georgia, South Carolina, North Carolina, Louisiana	New Jersey
1959	Georgia, South Carolina, North Carolina, Virginia, West Virginia, Louisiana, Mississippi, Texas, New Mexico	New Jersey
1960	South Carolina, North Carolina, Louisiana, Texas	New Jersey
1963	Georgia, Alabama, South Carolina, Florida	New Jersey
1964	South Carolina, Florida, Georgia, Alabama, Mississippi, Texas*, Oklahoma, Colorado*, Missouri*, Arkansas*	New Jersey Indiana
1965	Mississippi, Alabama, Arkansas, Texas*, Oklahoma, New Mexico*, Colorado*, Arizona*, Utah*	New Jersey Indiana

Table 2.1. continued

Year of Occurrence	States Affected	Serotype
1966	Texas, Colorado*, New Mexico*	New Jersey Indiana
1967	Louisiana	New Jersey
1968	Louisiana, Colorado	New Jersey
1969	Georgia, Alabama, Mississippi, Louisiana	New Jersey
1970	North Carolina, South Carolina, Georgia, Colorado	New Jersey
1971	Louisiana, Colorado, New Mexico	New Jersey
1972	Louisiana, Colorado, New Mexico	New Jersey
1973	Louisiana	New Jersey
1974	North Carolina	New Jersey
1981	Texas	New Jersey
1982-1983	Arizona, Colorado, New Mexico, California, Washington, Oregon, Kansas, Missouri, Montana, South Dakota, Nebraska, Idaho, Wyoming, Utah	New Jersey
1984	Texas	New Jersey
1985	Colorado, New Mexico	New Jersey

\*States where VSV-IN occurred in that year.

Table 2.2. Insect genera from which vesicular stomatitis viruses have been isolated.

Insect genera	Transmission shown	References
Tabanus	Yes	33
Chrysops	Yes	33
Aedes	Yes	11,33
Culex	Yes	33
Culicoides	Yes	56
Musca	No	37
Hippelates	No	96
Simulium	Yes	37
Lutzomyia	Yes	22
Stomoxys	Yes	33

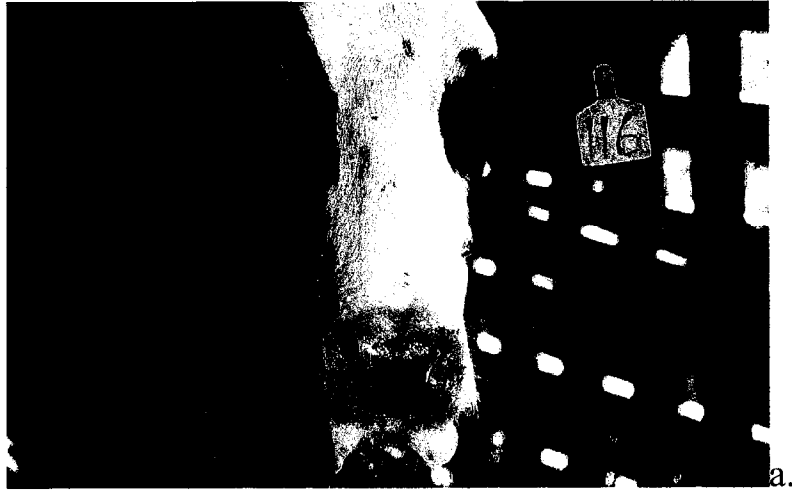
Table 2.3. Wild vetebrates identified to have antibodies to vesicular stomatitis viruses.

<b>Species</b>	<b>Common name</b>
<i>Alouatta villosa</i>	Howler monkey
<i>Antilocapra americana</i>	Pronghorn antelope
<i>Antilope cervicapra</i>	Blackbuck
<i>Aotus trivirgatus</i>	Night monkey
<i>Artibus spp.</i>	Fruit bat
<i>Baiomys taylori</i>	Pygmy mouse
<i>Bassaricyon gabii</i>	Olingo
<i>Bradypus infuscatus</i>	Sloth
<i>Canis latrans</i>	Coyote
<i>Cervus elaphus</i>	Elk
<i>Coendu rothschildi</i>	Porcupine
<i>Cynomys gunnisoni</i>	Prairie dog
<i>Dasypus novemcinctus</i>	Armadillo
<i>Didelphis virginianus</i>	Opposum
<i>Felis rufus</i>	Bobcat
<i>Lepus californicus</i>	Jackrabbit
<i>Lynx rufus</i>	Lynx
<i>Meleagris gallopavo</i>	Wild turkey
<i>Mus musculus</i>	House mouse
<i>Mephitis mephitis</i>	Skunk
<i>Myocastor coypu</i>	Nutria
<i>Neotoma mexicana</i>	Wood rat
<i>Odocoileus virginianus</i>	White tail deer
<i>Odocoileus hemionus</i>	Mule deer
<i>Ovis canadensis</i>	Bighorn sheep
<i>Peromyscus maniculatus</i>	Deer mouse
<i>Procyon lotor</i>	Raccoon
<i>Saguinus geoffroyi</i>	Marmoset
<i>Sciurus carolinensis</i>	Gray squirrel
<i>Sus scrofa</i>	Feral swine
<i>Sylvilagus auduboni</i>	Rabbit
<i>Tamandua tetradactyla</i>	Anteater
<i>Ursus americana</i>	Black bear

Table 2.4. Summary of financial impact studies of vesicular stomatitis on New Mexico livestock owners during the 1995 vesicular stomatitis outbreak.

Premises type	Survey popn. impact	No. of cases	Per case impact	Whole state estimated impact
Horse	\$10,311	27	\$382	\$86,138
Beef cattle	\$1,061	20	\$53	\$5,703
Dairy cattle	\$25,814	27	\$956	\$25,814
<b>Total</b>	<b>\$37,186</b>	<b>74</b>	<b>\$496</b>	<b>\$117,655</b>

Figure 2.1. Lesions caused by vesicular stomatitis viruses in cattle: a) ptyalism, b) severe ulceration of the oral mucosa, and c) vesiculation and ulceration of the teats and udder.



a.



b.

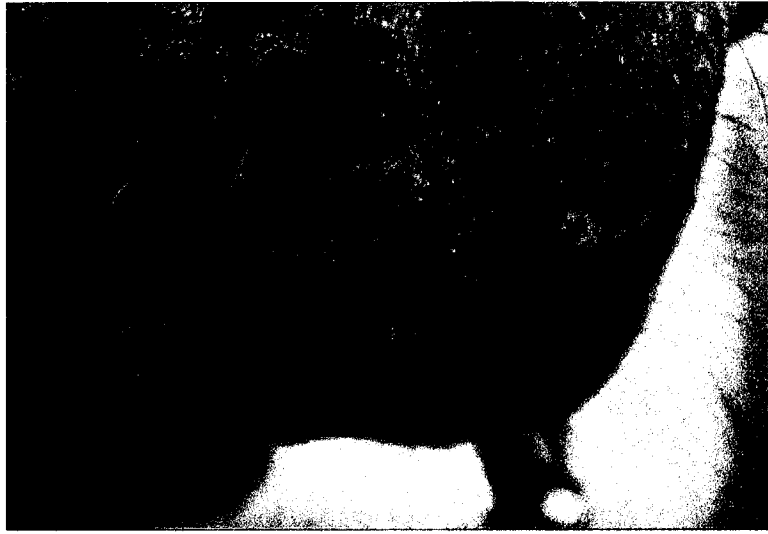


c.

Figure 2.2. Lesions caused by vesicular stomatitis viruses in horses: a) ulceration of the oral mucosa at the mucocutaneous junction, b) crusting lesion of the muzzle, and c) coronitis.



a.



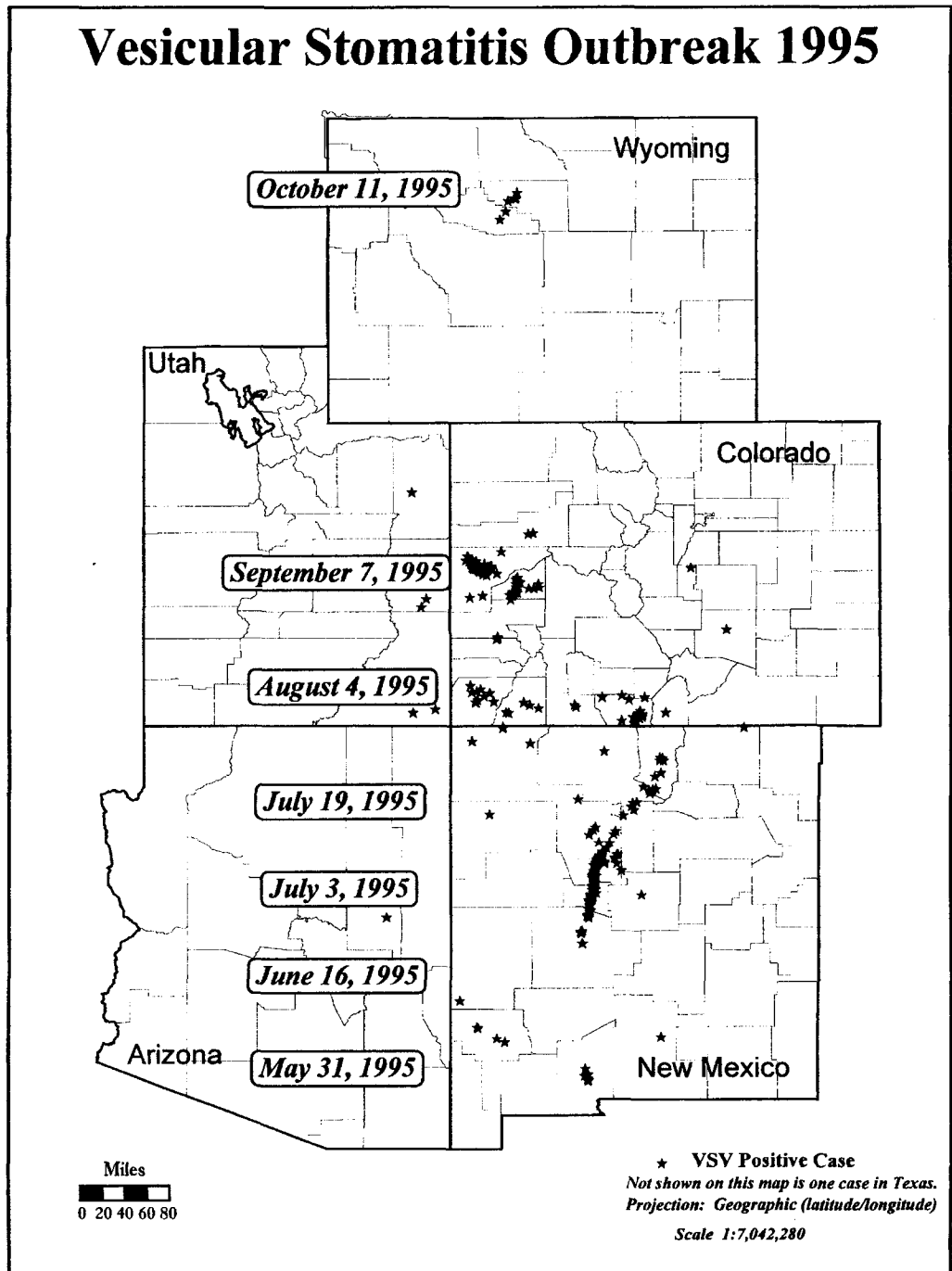
b.



c.

Figure 2.3. Chronology of identification of vesicular stomatitis positive premises during the 1995 outbreak in the southwestern United States.

# Vesicular Stomatitis Outbreak 1995



## Chapter 3.

### Use of Sentinel Herds in Monitoring and Surveillance Systems\*

#### Introduction

Sentinel surveillance is used to monitor or identify outbreaks and epidemics of disease, to investigate changes in the amount or severity of endemic diseases, to evaluate the effectiveness of newly instituted disease control programs, or to confirm a hypothesis about the ecology or epidemiology of a disease agent. This concept is one in which the health status of populations is periodically assessed. Sentinel surveillance has been applied liberally in development of public health surveillance systems (Thacker, 1983; Parrish and McDonnell, 1994). Applications of sentinel surveillance for animal health have generally been successful although applied in only limited cases.

Public health applications of sentinel surveillance have been used to monitor or identify epidemics of infectious diseases or to monitor the activity of conditions that change due to environmental conditions such as asthma. These systems cannot measure the magnitude of disease incidence or prevalence as they are not population based. Accurate incidence and prevalence estimates require knowledge of the population at risk for which

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the estimate is made. The French Communicable Disease Network (Sentiweb) has linked physicians in general practice throughout France by terminals supplied by the telephone company or through an Internet website (Valleron, 1992). The United States Influenza Sentinel Physicians Surveillance Network enlists approximately 260 physicians around the country who provide weekly reports on the total number of patients seen and the number of those patients with influenza-like illness by age group. Other sentinel systems employing physicians, hospitals, and laboratories reporting on a variety of diseases or identification of disease agents, exist in the United States and in other countries.

The list of sentinel surveillance systems in animal health is much shorter. The most well known and developed animal health sentinel surveillance system is the National Arbovirus Monitoring Program (NAMP) in Australia (Animal Health Australia, 2001). NAMP is a program managed by Animal Health Australia, a public company formed by the Commonwealth, State and Territory ministers of agriculture and the presidents of the national councils of Australia's livestock industries. The NAMP is funded by industry and government agencies and its goal is to monitor the distribution of certain arthropod borne livestock viruses and their vectors. The viruses of interest include Akabane, bluetongue and bovine ephemeral fever viruses. Data for this system are collected in sentinel cattle herds throughout Australia. Ten or more immunologically naïve young cattle on each sentinel location are blood tested at prescribed intervals to detect seroconversion to the viruses. Additional details of this system will be presented in later sections of this chapter.

In Canada, sentinel herds are also employed for bluetongue surveillance. Five sites were established in the Okanogan Valley of British Columbia in 1988. These sites were chosen purposefully as they had been identified historically to experience incursions of bluetongue (CAHNet Bulletin, 1999). A consortium of government agencies, producer groups and a university in Canada also established the sentinel herd mastitis project. This project used 27 sentinel veterinary practices, 40 veterinarians and 60 dairy herds to investigate the incidence of mastitis and monitor changes in incidence and management practices on dairies in Ontario (CAHNet Bulletin, 1999).

No doubt there are additional examples of sentinel surveillance systems or projects initiated by animal health officials or academic institutions in many countries. The purpose of this chapter is to provide guidance in the establishment of such systems. The rationale and limitations of sentinel surveillance systems are discussed as are specific procedures necessary for their implementation.

### **Rationale of Sentinel Surveillance**

The need for and uses of animal health surveillance are addressed in other chapters of this book. Improvements in animal health and livestock production and direction in appropriately allocating normally limited resources are all encompassing goals of surveillance. The international expectations of scientifically based disease risk management strategies require accurate and timely surveillance data.

Many countries have government sponsored systems for the routine collection of animal health information. Systems may include requirements for routine testing of all herds for a particular disease agent or testing of animals at slaughter. Determining how many animals in how many herds in what geographic area to provide some statistically estimable level of confidence that the disease does not exist or exists at some pre-determined level has been the difficult task of many veterinary epidemiologists and biostatisticians in the last few years. In most cases, the number of animals and herds required are large resulting in substantial investment of animal health resources. Surveillance systems are also implemented to monitor changes in occurrence of disease by time, place and species. It is in this application that sentinel surveillance systems have been of greatest value and their advantage is in the ability to target sampling and reduce costs.

Sentinel surveillance promotes targeting of herds or areas with higher probabilities of disease. Prior knowledge of disease distribution allows surveillance activities to focus on the margins of disease free and endemic areas or the borders between high prevalence and low prevalence areas. Absence of prior knowledge of disease distribution would necessitate a more random distribution of sentinel herds.

The cost effectiveness of sentinel surveillance systems makes them an attractive alternative to slaughter surveillance and cross-sectional surveys where sentinel surveillance systems are an appropriate option. Slaughter surveillance systems sample animals at the time of slaughter with subsequent testing of samples for antibodies to

disease agents of interest, the disease agents themselves or for residues. The USDA's Animal and Plant Health Inspection Service, Veterinary Services, estimates per animal collection and testing costs for bovine brucellosis slaughter surveillance to range from US\$0.60 – 1.50 (personal communication, Dr. Mike Gilsdorf, USDA, Veterinary Services, National Animal Health Program staff). An estimated 12 million samples from test eligible animals are required by program goals and to reach pre-determined statistically valid estimates of disease prevalence resulting in total slaughter surveillance costs of US\$7.2 – 18 million. Slaughter surveillance for bovine tuberculosis has estimated per animal sampling and testing costs between US\$31-34 and total annual testing costs ranging from US\$124,000-127,000.

Population based surveys with random sampling are similarly expensive and often difficult to conduct if adequate list frames of operations are not available. Surveys generally provide one time sampling (point estimates), thus requiring that they be repeated to assess changes in disease prevalence.

### **Establishing Sentinel Surveillance**

#### *Herd or site selection*

Goals of the sentinel system under consideration will certainly influence how herds or sites are selected. In the case of the NAMF in Australia, the primary goal is to facilitate plotting of the distribution of arboviral infections in cattle (Animal Health in Australia, 2001). Initially herds were selected to provide a random distribution over the country. True random selection of herds requires a deliberate process in which each herd has an

equal probability of being selected (Thrusfeld, 1995). This type of sampling uses a “list” of all herds in the population that meet the criteria of the study and a random number generator to assist in selection. In the NAMP, site selection now depends largely on the availability and interest of the regulatory field veterinarian in the area and the willingness of the operation’s owner (Table 3.1). Sentinel surveillance calls for recurring visits to the sentinel sites and consequently a commitment from the operations owner to allow such visits or regulatory authority to make such visits. Incentives to operation owners, including cash compensation or free or reduced diagnostic testing, may help in retention of sentinel surveillance sites.

As mentioned previously, targeting of surveillance is an advantage of sentinel surveillance and may impact site selection. The longevity of the NAMP in Australia has provided data indicating virus distribution so that program coordinators locate sites where previous virus activity has occurred, along the margins of previous virus activity to detect incursions of virus into new areas and in “free” areas to ensure they remain “free” areas.

Determining how many herds or operations to include in a sentinel system again depends on the goals of the system and in many situations on available resources. Using sentinel herds to determine freedom from a particular disease in a specific geographical area is a valid approach.

### *Animal Selection*

How many and which animals to include as sentinels is an important consideration when developing sentinel surveillance systems. The sentinel animals selected must be susceptible to the disease agent or agents of interest and generate a measurable clinical or immunological response to the agent. A cohort of young, immunologically naïve animals often make the ideal sentinels although adult animals may also be used. If young animals are to be marketed after initial sampling they would not be the best choice for sentinels. Although cohorts are followed through time, sentinel surveillance is different from a cohort study. A cohort study is designed to find risk factors associated with the occurrence of disease by following two separate groups of individuals, one group with exposures to suspected risk factors one group without these exposures. Cohorts used in sentinel systems are used as disease detectors and although risk factors of disease can be assessed, disease monitoring is the primary purpose of the cohorts. The sentinel animals chosen should be available for examination and/or sampling at each visit in order to monitor responses to the agent. This requirement often results in the owner of the operation choosing sentinel animals by convenience, that is, those that can be made available at each visit or those that can be easily handled. Due to the herd being the unit of observation and not the individual animal, random selection of sentinel animals is not imperative as long as those chosen are susceptible and likely to be exposed to the agent if it is present on the site.

Sample size estimates for sentinels on each site are based on standard formulas for detection of disease at predetermined prevalences and confidence levels. FreeCalc is a

free software package that assists in the planning and analysis of surveys to detect disease or prove freedom from disease (Cameron, 1998). FreeCalc calculates sample size requirements based on diagnostic test sensitivity and specificity, taking population size into account and can be used to determine the number of sentinel animals required on each site. Sample size estimates must consider the likelihood of dropouts. Sentinel animals may be sold, die or no longer be available for other reasons and thus lost to follow-up sampling.

### *Frequency of Sampling*

The frequency of sentinel site visits and subsequent sampling of sentinel animals is based on the goals of the surveillance and on the biology of the disease agent of interest. Table 3.2 suggests sampling frequency of sentinel sites for various frequencies of disease occurrence and by the effects the occurrence may have on animal populations. These effects may be direct (e.g loss of production or death) or indirect (restrictions on trade of live animals or animal products).

As mentioned previously, sampling frequency is also determined by the biology of the disease agent. For example, transmission of bluetongue virus (BTV) occurs almost exclusively by species of *Culicoides*. In the United States *Culicoides sonorensis* are the primary vectors. These vectors hatch, feed and breed from March through October with peak activity in June through September (Gibbs, 1987). Monitoring transmission of BTV through sentinel surveillance should most appropriately occur during the months of vector activity.

Sentinel sites used for research purposes could be sampled more or less often than suggested in Table 3.2, depending on the disease agent under investigation and on the resources available for the study.

### *Testing*

Assessing sentinel animal's response to the disease agent of interest is accomplished through clinical examination, serologic testing and/ or identification of the disease agent itself. Utilizing clinical examinations as the sole determinant of a positive sentinel animal is tenuous. Different disease agents may express very similar clinical manifestations (e.g. foot and mouth disease and vesicular stomatitis) requiring more specific discernment than a clinical examination can provide.

Various serologic procedures to detect antibodies to disease agents are available including numerous ELISA techniques, serum neutralization assays, complement fixation tests, agglutination tests, and many others. Availability is dependent on the agent of interest. Immunologically naïve animals would be expected to respond to the disease agent of interest in a measurable way. The primary immune response would be measurable by most if not all of the available serologic tests for that agent. However, animals already positive by one or more of the serologic tests need not be excluded as sentinel animals. Many assays can detect immunoglobulins of the IgM class. These antibodies are usually the earliest antibodies to appear after an antigenic stimulus but tend to decline rapidly and then disappear. Re-infection with the identical agent may stimulate

a new, measurable IgM response. Serologic tests that allow quantification of the amount of antibody can also be useful in animals previously exposed to the agent of interest. It is generally accepted that a four-fold rise in antibody titer suggests recent exposure to an infectious agent. For some agents this may indicate a recrudescence of a latent pathogen and for others re-exposure to the pathogen from the environment.

Isolation of the agent of interest or detection of genetic material from the agent (e.g. polymerase chain reaction techniques) from sentinel animals or from the environment of the sentinel site provides the best indication of the presence of the agent although these techniques are often the most expensive and difficult to conduct.

### **Examples**

In order to highlight the steps necessary in developing sentinel surveillance systems, two examples of sentinel herd projects will follow. In both examples the disease agents of interest are vesicular stomatitis viruses.

Vesicular stomatitis viruses (VSV) cause clinical disease in cattle, pigs and horses (McCluskey, 1999) and there are two serotypes of concern, vesicular stomatitis virus – New Jersey (VSV-NJ) and vesicular stomatitis virus –Indiana (VSV-IN). Clinical signs of disease may include vesicles and erosions of the oral and nasal mucosa, coronitis, vesicles or erosions of the udder and teats, and crusting lesions of the muzzle, ventral abdomen and external genitalia. Vesicular stomatitis (VS) is endemic in South America, Central America, parts of Mexico and on Ossabaw Island, a small barrier island off the

coast of the state of Georgia in the United States. The disease has been considered epidemic in the southwestern United States with sporadic outbreaks of VS occurring in this region. Outbreaks appear to be associated with arthropod transmission of the virus. Researchers with the Center for Veterinary Epidemiology and Animal Disease Surveillance Systems at Colorado State University and the USDA's Centers for Epidemiology and Animal Health, hypothesized that VSV were present in the southwestern United States in non-outbreak years and determined the best way to test this hypothesis was through sentinel herd testing of operations in the southwestern United States as well as operations in endemic areas of Central America as a comparative group.

#### *Sentinel Herds in El Salvador*

A total of 14 sentinel farms were selected from four different regions of El Salvador. These farms were selected based on samples of farms that were confirmed to have housed VS-positive cattle in one or more outbreaks during the years 1997 through 2000, or were adjacent to farms with positive cases during the outbreaks. These farms must have housed at least twenty dairy cows. Vesicular cases must have been confirmed as positive by the presence of clinical signs of VS and positive virus isolation and/or positive serologic test results. The confirmed positive cases did not necessarily still reside on the farms at the initiation of the sentinel study.

The distribution of the farms by regions was as follows (Figure 3.1):

The Department of Sonsonate – 4 farms

The Department of Chalatenango – 6 farms (2 dropped out from study)

The Department of San Miguel – 2 farms

## The Department of La Union – 2 farms

The selection of the sentinel cohorts was based on the population of cows on each farm. All heifers from 6 to 12 months of age were sampled from those farms with 30 or less heifers. On dairies with more than 30 cows, 15% of their additional heifers (above 30) were sampled.

The farm owner was required to commit to a minimum of two years of participation in the study and to monthly collection of biological samples from a cohort of cattle. Those farms having more than one susceptible animal species (equine, swine or bovine) or neighboring a farm with other species were preferentially selected to participate in the study.

Biological samples were collected monthly from heifers enrolled in the study. A serum sample and oral swab were collected from each heifer. At the same time, an examination of the oral cavity, nasal mucosa, coronary bands, mammary gland and genitalia was conducted. The cohorts were each assigned a score of 0 to 5 based on the presence of clinical lesions, body condition and activity level (0 = no lesions, 1 = lesions healing, 2 = mild ulceration with no affect on appetite or activity, 3 = moderate ulceration or crusting with mild affect on appetite or activity, 4 = extensive ulceration or crusting with noticeable weight loss and reduction in activity, 5 = active vesicles or severe loss of mucosal surfaces with marked reduction in weight and activity).

Descriptive analyses were performed at cow and farm levels. Overall, prevalence of VSV-NJ and VSV-IN was calculated as well as prevalence of each serotype by farm. For each farm, the percent positive by date was organized and graphed. Also, the dates of seroconversion for each positive animal were analyzed. Finally, the frequency of each serotype by age for each farm was calculated.

This sentinel project served a dual purpose. The epidemiology of VS had not been previously investigated in El Salvador. Peak time of transmission, potential vectors and reservoirs, and disease prevalence in general were unknown. The primary purpose of the design of this sentinel project was in attempting to investigate the ecology and epidemiology of VS in endemic areas of El Salvador and to compare this information with similar data collected in the sporadically epidemic regions of the United States. Secondly, monthly visits by veterinarians on operations that may not ordinarily have a veterinarian ever visit, establishes a disease surveillance system for all diseases potentially affecting cattle and other livestock species present on the operation.

#### *Sentinel Herds in Colorado and New Mexico, USA*

Vesicular stomatitis outbreaks in the United States are closely monitored by the USDA's Animal and Plant Health Inspection Service, Veterinary Services (APHIS-VS) and during the last three outbreaks (1995, 1997, 1998) premises and animal information was maintained in a database. There are many individual premises that have been designated as positive during more than one outbreak and a majority of positive premises primarily maintained horses and not cattle (McCluskey, 1999). The primary purpose of

establishing sentinel research herds was to test the hypothesis that VSV were present in the southwestern United States in non-outbreak years and was not an attempt to detect VS at a predetermined level or to conduct surveillance for other diseases or disease agents.

Only sentinel equine herds were selected because horses were more accessible on a routine sampling scheme and greater than 75%, 97% and 98% of the positive premises were identified as equine premises in the 1995, 1997 and 1998 outbreaks respectively. To improve the chance of detection of viral activity in the sentinel herds, herds that had been designated positive in at least one but preferably more than one of the last three outbreaks, had at least 2 horses, and were located in areas that had experienced extensive viral activity in one of the last three outbreaks were purposefully selected from outbreak databases (Figure 3.2). A total of 20 herds in Colorado and 20 herds in New Mexico were initially enlisted to participate. Once herds were selected from the outbreak databases, herd owners were contacted and their willingness to participate in a three year study determined. Paste anthelmintics were offered during each visit for each horse in the study as incentives to continue in the study. Quarterly visits were made to each sentinel premises in which between 2 and 20 sentinel horses were given examinations of the mouth, nasal cavity, feet and external genitalia. In addition, blood samples were drawn by jugular venipuncture and swabs were collected from the oral cavity. Information about premises management practices, animal movement history and other potential risk factors was collected by a standardized questionnaire at each visit.

All serum samples were tested by the competitive ELISA (cELISA) for antibody to both VSV serotypes. Samples positive by one or both cELISAs were tested by IgM capture ELISA, complement fixation tests and serum neutralization tests for each serotype of the virus. Oral swab samples were frozen at  $-70^{\circ}\text{C}$  until the completion of serologic testing.

Data collected through sentinel herd surveillance can be analysed by a number of methods, dependent upon either the epidemiologic question of interest or the surveillance need. In this study for example, descriptive statistics including overall farm-level prevalence and incidence densities were calculated; survival analysis was used to allow for inclusion of censored horses with a seroconversion considered a “failure” in the survival analysis; Kaplan-Meier curves were generated for those premises with horses that seroconverted and mean survival times calculated with mean survival times for each premises were used in a general linear model to evaluate management, environmental and other factors associated with variations in survival times.

## **Conclusions**

Sentinel herd surveillance can be an appropriate and economical alternative to more conventional methods of animal health surveillance. The key to success of any surveillance system is the establishment of goals prior to its development. Sentinel surveillance holds no exception to this. The steps necessary in establishing sentinel herd surveillance are summarized in Figure 3.3. Effective application of sentinel herd surveillance can enhance the overall animal health monitoring and surveillance programs of any country ultimately leading to healthier livestock and more competitive animal livestock industries.

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Table 3.1. National Arbovirus Monitoring Program (NAMP): Numbers of sites and collections – 1999-2000\*

State/Territory	<u>Serology and/or virology</u>					
	<u>Bluetongue</u>		<u>Akabane</u>		<u>Ephemeral Fever</u>	
	Sites	Collections	Sites	Collections	Sites	Collections
New South Wales	31	123	31	123	31	123
Northern Terr.	14	97	13	97	13	97
Queensland	23	76	23	71	23	71
South Aus.	4	8	4	8	1	2
Tasmania	3	6	3	6	3	6
Victoria	5	10	5	10	5	10
Western Aus.	10	30	10	31	10	30

\* Used with permission from Dr M.J. Nunn, Manager (Animal Health Science)  
Office of the Chief Veterinary Officer, Agriculture, Fisheries and Forestry - Australia

Table 3.2. Suggested frequency of sampling for sentinel herd surveillance applications.

<b>Disease Occurrence</b>	<b>Disease Affect</b>	<b>Example</b>	<b>Sampling Frequency</b>
Very Rare	Severe	Foot and Mouth Disease in a nearby country	Weekly
Rare	Severe	Bovine Brucellosis in free countries	Annually
Sporadic	Severe	Neosporosis Outbreaks	Quarterly
Sporadic	Moderate	Vesicular Stomatitis in the SW U.S.	Semi-annually
Endemic	Severe	Bluetongue in endemic countries	Semi-annually
Endemic	Moderate	Vesicular Stomatitis in Central America	Annually

Figure 3.1. Location of sentinel dairy herds in El Salvador to study the epidemiology of vesicular stomatitis.

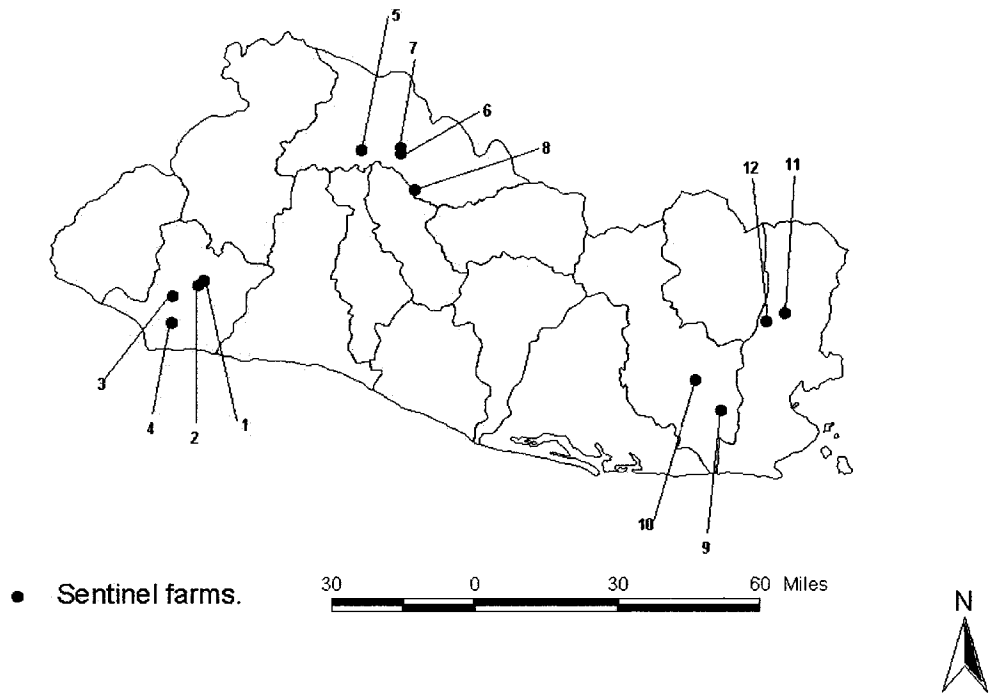


Figure 3.2. Location of sentinel equine operations in Colorado and New Mexico to study the epidemiology of vesicular stomatitis.

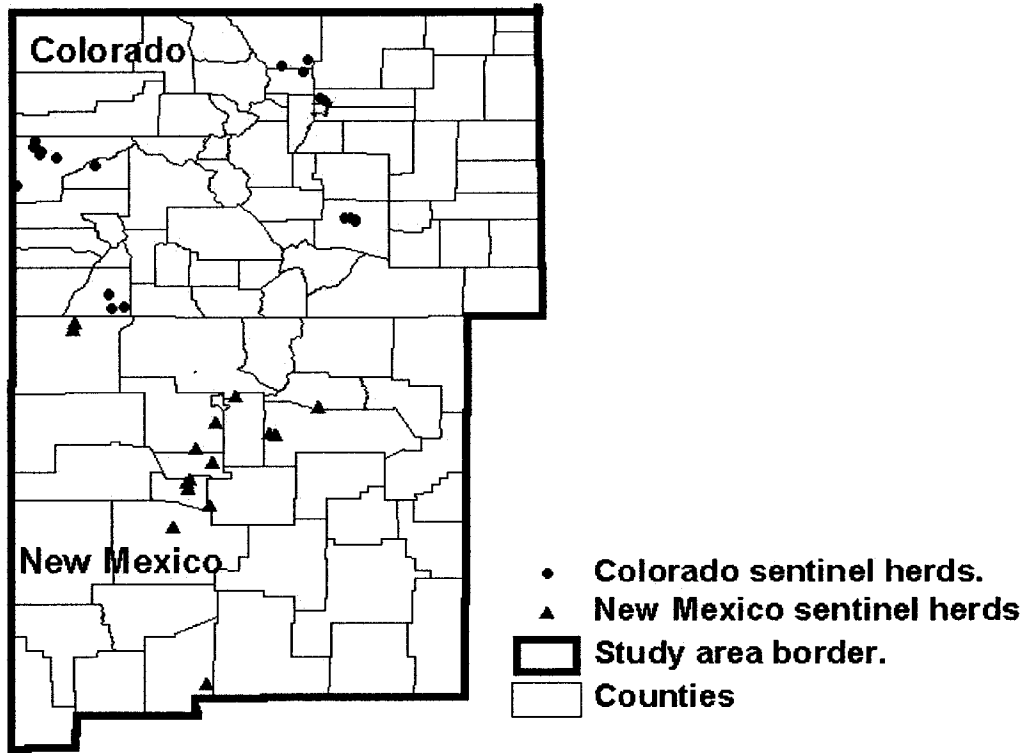
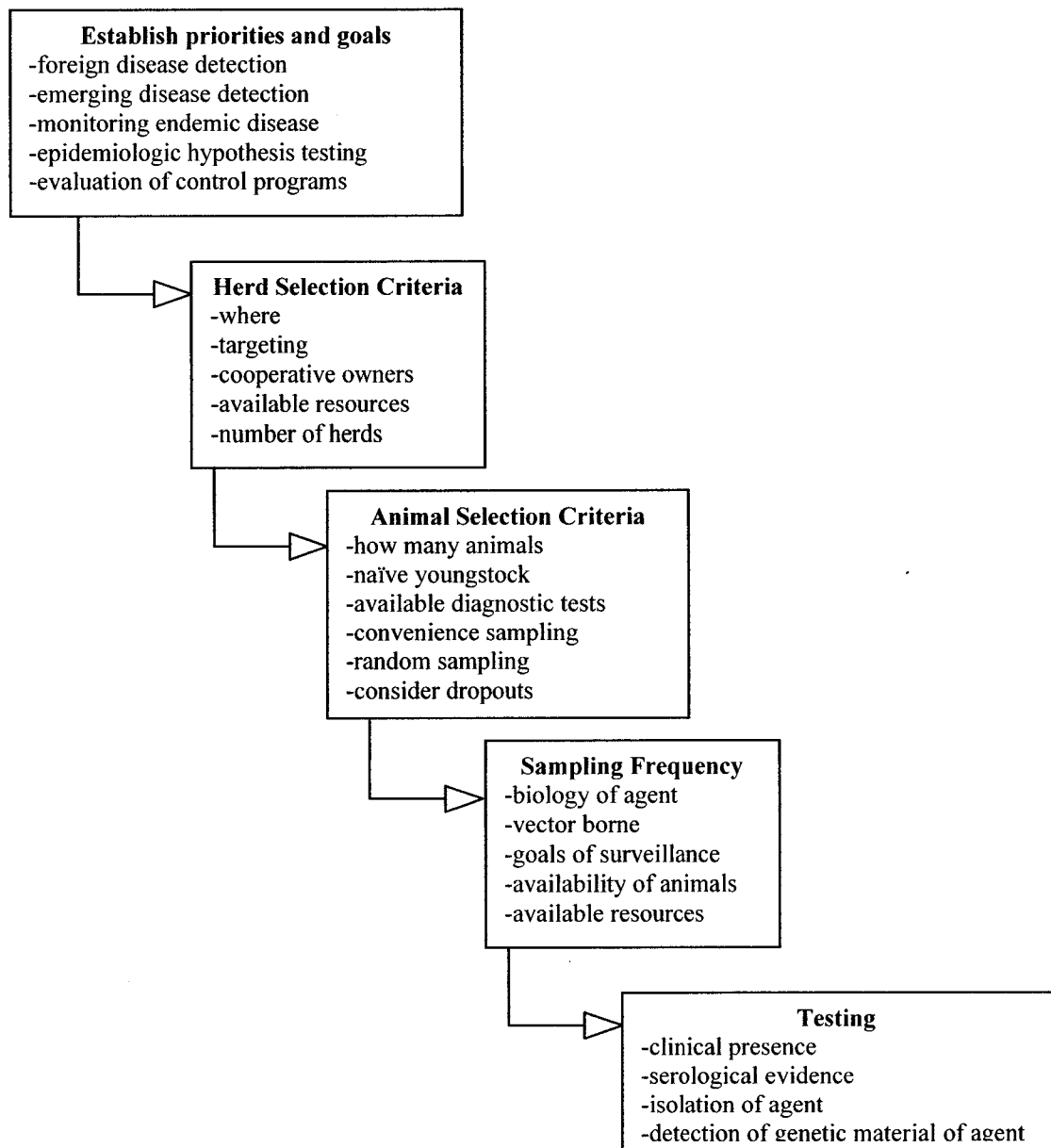


Figure 3.3. Considerations for establishing sentinel sites as a component of animal health monitoring and surveillance systems.



## **Chapter 4.**

### **Part I.**

#### **Vesicular Stomatitis in the Southwestern United States: Observations from a 3-Year Study of Sentinel Equine operations in Colorado and New Mexico**

##### **Introduction**

Vesicular stomatitis (VS) in the southwestern United States is caused by either of two distinct serotypes of the virus, VS virus New Jersey (VSV-NJ) or VS virus Indiana (VSV-IN). Both viruses are members of the Rhabdoviridae family, genus *Vesiculovirus*. Clinical VS in the southwest is observed almost exclusively in cattle and horses with only rare occurrences in pigs and llamas. During the last 3 outbreaks in the southwest most cases of VS occurred in horses.<sup>1</sup> Serologic evidence of viral exposure has been observed in many species including coyotes, deer mice, mule deer, pronghorn antelope, raccoons, white-tailed deer and wild turkeys.<sup>2-4</sup> Clinical signs of VS in livestock include vesicles and ulcers on the mucosal surfaces in the nasal and oral cavities (particularly lingual and gingival surfaces), on teats or at the coronary band. Crusting lesions of the muzzle, ventral abdomen and external genitalia are commonly seen in clinically affected horses. Ptyalism, anorexia and weight loss are sequelae of the oral lesions. Lameness and occasionally sloughing of the hoof are sequelae of coronary band lesions.

Sporadic outbreaks of VS occur in the southwestern United States with 5 such outbreaks occurring since 1980.<sup>1,5,6</sup> Outbreaks typically begin in the late spring. The index cases for the 1982, 1995, 1997 and 1998 outbreaks were diagnosed in the first week of June, May 9, May 27 and May 14, respectively (McCluskey, B., USDA, APHIS, Veterinary Services, 1998, unpublished data).<sup>1,6,7</sup> Premises with livestock positive for VS are then diagnosed throughout the summer with the last cases normally identified in the late fall. Also typical is the northward progression of the outbreak through time. Index cases for outbreaks in the United States are normally identified in southern New Mexico or Arizona. The index cases for the 1982, 1995, 1997 and 1998 outbreaks were found in Camp Verde, Arizona; Las Cruces, New Mexico; Taylor, Arizona; and Tularosa, New Mexico respectively. Both the temporal and spatial characteristics of VS outbreaks are suggested to be associated with arthropod abundance with identification of new cases occurring as warmer weather induces insect hatches and ceasing when cold weather predominates, inhibiting hatches.

The sporadic occurrence of VS outbreaks is perplexing. An animal reservoir has not been identified. Risk factors associated with the occurrence of VS on individual premises suggest that arthropods play a role in the epidemiology of VS in the southwest.<sup>8</sup> However, arthropod vectors competent to transmit VS viruses (VSV) are abundant in the southwest every year. Phylogenetic analysis of VSV-NJ indicated that virus causing the 1995 outbreak in the southwest belonged to a lineage distinct from that of the 1982 and 1985 viruses and was also distinct from Central America and Ossabaw Island, Georgia strains.<sup>9,10</sup> Additional phylogenetic analysis indicated that VSV-NJ and VSV-IN isolates from the 1995, 1997, and 1998 outbreaks in the southwest were closely related to viral

lineages circulating in Mexico and were not closely related to viruses from previous outbreaks in the southwest thereby suggesting the viruses causing several of the most recent outbreaks in the southwest originated in Mexico.<sup>11</sup> However, VSV-IN virus isolated from cases in the southwest in 1997 was identical to virus isolated in 1998 indicating that the virus is capable of persisting and inducing an outbreak in successive years. A serologic evaluation of VS virus exposure in horses and cattle in 1996 indicated that livestock may be exposed to VS viruses between outbreaks.<sup>12</sup>

We hypothesize that VSV are endemic in the southwestern United States and thus the objectives of this study were to investigate the persistence of vesicular stomatitis viruses over a 3-year period on equine operations in Colorado and New Mexico and to identify potential factors associated with viral infections in horses. The first paper in this series of two will present descriptive statistics and observations from the three year study. The second paper in the series will investigate risk factors potentially associated with serologic responses of horses to VS viruses on sentinel equine operations in the southwest.

## **Materials and Methods**

### *Sentinel Operation Selection and Data Collection*

Outbreak databases were maintained by the United States Department of Agriculture during the 1995, 1997 and 1998 outbreaks and included operation level and animal level data for each investigation conducted during the outbreaks. Sentinel equine operations (SEO) were selected from outbreak databases in May 1998 for Colorado operations and January 1999 for New Mexico operations. Operations in Colorado and

New Mexico having maintained at least one animal meeting the outbreak case definition during one or more of the outbreaks (positive operations) were eligible for selection. The index case definition for the United States was detection of clinical signs in the affected animal consistent with VS accompanied by virus isolation or a four-fold rise in titer (complement fixation test [CFT] or serum neutralization test [SNT]) in paired sera collected 7 days apart. The definition for subsequent cases was the detection of clinical signs and a positive result for a competitive ELISA (cELISA), clinical signs and a positive result for detection of antibodies by a CFT, or clinical signs and a SNT with titers greater than 1:32. A non-random selection of eligible SEO was made from the outbreak databases that would provide geographic representation similar to where clusters of positive operations occurred during the three outbreak years (see Chapter 2). Sentinel equine operations considered positive in more than one outbreak year or maintaining other susceptible livestock species were selected preferentially. A letter describing the study and an informed consent form were mailed to selected operations. Operations could participate if at least 2 horses routinely resided on the operation during the study period, owners agreed to allow up to 20 horses to be examined and have blood and swab samples collected approximately every three months for three years and a signed informed consent letter was received by the study coordinators.

A standardized questionnaire used in previous epidemiologic studies during outbreaks was modified for this study to collect data regarding operation VS history, management practices, livestock movement history and operation-level ecological characteristics. The questionnaire was administered for this study during the initial visit to the SEO. An additional questionnaire was developed to assess changes in

management, additions of livestock to the operation, movement of livestock and changes in ecological characteristics and was administered on each subsequent visit (Appendix 3).

Geographic positioning system instruments were used to obtain geographic coordinates for each SEO. Latitude and longitude readings were obtained from the location on the premises where horses spent the majority of time. A database containing the coordinates for each SEO was used to create base maps on relief, geology, soil, land cover, plant hardiness and ecoregions.<sup>a,b</sup> Data on elevation, slope and aspect were obtained from digital elevation models.

#### *Sentinel Horse Selection and Sampling*

Non-random selection of horses to be enlisted as sentinels was conducted on each participating SEO by the owner. Horses selected did not necessarily reside on the SEO when the operation was considered positive for VS during an outbreak. Between two and 20 horses on each SEO were enlisted as sentinels on the initial visit. Most participating SEO were small, non-commercial operations; therefore, on most operations all horses were included as sentinels.

Each sentinel horse was identified by name and then assigned an operation and horse number. Breed, gender, age, VS clinical history and VS vaccination history data were collected for each horse at the initial visit. New horses were added as sentinels throughout the study either through foals being born to currently enlisted sentinels or through purchase of new additions. Sentinels added during the study were also assigned an operation and horse number and signalment and VS history data were collected.

Clinical examination of the oral and nasal cavity, ventral abdomen, external genitalia and coronary bands was conducted on each sentinel horse at each visit. Horses

were assigned a score of 0 to 5 based on the presence of clinical lesions, body condition and activity level (0 = no lesions, 1 = lesions healing, 2 = mild ulceration with no affect on appetite or activity, 3 = moderate ulceration or crusting with mild affect on appetite or activity, 4 = extensive ulceration or crusting with noticeable weight loss and reduction in activity, 5 = active vesicles or severe loss of mucosal surfaces with marked reduction in weight and activity).

Blood samples were collected by jugular venipuncture at each visit from all available sentinel horses into a sterile tube without anticoagulant. If lesions were present, tissues and swabs from the lesions were collected and placed in tubes of Tris-buffered tryptose broth (TBTB). In the absence of lesions, swabs of the oral cavity were collected and also placed in tubes of TBTB. Blood samples were centrifuged and serum was separated and then frozen at -70 C. Swab and tissue samples were also frozen at -70 C.

#### *Serologic Testing*

Serum samples were tested by two competitive ELISAs, for antibodies to VSV-NJ and VSV-IN, respectively. Sensitivity and specificity of the VSV-NJ cELISA, relative to the serum neutralization test, are 98% and 93%, respectively.<sup>13</sup> Sensitivity and specificity of the VSV-IN assay, relative to the serum neutralization test, are 85% and 94%, respectively.<sup>13</sup> Laboratory procedures for the cELISA have been previously published.<sup>12,13</sup> The optical density of each well was read with an ELISA plate reader at 450 nm.<sup>12</sup> The optical density was used to calculate the percent reduction of each sample. The percent reduction in absorbance was used to determine the positive and negative classifications, with 50% serving as the cutoff value for positive reactions based on Office International des Epizooties (OIE) standards.<sup>14</sup>

Samples positive to either of the cELISAs were further tested by IgM capture ELISA and SNT for each virus serotype. The cELISA and IgM capture ELISA were performed at the Rocky Mountain Regional Animal Health Laboratory, Denver, Colorado. The SNT was performed at the National Veterinary Services Laboratories (NVSL), Ames, Iowa, the OIE reference laboratory for vesicular stomatitis. Laboratory procedures for the IgM ELISA and SNT have been previously published.<sup>14,15</sup> Samples to be tested by the SNT were stored at -70 C until all samples were collected. Samples from each visit for each sentinel horse positive by cELISA were then tested by the SNT side by side on the same day to control for as much laboratory variability as possible.

#### *Virus Isolation*

Frozen swab and tissues samples in TBTB from sentinel horses with SNT titers of 1:32 or greater were sent to the NVSL for virus isolation. Samples were pooled by visit number for each SEO with total pool size not exceeding 5 samples per pool. Sample pools were created before processing. Sample pools were vigorously agitated and then centrifuged at 1900 x g for 20 minutes. The sample fluid was then diluted 1:2 in 3X antibiotics f-15 media (MEM with Earles with 0.5% EDAMIN). One ml of inoculate suspension was added to 4 Leighton tubes and 2.0 to 3.0 ml added to one 25 cm<sup>2</sup> flask containing confluent VERO cells. The remaining inoculate suspension was stored at -70 C. Inoculated cell cultures were incubated at 37 C for 1 hour. Inoculum fluid was discarded and cell cultures washed 3 times with pre-warmed 3X penicillin-streptomycin solution (1.0 ml for Leighton tubes and 3.0 ml for flasks). Pre-warmed fetal bovine serum with penicillin-streptomycin solution added (2.5%) was added to Leighton tubes (1.5 ml) and flasks (8.0 to 10.0 ml). Leighton tube cell cultures were incubated at 33 to 35 C and

observed for cytopathic effect (CPE). After 18 to 24 hours of incubation, the coverslip from one Leighton tube culture per sample inoculated was stained with VSV serotype specific fluorescent antibody conjugate. Remaining Leighton culture tubes were returned to the incubator. Inoculated flasks were incubated at 35 to 37 C and observed daily for CPE. Samples with the presence of fluorescence were considered positive. Samples without fluorescence were incubated for an additional 6 days and fluorescent antibody conjugate staining performed again. If no fluorescence on stained slides or CPE in flask cultures were observed after 7 days post-inoculation samples were considered negative.

#### *Single-Tube Multiplex RT-PCR*

Aliquots of 240 swab samples submitted for virus isolation were subjected to a multiplex polymerase chain reaction assay (PCR) capable of detecting both serotypes of VSV in a single tube. Assay procedures have been previously described.<sup>16</sup>

#### *Data Analysis*

Descriptive statistics regarding sampling (visit numbers, horses sampled) were generated. Seroprevalence was calculated by dividing the number of horses positive at least once by the total number of horses tested on that farm. Apparent seroprevalence based on results of the cELISA for each serotype were adjusted to true seroprevalence according to the formula of Rogan and Gladen given as:

$$\text{True prevalence} = \frac{(\text{apparent prevalence} + (\text{specificity} - 1))}{(\text{sensitivity} + (\text{specificity} - 1))}.$$

This formula considers the imperfect sensitivity and specificity of the diagnostic tests used.<sup>17</sup> Changes in geometric mean titers (GMT) of the SNT over time for each serotype were calculated. Geometric mean titers were calculated by taking the log of SNT titers for each sentinel horse at each visit. Log transformed titers for all horses at each visit

were summed and divided by the total number of horses sampled at that visit for each SEO. This provided the average GMT for that SEO at that visit. The average GMT for each month for each serotype across all SEO was then plotted. Fisher's least significant difference test was used to make pairwise comparisons of GMT by month for each serotype.

The total number of seroconversions to either VS virus serotype was determined for each SEO. Seroconversions were considered to have occurred if: a) the IgM capture ELISA was positive, b) there was a four-fold rise in SNT titer between consecutive visits, or c) two consecutive negative cELISA tests were followed by a positive test that remained positive for two consecutive visits. An individual horse could seroconvert more than once if it met the established criteria. Average incidence density rates (IDR) for seroconversions to either VS virus serotype were also calculated for each SEO by determining the number of seroconversions occurring during the period between visits and dividing by the total number of horses at risk on the sentinel operation during that time period. Incidence densities were then averaged for all visits for which seroconversions occurred for each SEO. Incidence densities are reported as seroconversions per 1000 horse days at risk. All statistical procedures were conducted using statistical software.<sup>c</sup>

## **Results**

A total of 38 SEO (21 in Colorado, 17 in New Mexico) were enlisted in the study. Figure 4.1.1 shows the location of the 38 SEO. Overall, 180 and 93 SEO visits were conducted in Colorado and New Mexico, respectively. Sampling in New Mexico was

initiated approximately 1 year after sampling was begun in Colorado. An unequal number of visits were made to SEO due either to dropout or lack of access to operations at scheduled visit times. Sampling statistics including total number of visits made, total number of horses sampled, median number of samples collected per horse and the mean number of days between visits for each SEO are provided in Table 4.1.1.

During 33 (1.6%) of the clinical examinations, lesions consistent with VS were observed. There were 16, 14, 2 and 1 clinical examinations scored as a 1, 2, 3 and 4, respectively.

Overall, 1,984 samples were tested by the cELISA for both serotypes. There were 537 (27.1%) and 889 (44.8%) positive cELISA tests for the VSV-NJ and VSV-IN serotypes, respectively. The percentage of cELISA positive samples was statistically different for the two serotypes (z-test for proportions,  $p < .0001$ ). Adjusted seroprevalences for each SEO with 95% confidence intervals are given in Table 4.1.2. Seroprevalences were determined by dividing the total number of horses tested at least once on each SEO by the total number of horses that tested positive at least once.

Figure 4.1.2 and 4.1.3 show the GMT variation by month for VSV-NJ and VSV-IN, respectively. The Fisher's least significant difference test for pairwise comparisons, indicated that for VSV-IN, the GMT for June was significantly different ( $p < .05$ ) than the GMT for February, March, August, September, November and December. There were no significant differences for monthly GMT for VSV-NJ.

Figures 4.1.4 through 4.1.7 provide examples of SNT titers through time for horses on select SEO. In figure 4.1.4, horse 1 shows the classic anamnestic response to an antigen with a slow decline in antibody titer followed by an increase in titer at visit 6.

Horse 2 in this figure is representative of horses in this study whose titers fluctuated through time. It is notable that the two horses represented in this figure were the only two horses on this SEO and were both initially determined to be positive to VS in October, 1995. Thus, horse 1 for example, had maintained a titer to VSV-NJ of at least 1:512 for over 5 years. Horse 5 in figure 4.1.5 similarly showed fluctuations in titer while horse 6 from this SEO maintained a stable titer of 1:512 throughout almost the entire study. Horse 3 in figure 4.1.6 shows dramatic fluctuations in titer while horse 4 from this SEO shows a typical pattern of titers in response to the antigen tested for. This horse had a stable titer for the first 5 visits followed by a slight decline in titer with a subsequent 4 fold increase in titer at visit 8. Both horses in figure 4.1.7 maintained stable titers throughout the 5 visits. It is important to note that horse 3 in figure 4.1.7 maintained a titer of 1:8192 for the entire time it was enrolled in the study. None of the horses enrolled in the study from the SEO represented in figure 4.1.7 were present on the SEO during any of the last three VS outbreaks in the SW and yet substantial titers were retained.

Based on the established criteria, a total of 48 and 89 seroconversions occurred to VSV-NJ and VSV-IN respectively. At least one seroconversion occurred to VSV-NJ and VSV-IN on 23 (60.5%) and 27 (71.1%) of the 38 SEO respectively. The total number of seroconversions and number of horses seroconverting for each SEO with at least one seroconversion are presented in Table 4.1.3. Incidence density rates (IDR) were calculated as the number of horses seroconverting on each SEO per 1000 horse days at risk. The IDR ranged from 0.60 to 4.75 for VSV-NJ and from 0.79 to 4.50 for VSV-IN (Table 4.1.4).

A total of 156 pools for virus isolation were created representing 390 samples from 114 individual horses. All pools were negative for both VSV-NJ and VSV-IN.

The multiplex PCR identified four samples to be positive for VSV-NJ and one sample positive for VSV-IN. Positives were identified by identity with a band at the location of the control virus sample. The four positive VSV-NJ samples were collected from three separate SEO in Colorado (CO-7, CO-14 and CO-17) and the VSV-IN positive sample was also collected from an SEO in Colorado (CO-16).

## **Discussion**

Sentinel equine operations were purposefully selected from locations in which clusters of positive premises during outbreaks had been identified. The SEO were also required to have been considered a positive premises in one or more outbreak years. Both of these selection criteria were established in order to maximize the opportunity to find horses exposed to VS viruses during the course of the study. It was assumed that if the characteristics, both management and ecological, of the SEO that made horses on them more susceptible to VS infection during previous outbreaks, would also make them more susceptible to infection in non-outbreak periods. It was anticipated SEO would drop out of the study over time and that individual horses would be lost to follow-up. Even so, there were a total of 273 visits with 363 horses sampled at least once over a 40 month period. This was the largest longitudinal study of vesicular stomatitis ever conducted in the southwestern United States.

In other studies in which serologic evidence of exposure to VSV-NJ and VSV-IN was investigated, a larger percentage of serum samples was positive for VSV-NJ than

VSV-IN (Chapter 6).<sup>18</sup> These studies were conducted in VS endemic areas and the differences in seropositive percentages between VSV-NJ and VSV-IN were suggested to be associated with the different ecologies required of the two serotypes. The findings of these studies also agree with reports that over 80% of vesicular stomatitis cases in Costa Rica are caused by VSV-NJ as compared to VSV-IN.<sup>19</sup> In the study reported here, the percentage of total samples serologically positive for VSV-IN, as determined by the cELISA, was approximately 1.5 times greater than those positive for VSV-NJ. This can be attributed to VSV-IN being the last VSV serotype to have been isolated and to have caused an outbreak in the southwestern United States (1998). Prior to the 1997 VS outbreak in the southwest, in which both VSV-IN and VSV-NJ were isolated, VSV-IN had not been isolated from the United States since 1966.<sup>20</sup>

Seroprevalences of horses on individual SEO ranged from 0% to 92% for VSV-NJ and VSV-IN. Confidence intervals for the point estimates were wide due to the small sample sizes on each SEO. Seroprevalence estimates were high on some SEO but clinical signs of disease were rare. In most cases where lesions were observed they were scored as a 1 or 2 indicating that lesions were healing or that only mild ulceration was present. There are other pathogens, toxicants and traumas that can cause lesions that mimic those of VS in horses. An extensive review of these differentials has been published by this author.<sup>21</sup> In addition, subclinical disease during outbreaks is a common finding with seroprevalence often 3 to 9 times higher on affected premises than clinical prevalence (McCluskey, B., USDA, APHIS, Veterinary Services, 1995, unpublished data). In this study all horses with lesions were negative for virus isolation.

Observations of SNT titers over time for individual horses indicated that for many, titers remained stable, often maintained at high levels with only moderate decays in titer through time. However, there were other horses whose titers fluctuated through time. Both of these patterns of serologic response to VSV have been previously reported to occur in cattle.<sup>18,22</sup> A small laboratory study designed to compare serologic responses of cattle, swine and horses experimentally infected with VSV-NJ, indicated that horses maintained stable SNT titers.<sup>23</sup> This study, however, was of very short duration, lasting only 80 days.

Maintenance of positive antibody titers for many months or years, without evidence of clinical disease and without virus being isolated from the horses in this study indicated that they posed minimal risk of transmission of virus to other horses or livestock. Thus the movement restrictions, either international or within the United States, imposed on horses found to be seropositive for VS have little scientific justification.

The level of virus neutralizing antibody required to afford protection from infection is unknown. In a Costa Rican study, cattle were found with clinical disease that had neutralizing antibodies to VSV-NJ prior to disease indicating that even with neutralizing antibody present cattle remained susceptible to infection and development of disease.<sup>18</sup>

Studies in cattle in areas endemic for VS, suggested that fluctuations in antibody titers were due to constant antigenic stimulation by virus circulating in nature or by persistence of virus in cattle with occasional reactivations resulting in antibody responses.<sup>18</sup>

All SNT titers from samples collected from each SEO were added together and divided by the total number of samples collected from each SEO in each month, providing the average SNT titer by month for each SEO. The GMT was then calculated for each month among all the SEO for the entire study period. The GMT for both serotypes showed an increasing trend from early in the year to peak in the late spring and early summer. The GMT for VSV-IN in June was significantly different ( $p < 0.05$ ) than titers for 6 of the other months in the year. In addition, there was a spike in GMT for VSV-IN in October but this was not statistically different than other months. The time of the summer peaks in GMT is similar to when cases of VS are initially being identified in the southwest during outbreaks.<sup>1,6</sup> It would be remiss to assume that if titers in horses on individual SEO were relatively stable over time or fluctuated indiscriminately in non-outbreak periods that a trend towards increasing titers would be observed at any time of year. The observation of GMT rising, peaking in the early summer and then falling again, suggests that horses in the study were either being exposed to virus in the environment or were recrudescing at a similar time (late spring or early summer). An explanation for a timed recrudescence is difficult to develop although similar stresses among horses during the early summer might be proposed (e.g. heat stress, changes in feed, insect bite density). The temporal characteristics of VS outbreaks, and hence exposure to virus, is suggested to be associated with arthropod abundance where identification of new cases occur as warmer weather induces insect hatches and ceases when cold weather predominates, inhibiting hatches.

By the criteria established, at least one seroconversion to VSV-NJ and VSV-IN occurred on approximately 61% and 71% of SEO, respectively. Seroconversions

occurred on SEO from all geographic areas where clusters of positive operations occurred during the three outbreak years. There were a total of 7 and 1 seroconversions to VSV-IN and VSV-NJ as determined by a positive IgM ELISA, respectively. Five out of the seven VSV-IN seroconversions and the single VSV-NJ seroconversion as determined by IgM ELISA were detected at the first visit on Colorado premises (between May and September of 1998). There was an outbreak of VS occurring in Colorado during that time. Thus, these seroconversions are considered to have occurred due to exposure to virus during an outbreak. There were a total of 3 and 2 seroconversions to VSV-IN and VSV-NJ as determined by the cELISA. Seroconversions, as determined by this assay, were considered to have occurred if two consecutive negative cELISA tests were followed by a positive test that remained positive for two consecutive visits. Seroconversions, as determined by a test with a binomial outcome are tenuous. The sensitivity of the cELISA for VSV-NJ and VSV-IN, relative to the SNT, are 98% and 85%, respectively. Thus for the VSV-NJ cELISA in particular, there will be very few false negative assays (2%) but there will be a number of false positives.

During the last 3 outbreaks of VS in the southwestern United States, the index case definition for the United States was detection of clinical signs in the affected animal consistent with VS accompanied by virus isolation or a four-fold rise in titer, either by CFT or SNT in paired sera collected 7 days apart. In the study reported here, a four-fold increase in SNT titer between consecutive visits was considered a seroconversion. For example, a change in SNT titer from 1:32 to 1:128 from one visit to the next, would be considered a four-fold increase in titer and thus a seroconversion. As previously mentioned very few horses showed clinical signs consistent with VS and virus was not

isolated from any of the horses tested. Considering four-fold increases in SNT titers alone as the criterion for seroconversion status, there were 79 and 45 seroconversions to VSV-IN and VSV-NJ, respectively. There were a number of changes in SNT titers between consecutive visits beyond a four-fold increase for both VSV-IN and VSV-NJ. For example, for VSV-IN there were 27 increases in titer between consecutive visits greater than or equal to a 10 fold increase. For VSV-NJ there were 21 such increases. It is difficult to attribute these levels of titer increase to normal antibody fluctuations in a horse previously infected with a VS virus.

The four VSV-NJ PCR positive swab samples were obtained from horses on separate SEO in February 1999 (n=1), May 1999 (n=2) and August 1999 (n=1). The VSV-IN positive swab sample was collected from a horse in May 1999. No clinical signs were exhibited by any of the horses at the time samples were collected. Swab samples positive by PCR to VSV-NJ and VSV-IN suggest that these viruses were in the environment of the horses on these four SEO and that these horses were exposed to the virus. Horses PCR positive to VSV-NJ in 1999 in Colorado is of particular interest as the outbreak of VS in the previous year had only VSV-IN isolated from clinical cases. Thus the VSV-NJ identified by PCR may have persisted for up to two years.

Four of the SEO in this study were included in a separate study designed to investigate the role of small terrestrial rodents in the epidemiology of VS in the southwest (Dr. Todd Cornish, University of Wyoming, personal communication). Rodents of various species were trapped and blood samples were collected over the duration of the study. Blood samples from a total of seven pinon mice (*Peromyscus truei*) and two deer mice (*Peromyscus maniculatus*) trapped on two of the sentinel sites were found to have

neutralizing antibodies to VSV-NJ. The short lifespan of these rodents would exclude them from having retained antibody due to exposure to VSV during a previous outbreak. These findings suggest exposure to VSV or to a virus inducing cross-reacting antibodies to VSV in a non-outbreak period.

Seroconversions occurring in non-outbreak periods, as observed in this study, suggest that horses on SEO were either being exposed to one or the other of the VSV serotypes in non-outbreak periods, that horses may have been infected previously with VSV and seroconversions represent recrudescence of virus or, that a virus inducing cross reacting antibodies to VSV infected horses. Studies examining the persistence of virus in pigs have indicated that virus is only shed from animals with lesions and that once shedding ceases viable virus can no longer be isolated or detected by molecular techniques from blood, saliva or tissues.<sup>23</sup> Thus, there is currently no evidence of latent or persistent infections in livestock. There are many vesiculoviruses found in nature including numerous plant viruses and horses may have been responding serologically to exposure to one of these plant viruses through grazing or were exposed to an unidentified, yet antigenically similar, VSV.<sup>24</sup> We suggest that the most likely antigenic stimulus causing seroconversions to VSV-IN and VSV-NJ in the horses in this study were exposures to wild-type VSV in the environments of sentinel horses.

The observation that at least one seroconversion occurred on a majority of the SEO indicates that exposures, from whatever source, are not rare events. Considering the small number of equine operations and horses included in this study, relative to the large numbers of equine operations and horses located in Colorado and New Mexico, observations of seroconversions on any of them is significant. The SEO were chosen by

criteria that would maximize the opportunity to observe exposures of horses if they were to occur. However, the management and ecology of the 38 SEO in this study would be considered similar to many other equine operations in these two states suggesting that exposure to virus, by whatever mechanism is occurring on many equine operations in Colorado and New Mexico.

Seroconversions occurring due to exposure to wild-type virus in the environment during non-outbreak years suggest that VSV may be present in the southwestern United States in all years. Although presence of VSV in all years may require that VSV be redefined as endemic infectious agents with concomitant changes in the response to outbreaks and restrictions placed on infected horses, VS as a disease entity should continue to be considered to occur only sporadically. The response by animal health authorities to outbreaks of VS in horses in the southwestern United States should be reviewed based on the findings of this study. However, due to the clinically indistinguishable lesions of foot and mouth disease (FMD) and VS in those species susceptible to both (cattle and pigs), the regulatory response to disease in ruminants and swine must be to assume that the disease observed is potentially FMD, a devastating livestock disease not seen in the United States since 1929.

## Endnotes

<sup>a</sup> Arc View ver. 3.2, ESRI, Redlands, CA.

b

DATA	ON LINE DATA
Relief map	<a href="http://www.usgs.gov">www.usgs.gov</a>
Geology	<a href="http://www.geology.cr.usgs.gov/pub/National_Atlas">www.geology.cr.usgs.gov/pub/National Atlas</a>
Soil	<a href="http://www.ftw.nrcs.usda.gov/statgo2_ftp.html">www.ftw.nrcs.usda.gov/statgo2_ftp.html</a>
Land cover	<a href="http://www.nationalatlas.gov/atlasftp.html">www.nationalatlas.gov/atlasftp.html</a>
Hardiness	<a href="http://www.usns.gov/hardzones">www.usns.gov/hardzones</a>
Ecoregions	<a href="http://www.fs.fed.us/land/ecosysmgmt/ecoreg1_home.html">www.fs.fed.us/land/ecosysmgmt/ecoreg1_home.html</a>

<sup>c</sup> SAS ver. 8.02, SAS Institute Inc, Cary, NC.

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Table 4.1.1. Sampling statistics for sentinel equine premises in Colorado and New Mexico.

<u>Premises No.</u>	<u>Total Visits</u>	<u>Total horses sampled</u>	<u>Median number of samples per horse</u>	<u>Mean number of days between visits</u>
CO-1	2	4	1.5	147
CO-2	9	10	8.0	128
CO-3	9	2	9.0	128
CO-4	10	2	10.0	121
CO-5	5	7	5.0	102
CO-6	12	9	8.0	106
CO-7	12	11	12.0	109
CO-8	9	5	9.0	112
CO-9	12	4	9.0	104
CO-10	12	11	11.0	106
CO-11	12	9	5.0	107
CO-12	3	3	2.0	100
CO-13	9	8	7.0	126
CO-14	9	13	6.0	123
CO-15	4	14	4.0	98
CO-16	6	12	5.5	95
CO-17	12	6	11.0	105
CO-18	12	19	7.0	103
CO-19	7	10	6.5	111
CO-20	12	38	3.0	110
CO-21	5	4	5.0	153
NM-1	6	7	6.0	107
NM-2	7	17	6.0	112
NM-3	5	6	4.0	126
NM-4	6	9	5.0	97
NM-5	3	3	3.0	106
NM-6	7	23	6.0	95
NM-7	1	9	1.0	NA
NM-8	5	20	5.0	105
NM-9	6	20	6.0	91
NM-10	6	9	6.0	105
NM-11	7	8	6.5	123
NM-12	6	5	5.0	106
NM-13	7	7	5.0	135
NM-14	5	6	5.0	123
NM-15	5	6	3.0	89
NM-16	5	4	3.5	118
NM-17	6	3	5.0	90
<b>Total</b>	<b>273</b>	<b>363</b>	-	-

Table 4.1.2. True seroprevalence estimates with 95% confidence intervals based on cELISA for vesicular stomatitis New Jersey serotype (VSV-NJ) and vesicular stomatitis Indiana serotype (VSV-IN) for sentinel equine operations in Colorado and New Mexico.\*

<u>Operation No.</u>	Seroprevalence VS-New Jersey		Seroprevalence VSV-INdiana	
	<u>(%)</u>	<u>95% C.I.</u>	<u>(%)</u>	<u>95% C.I.</u>
CO-1	67	21,100	0	NA
CO-2	32	3,61	0	NA
CO-3	92	55,100	92	55,100
CO-4	92	55,100	92	55,100
CO-5	78	47,100	49	12,86
CO-6	14	0,37	69	39,99
CO-7	55	25,84	73	47,99
CO-8	52	8,96	32	0,74
CO-9	17	0,54	67	21,100
CO-10	0	NA	37	8,66
CO-11	14	0,37	80	54,100
CO-12	92	62,100	92	62,100
CO-13	5	0,21	30	0,62
CO-14	15	0,35	15	0,35
CO-15	34	9,59	34	10,59
CO-16	8	0,24	42	14,70
CO-17	58	19,98	75	41,100
CO-18	18	1,36	13	0,29
CO-19	2	0,12	52	21,83
CO-20	0	NA	42	27,58
CO-21	67	21,100	92	66,100
NM-1	77	46,100	77	46,100
NM-2	50	27,74	44	21,68
NM-3	52	9,96	72	33,100
NM-4	47	15,80	36	5,68
NM-5	92	62,100	92	62,100
NM-6	20	3,38	49	28,71
NM-7	25	0,54	36	5,68
NM-8	12	0,27	12	0,27
NM-9	7	0,19	52	31,74
NM-10	0	NA	36	5,68
NM-11	5	0,21	42	8,77
NM-12	32	0,73	52	9,96
NM-13	49	12,86	63	28,99
NM-14	58	19,98	75	41,100
NM-15	8	0,30	75	41,100
NM-16	42	0,91	99	NA
NM-17	92	62,100	25	0,75

\*Apparent seroprevalence adjusted to true seroprevalence based on the formula of Rogan and Gladen.<sup>16</sup>

Table 4.1.3. Number of seroconversions and number of horses seroconverting to vesicular stomatitis New Jersey serotype (VSV-NJ) or vesicular stomatitis Indiana serotype (VSV-IN) on sentinel equine operations in Colorado and New Mexico.

<u>Operation Number</u>	<u>Number VSV-NJ seroconversions (number horses seroconverting)</u>	<u>Number VSV-IN seroconversions (number horses seroconverting)</u>
CO-2	3 (1)	0 (0)
CO-4	2 (1)	3 (2)
CO-5	1 (1)	1 (1)
CO-6	2 (2)	1 (1)
CO-7	4 (4)	4 (2)
CO-8	3 (3)	1 (1)
CO-10	0 (0)	7 (4)
CO-11	1 (1)	7 (4)
CO-12	1 (1)	1 (1)
CO-13	2 (1)	4 (3)
CO-14	0 (0)	2 (1)
CO-15	1 (1)	2 (2)
CO-16	3 (2)	6 (5)
CO-17	3 (2)	6 (3)
CO-18	3 (2)	2 (2)
CO-19	0 (0)	2 (2)
CO-20	0 (0)	4 (4)
CO-21	2 (2)	1 (1)
NM-1	6 (5)	6 (5)
NM-4	1 (1)	2 (1)
NM-6	2 (2)	7 (6)
NM-9	2 (1)	7 (6)
NM-10	0 (0)	1 (1)
NM-11	1 (1)	2 (1)
NM-12	0 (0)	3 (2)
NM-13	1 (1)	4 (4)
NM-14	2 (2)	2 (2)
NM-15	1 (1)	1 (1)
NM-17	1 (1)	0 (0)
<b>Total</b>	<b>48 (39)</b>	<b>89(68)</b>

Table 4.1.4 Average incidence density rates (number of horses seroconverting per 1000 horse days at risk) for vesicular stomatitis New Jersey serotype (VSV-NJ) and vesicular stomatitis Indiana serotype (VSV-IN) on sentinel equine operations in Colorado and New Mexico.

<u>Operation Number</u>	<u>Incidence density rate VSV-NJ</u>	<u>Incidence density rate VSV-IN</u>
CO-2	0.92	NA
CO-4	4.75	4.47
CO-5	1.28	1.57
CO-6	2.55	NA
CO-7	0.99	1.12
CO-8	2.73	1.68
CO-10	NA	1.75
CO-11	1.50	2.83
CO-12	4.50	4.50
CO-13	1.13	1.95
CO-14	NA	0.83
CO-15	0.69	1.37
CO-16	1.32	1.98
CO-17	2.27	3.02
CO-18	0.97	1.12
CO-19	NA	1.39
CO-20	NA	0.79
CO-21	3.85	1.63
NM-1	1.87	2.33
NM-4	NA	1.58
NM-6	0.60	1.68
NM-9	0.57	1.05
NM-10	NA	1.05
NM-11	2.22	1.25
NM-12	NA	2.05
NM-13	1.23	2.59
NM-14	1.36	2.71
NM-15	3.21	3.21
NM-17	3.70	NA

Figure 4.1.1. Location of sentinel equine operations in Colorado and New Mexico.

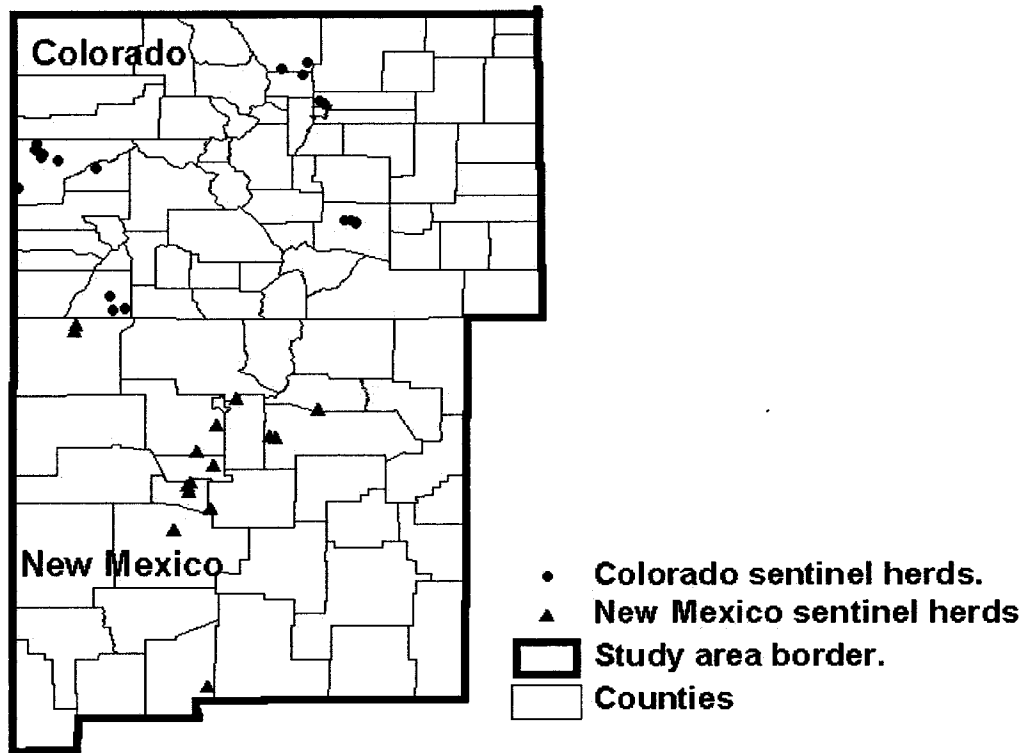


Figure 4.1.2. Geometric mean serum neutralization titers (and 95% confidence intervals on the mean estimate) for vesicular stomatitis New Jersey serotype by month for horses on 38 sentinel equine operations in Colorado and New Mexico.

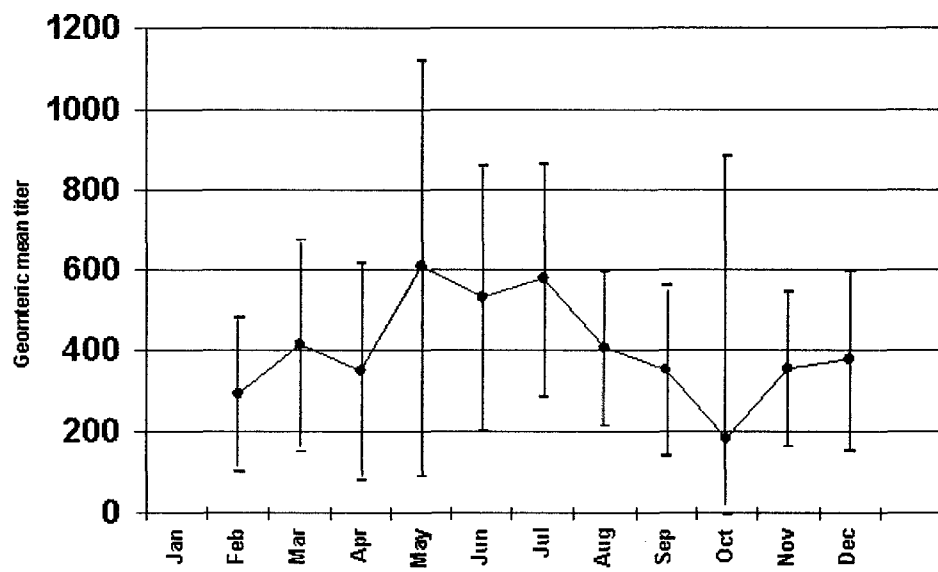


Figure 4.1.3. Geometric mean serum neutralization titers (and 95% confidence intervals on the mean estimate) for vesicular stomatitis Indiana serotype by month for horses on 38 sentinel equine operations in Colorado and New Mexico.

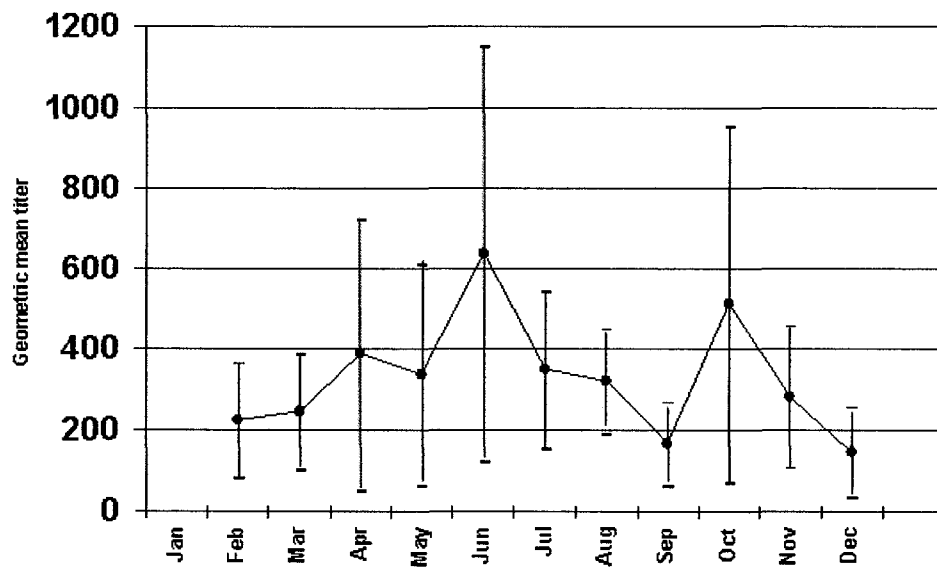


Figure 4.1.4. Patterns of  $\log_2$  transformed serum neutralization titers for vesicular stomatitis New Jersey serotype for 2 horses on sentinel equine operation CO-4.

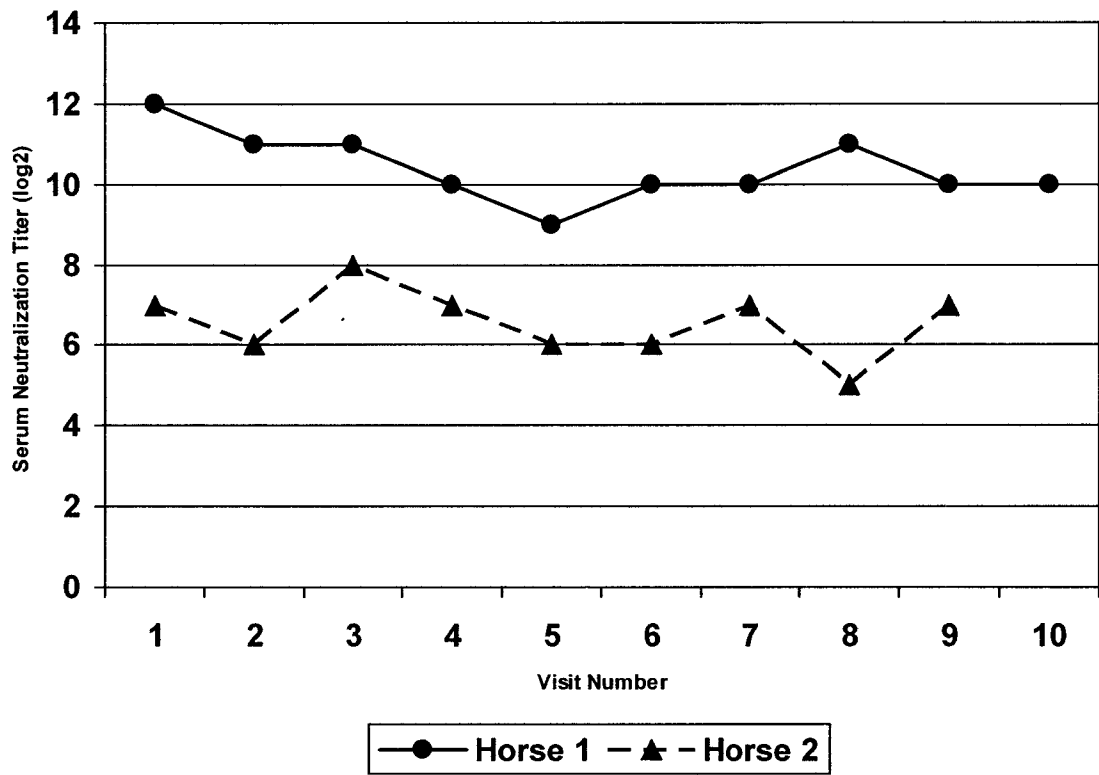


Figure 4.1.5. Patterns of  $\log_2$  transformed serum neutralization titers for vesicular stomatitis New Jersey serotype for 2 horses on sentinel equine operation CO-7.

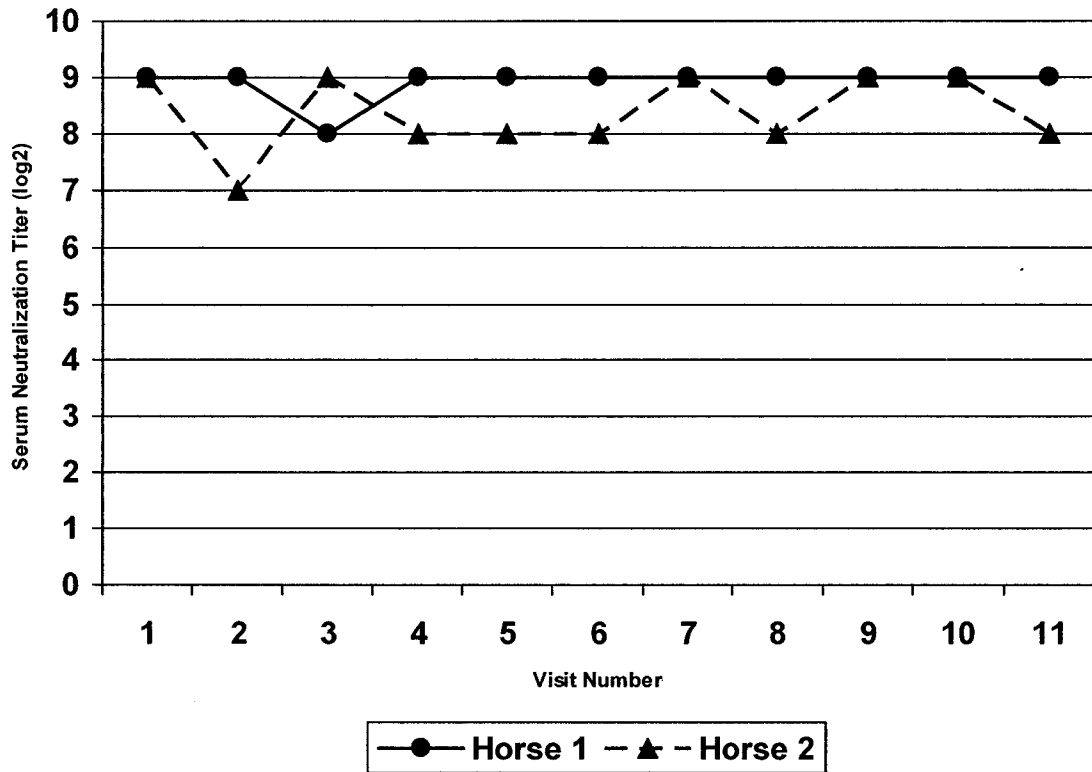


Figure 4.1.6. Patterns of  $\log_2$  transformed serum neutralization titers for vesicular stomatitis New Jersey serotype for 2 horses on sentinel equine operation CO-17.

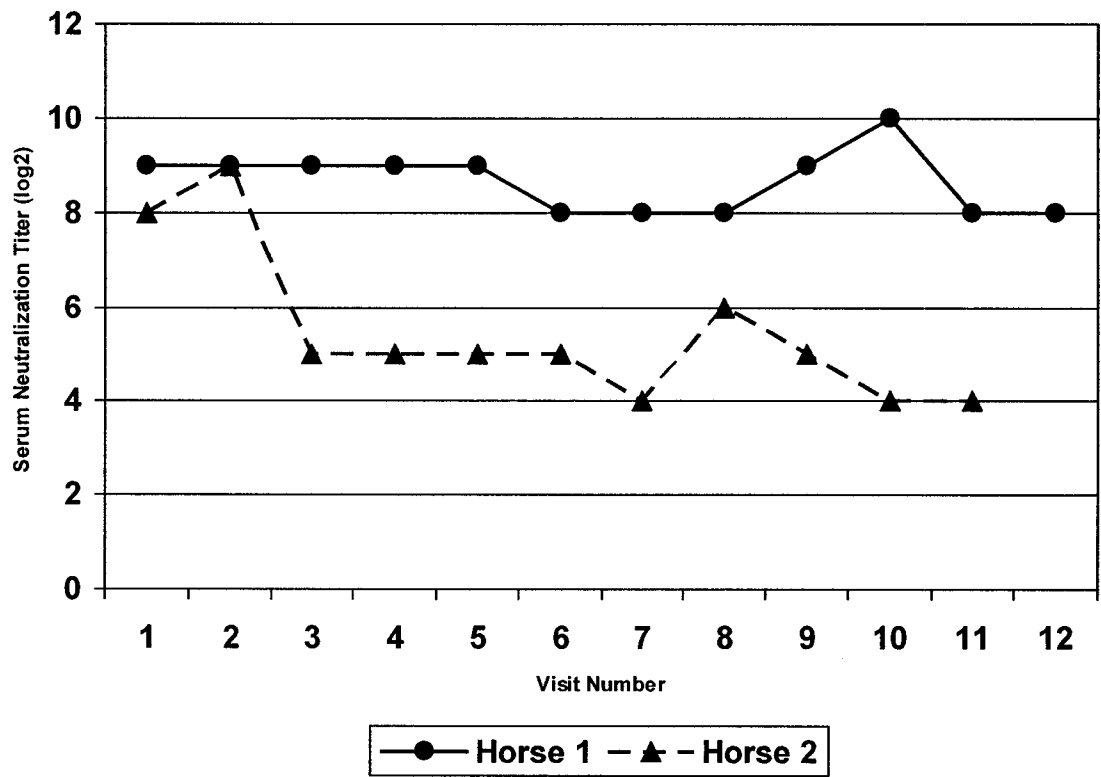
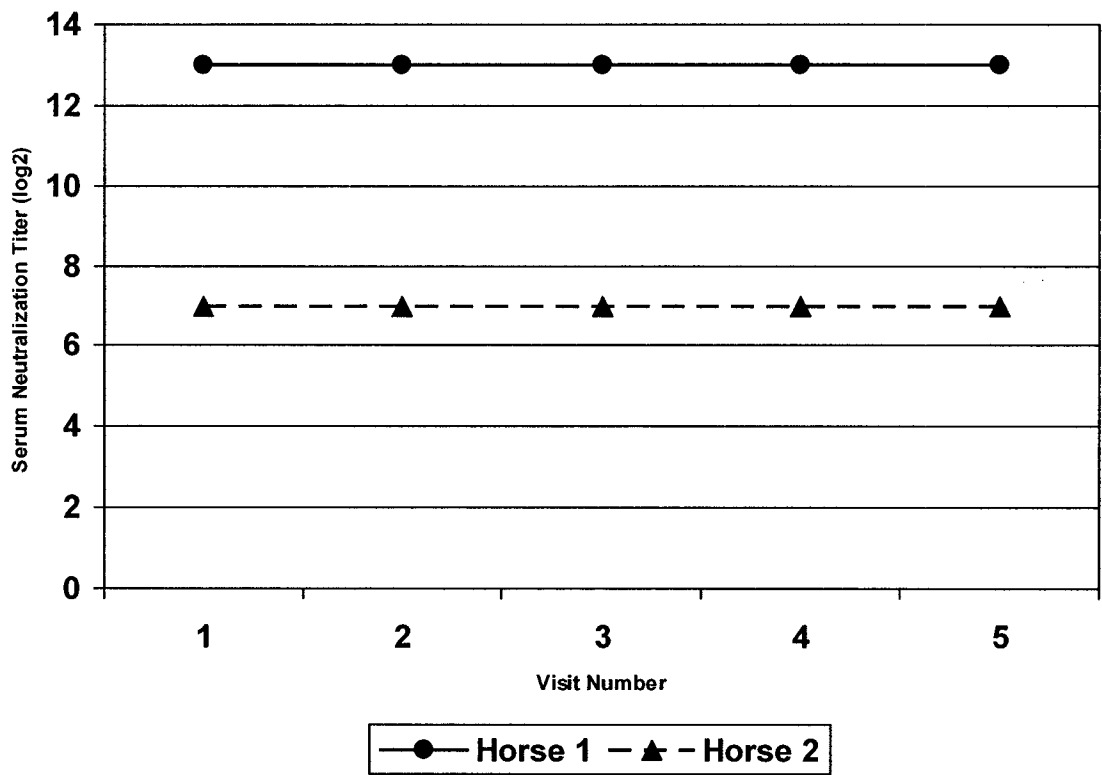


Figure 4.1.7. Patterns of  $\log_2$  transformed serum neutralization titers for vesicular stomatitis New Jersey serotype for 2 horses on sentinel equine operation CO-21.



## **Part II.**

### **Investigation of Operational and Horse Level Factors Associated with Changes in Serum neutralization Titers to Vesicular Stomatitis Viruses on Sentinel Equine Operations in Colorado and New Mexico**

#### **Introduction**

Vesicular stomatitis (VS) is a viral disease of the western hemisphere caused by either of two serotypes, vesicular stomatitis virus New Jersey (VSV-NJ) or vesicular stomatitis virus Indiana (VSV-IN). The viruses primarily affect livestock, although human infections are possible.<sup>1</sup> Vesicular stomatitis occurs as an endemic disease in South America, Central America, Mexico and on Ossabaw Island, Georgia, in the United States but only occurs sporadically in other regions of the United States.<sup>1</sup> Numerous studies investigating factors associated with the occurrence of VS have been conducted in both endemic and non-endemic areas.

A study of the ecological associations of VS virus exposure in human populations in Central America indicated that virus neutralizing antibodies to VSV-NJ were greatest for persons living at elevations between 350 and 649 meters, with open and dry vegetation and distinct seasonal changes in ground conditions from dry to moist.<sup>2</sup> Similar associations were found for VSV-IN with the addition of moist environments with dense tree cover as a risk for infection. It was concluded from this study that VSV-NJ and VSV-IN have similar but not identical transmission and maintenance cycles.

A cross-sectional study conducted on cattle farms in Costa Rica examined both environmental and host factors and their association with seropositivity to VSV-NJ and VSV-IN.<sup>3</sup> Cattle residing at elevations between 500 and 1500 meters in premontane or lower montane forest had an increased risk of being seropositive to VSV-NJ compared with cattle housed at lower elevations. Cattle residing at elevations of 0 to 500 meters and with less than 2 meters of rainfall were also at higher risk of seropositivity to VSV-NJ. Antibody prevalence also increased as the age of the cattle tested increased. There were no factors identified that were associated with VSV-IN seropositivity. These researchers suggested that at least two transmission cycles may exist for VSV-NJ, one at higher elevations and one at lower elevations indicating the potential for different arthropod vectors in the two ecological zones. They also suggested that because no factors were identified for VSV-IN that the transmission cycle was most likely different than that for VSV-NJ.

Cow, farm and ecological risk factors associated with clinical VS were investigated on Costa Rican dairy farms.<sup>4</sup> Clinical VS was associated with the presence of poultry and a longer calving interval and affected cattle were generally older, with seven-year old cows having the highest age specific incidence rate. Two ecological variables were forced into the farm model, the presence of sandflies (Diptera: Psychodidae) and the higher proportion of the farm being in forest land. It was concluded from this study that clinical VS was more likely related to ecologically dependent vector transmission to older lactating animals.

The factors associated with the spread of VS on four California dairies affected in 1982-1983 were investigated.<sup>5</sup> The use of course roughage and hard-pelleted

concentrates, the presence of uneaten feed, increased inter-pen movement of cows, poor ground surface conditions, poor milking hygiene and poor teat sanitation were the management factors associated with the presence of clinical disease. In addition the level of milk production and days in milk of individual cows also was important in the spread of clinical disease.

A prospective case control study was conducted in the southwestern United States following the 1997 outbreak of VS to identify management factors affecting the risk of developing VS.<sup>6</sup> Animals that had access to a shelter or barn had a decreased risk of developing VS and this was more pronounced for premises with horses. An adjusted analysis that examined equine premises only found an increased disease risk where animals had access to pasture and on premises where owners reported insects were greater than normal, odds of developing disease were also higher. Premises with animals within 0.25 miles of a source of running water were more than twice as likely to have VS. The findings of this study were similar to a case study of a single equine ranch conducted in 1982 where horses on pasture had 100% infection rates whereas horses in open pens and barns had much lower infection rates.<sup>7</sup>

Sentinel equine operations (SEO) in the study reported here were used previously in an approach to identify factors associated with seroconversions to VS viruses.<sup>8</sup> Kaplan-Meier curves were generated for premises with horses that seroconverted and mean survival times were generated. Mean survival times were used as the outcome variable in a general linear model to evaluate operation level management and environmental factors associated with variations in survival times. Although the factors retained in the final model were similar to those identified in the case control study

conducted in the southwest described previously, odd ratios were not significant. Only operation level data from sentinel operations in Colorado for the first two years of the study were used, resulting in a notable shortcoming of this study, a small number of operations with seroconversions.

An alternative approach to analyzing data that have multiple levels, in this case operation level, animal level and repeated sampling, is mixed models or hierarchical models. Mixed models have two primary applications, the first is to study the effects of independent variables on an outcome variable of interest and the second involves the estimation of the contribution of various levels to the total variance of the parameter of interest.<sup>9</sup> Mixed models allow researchers to consider the clustered structure of the data. This approach allows the simultaneous consideration of operational level variables, animal level variables and the multiple samples collected from each subject over time.

The primary purpose of epidemiologic research in general is to identify factors associated with occurrence of disease in specific populations. Ideally these factors are ones in which some control can be applied to reduce or eliminate the influence they have on disease occurrence. All of the studies mentioned previously suggest that there are factors associated with both the occurrence of clinical disease and with exposure to VS viruses. Many environmental factors (e.g. elevation, rainfall) cannot be controlled but many other factors (e.g. distance to running water, time on pasture, type of feed provided) can be influenced. We hypothesize that there are factors associated with variations in exposure and hence antibody responses to VSV-NJ and VSV-IN. Thus the objective of this study was to identify factors associated with changes in serum neutralization titers to VSV-NJ and VSV-IN in horses located on SEO in Colorado and New Mexico.

## **Materials and Methods**

### *Study Design*

Sentinel equine operation selection, sentinel horse selection, examination procedures and serologic testing procedures for this study have been described in Chapter 4, Part I. On average, SEO were visited approximately every 100 days for up to three years. At each visit, physical examinations, blood sample collections and oral swab collections were conducted on horses included in the study. Operational level management and ecological data were collected through the administration of a questionnaire on the initial visit and then changes in management factors were assessed through another questionnaire administered on subsequent visits (Appendix 3). Geographic positioning system instruments were used to obtain geographic coordinates for each SEO. A database containing the coordinates for each SEO was used to create base maps on relief, geology, soil, land cover, plant hardiness and ecoregions and data on elevation, slope and aspect were obtained from digital elevation models.

Horse level data including age, breed, gender, history of clinical VS and history of VS vaccination were collected for each sentinel horse the first time they were examined and sampled.

### *Statistical Analysis*

Serum neutralization titers were  $\log_2$  transformed and the differences or changes in transformed titers between consecutive visits starting with visit two calculated. A mixed model was developed using the change in SNT titer as the outcome variable and operational level and animal level variables collected through questionnaires and through

the geographic information system as independent or explanatory variables. All management, ecological and animal level variables were considered fixed effects. The SEO were considered a random effect as they represent a random sample of a larger set of potential levels. Separate models were developed for changes in titer to VSV-NJ and VSV-IN.

The mixed model procedure in SAS (proc mixed) was used. Different covariance structures were assigned until model convergence was achieved using the first order autoregressive covariance structure. Candidate fixed effect variables were entered individually into the model. Variables in which the type 3 test of fixed effects were significant at  $p < 0.25$  were retained and used to build the multilevel model. The Akaike's information criterion (AIC) was used to assess model fit as variables were entered into and removed from the model.

## **Results**

Table 4.2.1 provides the frequencies of the differences in SNT titers between consecutive visits. Changes in titer of +2 or higher indicate a four-fold or greater increase in titer, the standard by which a horse is considered to have seroconverted to a VS virus.

Candidate fixed effect variables and the results of their entry as individual variables into the model are presented in Table 4.2.2. There were no variables in the VSV-NJ model that were significant at  $p < 0.25$ . Ecological section, plant hardiness zone, whether insect populations were higher than normal at the initial visit, whether horses have access to a barn and the number of hours horses spent in a barn were statistically significant for changes in titer to VSV-IN. The AIC was lowest for the model that

included the variables ecological section and plant hardiness zone. Final model statistics are presented in Table 4.2.3. The least squares means differences of serum neutralization titers to VSV-IN between ecological sections and plant hardiness zones were determined and differences were considered significant at  $p < 0.05$  (Table 4.2.4).

## **Discussion**

There were numerous changes in serum neutralization titers observed between consecutive visits. A one-step change represents a one dilution difference in titer. For example a difference of +5 could indicate an increase in titer from 1:32 to 1:1024 and conversely a difference of -5 could indicate a decrease in titer from 1:256 to 1:8.

Increases in serum neutralization titers, as described in Chapter 4, Part I, may be due to: recrudescence of latent or persistent infections in horses; exposure to a virus that is induces cross-reactive antibodies to vesicular stomatitis viruses; or to exposure to either VSV-NJ or VSV-IN in the environments of the SEO.

There were no animal level variables found to be significant in either the VSV-NJ or VSV-IN models. Predilections to VS virus infections in horses due to breed, gender or age have not been reported. Seven year old cattle were shown to have the highest age specific incidence rate on dairy farms in Costa Rica.<sup>4</sup> Percentage of seropositive horses would be expected to be higher for older horses as they would have had more opportunity for exposure to virus, but an association with a change in titer and age would not be expected. Clinical history of VS was not shown to be associated with changes in neutralization titers. Prevalence of clinical VS on operations with infected horses has been shown to be much lower than seroprevalence (Chapter 2). Because horses in this

study were from operations that were known to have been positive for VS in one or more outbreaks, horses without a history of clinical VS were still very likely to have been exposed to virus and would have generated and maintained antibodies.

The ecological section and plant hardiness zone were found to be variables that explained titer differences in the VSV-IN model (Table 4.2.3). The primary purpose for delineating areas into ecological units is to identify land and water areas at different levels of resolution that have similar capabilities and potentials for land management. Depending on the scale, ecological units are designed to exhibit similar patterns in: potential natural communities, soils, hydrologic function, landform and topography, lithology, climate, and natural processes such as nutrient cycling, productivity, succession, and natural disturbance regimes associated with flooding, wind, or fire.<sup>10</sup> Ecological sections specifically, are broad areas of similar sub-regional climate, geomorphic process, stratigraphy, geologic origin, topography, and drainage networks. Such areas are often inferred by relating geologic maps to potential natural vegetation groupings. Numerical analyses of weather station and remotely sensed climatic information also determine section boundaries. Boundaries of some sections approximate geomorphic provinces as recognized by geologists. Section names generally describe the predominant geomorphic type or feature upon which the ecological unit delineation is based.

Horses on SEO in the Northern Canyon Lands (NCL) ecological section had significantly larger changes in serum neutralization titers to VSV-IN than SEO in both the Sacramento-Monzano Mountain (SMM) and Upper Rio Grande Basin (URGB) sections (Table 4.2.4). The NCL includes sections of western Colorado, including Mesa

County. Mesa County was the location of many clusters of operations with confirmed VS positive livestock in both the 1995 and 1997 outbreaks (McCluskey, B., USDA, APHIS, Veterinary Services, 1995-1997, unpublished data). The NCL naturally contains desert shrub and woodland vegetation with some big sagebrush.<sup>11</sup> Precipitation ranges from 6 to 30 inches per year and average temperatures are 45 to 55 F. Grazing of sheep and cattle are common in this area as well as pasture and hay production where irrigation is available. The SMM is located in central and south-central New Mexico.<sup>11</sup> Vegetation consists of ponderosa pine, pinyon-juniper and oak at lower elevations. Precipitation ranges from 12 to 35 inches per year and average temperatures are 40 to 57 F. The URGB is located in north-central New Mexico and south-central Colorado.<sup>11</sup> This area includes valleys, lowlands, and elevated plains and hills. Precipitation ranges from 6 to 20 inches per year and average temperatures are from 39 to 57 F. Horses on sentinel equine operations in the South-Central Highlands (SCH) ecological section also had significantly larger changes in serum neutralization titers to VSV-IN than horses on SEO in the URGB section. The SCH includes the San Juan Mountain range of south-western Colorado and surrounding areas.<sup>11</sup> Vegetation includes shrub and grassland forests, oak scrub, juniper-pinyon and alpine tundra. Precipitation ranges from 15 to 30 inches per year and average temperatures are from 32 to 45 F.

Plant hardiness zones are delineated by the average annual minimum temperature range.<sup>12</sup> Hores on SEO in hardiness zone 6a (-5 to -10 F) had significantly larger changes in serum neutralization titers to VSV-IN than horses on SEO in hardiness zone 6b (0 to -5 F).

The difference observed in titer changes in horses on SEO between ecological areas suggests that certain ecologies support the transmission of VS viruses more efficiently than others, if exposure to wild-type virus in the environment is assumed to be the cause of titer changes. Studies conducted in endemic areas support the idea that environmental factors do effect the transmission cycles and evolution of VS viruses.<sup>3,4,13</sup> Although both serotypes of VS viruses have been identified in the southwestern United States, differences in the models developed for VSV-NJ and VSV-IN suggest that the ecologies for these two serotypes are different and may depend on specific local environments.

Three management variables were significant ( $p < 0.25$ ) when entered into the model individually but were not retained in the final model. Above normal insect population at the time of the initial visit, horses having access to a barn or shelter and the number of hours horses spent in a barn were significant variables. These variables have been identified in previous studies as being potentially associated with the occurrence of VS in the southwestern United States and suggest that arthropods are important in the epidemiology of VS in the southwest.<sup>6,8</sup>

Arthropod trapping to assess insect type and density was not conducted in this study. This is a major limitation in attempting to identify factors associated with changes in serum neutralization titers as other studies have shown that arthropods are capable of transmitting VS viruses in the southwest.<sup>14-16</sup> An association between the risk of seroconversion of sentinel cattle to bluetongue viruses and the number of *Culicoides wadai* caught by light trap was observed on sentinel operations in Queensland, Australia.<sup>17</sup> Vector trapping was conducted up to four times per month in this study with

each collection period made over three nights. Changes in titer to VS viruses, if due to virus exposure from the environment, may potentially be associated with the species and density of insects as well as with the number of bites experienced by each horse. Steers located in Wyoming were attacked by up to 100,000 hematophagous insects, including mosquitoes, black flies and stable flies, during a 4 week period.<sup>18</sup> Most of the species of biting flies found in the southwest will feed on horses and cattle, therefore similar levels of attack might be expected to occur on horses. Future studies of the factors associated with the occurrence of VS in the southwest should include arthropod trapping to assess the species and density of potential vectors.

As mentioned previously, the general goal of epidemiologic research is to identify factors associated with occurrence of disease in specific populations and hopefully to exert some controls to reduce or eliminate the influence the factors have on disease occurrence. The two significant ecological factors identified in this study, unfortunately, are not ones in which horse owners or animal health officials have any influence. Beyond moving horses out of those particular ecological sections or plant hardiness zones, an unlikely occurrence for a fairly innocuous disease, this study did not find alterable management factors that might influence the immunological response of horses to VS viruses.

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Table 4.2.1 Frequency of the differences in  $\log_2$  transformed serum neutralization test titers to vesicular stomatitis New Jersey serotype (VSV-NJ) and vesicular stomatitis Indiana serotype (VSV-IN) between consecutive visits on sentinel equine operations in Colorado and New Mexico.

Difference in titers	Frequency VSV-NJ	Frequency VSV-IN
-9	2	0
-8	2	5
-7	4	5
-6	7	15
-5	9	12
-4	7	11
-3	9	13
-2	13	33
-1	71	96
No difference	1447	1190
+1	65	90
+2	15	31
+3	8	14
+4	2	9
+5	6	8
+6	11	8
+7	3	5
+8	1	5
+9	0	1

Table 4.2.2. Operational and horse level variables potentially explaining changes in serum neutralization titers to vesicular stomatitis New Jersey serotype (VSV-NJ) and vesicular stomatitis Indiana serotype (VSV-IN) entered into a mixed model and their associated p values (type 3 fixed effects test).

Variable name	Level	Variable description	VSV-NJ	VSV-IN
Signsvs	Animal	History of clinical signs of VS	0.2936	0.5613
Breed	Animal	Breed of horse	0.9210	0.5512
Vacc	Animal	History of vaccination for VS	0.9677	0.9692
Landcode	Operation	Land cover type (e.g. grassland, shrubland)	0.8651	0.6926
Ecodivcode	Operation	Ecological division (e.g. temperate desert)	0.9577	0.5005
Ecoprovcode	Operation	Ecological province (e.g. great plains-palouse dry steppe)	0.9741	0.2532
Ecoseccode	Operation	Ecological section (e.g. central high plains)	0.9944	<b>0.0493*</b>
Hardicode	Operation	Plant hardiness (average min. winter temperature)	0.8996	<b>0.0845*</b>
State	Operation	Colorado, New Mexico	0.4653	0.4806
Chrodent	Operation	Does the rodent population appear above normal for this time of year?	0.4105	0.3227
Hchange	Operation	Change in the number of horses within a 1 mile radius since the last visit	0.7095	0.8729
Change	Operation	Change in the number of cattle within a 1 mile radius since the last visit	0.5579	0.8901
Chinsect	Operation	Does the insect population appear above normal for this time of year?	0.7070	0.9440
Wchange	Operation	Did the source of water for horses change since the last visit?	0.2745	0.4704
Nanim	Operation	Have any new animals been added to the operation since the last visit?	0.2656	0.3206
Horses	Operation	Are there any horses within a 1 mile radius at the time of initial visit?	0.7870	0.6859
Cattle	Operation	Are there any cattle within a 1 mile radius at the time of the initial visit?	0.7759	0.4018
Rodent	Operation	Was the rodent population above normal at the time of the initial visit?	0.4019	0.4463
Insect	Operation	Was the insect population above normal at the time of the initial visit?	0.6384	<b>0.2500*</b>
Barn	Operation	Did the horses have access to a barn?	0.3380	<b>0.1909*</b>
Past	Operation	Did the horses have access to pasture?	0.3992	0.4246
Dlot	Operation	Did the horses have access to a drylot?	0.3589	0.2277
Nresa	Operation	Did horses have contact with non-resident animals in the last 3 months?	0.7425	0.6440
Rwater	Operation	Distance in feet of horses to source of running water (e.g. stream, river)	0.5429	0.7903
Swater	Operations	Distance in feet of horses to source of standing water (e.g. pond, lake)	0.3553	0.3064
Hbarn	Operation	Number of hours per day horses spend in a barn or shelter	0.3251	<b>0.0563*</b>
Hpast	Operation	Number of hours per day horses spend in on pasture	0.8397	0.9637

\* Statistically significant at p<0.25)

Table 4.2.3 Final mixed model statistics for variables associated with changes in serum neutralization titer to vesicular stomatitis Indiana serotype in horses on sentinel equine operations in Colorado and New Mexico.

<b>Variable name</b>	<b>Degrees of freedom</b>	<b>F value</b>	<b>Pr&gt;F</b>
Ecoseccode	10	1.78	0.0593
Hardicode	4	1.93	0.1026

Akaike's Information Criteria = 3604.2

Table 4.2.4 Significant least squares means differences of serum neutralization titers to vesicular stomatitis Indiana serotype, between ecological sections and plant hardiness zones, with significance considered at  $p < 0.05$ .

Variables compared	Estimated difference	P > t
<i>Ecological Sections</i>		
South Central Highlands vs Upper Rio Grande Basin	0.7070	0.026
Northern Canyonlands vs Upper Rio Grande Basin	0.6598	0.042
Northern Canyonlands vs Sacramento-Monzano Mountain	0.7045	0.021
<i>Plant Hardiness Zones</i>		
Zone 6a vs Zone 6b	0.7144	0.037

## **Chapter 5.**

### **Part I.**

#### **Climatic Factors and the Occurrence of Vesicular Stomatitis in New Mexico\***

##### **Summary**

Vesicular stomatitis (VS) outbreaks occurred in the southwestern United States in 1995, 1997 and 1998. The epidemiology of VS is not understood completely and some of the epidemiologic aspects of this disease are currently under investigation. In this study daily maximum temperature, daily minimum temperature, daily mean temperature, daily mean relative humidity and daily total precipitation were collected at the Sevilleta Long Term Ecological Research (SLTER) site in central New Mexico. Discriminant analysis was used to identify the climatic variables best able to classify in which months VS would occur. The study found the amount of precipitation occurring 2, 10, 11 and 12 months previous to the month the cases were diagnosed were the climatic variables that best described the occurrence of VS cases. The association of VS cases and precipitation suggests that, like numerous other arthropod-borne diseases, transmission of the disease-causing pathogen is linked to variations in climate.

**Keywords:** Vesicular stomatitis – Climate – Vector – Arbovirus – Precipitation – Discriminant analysis – New Mexico - Epidemiology

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\* Part I of this chapter has been accepted for publication in the OIE Scientific and Technical Review Journal, submitted October 2002.

## **Introduction**

Vesicular stomatitis (VS) in the United States is caused by either vesicular stomatitis virus (VSV), New Jersey serotype (VSV-NJ), or VSV, Indiana serotype (VSV-IN). These viruses are members of the family Rhabdoviridae, genus *Vesiculovirus*. Clinical VS is seen in cattle, swine and horses in the United States. However, serologic evidence of virus exposure has been observed in many species (7, 28). Reviews of the biologic, pathologic and epidemiologic aspects of this disease have been published (12, 14).

Historically, outbreaks of VS in the southwestern United States are sporadic. Most recently, outbreaks have occurred in 1995, 1997 and 1998. Outbreaks typically begin in the late spring and end at the first frost. There is also typically a northward progression of disease through time with the first positive premises in an outbreak identified in southern New Mexico and the last positive premises identified in Colorado. Recent outbreaks in the southwestern United States have resulted in clinical disease in greater proportions in horses than in cattle (14). In areas where VS is endemic (including Central America and Ossabaw Island, Georgia, USA) VS viruses are transmitted by arthropod vectors (2, 3). Arthropods apparently transmit VS viruses in the southwestern United States (15, 26).

The ecologic and epidemiologic factors associated with both the sporadic temporal and geographic nature of VS in nonendemic areas are poorly understood. Anecdotal reports of associations of VS outbreaks with certain climatic patterns, primarily above-average

winter or spring precipitation, are not uncommon. Seasonality in disease occurrence suggests an association with specific weather conditions. Numerous arthropod-borne diseases are associated with climatic events including dengue, malaria, St. Louis encephalitis and Ross River virus (10, 13, 22, 25). In the southwestern United States, Sin Nombre virus infections of humans have been linked to the effects of the El Niño Southern Oscillation (ENSO) on rodent populations (19, 20). The number of human plague cases in New Mexico was found to be associated with higher than normal winter-spring precipitation at a local level of measurement (21). A strong association between summer temperature and rainfall and seroconversion to bluetongue virus by cattle was found in Australia (27). The spread of VS has been proposed to occur through transport of virus-infected vectors during appropriate climatic conditions (24). Apart from this study, we are unaware of any other investigations of climatic factors and their potential association with outbreaks of VS in the southwestern United States. Our objective was to examine the effects of climatic factors on the incidence of VS outbreaks on premises in New Mexico. This information could be included in future outbreak databases, used in risk assessments or used to assist researchers and animal health regulatory personnel in predicting the occurrence of VS outbreaks. The relationship of local climate variables and outbreaks at the state level were examined.

## **Materials and Methods**

### *Identification of positive premises*

Information from the 1995, 1997 and 1998 VS outbreak databases was used for this study. A case in these outbreaks was defined as a premises that housed at least one

animal positive to one or more VS virus serologic tests, exhibiting clinical signs consistent with VS and/or from which VS virus was isolated from submitted tissue or swab samples (1, 14). We did not differentiate positive premises by the species affected.

#### *Climatic variables*

Information on climatic variables was collected at the Sevilleta Long Term Ecological Research site (SLTER). The SLTER is located in Socorro County in central New Mexico and was established in October 1988. The SLTER is unique in that it straddles several major biomes of the southwest (8). The SLTER region is influenced by the ENSO, with major fluctuations in precipitation occurring on semidecadal time scales. Hourly readings of total precipitation, maximum temperature, minimum temperature, mean temperature, mean relative humidity, mean vapor pressure, mean and maximum wind speed and daily solar radiation are collected at seven weather stations located within the SLTER. For this study, daily summaries of maximum temperature (mxt), minimum temperature (mit), mean temperature (mnt), mean relative humidity (mnrh) and total precipitation (precip) were obtained from the SLTER for the years 1989 through 1999.

#### *Descriptive statistics*

Monthly mean values of mxt, mit, mnt, mnrh and the total monthly precipitation were calculated. The average of each climatic variable for the 11-year period (period average) also was calculated. The period average was used as a baseline to detect differences in climate variables. Each climatic variable and its period average were plotted for each year by month. Climatic variables with large graphical displacements from the period average

were examined in more detail by plotting the climatic variable by month for the year of a VS outbreak and the year prior to a VS outbreak along with the period average.

### *Multivariable analysis*

A total of 132 months of data were available for analysis. The STEPDISC procedure in SAS was used to produce a discrimination model through stepwise selection of continuous variables that proved useful in finding differences between classes. Two classes were established in this study, representing months in which VS cases were reported and confirmed (positive months) or were not reported (negative months). Variables entered the model if they were significant at the  $p \leq 0.05$  level. Only those variables significant at  $p \leq 0.10$  were retained in the model. The model considered all months in the years of 1989 through 1999. For all climatic variables, lag period variables were created that represented months 1 to 12 months previous to the monthly values calculated. Forward selection of variables into the discriminant model was conducted.

The significant variables identified in the discriminant model were used in the SAS DISCRIM procedure to create classification tables. Proportional prior probabilities were assigned due to the large number of months without cases of VS. Error count estimates were also provided by this procedure.

### **Results**

Simple plots of weather variables by month for each year showed only minor displacements from the 11-year average for all variables except total monthly precipitation. Total monthly precipitation for the year of a VS outbreak and the year

prior to a VS outbreak along with the period average and number of VS cases are presented in Figures 5.1.1, 5.1.2 and 5.1.3.

Eight observations had missing data for one or more variables and were not included in the analysis. Overall, there were 17 positive and 107 negative months, respectively.

A total of 65 climatic variables were available for modeling (Table 5.1.1). The stepwise selection of variables into the model resulted in the 12-, 11-, 10- and 2-month lag period variables of total monthly precipitation (L12PRECIP, L11PRECIP, L10PRECIP, L2PRECIP) remaining in the model (Table 5.1.2).

The number of observations and percent classified as either a positive or negative month are presented in Table 5.1.3. Prior probabilities were 0.8629 for negative months and 0.1371 for positive months. The discriminate function incorrectly classified 3.7% of the negative months as positive (false positives) and 35.3% of the positive months as negative (false negatives).

### **Discussion**

The lag period precipitation variables identified as the best predictors incorrectly classified only 10 months out of 124 for an overall error rate of 8%. Discriminant analysis indicated that 10-12 month lag periods in total monthly precipitation and a 2-month lag in total monthly precipitation were significant predictors of which months VS cases would occur. Examination of the graphical displays of precipitation that

included the year prior to an outbreak, the year of an outbreak and the 11-year average indicated that in all years prior to outbreak years there were multiple months that had above-average precipitation. In 1994 (Figure 5.1.2), the year prior to the 1995 outbreak, 6 out of 12 months had above-average precipitation with 5 out of those 6 months occurring in the 10-12 month lag period. However, precipitation 2 months prior to the first month with cases in 1995 was below average and remained below average throughout the outbreak. In 1996 (Figure 5.1.3), one year prior to the 1997 outbreak, only 3 out of 12 months had above-average precipitation but all 3 occurred in the 10-12 month lag period. Two months prior to the initial month with cases in 1997 precipitation was over 100 mm above average and remained above average in all months of the 1997 outbreak except the last two (October and November). In 1997 (Figure 5.1.4), one year prior to the 1998 outbreak, 8 out of 12 months had above-average precipitation and 4 out of the 7 months were in the 10-12 month lag period. Precipitation in the 2 months prior to the initial cases was approximately 200 mm above average. In each of the three outbreak years, the month with the highest total monthly precipitation occurred 10 months prior to the peak number of cases. Above-average levels of precipitation may allow for increases in reservoir and/or amplifying host populations necessary for VS outbreaks.

The role of precipitation variability in influencing levels of total biomass (both plant and animal) is well established. Large increases in rodent populations have been observed in response to the increased rainfall associated with El Nino events in the southwestern United States (6). These increases were shown to be associated with 3-6 month lag periods in precipitation. Rodent population increases have been correlated with

precipitation increases in both the southwestern United States and South America (10, 13, 16). Rodent population increases also have been correlated with disease (11, 17).

A vertebrate reservoir of VS viruses has yet to be discovered although numerous hosts have tested positive for antibodies to VS viruses. This list includes livestock species, birds and a host of small mammals including bats, deer mice, house mice, opossums, rabbits, raccoons, rock mice, skunks, squirrels, white-footed mice and wood rats. Viremia has not been found in any field-collected potential reservoirs (12). Many arthropod-borne viruses have small mammals as either reservoir or amplifying hosts in their life cycles. It would not be unreasonable to assume that VS viruses also use a small mammal as their reservoir host.

Many vectors of arthropod-borne diseases have life cycles intimately dependent on water. Rain provides the breeding sites for many vectors and also helps to create a humid environment that assists in prolonging the life of vectors. The competency of some arthropods to serve as biological vectors of VS viruses in the southwestern United States has been demonstrated in laboratory settings. *Simulium* spp. (black flies) and *Culicoides* spp. (midges) can transmit VS viruses biologically (5, 15, 18). Members of these groups are abundant in the southwestern United States during VS outbreaks, had VSV isolated from field collections and depend on aquatic systems for propagation (26). Black flies lay their eggs in water and with rare exceptions, their larvae are filter-feeding organisms found strictly in running-water habitats (4). *Culicoides* spp. typically lay eggs in damp areas with decaying organic matter (9). The larvae of different *Culicoides* spp. can be

found at the edges of ponds and streams or in areas around water troughs. Over-wintering in the larval stage may occur in temperate climates.

Above-average precipitation during the vector season might provide larger numbers of potential breeding sites and therefore larger populations of arthropod vectors. Increased populations during a vector season may result in more larvae overwintering. This would result in larger initial insect populations during the next year's transmission season. A large initial hatch of arthropods may be the trigger necessary to begin transmission of virus if it is present. If virus persists in only a small number of reservoirs, arthropod vectors may be necessary to move the virus into larger numbers of reservoirs and amplifying hosts or act as the amplifying hosts themselves, resulting in spillover of virus into livestock species. Virus might survive in the southwestern United States through the winter months. Five virus isolates obtained in New Mexico in the spring of 1997 and in the summer of 1998 were identical to two virus isolates obtained in Colorado in 1997. This indicated that the same VSV-IN virus caused outbreaks in the spring and summer of 1997 and 1998 (23). No specific evidence exists to indicate VSV over-winter in arthropods. However, without identification of a vertebrate species that exhibits viremia, the over-wintering of VS virus in arthropods is plausible.

The limitations of this study are important to note. The model used accurate and specific climatic information but from a limited geographic area. A major point of a recent paper on the incidence of plague and its relationship to precipitation was that local climate effects are the best predictors of disease occurrence. In the study reported here, we used

climate data from seven weather stations located on the SLTER. Cases of VS between 1989 and 1999 occurred from southern New Mexico, near the Texas border, to northern New Mexico, near the Colorado border. Climate is generally similar across New Mexico, but local differences do occur and should be considered.

It is unlikely that all cases of VS are either detected or reported. Therefore, the dependent variable in the model is underestimated. It is reasonable to assume that undetected or unreported cases occur in the months observed in these data sets. However, cases may be occurring in low numbers in nonoutbreak years. This would have a significant effect on the association of lag period precipitation and when cases occur.

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Table 5.1.1. Variable labels and descriptions included in discriminant analysis.

Variable Name	Variable Description
MXT	Monthly mean value of daily maximum temperatures
MIT	Monthly mean value of daily minimum temperatures
MNT	Monthly mean value of daily mean temperatures
MNRH	Monthly mean value of daily mean relative humidity
PRECIP	Total monthly precipitation
L1MXT to L12MXT	Monthly mean value of daily maximum temperatures 1 to 12 months previous to current month
L1MIT to L12MIT	Monthly mean value of daily minimum temperatures 1 to 12 months previous to current month
L1MNT to L12MNT	Monthly mean value of daily mean temperatures 1 to 12 months previous to current month
L1MNRH to L12MNRH	Monthly mean value of daily mean relative humidity 1 to 12 months previous to current month
L1PRECIP to L12PRECIP	Total monthly precipitation 1 to 12 months previous to current month

Table 5.1.2. Results of step-wise selection of climatic variables that explain differences in incidence of VS cases by month.

Variable	Partial R-square	F value	Pr > F
L12precip	.1964	29.82	.0001
L10precip	.0859	11.37	.001
L11precip	.0481	6.07	.0152
L2precip	.0390	4.83	.0299

Significance level to stay in the model  $p < .10$

Table 5.1.3. Number of observations and percent classified as either months with VS cases or months without VS cases.

	<b>Number of observations classified as negative (%)</b>	<b>Number of observations classified as positive (%)</b>	<b>Total</b>
<b>Negative months</b>	103 (96.26)	4 (3.74)	107
<b>Positive months</b>	6 (35.29)	11 (64.71)	17
<b>Total</b>	109 (87.90)	15 (12.10)	124

Figure 5.1.1. Total monthly precipitation for 1994 and 1995, the 11-year average monthly precipitation and the total number of vesicular stomatitis cases by month for 1995.

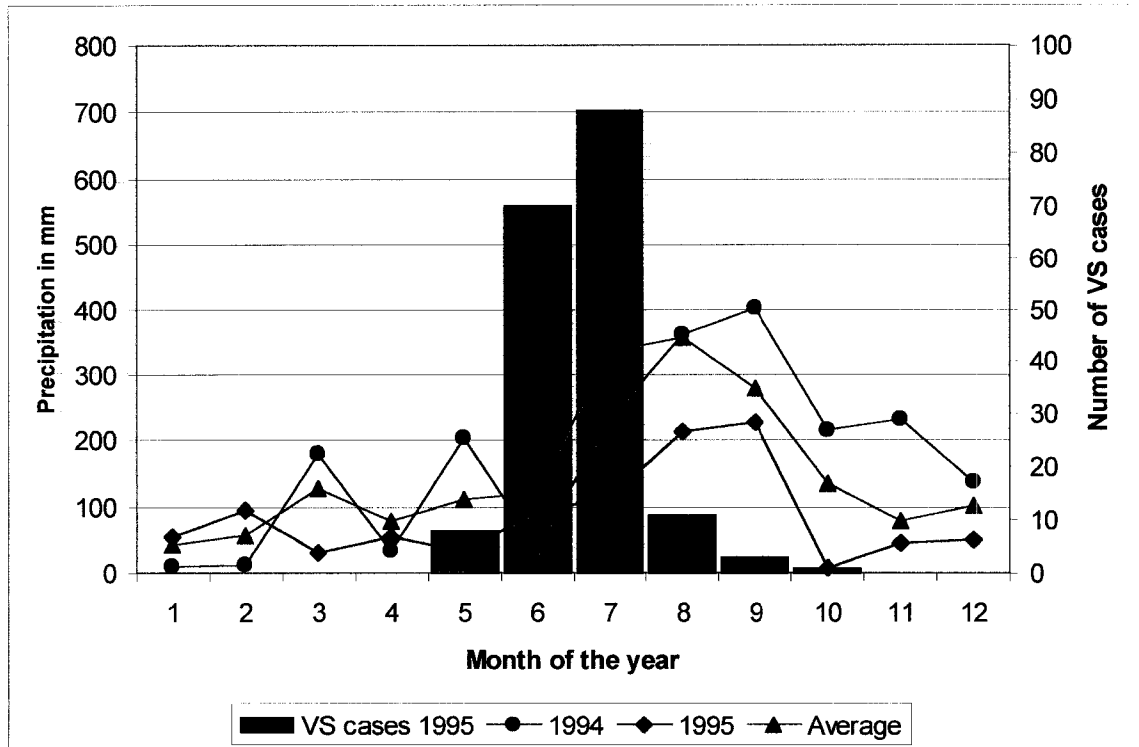


Figure 5.1.2. Total monthly precipitation for 1996 and 1997, the 11-year average monthly precipitation and the total number of vesicular stomatitis cases by month for 1997.

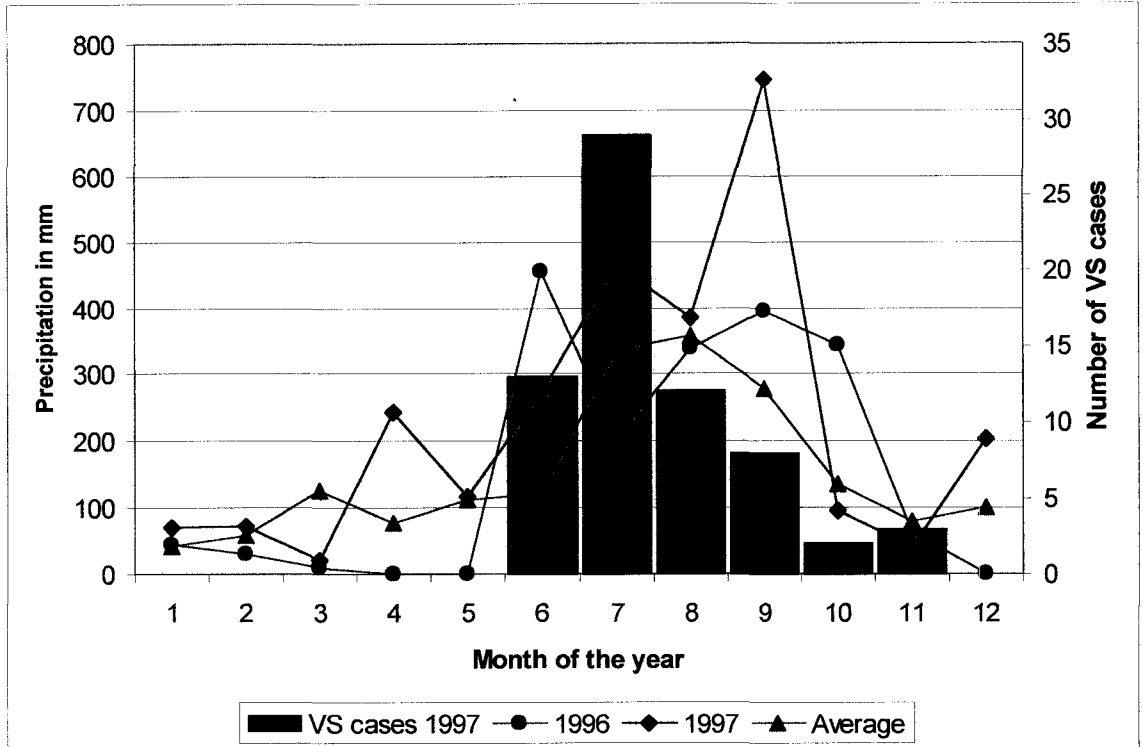
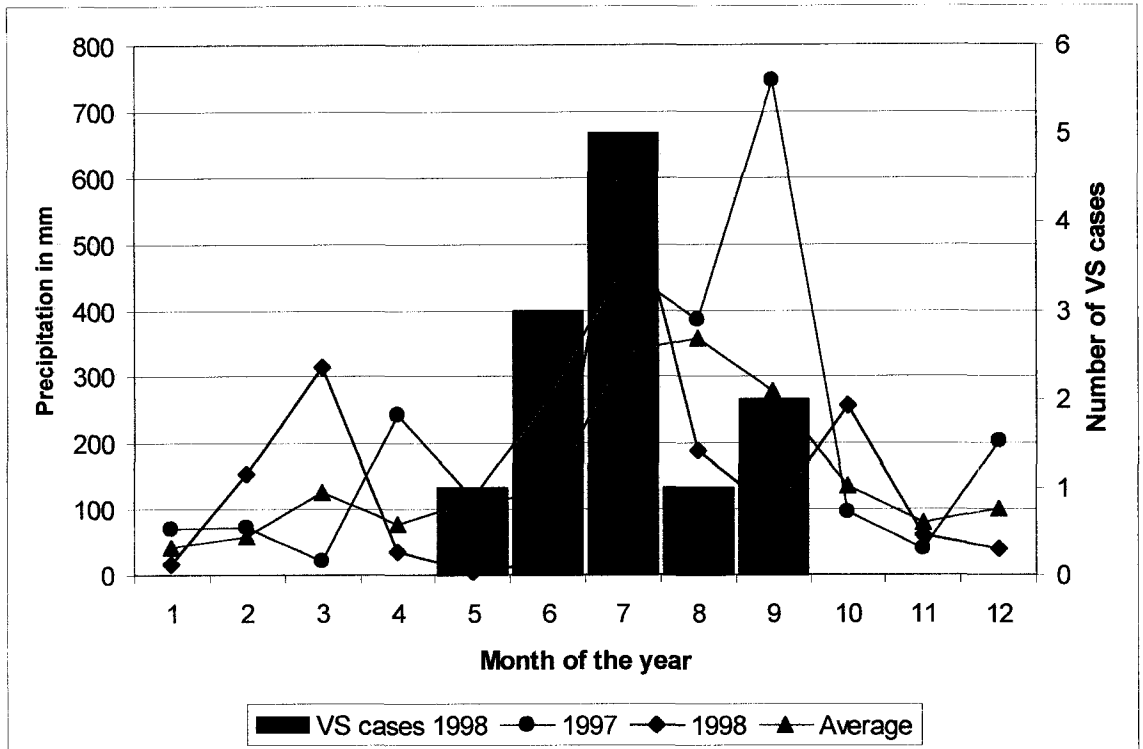


Figure 5.1.3. Total monthly precipitation for 1997 and 1998, the 11-year average monthly precipitation and the total number of vesicular stomatitis cases by month for 1998.



## **Part II.**

### **Climatic Factors and Remote Sensing Data and Their Association with Vesicular Stomatitis Virus Exposure on Sentinel Equine Operations in Colorado**

#### **Introduction**

Vesicular stomatitis (VS) in the United States is caused by either vesicular stomatitis virus New Jersey serotype (VSV-NJ) or vesicular stomatitis virus Indiana serotype (VSV-IN). These viruses are members of the family Rhabdoviridae, genus *Vesiculovirus*. Clinical VS has been seen in cattle, swine, llamas and horses in the United States. However, serologic evidence of virus exposure has been observed in many more species.<sup>1,2</sup> Reviews of the biological, pathologic and epidemiologic aspects of this disease have been published.<sup>3,4</sup>

Outbreaks of VS in livestock in the southwestern United States occur sporadically. Most recently, outbreaks occurred in 1995, 1997 and 1998. Outbreaks typically begin in the late spring and end at the first frost. There is typically a northward progression of disease over time with the first positive premises in an outbreak typically identified in southern New Mexico and with the last positive premises occurring in Colorado. The most recent outbreaks in the southwestern United States resulted in clinical disease in greater proportions in horses than cattle.<sup>3</sup> In VS endemic areas, including Central America and

Ossabaw Island, Georgia, USA, virus is transmitted by arthropod vectors.<sup>5,6</sup> Arthropods apparently transmit VS viruses in the southwestern United States.<sup>7,8</sup>

The epidemiologic and ecologic factors that influence the occurrence of VS in the southwestern United States, although investigated for decades, remain elusive. The seasonality in disease occurrence suggests that climatic factors may influence virus transmission. The late spring and summer occurrence of cases supports the hypothesis of virus transmission by arthropod vectors. Examples of arthropod disease transmission and associated climate events are well documented.<sup>9-15</sup>

In a previous study, the effects of climatic factors on the incidence of VS outbreaks in New Mexico were examined through discriminant analysis (Chapter 5, Part I). This study found the amount of precipitation occurring 2, 10, 11 and 12 months previous to the month the cases were diagnosed were the climatic variables that best described the occurrence of VS cases. A limitation identified by this study was the application of local climatic variables to the incidence of state-level cases of disease.

Identification of a key indicator or predictor of VS outbreaks would assist animal health officials in initiating prevention strategies or preparing appropriate responses to the outbreak. The study mentioned previously identified increases in precipitation above normal as the climatic factor best able to predict the occurrence of VS cases. The application of spatial resolution imagery has been successful in providing a key to identifying vector habitats and in monitoring changes in habitat so that predictions of

disease occurrence are possible. An outbreak of Rift Valley Fever was correctly predicted in central Kenya using data from the National Oceanic and Atmospheric Administration's (NOAA) Advanced Very High Resolution Radiometer (AHVRR).<sup>16</sup>

The U.S. Geological Survey's (USGS) EROS data center has used the AHVRR data to produce weekly and biweekly normalized difference vegetation index (NDVI) composites for the coterminous United States since 1990. The consistent collection of these data provides opportunities to monitor the vegetation conditions of different ecosystems in the United States and allows assessment of variations in vegetation conditions. The objective of this study was to investigate the use of NDVI and other climatic factors as key indicators or predictors of exposure to VS virus (VSV) as determined by clinical signs of disease with serologic and/or virologic confirmation or seroconversion to either of the two serotypes of VSV without clinical signs in horses on sentinel equine operations in Colorado.

## **Materials and Methods**

### *Sentinel Operation and Horse Selection and Characterization*

Colorado equine operations known to have had VS-positive horses during the VS outbreaks of 1995, 1997, and/or 1998 were asked to participate in this study. The criteria for sentinel operation selection included: 1) at least 1 horse on the operation meeting the USDA:APHIS Veterinary Services VS case definition (the presence of clinical signs of VS and positive virus isolation, and/or positive serologic test result) in 1995, 1997, and/or 1998; 2) at least 2 horses routinely residing on the operation and available for biologic

sampling and; 3) operation owners agreed to participate in the study for a minimum of 3 years and to the collection of biologic samples from horses 4 times per year. Sentinel operations were selected to provide a geographic distribution of sentinel locations that was similar to the distribution of where cases of VS occurred during the three outbreak years in Colorado. Seven regions within Colorado were created with sentinel operations placed in one of the seven regions.

Sentinel horses on each operation were selected by the operation owner. The only criterion for inclusion was that each sentinel horse would be likely to reside primarily on the sentinel operation for the 3 years of the study. Between 2 and 20 horses were examined and sampled at each visit. Drop-out of study horses due to death and sale was anticipated.

A standardized questionnaire was administered at the initial premises visit addressing potential risk factors for VS on the premises (including physical layout of the premises, rodent/insect control measures, and housing). Each quarterly premises visit had a follow-up visit questionnaire administered to assess any changes in management during the past quarter. A Geographic Positioning System (GPS) receiver was used to acquire coordinates for each operation. These coordinates were entered into a geographic information system (GIS) database (ArcView ver 3.2).

Blood samples and oral swabs were collected from each study horse at each premises visit. Sera were tested for the presence of antibodies to both VSV-NJ and VSV-IN by one or more tests. The cELISA was conducted on all samples. Procedures for the

cELISA have been described previously.<sup>17</sup> The complement fixation test (CFT), IgM capture ELISA (IgM) and serum neutralization test (SNT) were conducted on all samples positive by cELISA. Procedures for the CFT, IgM and SNT have been previously described.<sup>18,19</sup>

A case in this study was defined as either an operation that housed at least one horse positive to one or more VSV serologic tests, exhibiting clinical signs consistent with VS and/or from which VSV was isolated from submitted tissue or swab samples during one of the three outbreaks (1995, 1997, 1998) or seroconversion to either of the two serotypes by sentinel horses during the quarterly visit. A seroconversion was considered to have occurred if a sample had one or more of the following results: 1) was positive by the IgM; 2) had a CFT titer of 1:20 or higher or; 3) had a four-fold rise in SNT titer between 2 consecutive visits.

#### *NDVI Data Collection*

United States Geological Survey Coterminous AVHRR biweekly composites were obtained on compact discs for the years 1991 through 1999. These datasets are typically composed of twenty-one to twenty-six 14-day maximum NDVI composites created from satellite images. The NDVI was calculated as the difference of near infrared (AVHRR channel 2) and the visible (AVHRR channel 1) reflectance values divided by the total reflectance. Values are generated in the range -1.0 to 1.0 where negative values represent non-vegetated surfaces and positive values represent surfaces with vegetation. NDVI values were scaled to the range of 0 to 200 where computed -1.0 equals 0 and 1.0 equals 200.

Images were imported into the GIS and coordinates of sentinel operations applied to the images. Each pixel on the image has an associated NDVI value and represents a square kilometer. Each sentinel operation location was placed in the center of 9 adjoining pixels. The NDVI value for each of the 9 pixels was recorded and the mean of the 9 pixels was calculated. This was recorded as the NDVI value for that sentinel operation for that specific two week period. The mean NDVI value, as described, was recorded for all sentinel operations for each two week period between 1991 and 1999.

### *Climate Data Collection*

To facilitate matching of climate data with NDVI data, specific time periods were established (Table 5.2.1). To account for differences in NDVI collection dates as provided by USGS and calendar dates, periods 9 and 10 represent the same time period, June 2 through June 15. Sentinel operation coordinates were plotted onto a regional map in the GIS. Weather station information was obtained from the National Climate Data Center (NCDC) (<http://lwf.ncdc.noaa.gov/oa/ncdc.html>). Longitude, latitude and elevation for every station in the sentinel's region were imported into the GIS and overlaid on the sentinel operation map. Distances between stations and sentinel operations were calculated and the nearest three weather stations to each operation were identified. Elevation and complete climate information were then assessed for each of the chosen stations. In some instances, the second or third farthest station was selected to either be compatible in elevation with the sentinel operation or have adequate data for the entire period of the study. For each station selected, daily surface data including minimum temperature, maximum temperature and total precipitation were obtained from the NCDC from January 1991 through December 1999. Using the daily values as inputs,

the mean maximum and minimum temperatures, the mean overall temperature and the total precipitation were calculated for each time period.

### *Data Analysis*

The number of cases occurring over the nine year period was plotted by period. In addition the average NDVI value and standard error for each period for each sentinel operation was calculated and average NDVI values plotted for each sentinel by region to assess changes in NDVI through time.

The correlation between the NDVI value and other climatic variables (minimum temperature, maximum temperature, mean temperature and precipitation) was tested by the Proc Corr procedure in SAS<sup>a</sup> (Pearson correlation coefficient with variables considered correlated if  $p < .05$ ).

Two approaches to modeling were used. A logistic regression model was used to evaluate the NDVI's association with cases (as defined previously). The Proc Logistic procedure was used in SAS with cases as the outcome variable and NDVI as the explanatory variable. Period was entered into the model as a confounding variable. The Hosmer-Lemeshow goodness of fit test was performed to assess the fit of the model to the predicted model.

The Proc Stepdisc procedure in SAS was used to create a discrimination model. Two classes, representing periods when cases occurred and did not occur were established. Variables were entered into the model in stepwise fashion if they were significant at  $p < .05$  and remained in the model if they were significant at  $p < .10$ . The model considered

all periods between 1991 and 1999. For all climatic variables and NDVI values, lag period variables were created that represented 1 to 12 periods previous to the period values calculated.

The variables statistically significant in the discriminant model were used in the Proc Discrim procedure in SAS to create classification tables. Proportional probabilities were assigned due to the large number of periods without cases. Error count estimates were also provided by this procedure.

## **Results**

The first and second greatest number of cases occurred in periods 7 (May 1 through May 15) and period 17 (September 16 – October 1) (Figure 5.2.1). Most cases occurred during the summer months, as expected, but cases did occur in November and in February. The standard errors for average NDVI values were generally small, indicating very little variation of NDVI values in the same period across different years. NDVI values were highly correlated with all climatic variables including lag period variables (Pearson correlation coefficient,  $p < 0.001$ ).

Results of logistic regression are presented in Table 5.2.2. The point estimate for the odds ratio for NDVI was 1.032 (95% Wald Confidence Limits, 1.003 – 1.061) indicating that for each 1 unit increase in NDVI the odds of a case occurring on that sentinel operation increases by .0312. The Hosmer-Lemeshow goodness of fit test was not significant ( $\chi^2 = 7.7685$ ,  $p = 0.4564$ ) indicating the model was not significantly different from the predicted model.

Stepwise selection of variables into the discriminant model resulted in the 10 period lag in total precipitation and the 11 period lag in NDVI values remaining in the model (Table 5.2.3). The discriminant function provided the number of observations and percent classified as either a positive or negative period (Table 5.2.4). The discriminant function incorrectly classified 30% of negative periods as positive and 55% of positive periods as negative for an overall error rate of 43%.

### **Discussion**

During recent outbreaks of VS in the southwestern United States, most cases have occurred in the spring and summer months with a few cases occurring into the early fall (Figure 5.2.1). Cases identified in this study also occurred primarily during this time period. However there were 13 cases that occurred outside of the timeframe identified by the 1995 and 1997 outbreaks. Although unusual, this is not unique to this study. During the VS outbreak of 1982-1983, cases occurred through the winter months and into the next spring.<sup>20</sup> Cases occurring during the warmer periods of the year suggest an arthropod component in the ecology of this disease in the southwest. Studies of arthropods in the southwest have resulted in the isolation of VS viruses from numerous different species of arthropods.<sup>7</sup> Cases occurring in traditionally what is considered the non-vector season suggests non-vector mediated routes of virus transmission and or recrudescence of virus within host species. Contact transmission of VSV has been observed experimentally and field investigations during outbreaks have supported these findings.<sup>21</sup> There are no published reports of VSV recrudescence in horses or other species of livestock. In addition, viremia and/or viral latency have not been shown to occur in livestock.

An objective of this study was to identify one variable that would provide adequate predictive power of the occurrence of VS cases. The application of NDVI values was thought to be a good candidate variable. The NDVI was shown in other studies of vector borne disease to function reasonably well as an indicator of vector habitats and through monitoring changes in NDVI was able to predict the occurrence of disease.<sup>16</sup> NDVI values are dependent on the type, quantity and density of vegetation and these vegetation characteristics are obviously dependent on local climatic factors. The analysis of the correlation of climatic factors with NDVI indicated that NDVI values were highly correlated with temperatures and precipitation. This suggested that NDVI might be the one indicator variable sought. However, when NDVI values were averaged for each period for each sentinel over the 9 years of data examined, only minor fluctuations in NDVI were observed. Standard errors were small indicating that vegetation type, quantity and density are generally insensitive to short term climatic changes in the southwestern United States. The logistic regression model indicated that a unit increase in NDVI increased the odds of a case of VS by .0312. For example, Figure 5.2.2 shows the NDVI values of 4 sentinel operations and indicates that NDVI increases beginning in January, peaks in August and then slowly declines through the end of the year. Cases of VS also peak in the summer so that the logistic model appears correct in establishing increased risk as NDVI values increase.

The similar finding of the significance of a 10 month lag in precipitation in this study to a previous study (Chapter 5, Part 1) supports its validity as a predictor of VS cases. There

is biological plausibility to a lag period in precipitation and its association with cases. Above average precipitation occurring during the vector season may provide larger numbers of breeding sites for arthropods resulting in larger populations of vectors. Increased populations during vector seasons may result in a larger population of overwintering arthropods. This would result in a larger initial arthropod population during the next season's transmission season. This large initial hatch or bloom of insects may be the environmental trigger necessary to initiate viral transmission and start an outbreak.

Advances in GPS and GIS technology facilitate the integration of remote sensing data with disease occurrence so that prevention strategies can be developed. As has been the situation repeatedly with the epidemiology of vesicular stomatitis, an easy route to identify environmental associations is not forthcoming. In neither of the two studies examining climatic variables and the occurrence of VS cases or VSV exposure were models able to predict with adequate accuracy when these events might occur. The body of evidence that arthropods are involved in the epidemiology of VS in the southwest continues to mount and it is now assumed that arthropods do transmit VSV during outbreaks in the southwestern United States. However, the associations of increased numbers of arthropods, climatic factors and VS cases must still be considered weak. During outbreaks of VS in the southwestern United States, Mexico as a source of virus for United States outbreaks is suggested routinely. A study of the climatic factors and VS occurrence in Mexico may result in improved predictive capability for VS outbreaks in the southwestern United States.

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Table 5.2.1. Time periods used for comparison of normalized difference vegetation index and climatic variables on sentinel equine operations in Colorado.

<b>Period</b>	<b>Dates included in time period</b>
1	January 1 to January 31
2	February 1 to February 29
3	March 1 to March 15
4	March 16 to April 1
5	April 2 to April 15
6	April 16 to April 30
7	May 1 to May 15
8	May 16 to June 1
9	June 2 to June 15*
10	June 2 to June 15*
11	June 16 to July 1
12	July 2 to July 15
13	July 16 to August 1
14	August 2 to August 15
15	August 16 to September 1
16	September 2 to September 15
17	September 16 to October 1
18	October 2 to October 15
19	October 16 to November 1
20	November 2 to November 15
21	November 15 to December 1
22	December 1 to December 31

\* To facilitate matching of climate data with NDVI data, specific time periods were established. To account for differences in NDVI collection dates as provided by USGS and calendar dates, periods 9 and 10 represent the same time period.

Table 5.2.2. Results of logistic regression modeling with the number of vesicular stomatitis cases as the outcome variable, Normalized Difference Vegetation Index as the explanatory variable and period entered as a covariate for sentinel equine operations on Colorado.

Parameter	Estimate	Wald Chi-Square (p=)	Odds Ratio (95% C.L.)
Intercept	-8.7180	21.71 (p<.0001)	
NDVI	0.0312	4.47 (p=.029)	1.032 (1.003-1.061)
Period	0.00936	0.142 (p=0.71)	1.009 (0.961-1.060)

Table 5.2.3. Results of stepwise regression of variables that explain differences in occurrence of vesicular stomatitis cases by period for sentinel equine operations in Colorado.

Variable	Partial R-square	F value	Pr>F
10 period lag in precipitation	0.0141	30.79	<0.0001
11 period lag in NDVI value	0.0077	16.85	<0.0001

Significance level to stay in the model  $p < 0.10$

Table 5.2.4. Number of observations and percent classified as either periods with vesicular stomatitis cases or periods without vesicular stomatitis cases for sentinel equine operations in Colorado.

	Number of observations classified as negative (%)	Number of observations classified as positive (%)	Total
Negative periods	2594 (69.32)	1148 (30.68)	3742
Positive periods	23 (54.76)	19 (45.24)	42
Total	2617 (69.16)	1167 (30.84)	3784

Figure 5.2.1 Number of cases of vesicular stomatitis or seroconversions to vesicular stomatitis viruses occurring on Colorado sentinel equine operations by established time periods.

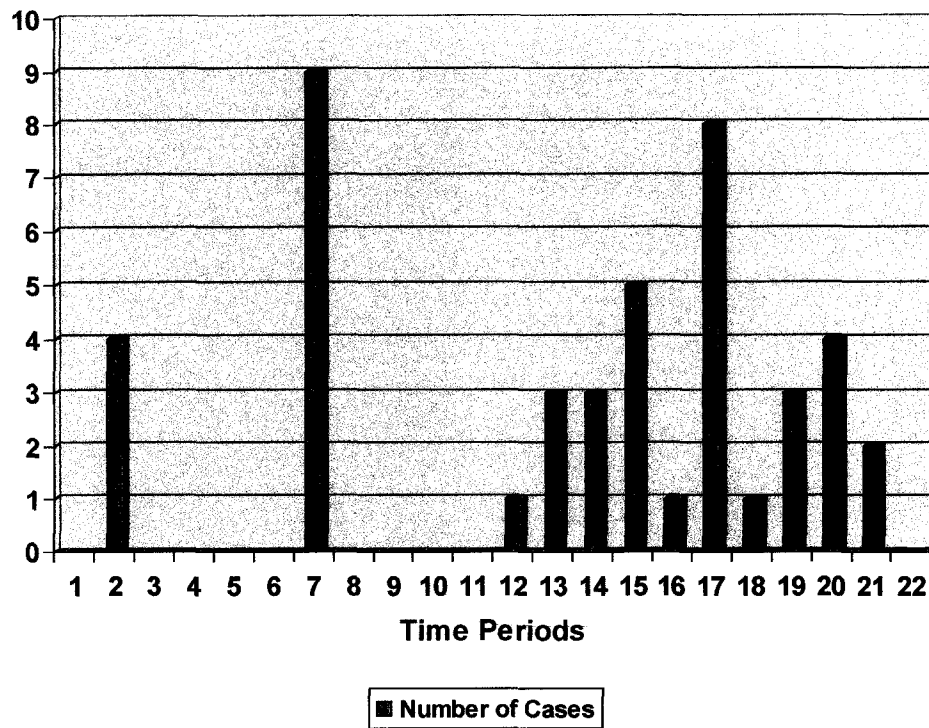
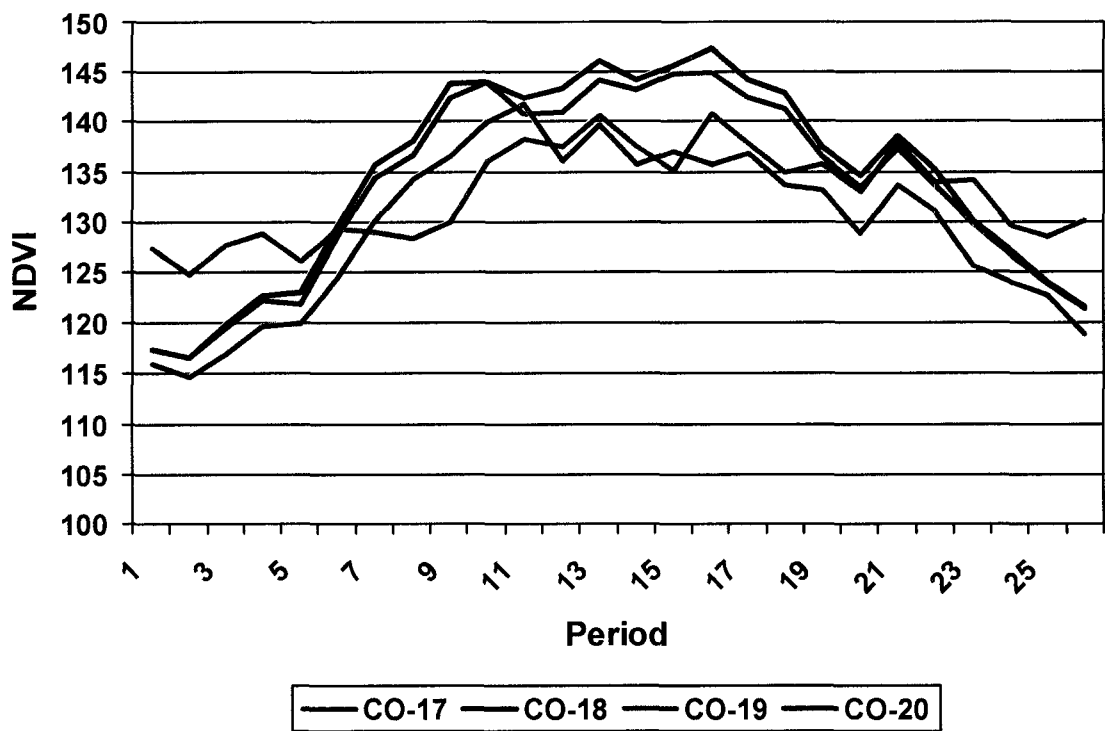


Figure 5.2.2 Average normalized difference vegetation indices for 1991 to 1999 for four sentinel equine operations in Colorado by period.



**Chapter 6.**  
**Vesicular Stomatitis in El Salvador:**  
**Observations from a 3-Year Pilot Study of**  
**Sentinel Dairy Herds\***

**Abstract**

The occurrence of vesicular stomatitis (VS) was investigated in El Salvador through monthly visits to 12 sentinel cattle operations located in 4 different departments. Management, environmental, and spatial data were collected. Heifers were enrolled on the operations and were examined and bled monthly for 3 years. Two competitive ELISAs were used to detect antibodies on each sample for each serotype of VS virus. On 8 of the 12 operations, small terrestrial rodents were trapped, blood samples collected, and antibodies to both VS serotypes evaluated using a serum neutralization test for each virus serotype.

Similar to other studies of VS in Central America, the seroprevalence of the New Jersey serotype was higher than the seroprevalence to the Indiana serotype. An outbreak of VS appeared to occur in the Department of Sonsonate in the summer of 1999. We confirmed that VS is endemic in the four departments investigated in El Salvador.

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Keywords: vesicular stomatitis; El Salvador; seroprevalence

## **1. Introduction**

Vesicular stomatitis viruses (VSV) are members of the genus Vesiculovirus in the Rhabdoviridae family. The two known serotypes are vesicular stomatitis-New Jersey serotype (VSV-NJ) and vesicular stomatitis-Indiana serotype (VSV-IN). There are endemic foci in South America, Central America, Mexico, and the United States (Mason 1954; Stallknecht et al., 1985; Rodriguez et al., 1990; Atwill et al., 1993; Vanleeuwen et al., 1995; Stallknecht et al., 1999). Viruses have been isolated from clinically affected animals during sporadic vesicular stomatitis (VS) outbreaks in the southwestern United States (Bridges et al., 1997).

Vesicular stomatitis viruses can infect many species of animals including (but not limited to) birds, rodents, deer, primates, and livestock (Letchworth et al., 1999). Infection in most of these species is subclinical. Clinical disease has been observed only in cattle, horses, pigs, and (rarely) llamas. Clinical manifestations include vesiculation and ulceration of the nasal mucosa, oral mucosa (both lingual and gingival surfaces), coronary bands, udder, and teats. Crusting lesions of the muzzle, ventral abdomen, prepuce, and external genitalia of horses are also common. Resolution of lesions generally occurs within 7 to 10 days without treatment (although secondary bacterial infections can prolong recovery).

Transmission of VSV occurs through contact and by mechanical and biological arthropod vectors (Rodriquez et al., 1993; Herrero et al., 1994; Mead et al., 1999; Stallknecht et al., 1999). Neither a reservoir nor an amplifying host for VSV has been

identified (although experimentally viremia was observed in bats and deer mice) (Donaldson, 1970; Cornish et al., 2001).

Published research on the epidemiology and ecology of VSV in Central America has been limited to studies in Costa Rica and Panama (Tesh et al., 1969; Tesh et al., 1971; Cline, 1976; Tesh et al., 1987; Webb et al., 1987; Rodriguez et al., 1990; Atwill et al., 1993; Herrero et al., 1994; Vanleeuwen et al., 1995; Rodriguez et al., 1996). A study was conducted in El Salvador: to investigate the persistence of VSV in cattle on farms previously known to be infected or in areas where infection had been identified; to follow a cohort of young animals serologically to determine infection and disease patterns; and to describe the ecological characteristics present on dairy farms in El Salvador previously identified as positive for VS. We report results of a 3-year study in 12 sentinel dairy herds. We believe this is the first published report on the epidemiology of VS in El Salvador.

## **2. Materials and Methods**

### *2.1. Sentinel farm selection*

The Ministry of Agriculture, through the Convenio Anti-aftosa Bilateral (CAB), established sentinel farms throughout El Salvador in 1985. Approximately 800 farms in the sentinel system receive routine visits from Ministry of Agriculture veterinarians. Eight species (cats, cattle, goats, horses, pigs, poultry, rabbits, sheep) and 14 diseases (anaplasmosis, anthrax, babesiosis, blackleg, brucellosis, classical swine fever, dermatobiasis, equine infectious anemia, fowl cholera, leptospirosis, Newcastle's disease, rabies, tuberculosis, vesicular stomatitis) are under surveillance. Twelve of these sentinel

farms were selected from four different departments for this study. Farms were selected in these departments because of their proximity to Ministry of Agriculture field veterinarians and because VSV had been recently diagnosed in all four departments. Farms within departments were selected based on confirmation that they had housed VS-positive cattle in one or more outbreaks during 1995-1997. Farms must have maintained  $\geq 20$  dairy cows. Cases were confirmed by the presence of clinical signs of VS and positive virus isolation and/or seropositivity (laboratory confirmation from Laboratorio de diagnóstico de enfermedades vesiculares – LADIVES, Panama). The confirmed positive animals did not necessarily still reside on the farms at the start of the study. The distribution of the farms by department is shown in Figure 6.1. Each farm's owner agreed to commit to a minimum of 2 years participation in the study and to the monthly collection of biological samples from cattle. Farms having more than one susceptible animal species (equine, swine, or bovine) or an adjacent farm with other species were selected preferentially. Participating farms benefited from monthly veterinary visits where examination of livestock for purposes other than this study could be conducted.

## *2.2. Sentinel-farm characterization*

A standardized questionnaire, adapted from a questionnaire used successfully in a similar sentinel study in the southwestern United States, was translated into Spanish and administered during the initial visit to the sentinel farm.<sup>1</sup> The questionnaire collected information about herd management practices, herd VS history, and farm ecological factors. A follow-up questionnaire, also adapted from a similar questionnaire used in the southwestern United States, was translated into Spanish and was to be administered on

each subsequent visit to assess any changes in management or movement of animals onto or off of the farm. Geographical positioning system units were used to acquire the coordinates of each sentinel farm. These coordinates were used to obtain geospatial characteristics of each sentinel farm.

### *2.3. Sentinel animal selection and sampling*

Selection of sentinel cattle was based on the population of heifers on each farm. Initially, all heifers from 6 to 12 months old were sampled from farms with  $\leq 30$  heifers. Heifers <6 months old can retain maternal antibody and thus serologic testing may not reflect their virus exposure. On dairies with >30 heifers, 15% of their additional heifers above 30 were added to the sample size without randomization. New heifers were added for each farm when they reached 6 months old. Heifers to be included in the study were identified either by numbered eartag or by name. All visits to sentinel farms were made by veterinarians from the El Salvador Ministry of Agriculture. Beginning in January 1998 and continuing until October 2001, blood samples were collected monthly from all sentinel cattle into a sterile tube without anticoagulant by jugular venipuncture. Examination of the oral cavity, nasal mucosa, coronary bands, mammary glands, and genitalia also was conducted at each visit. Several natural disasters (including hurricanes, earthquakes, and mudslides) resulted in missed visits to sentinel farms throughout the study and no samples were collected in 2000 due to interruptions in funding.

### *2.4 Rodent trapping*

Terrestrial species of small rodents were targeted specifically for trapping on sentinel farms. Eight of twelve sentinel farms agreed to rodent trapping. Ecological maps

of each farm were used to identify specific rodent habitats. Locations of habitat types were marked on the map and one location of each habitat type selected for trapping. The selected habitat location was divided into transects with 10 to 50 m between transects. One transect within each habitat location was selected and 10 Sherman live-traps were placed at the beginning of each transect and then every 10 to 50 m. Traps were set for 3 consecutive days every month during 1 year of the study. During placement, traps were checked two times per day. Traps were baited with a rodent-feed pellet mixed with peanut butter and vanilla. Blood samples were obtained from captured rodents by cardiac puncture or from the tail or eye. Blood samples were centrifuged and stored in liquid nitrogen and sent to the Universidad Nacional, School of Veterinary Medicine in Costa Rica for serum neutralization testing. The serum-neutralization methods used have been described (Mumford et al., 1998, OIE Standards Manual, 2000).

### *2.5 Serological testing*

Blood samples from cattle were centrifuged and serum was harvested. Serum was screened by a separate competitive enzyme-linked immunosorbent assay (cELISA) for each virus serotype. This assay was designed to detect specific IgG antibodies for each serotype. The two serotypes are distinct viruses and cross-reactivity between serotypes when tested by the cELISA is not believed to occur. Sensitivity and specificity of the VSV-NJ cELISA, relative to the serum neutralization test, is 98% and 93%, respectively (Alvarado, 2002). Sensitivity and specificity of the VSV-IN assay, relative to the serum neutralization test, is 85% and 94%, respectively (Alvarado, 2002). In short, recombinant antigen of both VSV-NJ and VSV-IN serotypes was added to separate plates. Control

sera were diluted 1:8. The test and control sera then were poured onto the treated plates and incubated for 30 min at 37°C. The unwashed plates were incubated for another 30 min at 37°C after the addition of a 50- $\mu$ l aliquot of polyclonal mouse ascites fluid. Plates then were washed with 0.05% polysorbate in phosphate-buffered saline solution. Fifty microliters of goat anti-mouse horseradish-peroxidase conjugate was added to each well. Plates were incubated 30 min and washed again with saline solution. A total of 50  $\mu$ l of tetramethylbenzidine substrate was added to each well. Plates were incubated at 25°C until the optical density for the control sample reached a value of 1.0. The reaction then was stopped using 50  $\mu$ l of 0.5M H<sub>2</sub>SO<sub>4</sub>. The optical density of each well was read with an ELISA plate reader<sup>a</sup> at 450 nm (Mumford et al., 1998). The optical density was used to calculate the percent reduction of each sample. The percent reduction was used to determine the positive and negative classifications, with 50% serving as the cutoff value for positive reactions based on Office of International Epizootics standards (OIE Standards Manual, 2000).

## *2.6 Data analysis*

Sampling statistics for each sentinel farm were calculated as well as seroprevalence for VSV-NJ and VSV-IN. A chi-square test for equality of proportions was conducted to examine differences in the seroprevalence of VSV-NJ and VSV-IN in cattle between farms. Significant differences were considered at  $p < 0.05$ . The number of seroconversions to VSV-NJ and VSV-IN was determined for each farm. A seroconversion was considered to have occurred if a heifer tested negative on the first test and then tested positive at one or more samplings during the study period. Three different

patterns of serologic responses were considered for cattle that were negative on the initial test. Cattle with pattern 1 initially tested negative and remained negative for the entire study period (no seroconversion). Cattle with pattern 2 initially tested negative, seroconverted at some point in the study and remained seropositive until the end of the study. Cattle with pattern 3 initially tested negative, seroconverted at some point in the study and then had variable test results until the end of the study. The percentage of cattle exhibiting each pattern of serologic response for each farm was determined. Statistical software (SAS v8.02) was used for all analyses.<sup>b</sup>

### **3. Results**

Ecological and management descriptors for each sentinel farm are presented in Table 6.1. Most of the sentinel farms in this study were primarily dairy operations but managed cattle for dairy and beef purposes. Sentinel farms in this study were found between 72 and 404 m above sea level. All but two of the sentinel farms used pasture as the primary source of forage with cattle spending between 6 and 22 hr on pasture. One sentinel farm managed cattle in loafing sheds and another in dry lots and both supplied cut grass as forage. Vegetation density varied among sentinel farms from thick, almost junglelike conditions to wide open grass pastures with a few shade trees.

Overall, 92% of sentinel farms reported that horses, other than those housed on their operation, were housed within 1.6 km of their operation with the number of horses within 1.6 km ranging from 0 to 500 (median=50). All of the sentinel premises reported that cattle, owned by other farms, were maintained within 1.6 km of their operations with the number of cattle within 1.6 km ranging from 100 to 2,000 (median=400).

A total of 8,416 serum samples, representing 1,220 animals, were tested by each cELISA for VSV-NJ and VSV-IN antibodies. Overall, 2,698 (32.1%) and 1,032 (12.3%) samples were positive for antibodies to VSV-NJ and VSV-IN respectively. Sampling statistics and seroprevalence for each serotype for each sentinel farm are reported in Table 6.2. Seroprevalence was calculated by dividing the number of heifers positive at least once by the total number of heifers tested on that farm. Apparent prevalences were corrected to true prevalences (Rogan and Gladen, 1978). The differences in seroprevalence for VSV-NJ and VSV-IN on each farm were statistically significant for 9 of 12 sentinel farms. There was an average of 234 total samples taken per year from each sentinel farm. Therefore approximately 2,800 samples failed to be collected during year 2000.

There was a total of 232 and 223 seroconversions to VSV-NJ and VSV-IN respectively. Incidence of seroconversions was highest in July and August and lowest in January and November for VSV-NJ and highest in July and December and lowest in March and June for VSV-IN (Figure 6.2).

Overall, 756 heifers initially tested negative for VSV-NJ with 524, 16, and 216 heifers exhibiting serologic response patterns 1, 2, and 3 respectively. Overall, 1,046 cohorts initially tested negative for VSV-IN with 823, 4, and 219 cohorts exhibiting patterns 1, 2, and 3 respectively. The number of times a heifer was sampled was associated with its serological response pattern for both serotypes (Kruskal-Wallis,  $p < 0.0001$ ).

Eight of the twelve sentinel farms participated in rodent trapping. A total of 194 rodents were trapped representing 11 different species. Results of rodent trapping are

provided in Table 6.3. Overall, 102 rodent samples were tested by the serum neutralization test of which 3 were positive. All three samples were from rodents trapped on farm number 9 and two of the three positives were from *Sigmodon hispidus* and one from *Neotoma mexicana*.

#### **4. Discussion**

Sentinel farms or livestock operations can be used to monitor or identify outbreaks and epidemics of disease, to investigate changes in the amount or severity of endemic diseases, to evaluate the effectiveness of newly instituted disease control programs, or to confirm a hypothesis about the ecology or epidemiology of a disease agent. This concept is one in which the health status of populations is periodically assessed. El Salvador has maintained a sentinel herd surveillance system since 1985. This system entails routine farm visits by veterinarians from the Ministry of Agriculture for observation of livestock and poultry for many diseases. Approximately 1,000 on-farm visits are made annually. We elected to use established sentinel herds in El Salvador to investigate the epidemiology of vesicular stomatitis virus in a purported endemic area.

The sentinel farms chosen were typical of dual-purpose cattle operations in El Salvador with most forage supplied through grazing, with animals spending little time in barns or other inside housing. A case-control study conducted in the southwestern United States identified pasture and housing livestock within 0.25 mi of a source of running water as risk factors for being a positive VS premises (Hurd et al., 1999); housing of livestock inside barns or other shelters was protective. Both the risk and protective factors were concluded to point to arthropod vectors as potential sources of transmission of VSV.

The high seroprevalences found on the sentinel farms of the study reported here may be due to the consistent exposure to pasture and running water and limited amounts of indoor housing although the study design and limited number of operations precludes a statistical evaluation of differences.

The large numbers of horses and cattle within a 1.6 km radius of the sentinel farms may also increase the risk of livestock being exposed on sentinel farms. Although no assessment of potential contact exposure to neighboring livestock or testing of livestock other than cattle on the sentinel farms was conducted, the high numbers of horses and cattle within a 1.6 km radius increase the feeding source for arthropods and thus may increase the amount of virus in local environments. There is no evidence however, that horses or cattle are amplifying hosts of VSV.

The percentage of VSV-NJ positive tests in this study was more than 2.5 times that of the percentage of VSV-IN positive tests. On an individual farm basis, the percent of VSV-NJ positives was significantly higher for 9 of the 12 sentinel farms. The greater rate of seropositivity for VSV-NJ has been observed in other studies in Central America (Rodriguez et al., 1990; Atwill et al., 1993) and has been attributed to different ecological cycles for the two viruses. In the United States, sporadic outbreaks of VS occurring in the southwest from 1962 to 1995 were all of the VSV-NJ serotype. In 1997, a VS outbreak in the southwestern United States was of both serotypes and in 1998 the outbreak was only of the VSV-IN serotype. The consistent finding in both endemic and nonendemic areas of a higher seroprevalence of VSV-NJ than VSV-IN suggests that livestock hosts are either more susceptible to VSV-NJ, specific arthropods are more competent vectors of VSV-NJ, there is a greater abundance of competent VSV-NJ vectors, or the virus is circulating in

greater abundance and thus livestock are more frequently exposed to it. The environmental factors that may be associated with greater abundance of one serotype over another have not been well described.

Seroconversions occurred in all months of the study (except January for VSV-NJ) for both VSV-NJ and VSV-IN (Figure 6.2). The large number of seroconversions occurring in July can be attributed to Farms 1, 2, 3, and 4, all located in the Department of Sonsonate. Serologic testing of cattle on these four farms found no seropositives from all test dates in 1998 and through June 1999. In July 1999 all four farms had cattle test positive for VSV-NJ and VSV-IN. The large number of seroconversions in one month indicates that an outbreak of VS was occurring in this area of the department. Data about changes in ecological and management factors were not collected so that conclusions about the source of the outbreak cannot be drawn. Interestingly, no clinical cases of VS were reported from any of the four farms during July 1999 although a clinical case was reported to the Ministry of Agriculture from Farm 1 in November 1999. It is also notable that Farm 1 routinely vaccinated cattle for VS prior to and during 1998 and yet no seropositive animals were identified until July 1999. The vaccine used was produced in Central America, presumably from local isolates of the virus. An investigation of a formalin-killed, cell-culture VSV-NJ vaccine indicated that serum-neutralizing antibodies were produced at group mean titers of greater than 1:500 by 21 days after a second vaccination, but decreased to a group mean titer of less than 1:66 by 175 days post-vaccination (Gearhart et al., 1987). An autogenous VSV-NJ vaccine was approved for use in the United States during the 1995 VS outbreak. Three dairies were included in a field study of this vaccine where it was found that all cattle administered the vaccine

generated an immune response as measured by the serum neutralization test, but the titers were short-lived with geometric mean titers falling below 1:20 on two dairies by 58 days post-vaccination and on one dairy by 105 days post-vaccination (McCluskey, 1997). Challenge with VSV-NJ, either naturally or experimentally, did not occur in either of these studies. Therefore, the efficacy of the vaccine was not determined. The lack of a detectable immune response in the cattle vaccinated on Farm 1 in the study reported here suggests that the vaccine was either administered incorrectly, or was not immunogenic based on samples tested by the cELISA.

Most heifers remained serologically negative to both serotypes throughout the study (pattern 1). There were very few heifers that seroconverted and then remained positive to the end of the study (pattern 2). Cattle have been reported to maintain antibody titers to VSV for extended periods; therefore the low number of heifers remaining positive from the point of seroconversion to the end of the study was unexpected (Katz et al., 1997). However, the occurrence of sporadic antibody responses in cattle has also been reported (pattern 3), most notably in cattle from sentinel herds in Costa Rica (Sorensen et al., 1958; Rodriguez et al., 1990).

Although no reservoir hosts of vesicular stomatitis viruses have been identified, many arthropod-borne viruses use small terrestrial mammals as hosts. Rodents trapped on sentinel farms in this study were those typically found in El Salvador. Previous field studies investigating the potential of small mammals as reservoir hosts of VSV have found serologic evidence of VSV exposure in a variety of species although no virus was isolated from any specimens tested (Webb et al., 1987; Aguirre et al., 1992).

Experimental infections of rodents in a number of studies indicated that rodents will

develop immune responses to VSV (Tesh et al., 1969). In both experimental inoculations of bats (*Myotis lucifigus lucifigus*) and deer mice (*Peromyscus maniculatus*) viremia was detected indicating that these species may have a role in the epidemiology of VS (Donaldson, 1970; Cornish et al., 2001). If, like other arthropod-borne viruses, VSV use rodents as reservoirs, the relative abundance of rodents on those sentinel farms conducting trapping indicates the presence of potential reservoir species.

## **5. Conclusion**

The investigation reported here suggests that VSV in El Salvador behave similarly as in other endemic areas in Central America. Also similar to both endemic and nonendemic areas, exposure to virus as measured by serum antibody responses appears to occur at high rates whereas clinical disease is a rare event. On dairy operations in El Salvador where clinical disease does occur there appear to be little direct effects on production. Investigations of VS outbreaks in United States dairies indicated losses of \$97 to \$253 per cow (Alderink, 1984; Goodger, 1985). The higher average production per cow and more intense management of dairies in the United States may result in the greater effects on production than observed in El Salvador.

The low number of clinically affected animals and small effects on production make VS of minimal concern to producers in El Salvador. However, it is of great concern to animal health officials as it is clinically indistinguishable from foot and mouth disease (FMD). Differentiation of FMD and VS can be accomplished only through laboratory testing. El Salvador is free of foot and mouth disease but is close enough to FMD-positive countries in South America to warrant constant surveillance for this disease.

The cost effectiveness of sentinel surveillance systems makes them an attractive alternative to slaughter surveillance and cross-sectional surveys where sentinel surveillance systems are an appropriate option. Population-based surveys with random sampling are similarly expensive and often difficult to conduct if adequate list frames of operations are not available. Surveys generally provide one-time sampling (point estimates) thus requiring that they be repeated to assess changes in disease prevalence. The sentinel farms established in this study were used to make observations about VS epidemiology in El Salvador. However, they could also be used as sites for routine surveillance not only for vesicular diseases but also for other diseases of importance to producers and animal health officials.

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**Footnotes**

<sup>1</sup> Questionnaire available from the corresponding author upon request.

**Manufacturers**

<sup>a</sup> Multiskan Plus MKII, Titertek, Huntsville, AL, USA

<sup>b</sup> SAS, Version 8.02, SAS Institute Inc, Cary, NC, USA

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Table 6.1. Management and ecological descriptors of 12 sentinel dairy farms in El Salvador.

<u>Farm Number</u>	<u>Operation Type</u>	<u>Year of Last VS Clinical Case</u>	<u>Elevation (meters)</u>	<u>Aspect</u>	<u>Number of Horses within 1.6 km of farm</u>	<u>Number of Cattle within 1.6 km of farm</u>	<u>Distance to Source of Running Water (m)</u>	<u>Distance to Source of Standing Water (m)</u>	<u>Time Cattle Spend on Pasture per Day (hrs)</u>
1	Dairy only	1997	404	219.08 SW	50	2000	100	10	10
2	Dairy only	1992	336	241.62 SW	50	100	5	20	0
3	Dairy and beef	1997	172	213.21 SW	200	1000	*	20	16
4	Dairy and beef	1997	72	176.85 S	10	2000	10	10	12
5	Dairy and beef	1997	301	154.04 SE	160	500	10	*	18
6	Dairy and beef	1997	254	171.68 S	25	150	*	20	20
7	Dairy and beef	1997	279	200.43	50	600	200	200	20
8	Dairy and beef	1997	277	282.10 W	115	300	500	500	19
9	Dairy only	1997	57	225.00 SW	50	1000	30	10	0
10	Dairy and beef	1997	72	116.56 SE	0	200	10	10	6
11	Dairy and beef	1997	85	122.66 SE	10	100	*	15	22
12	Dairy and beef	1996	195	3.03 N	20	250	*	*	20

\* None within 1000 meters.

Table 6.2. Sampling statistics and corrected seroprevalence estimates for vesicular stomatitis New Jersey serotype and vesicular stomatitis Indiana serotype on 12 sentinel dairy farms in El Salvador.

<b>Farm Number</b>	<b>Number of animals sampled</b>	<b>Median times each animal sampled</b>	<b>Seroprevalence (%)*</b>		<b>95% Confidence interval (%)</b>	
			<b>VSV-NJ</b>	<b>VSV-IN</b>	<b>VSV-NJ</b>	<b>VSV-IN</b>
1	131	4	<b>56</b>	<b>37**</b>	<b>47,65</b>	<b>29,46</b>
2	102	4	<b>11</b>	<b>16</b>	<b>5,17</b>	<b>9,24</b>
3	167	3	<b>2</b>	<b>9**</b>	<b>.03,4</b>	<b>4,14</b>
4	140	4	<b>9</b>	<b>9</b>	<b>4,14</b>	<b>4,14</b>
5	125	4	<b>85</b>	<b>22**</b>	<b>79,91</b>	<b>15,30</b>
6	50	4	<b>70</b>	<b>18**</b>	<b>57,82</b>	<b>7,29</b>
7	89	4	<b>76</b>	<b>21**</b>	<b>67,85</b>	<b>12,30</b>
8	94	4	<b>81</b>	<b>45**</b>	<b>73,89</b>	<b>35,55</b>
9	134	5	<b>59</b>	<b>30**</b>	<b>50,68</b>	<b>22,38</b>
10	107	4	<b>53</b>	<b>10**</b>	<b>43,63</b>	<b>4,16</b>
11	23	19	<b>83</b>	<b>70</b>	<b>68,99</b>	<b>51,89</b>
12	58	3	<b>90</b>	<b>70**</b>	<b>82,98</b>	<b>58,82</b>

\* Apparent prevalence adjusted to true prevalence (Rogan and Gladen, 1978).

\*\*  $p < 0.05$  for  $\chi^2$  test for proportions comparing seroprevalence of VSV-NJ and VSV-IN for each sentinel farm.

Table 6.3. Number and species of rodents trapped on 12 sentinel dairy farms in El Salvador.

<b><u>Species</u></b>	<b><u>Farm1</u></b>	<b><u>Farm2</u></b>	<b><u>Farm7</u></b>	<b><u>Farm8</u></b>	<b><u>Farm9</u></b>	<b><u>Farm10</u></b>	<b><u>Farm11</u></b>	<b><u>Farm12</u></b>
<i>Sigmodon hispidus</i>	0	1	1	3	38	14	2	3
<i>Neotoma mexicana</i>	1	4	0	0	28	0	0	0
<i>Liomys salvini</i>	7	4	4	5	0	0	1	10
<i>Rattus rattus</i>	10	15	3	4	1	5	3	0
<i>Rattus norvegicus</i>	1	1	0	0	0	0	0	0
<i>Peromyscus guatemalensis</i>	0	2	1	0	0	1	0	0
<i>Nyctomys sumichrasti</i>	0	0	0	1	0	0	0	0
<i>Mus musculus</i>	7	2	0	0	1	1	0	0
<i>Liomys albinus</i>	0	0	0	2	0	0	0	0
<i>Peromyscus mexicanus</i>	1	5	0	0	0	0	0	0
<i>Oryzomys alfaroi</i>	0	0	1	0	0	0	0	0
<b>Total</b>	27	34	10	15	68	21	6	13

Figure 6.1. Location of 12 sentinel dairy farms in El Salvador.

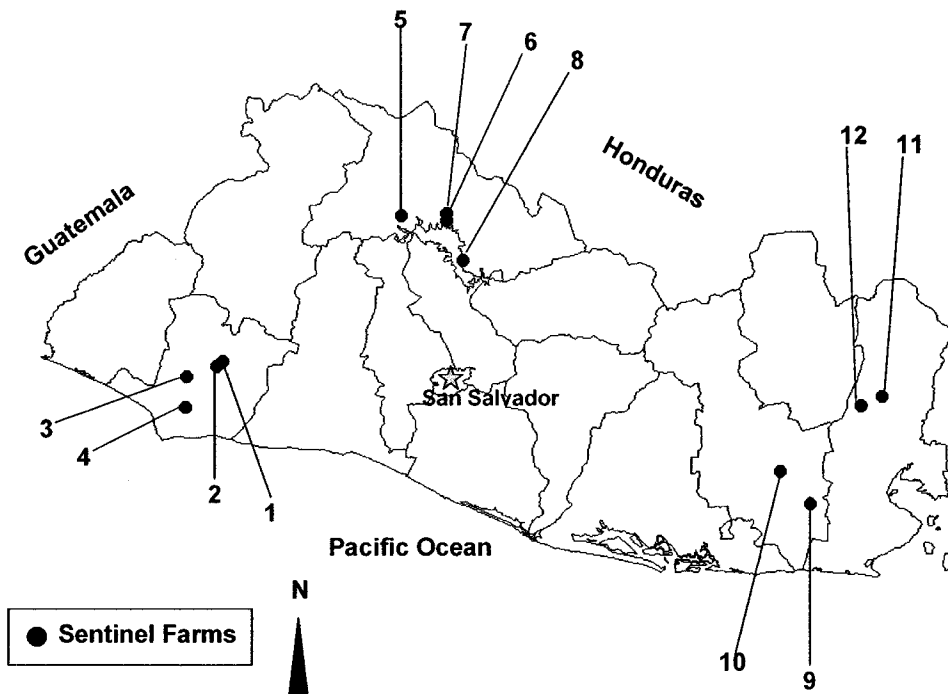
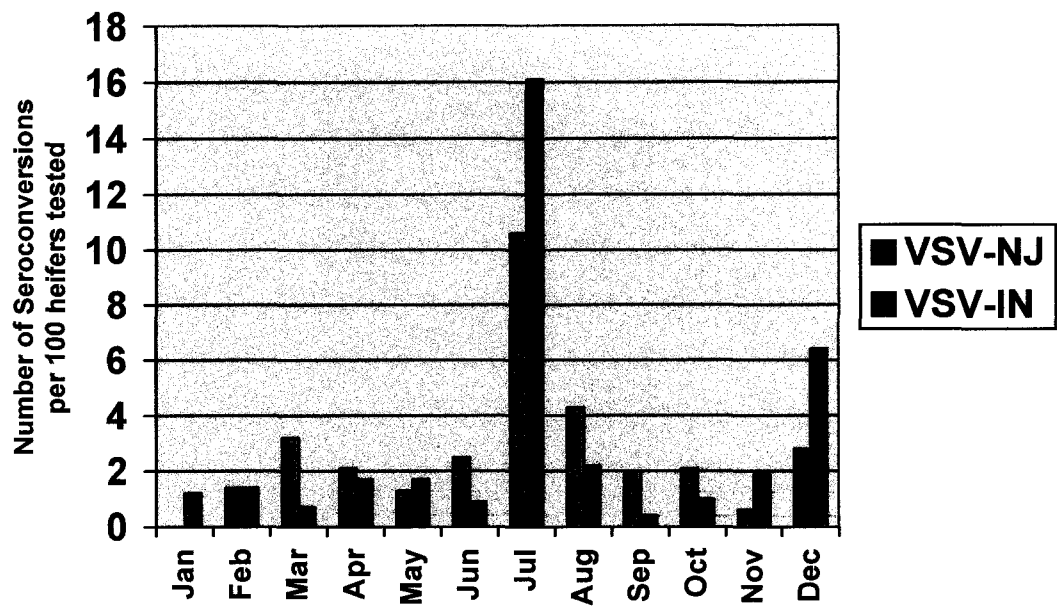


Figure 6.2. Number of seroconversions to vesicular stomatitis virus New Jersey serotype and vesicular stomatitis virus Indiana serotype per 100 heifers tested, by month on 12 sentinel dairy farms in El Salvador.



## **Chapter 7.**

### **Conclusions**

- Sentinel herd surveillance can be an appropriate and economical alternative to conventional methods of animal surveillance and is also a valuable tool in epidemiologic investigations. The steps required in the development of sentinel surveillance include:

- establishing priorities and goals
- establishing criteria for sentinel herd selection
- establishing criteria for sentinel animal selection
- establishing sampling frequency
- determining testing protocols

Effective application of sentinel herd surveillance can enhance the overall animal health monitoring and surveillance programs of any country.

- Seroconversions as determined by serum neutralization tests, competitive enzyme-linked immunosorbent assays or IgM capture enzyme-linked immunosorbent assays to both vesicular stomatitis virus New Jersey serotype (VSV-NJ) and vesicular stomatitis virus Indiana serotype (VSV-IN) occurred on a majority of the sentinel equine operations in Colorado and New Mexico.

Seroconversions are potentially due to:

- exposure to VSV-NJ or VSV-IN found in the environments of the sentinel equine operations.
- recrudescence of latent or persistent virus in previously infected horses
- exposure to virus(es) that are immunologically cross-reactive to the known VSV found in the environments of the sentinel equine operations.

Evidence of VS virus recrudescence in horses is lacking. The presence of one or more cross-reacting vesiculoviruses is plausible as many are known to infect plants and invertebrates. However, I conclude that the most likely cause of the seroconversions is the exposure of horses to wild-type VSV-NJ or VSV-IN.

- The identification of polymerase chain reaction positive swab samples collected from separate operations in 1999, supports the conclusion that VSV-NJ persists in the environment of equine operations in the southwest in non-outbreak years.
- Vesicular stomatitis (VS) as a disease entity must continue to be considered to occur only sporadically in the southwestern United States. However, sub-clinical infections of VSV do occur in horses in Colorado and New Mexico in non-outbreak years. Subclinical infections result in many seropositive horses. Maintenance of antibody titers in horses for many months or years, as observed in this study, without evidence of clinical disease indicates that they pose minimal risk of transmission of virus to other horses or livestock.
- The response by animal health officials to outbreaks of VS in the southwestern United States should be reviewed. As clinical disease in horses is in most cases innocuous and self-limiting and exposure to virus may be occurring in all years, the institution of quarantines and other movement restrictions on horses may not

be warranted. However, due to the clinically indistinguishable lesions of foot and mouth disease (FMD) and VS in cattle and other susceptible species, the response to a VS outbreak in these species must continue to be to assume that FMD has been introduced into the United States.

- The identification of alterable management factors associated with the occurrence of VS virus infection remains elusive. Two ecological factors, ecological section and plant hardiness zone, were shown to be associated with changes in serum neutralization titer to VSV-IN. These findings as well as the lack of factors associated with changes in titers to VSV-NJ suggest that the ecologies required for transmission of the two serotypes are different. This may be related to reservoirs of the viruses or to their arthropod vectors.
- The occurrence of VS in Colorado and New Mexico was successfully modeled when total precipitation occurring 10 to 12 months previous to the occurrence of cases was used as the predictor. However, the accuracy of prediction is not adequate enough to use as a management tool for animal health officials. The association of precipitation and the occurrence of VS further suggests that arthropods, either as vectors or reservoirs, are key components to understand the epidemiology of VS in the southwestern United States.
- Seropositivity to VSV-NJ was found to be greater than that for VSV-IN in the four departments studied in El Salvador. This finding is consistent with other studies of VSV in Central America and is attributed to the different ecological cycles of the two viruses.

- Both VSV-NJ and VSV-IN were found to be endemic in the Departments of Sonsonate, Chalatanengo, San Miguel and La Union in El Salvador. However, similar to findings in other endemic areas and in non-endemic areas, clinical disease was infrequent.
- The establishment of sentinel herds in El Salvador was shown to be useful in investigating the epidemiology of VS but also provided access to sites for routine surveillance for other diseases of importance to animal health officials.

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**Appendix 1.**

**SENTINEL EQUINE OPERATIONS IN COLORADO AND NEW MEXICO**

**CO-1**

*Initial Date:* 7/7/95

*Number of Visits:* 2

*County:* La Plata

*Coordinates:* 37.09033 N  
107.71362 W

*Slope:* 0

*Aspect:* -1°

*Elevation:* 2066 m

*Domain:* Dry

*Division:* Temperate Steppe  
Regime Mountains

*Province:* Southern Rocky Mountain Steppe-Open Woodland-Coniferous Forest-  
Alpine Meadow

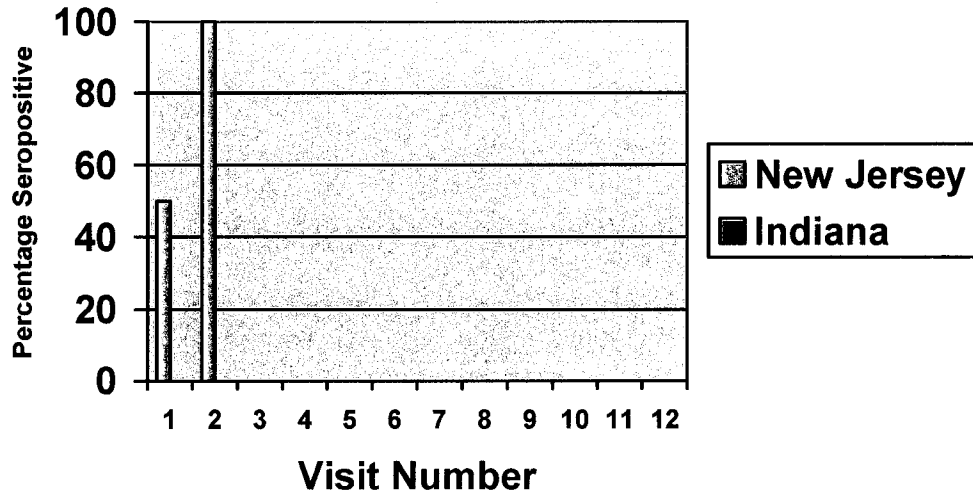
*Section:* South-Central Highlands

*Landcover:* Deciduous broadleaf forest

*Soil:* Zyme-Rock outcrop-ustic torriothents

*Plant Hardiness Zone:* 5b

# Premises 95CO0187



**CO-2**

*Domain:* Dry

*Initial Date:* 8/9/95

*Division:* Temperate Steppe  
Regime Mountains

*Number of Visits:* 9

*County:* Delta

*Province:* Southern Rocky Mountain  
Steppe-Open Woodland-Coniferous  
Forest-  
Alpine Meadow

*Coordinates:* 38.95601 N  
107.94708 W

*Section:* North-Central Highlands

*Slope:* 3.814075

*Landcover:* Evergreen needleleaf forest

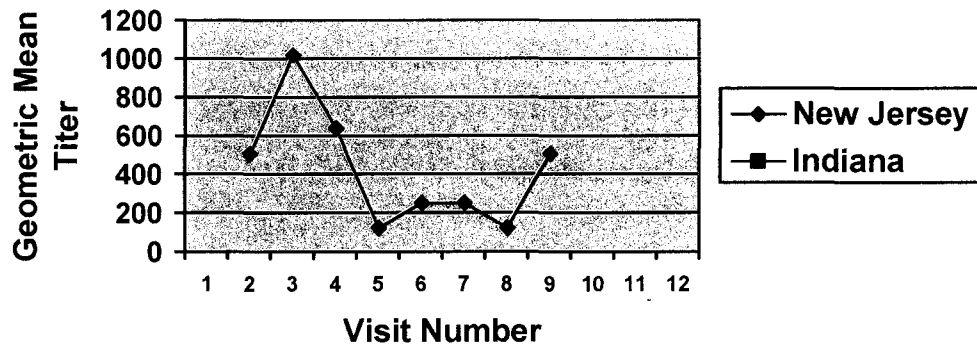
*Aspect:* 180 S

*Soil:* Agua fria-saraton colonia

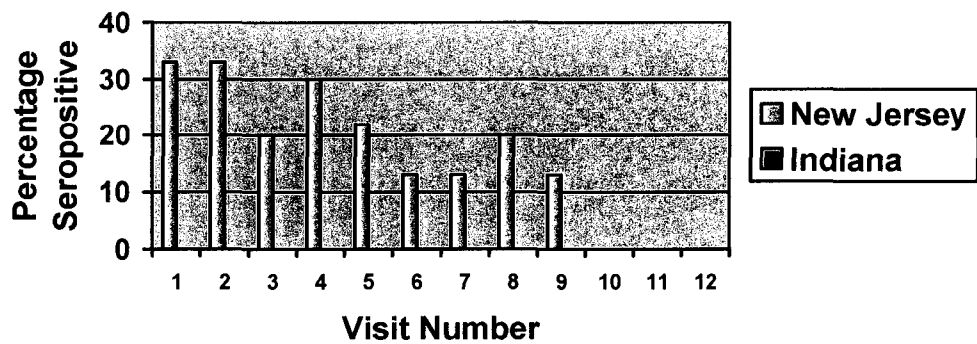
*Elevation:* 2169 m

*Plant Hardiness Zone:* 6a

### Premises 95CO0433



### Premises 95CO0433



**CO-3**

*Initial Date:* 9/15/95

*Number of Visits:* 9

*County:* Mesa

*Coordinates:* 39.05464 N  
108.44924 W

*Slope:* .0477454

*Aspect:* 0 N

*Elevation:* 1445 m



*Domain:* Dry

*Division:* Temperate Desert

*Province:* Intermountain Semi-Desert  
and Desert

*Section:* Northern Canyon Lands

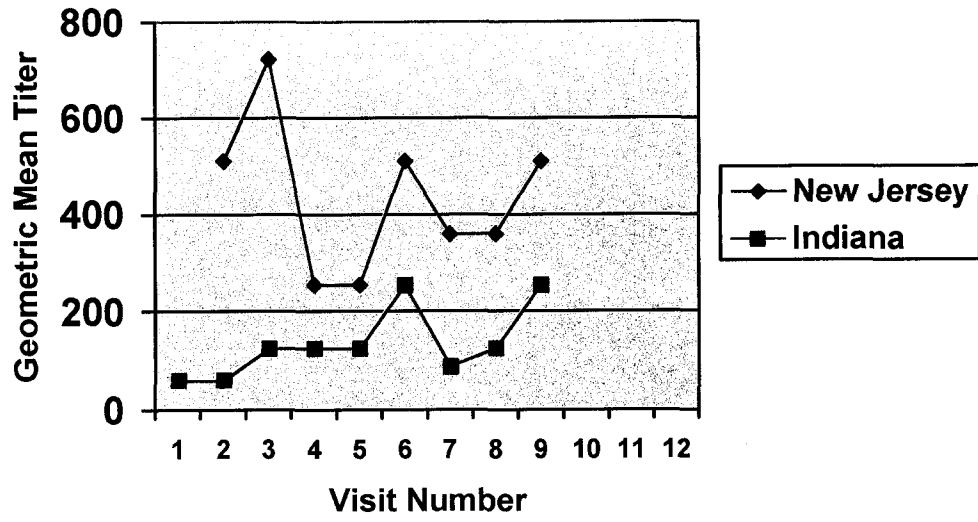
*Landcover:* Evergreen needleleaf  
forest

*Soil:* Utaline-shavano-Lazear

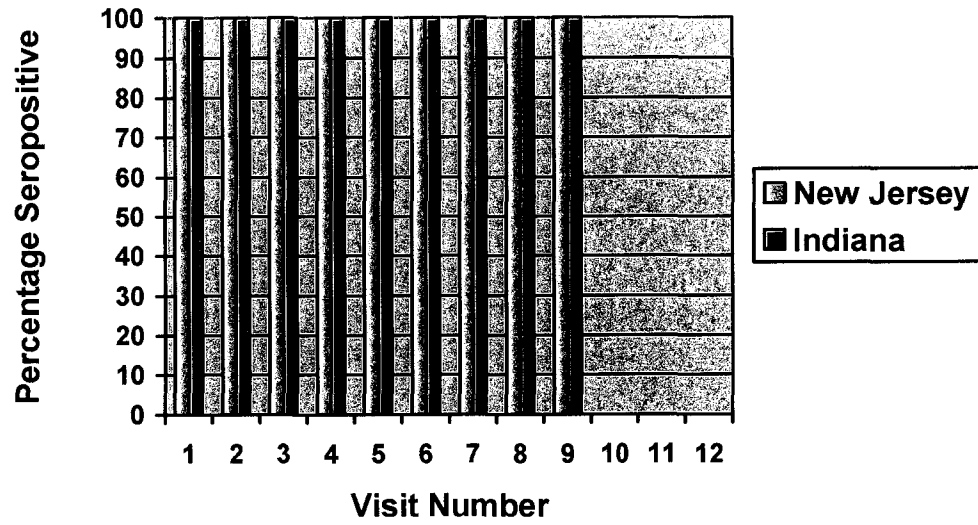
*Plant Hardiness Zone:* 7a



Premises 95CO0689



Premises 95CO0689



**CO-4**

*Initial Date:* 9/21/95

*Number of Visits:* 10

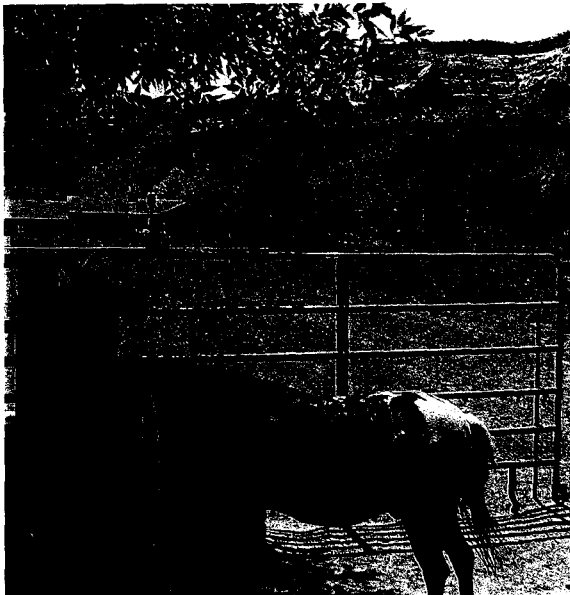
*County:* Mesa

*Coordinates:* 39.08529 N  
108.67932 W

*Slope:* 2.263636

*Aspect:* 18.434948 N

*Elevation:* 1450 m



*Domain:* Dry

*Division:* Temperate Steppe Regime  
Mountains

*Province:* Southern Rocky Mountain Steppe-  
Open Woodland-Coniferous Forest-  
Alpine Meadow

*Section:* South-Central Highlands

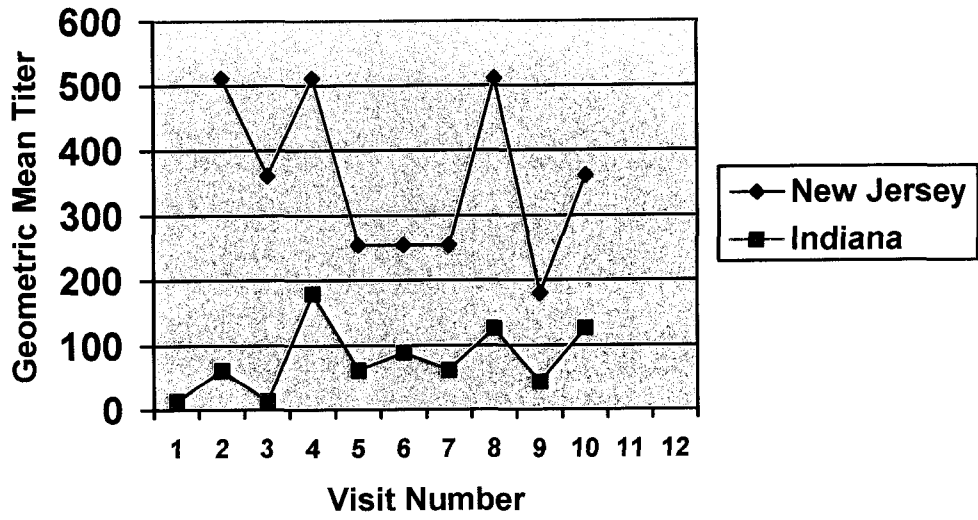
*Landcover:* Grassland

*Soil:* Rizno-Rock Outcrop-Ignacio

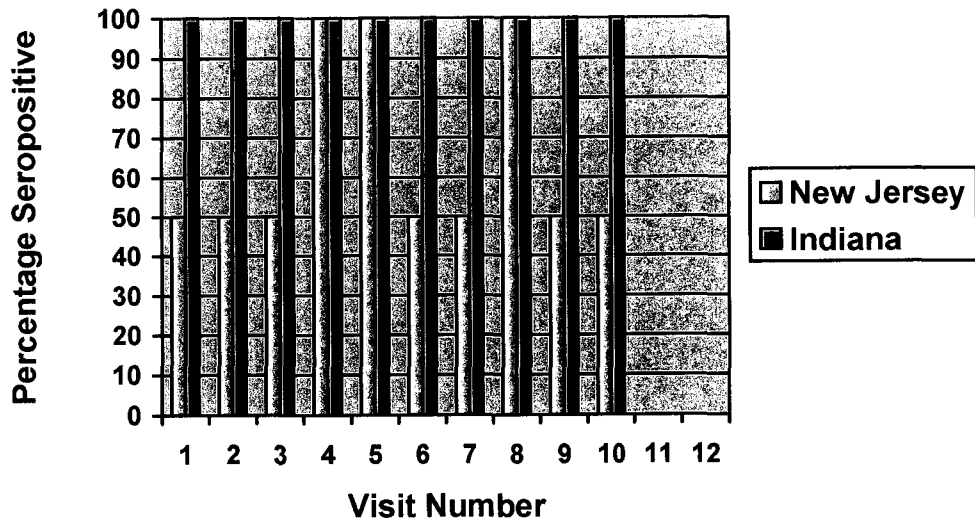
*Plant Hardiness Zone:* 6b



### Premises 95CO0749



### Premises 95CO0749



**CO-5**

*Initial Date:* 11/27/95

*Number of Visits:* 5

*County:* Mesa

*Coordinates:* 39.14764 N  
108.68324 W

*Slope:* 0

*Aspect:* -1°

*Elevation:* 1389 m

*Domain:* Dry

*Division:* Temperate Desert

*Province:* Intermountain Semi-Desert  
and Desert

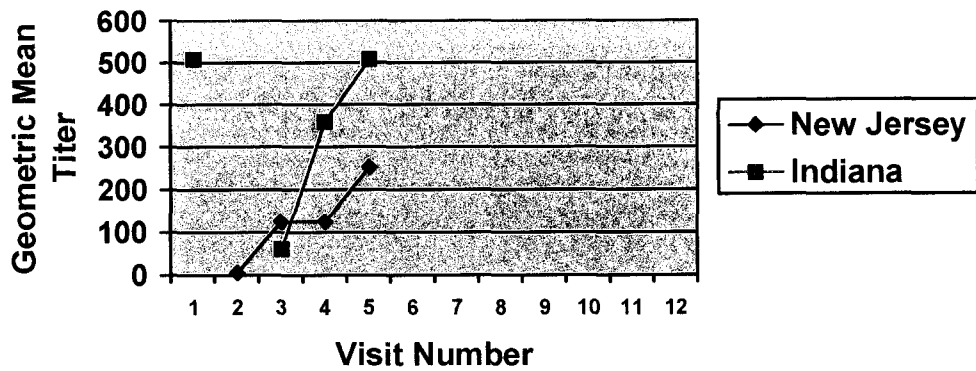
*Section:* Northern Canyon Lands

*Landcover:* Evergreen needleleaf forest

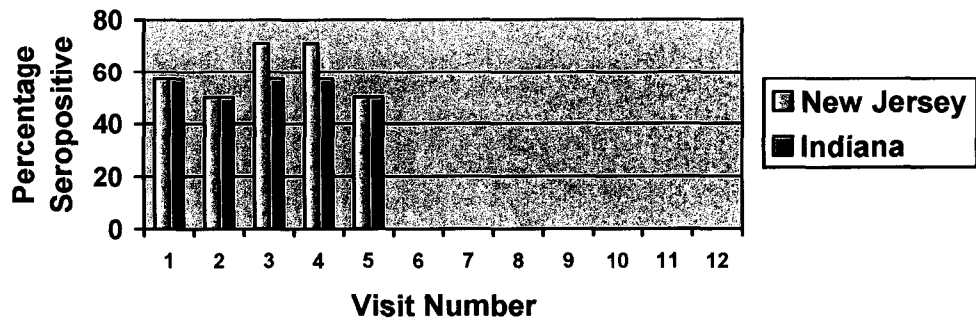
*Soil:* Billing-Rayola-Fruita

*Plant Hardiness Zone:* 6b

**Premises 95CO1111**



**Premises 95CO1111**



**CO-6**

*Initial Date:* 7/28/97

*Number of Visits:* 12

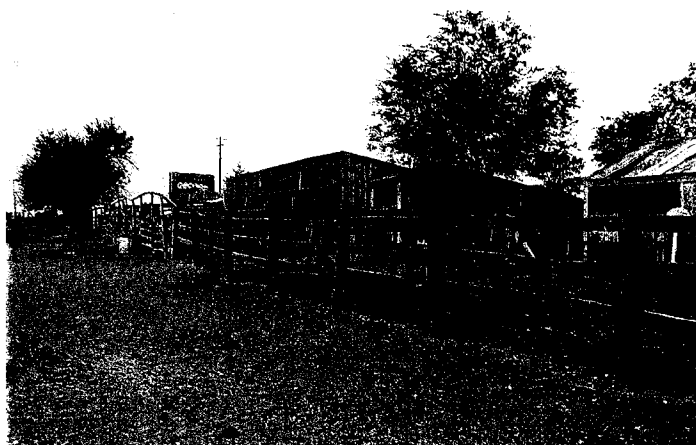
*County:* Pueblo

*Coordinates:* 38.27225 N  
104.54646 W

*Slope:* 1.909152

*Aspect:* 180 S

*Elevation:* 1723.9 m



*Domain:* Dry

*Division:* Temperate Steppe

*Province:* Great Plains  
Palouse Dry Steppe

*Section:* Arkansas  
Tablelands

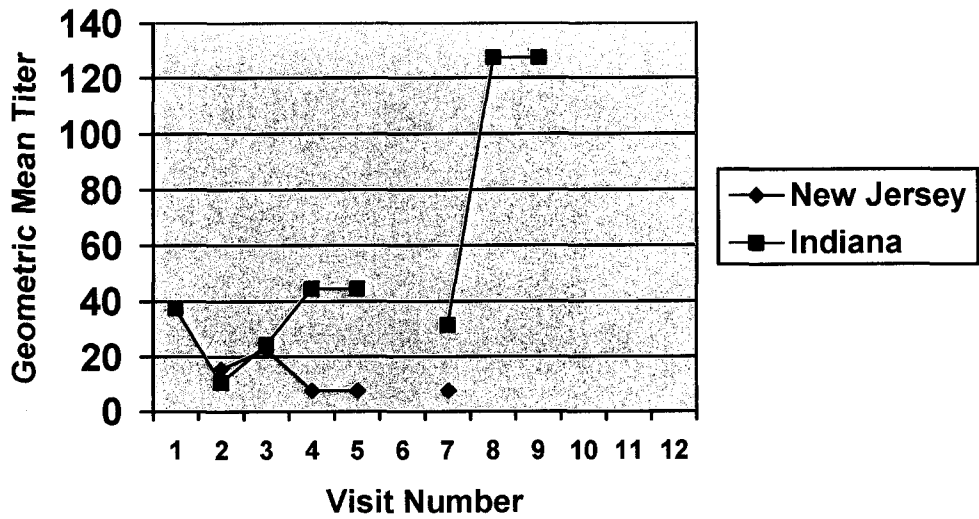
*Landcover:* Grassland

*Soil:* Razor-Midway-Limon

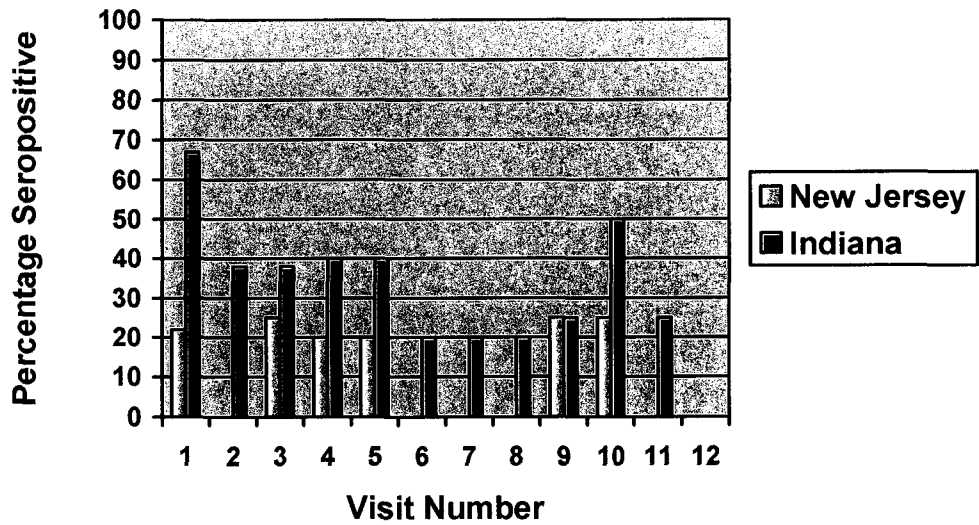
*Plant Hardiness Zone:* 5b



**Premises 97CO0034**



**Premises 97CO0034**



**CO-7**

*Initial Date:* 7/30/97

*Number of Visits:* 12

*County:* Pueblo

*Coordinates:* 38.26671 N  
104.64584 W

*Slope:* 4.393377

*Aspect:* 12.528808 N

*Elevation:* 1498.9 m



*Domain:* Dry

*Division:* Temperate Steppe

*Province:* Great Plains-Palouse  
Dry Steppe

*Section:* Arkansas Tablelands

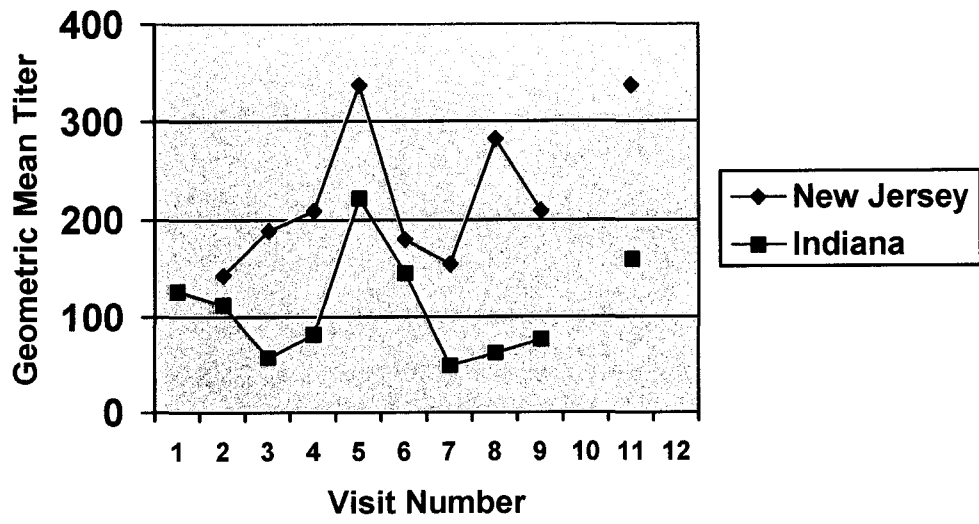
*Landcover:* Urban or built-up land

*Soil:* Cascajo-Limon-Glenberg

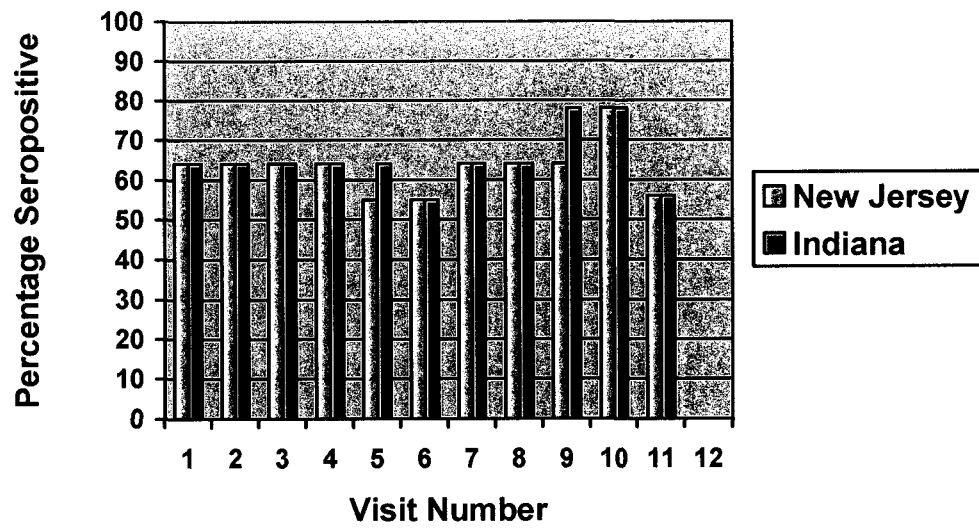
*Plant Hardiness Zone:* 5a



Premises 97CO0036



Premises 97CO0036



**CO-8**

*Initial Date:* 8/17/97

*Number of Visits:* 9

*County:* La Plata

*Coordinates:* 37.10971 N  
107.54827 W

*Slope:* 4.058664

*Aspect:* 229.763641 SW

*Elevation:* 2007 m



*Domain:* Dry

*Division:* Temperate Steppe  
Regime Mountains

*Province:* Southern Rocky Mountain  
Steppe-Open Woodland-Coniferous  
Forest-Alpine Meadow

*Section:* South-Central Highlands

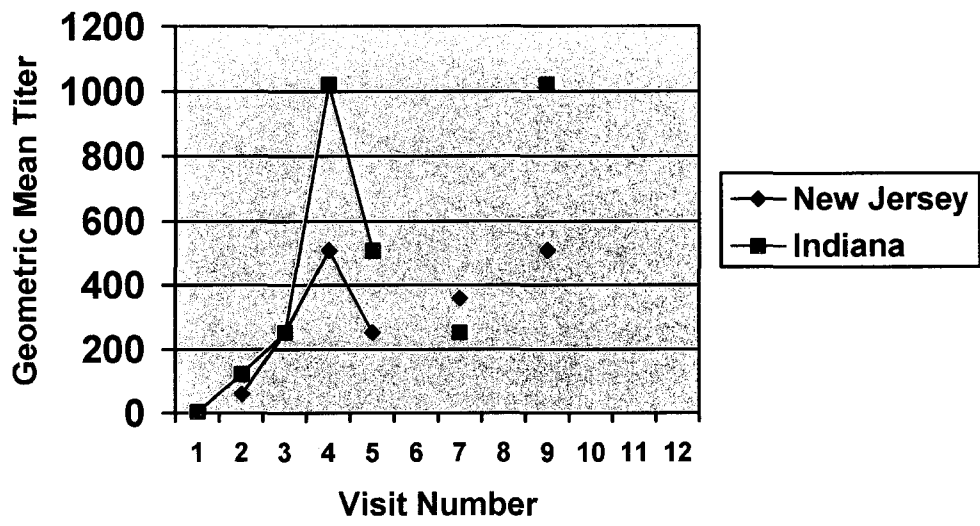
*Landcover:* Evergreen Needleleaf forest

*Soil:* Arboles-Bayfield-Zyme

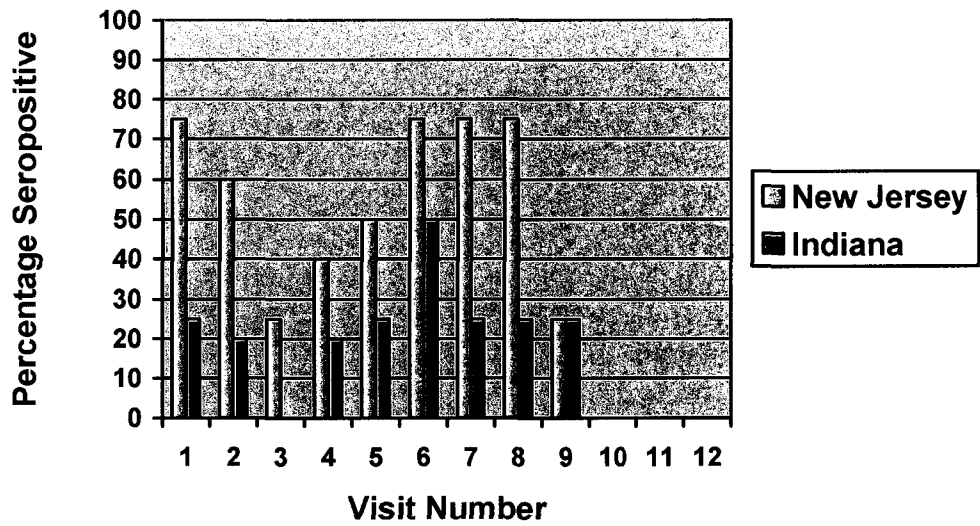
*Plant Hardiness Zone:* 5b



### Premises 97CO0075



### Premises 97CO0075



**CO-9**

*Initial Date:* 8/20/97

*Number of Visits:* 12

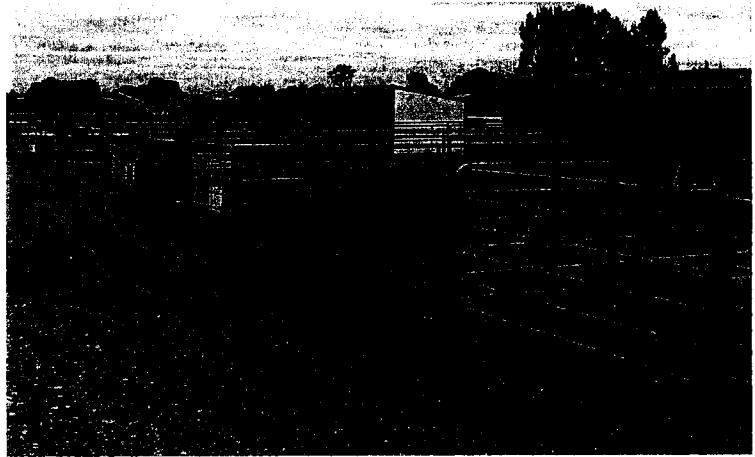
*County:* Pueblo

*Coordinates:* 38.242929 N  
104.49764 W

*Slope:* 1.217117

*Aspect:* 11.309933 N

*Elevation:* 1421 m



*Domain:* Dry

*Division:* Temperate  
Steppe

*Province:* Great Plains-  
Palouse Dry Steppe

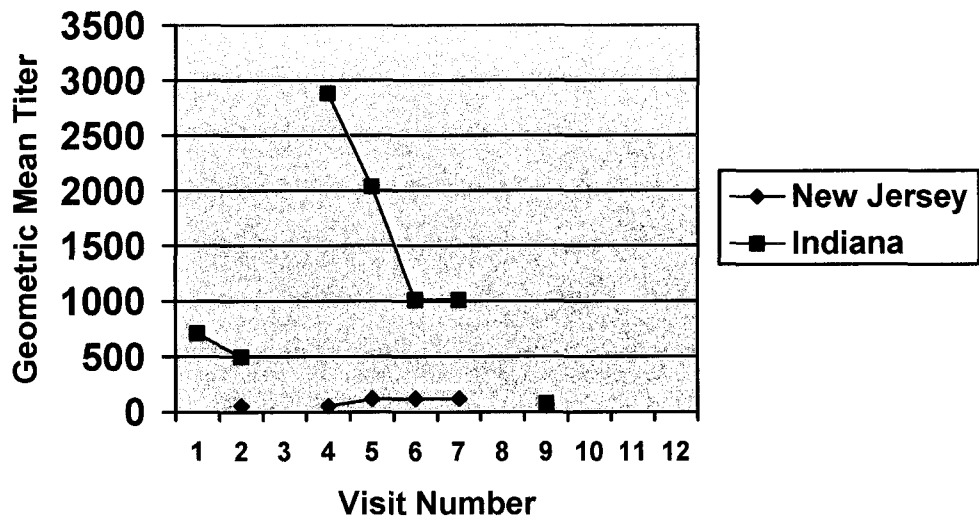
*Section:* Arkansas  
Tablelands

*Landcover:* Grassland

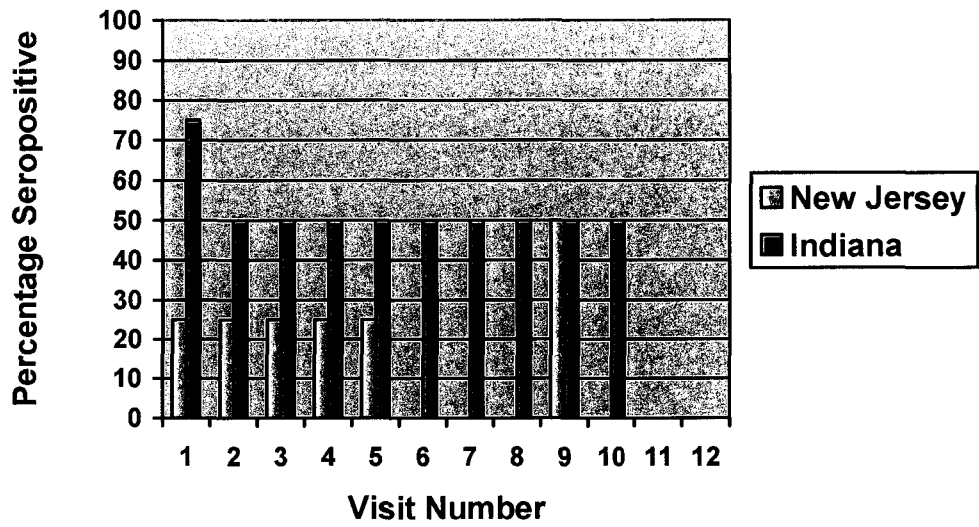
*Soil:* Rocky Ford-Numa-  
Nepesta

*Plant Hardiness Zone:*  
5b

### Premises 97CO0086



### Premises 97CO0086



**CO-10**

*Initial Date:* 9/24/97

*Number of Visits:* 12

*County:* Pueblo

*Coordinates:* 38.22202 N  
104.49744 W

*Slope:* 4.16874

*Aspect:* 300.96 NW

*Elevation:* 1417.33 m

*Domain:* Dry

*Division:* Temperate Steppe

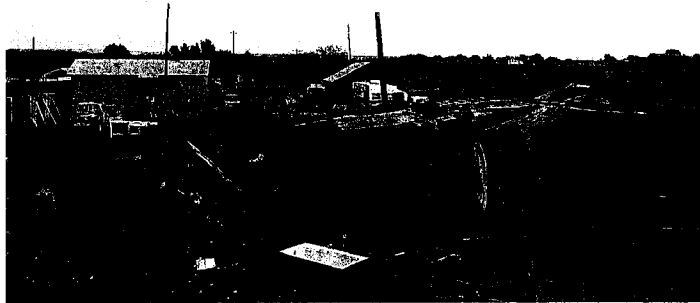
*Province:* Great Plains-  
Palouse Dry Steppe

*Section:* Arkansas  
Tablelands

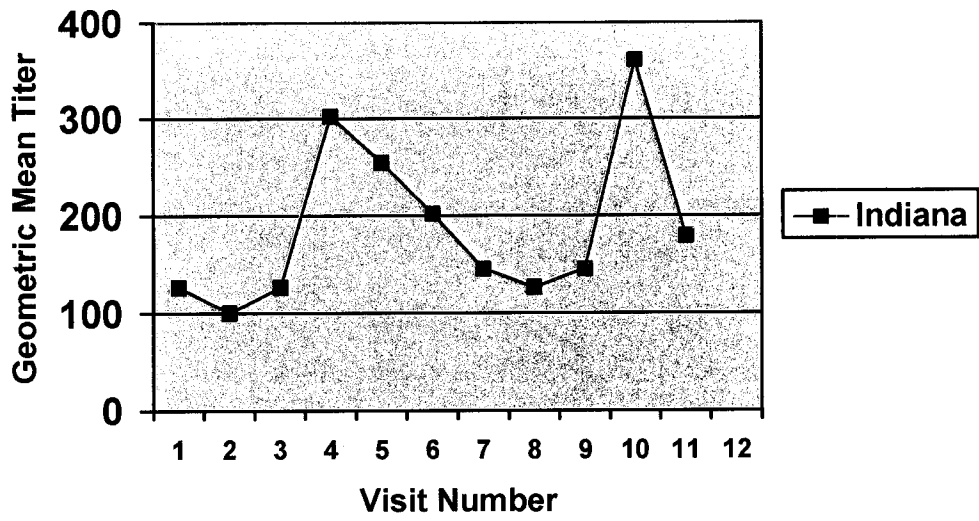
*Landcover:* Grassland

*Soil:* Cascajo-Limon-Glenberg

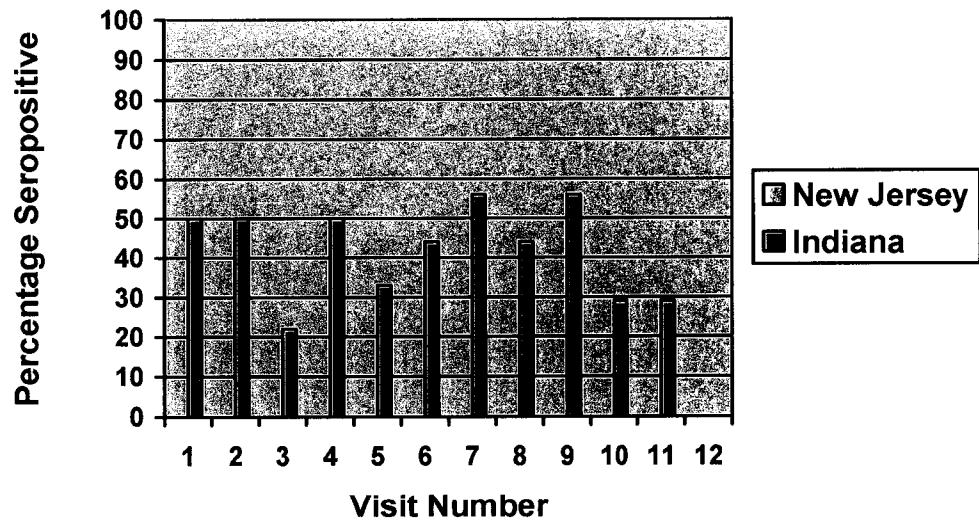
*Plant Hardiness Zone:* 5b



**Premises 95CO0183**



**Premises 97CO0183**



**CO-11**

*Initial Date:* 9/29/02

*Number of Visits:* 12

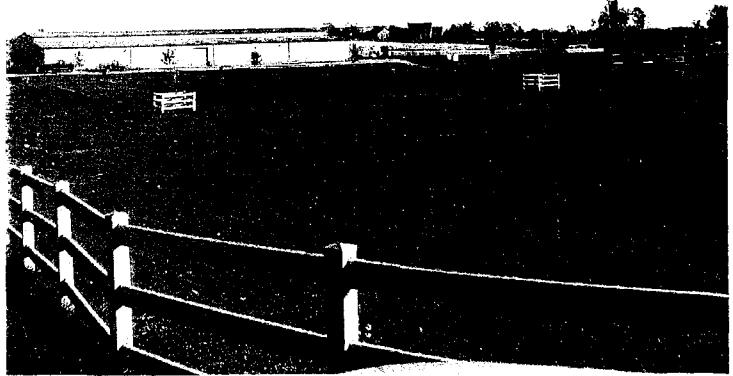
*County:* Larimer

*Coordinates:* 40.18856 N  
105.17622 W

*Slope:* 2.862405

*Aspect:* 180 S

*Elevation:* 1555 m



*Domain:* Dry

*Division:*  
Temperate

*Province:* Southern  
Rocky Mountain  
Steppe-Open  
Woodland-  
Coniferous Forest-  
Alpine Meadow

*Section:* Northern  
Parks and Ranges

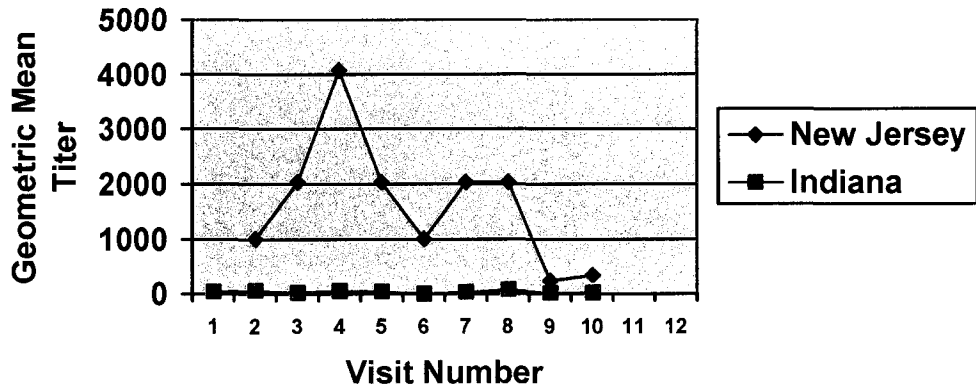
*Landcover:* Cropland/Grassland Mosaic

*Soil:* Fluvaquents-  
Alda-Bankard

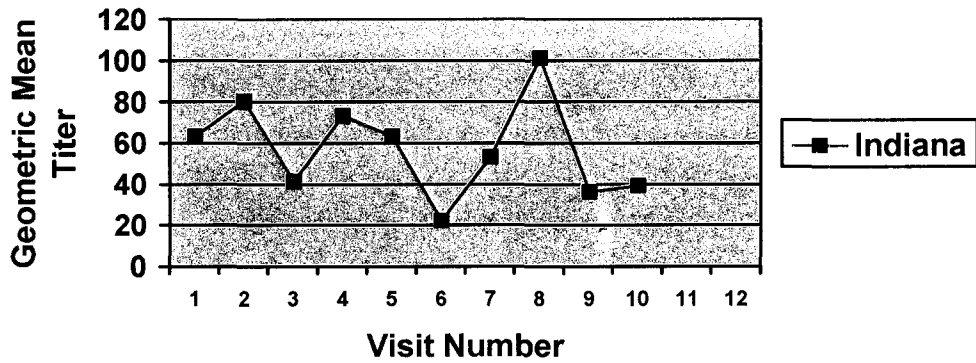
*Plant Hardiness Zone:* 5a



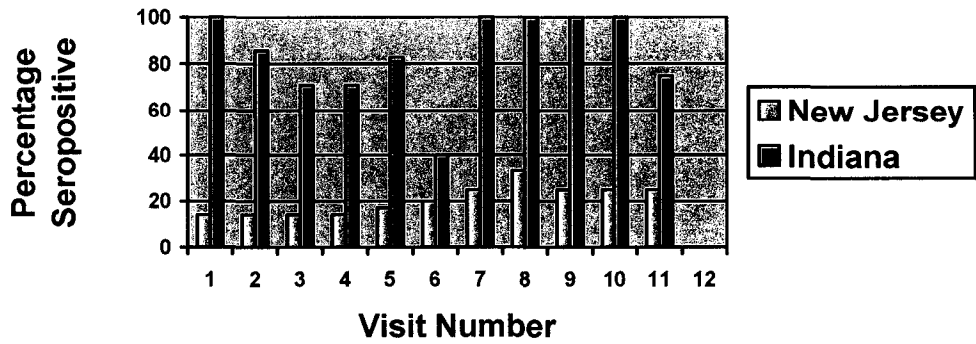
**Premises 97CO0205**



**Premises 97CO0205**



**Premises 97CO0205**



**CO-12**

*Initial Date:* 9/28/97

*Number of Visits:* 4

*County:* Mesa

*Coordinates:* 39.18476 N  
108.74568 W

*Slope:* 2.263636

*Aspect:* 198.43 S

*Elevation:* 1390 m

*Domain:* Dry

*Division:* Temperate Desert

*Province:* Intermountain Semi-Desert  
and Desert

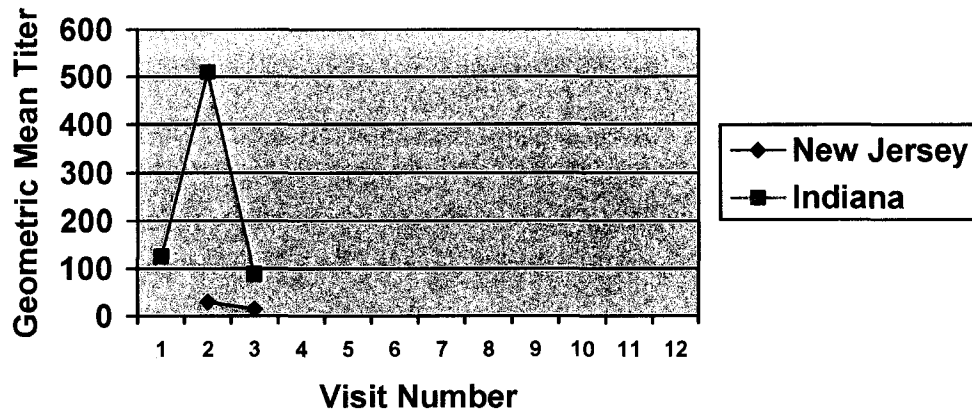
*Section:* Northern Canyon Lands

*Landcover:* Cropland/Grassland Mosaic

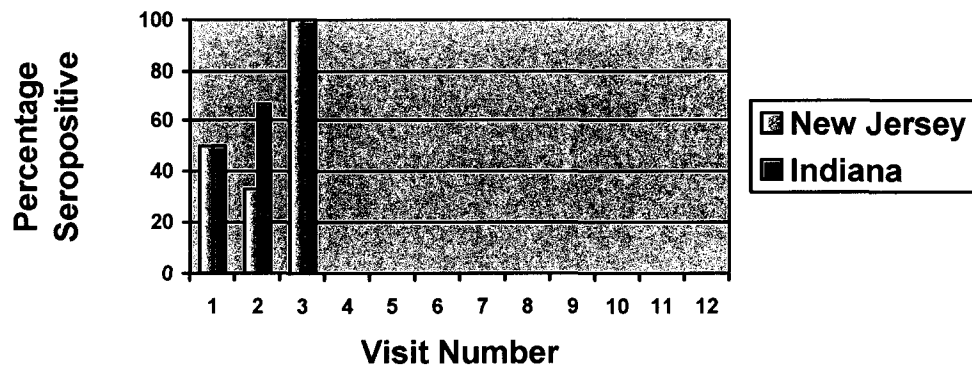
*Soil:* Billing-Rayola-Fruita

*Plant Hardiness Zone:* 6a

**Premises 97CO0212**



**Premises 97CO0212**



**CO-13**

*Initial Date:* 9/26/97

*Number of Visits:* 9

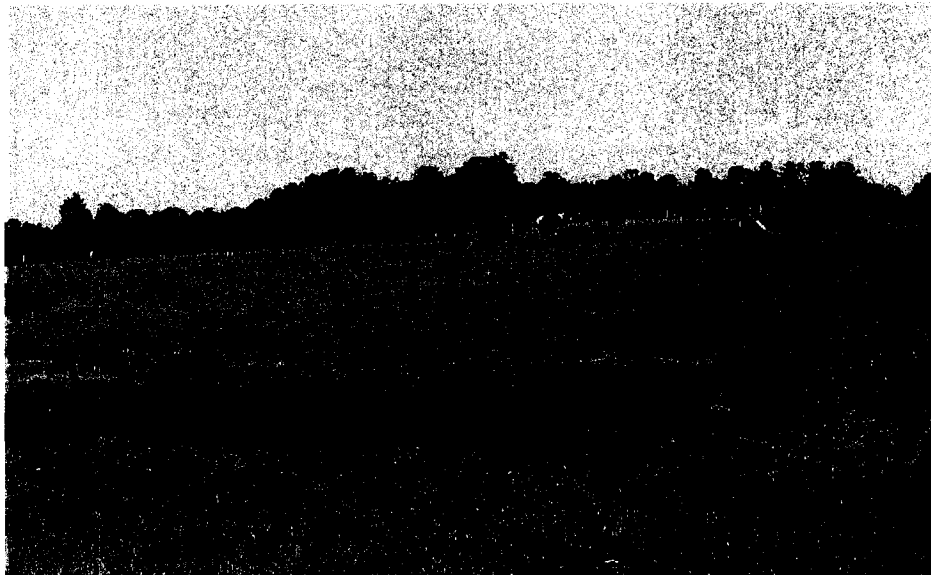
*County:* La Plata

*Coordinates:* 37.26832 N  
107.75026 W

*Slope:* 3.109633

*Aspect:* 212.471191 SW

*Elevation:* 2174 m



*Domain:* Dry

*Division:*  
Temperate  
Steppe Regime  
Mountains

*Province:*  
Southern Rocky  
Mountain  
Steppe-Open  
Woodland-  
Coniferous  
Forest-Alpine  
Meadow

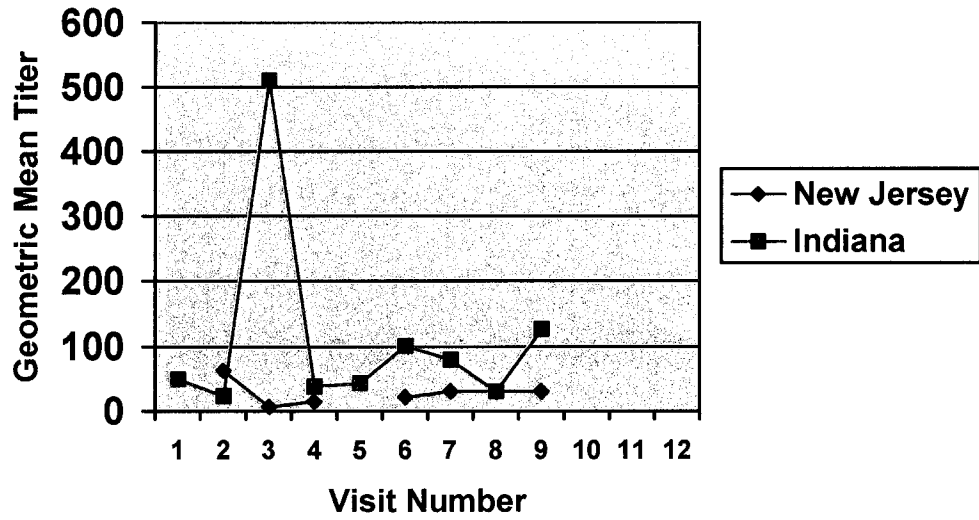
*Section:* South-Central Highlands

*Landcover:* Irrigated cropland and pasture

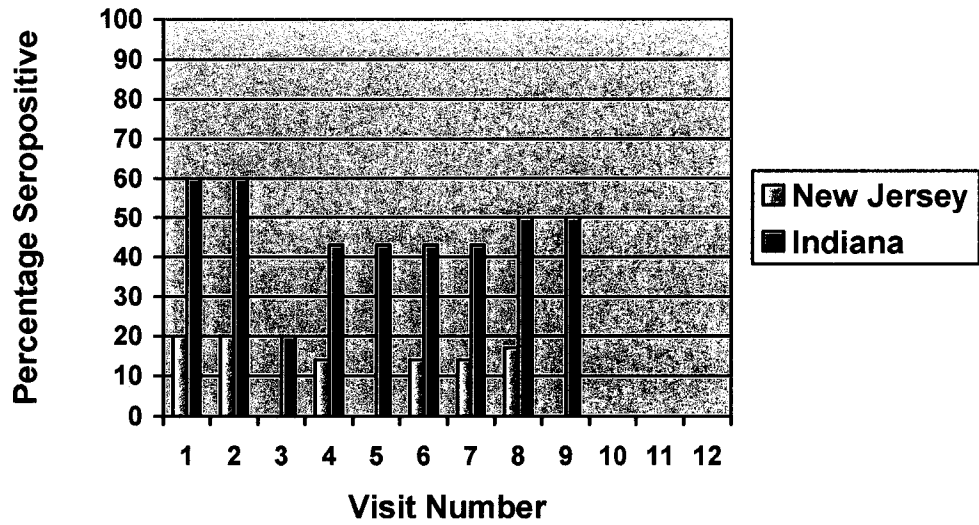
*Soil:* Archuleta-Goldvale-Sanchez

*Plant Hardiness Zone:*  
5a

### Premises 97CO0220



### Premises 97CO0220



**CO-14**

*Initial Date:* 10/01/97

*Number of Visits:* 9

*County:* Mesa

*Coordinates:* 39.13123 N  
108.65025 W

*Slope:* 0

*Aspect:* -1°

*Elevation:* 1386 m

*Domain:* Dry

*Division:* Temperate Desert

*Province:* Intermountain Semi-Desert and Desert

*Section:* Northern Canyon Lands

*Landcover:* Cropland/Grassland Mosaic

*Soil:* Billing-Rayola-Fruita

*Plant Hardiness Zone:* 6b



**CO-15**

*Initial Date:* 10/03/97

*Number of Visits:* 4

*County:* Mesa

*Coordinates:* 38.67885 N  
108.98167 W

*Slope:* 1.817522

*Aspect:* 336.80 NW

*Elevation:* 1386 m

*Domain:* Dry

*Division:* Temperate Desert

*Province:* Intermountain Semi-Desert  
and Desert

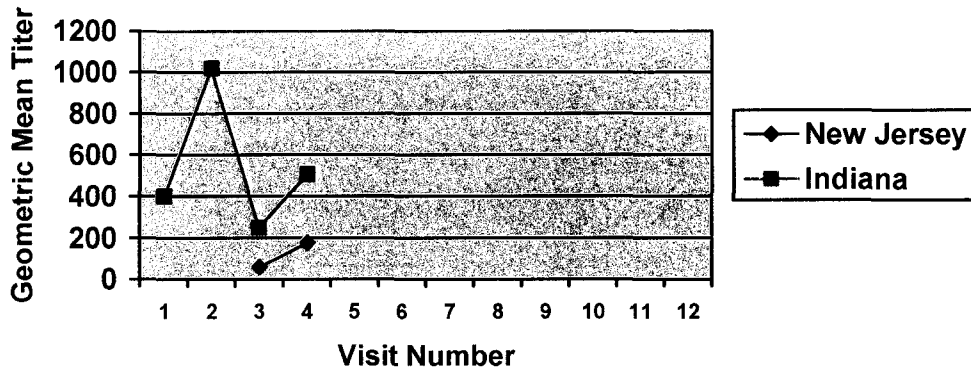
*Section:* Northern Canyon Lands

*Landcover:* Grassland

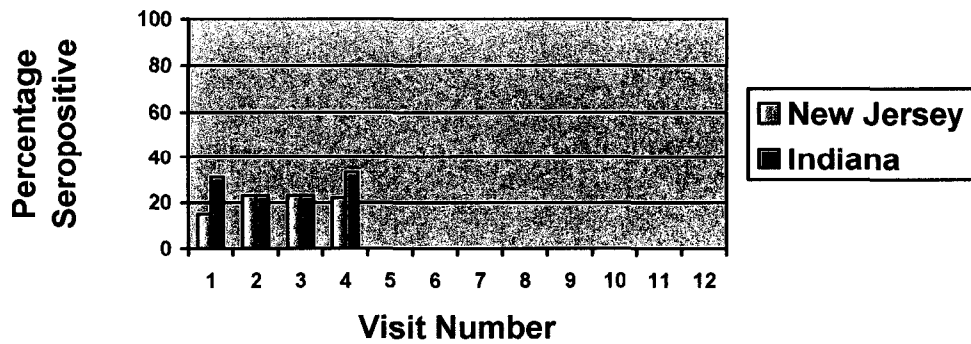
*Soil:* Begay-Moab-Redbank

*Plant Hardiness Zone:* 6b

**Premises 98CO0010**



**Premises 98CO0010**



**CO-16**

*Initial Date:* 10/16/97

*Number of Visits:* 6

*County:* Boulder

*Coordinates:* 40.18845 N  
105.17294 W

*Slope:* 0

*Aspect:* -1°

*Elevation:* 1554 m

*Domain:* Dry

*Division:* Temperate Steppe Regime  
Mountains

*Province:* Southern Rocky Mountain  
Steppe-Open Woodland-Coniferous  
Forest-Alpine Meadow

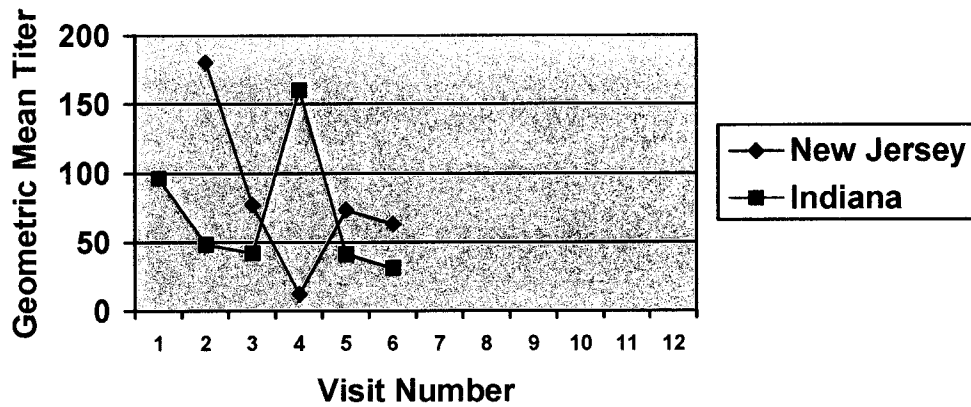
*Section:* Northern Parks and Ranges

*Landcover:* Cropland/Grassland Mosaic

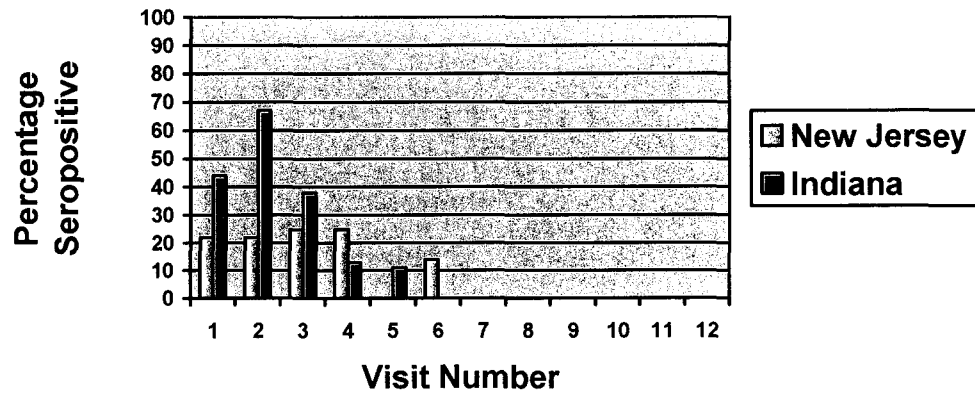
*Soil:* Fluvaquents-Alda-Bankard

*Plant Hardiness Zone:* 5a

**Premises 98CO0072**



### Premises 98CO0072



**CO-17**

*Initial Date:* 10/20/97

*Number of Visits:* 12

*County:* Jefferson

*Coordinates:* 39.80462 N  
104.90703 W

*Slope:* 2.7881886

*Aspect:* 329.04 NW

*Elevation:* 1583.6 m

*Domain:* Dry

*Division:* Temperate Steppe

*Province:* Great Plains-Palouse Dry Steppe

*Section:* Central High Plains

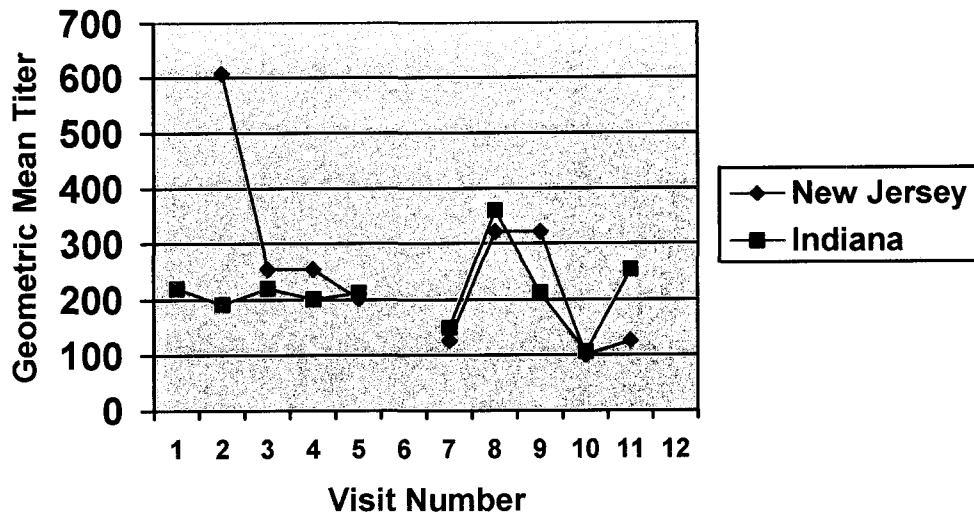
*Landcover:* Urban or built up land

*Soil:* Otero-Thedaluno-Olney

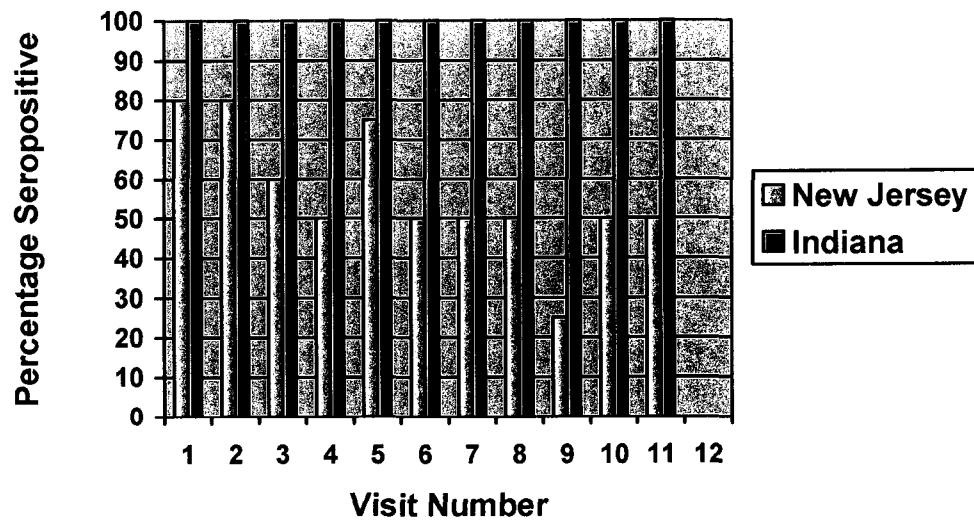
*Plant Hardiness Zone:* 5b



**Premises 98CO0107**



**Premises 98CO0107**



**CO-18**

*Initial Date:* 10/21/97

*Number of Visits:* 12

*County:* Jefferson

*Coordinates:* 39.84765 N  
104.95324 W

*Slope:* 8.792413

*Aspect:* 94.635460 E

*Elevation:* 1566.5 m

*Domain:* Dry

*Division:* Temperate Steppe

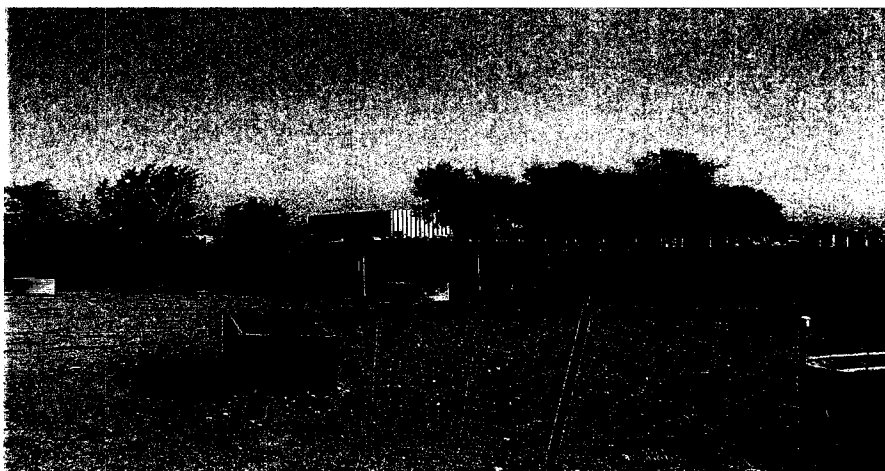
*Province:* Great Plains-Palouse Dry Steppe

*Section:* Central High Plains

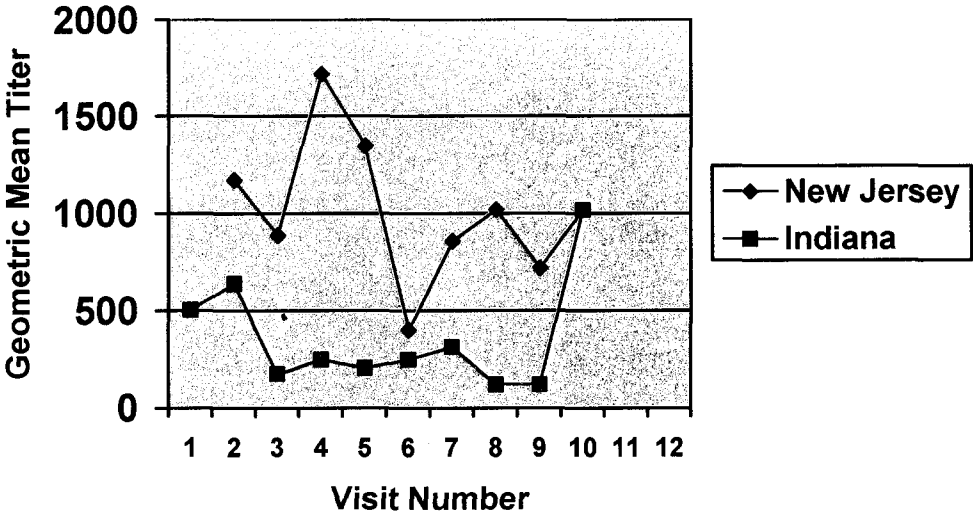
*Landcover:* Grassland

*Soil:* Nederland-Denver-Kutch

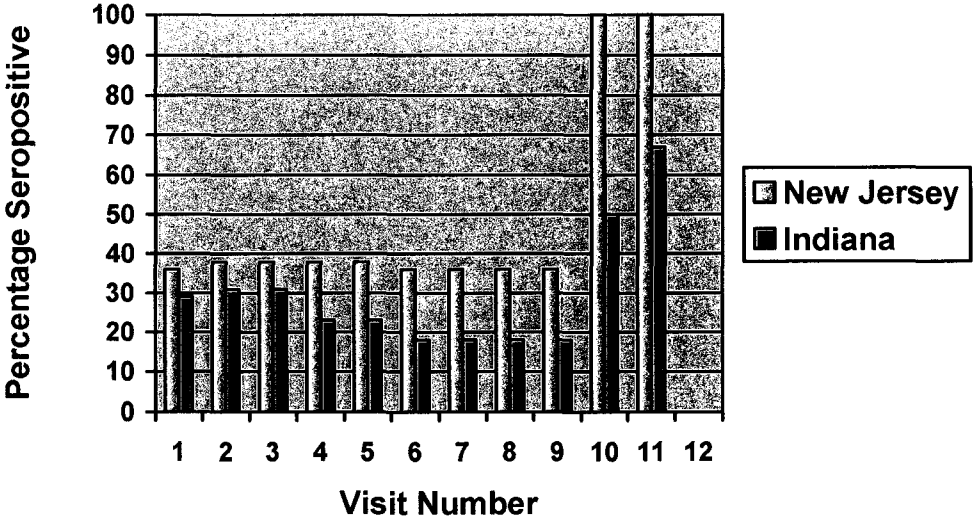
*Plant Hardiness Zone:* 5b



**Premises 98CO0113**



**Premises 98CO0113**



**CO-19**

*Initial Date:* 8/12/98

*Number of Visits:* 11

*County:* Weld

*Coordinates:* 40.35105 N  
105.12134 W

*Slope:* 8.792413

*Aspect:* 270 W

*Elevation:* 1546 m

*Domain:* Dry

*Division:* Temperate Steppe Regime  
Mountains

*Province:* Southern Rocky Mountain  
Steppe-Open Woodland-Coniferous  
Forest-Alpine Meadow

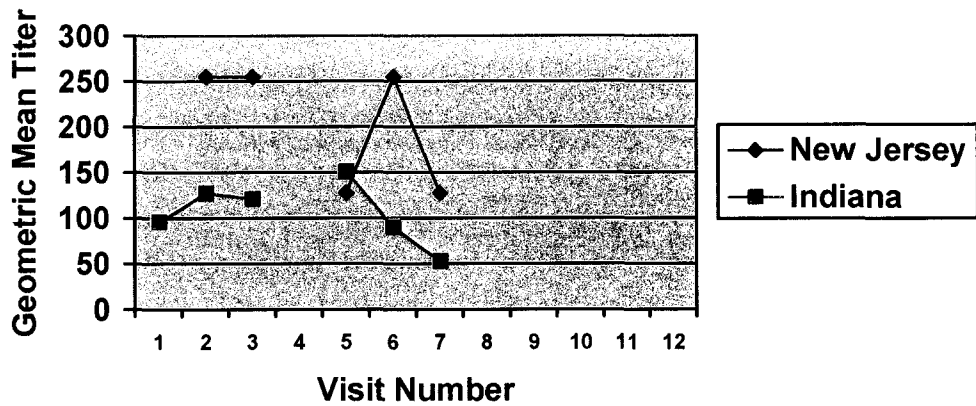
*Section:* Northern Parks and Ranges

*Landcover:* Cropland/Grassland Mosaic

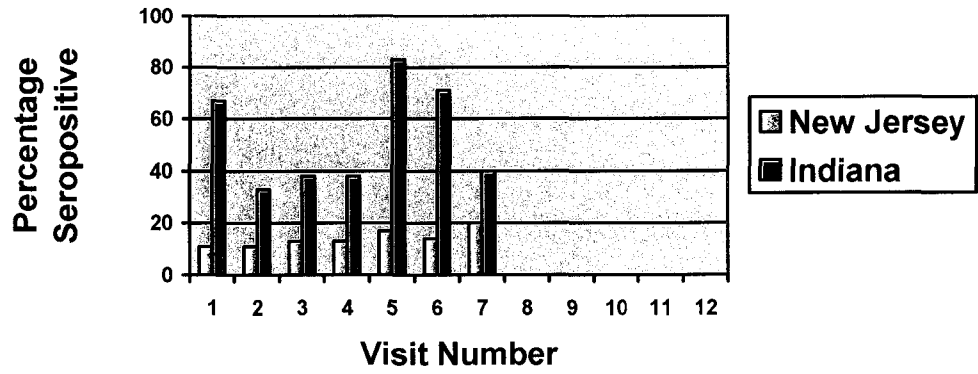
*Soil:* Nunn-Ulm-Englewood

*Plant Hardiness Zone:* 5a

**Premises 98CO0209**



### Premises 98CO0209



**CO-20**

*Initial Date:* 9/30/98

*Number of Visits:* 10

*County:* Larimer

*Coordinates:* 40.27404 N  
105.45963 W



*Slope:* 5.710593

*Aspect:* 180 S

*Elevation:* 2439 m

*Domain:* Dry

*Division:* Temperate Steppe Regime Mountains

*Province:* Southern Rocky Mountain Steppe-Open Woodland-Coniferous Forest-Alpine Meadow

*Section:* Northern Parks and Ranges

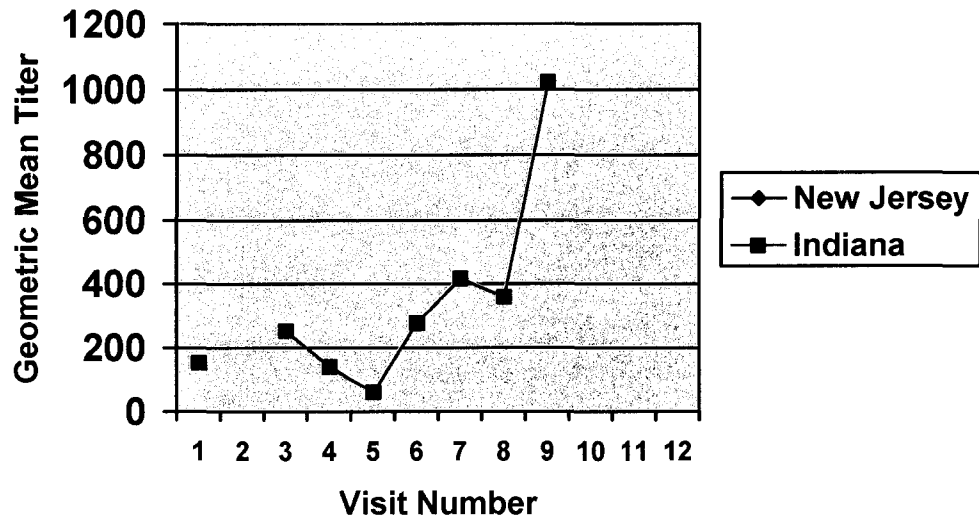
*Landcover:* Cropland/Grassland Mosaic

*Soil:* Ratake-Wetmore-Rock Outcrop

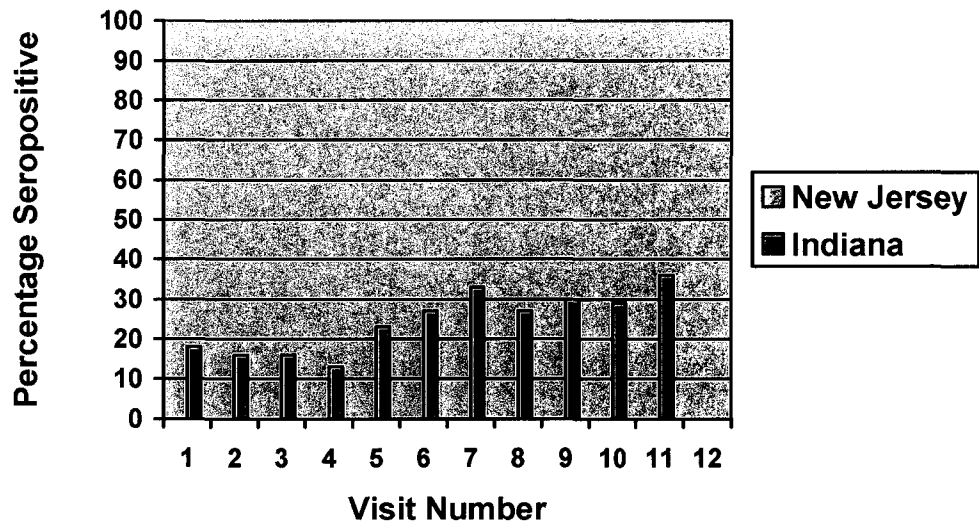
*Plant Hardiness Zone:* 5a



### Premises 98CO0304



### Premises 98CO0304



**CO-21**

*Initial Date:* 12/02/99

*Number of Visits:* 5

*County:* Mesa

*Coordinates:* 39.27257 N  
108.73865 W

*Slope:*

*Aspect:*

*Elevation:*

*Domain:* Dry

*Division:* Temperate Desert

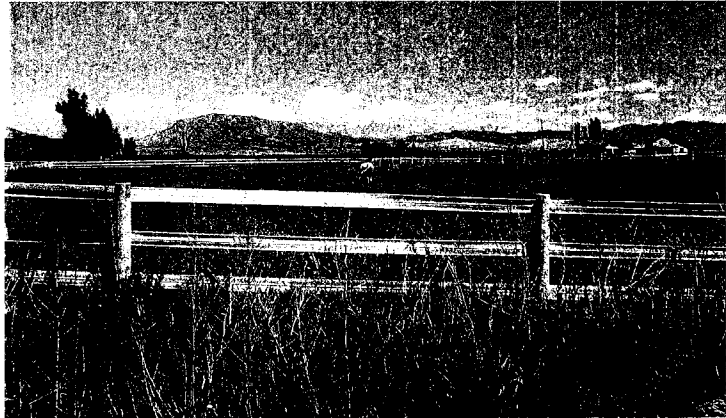
*Province:* Intermountain Semi-Desert and Desert

*Section:* Northern Canyon Lands

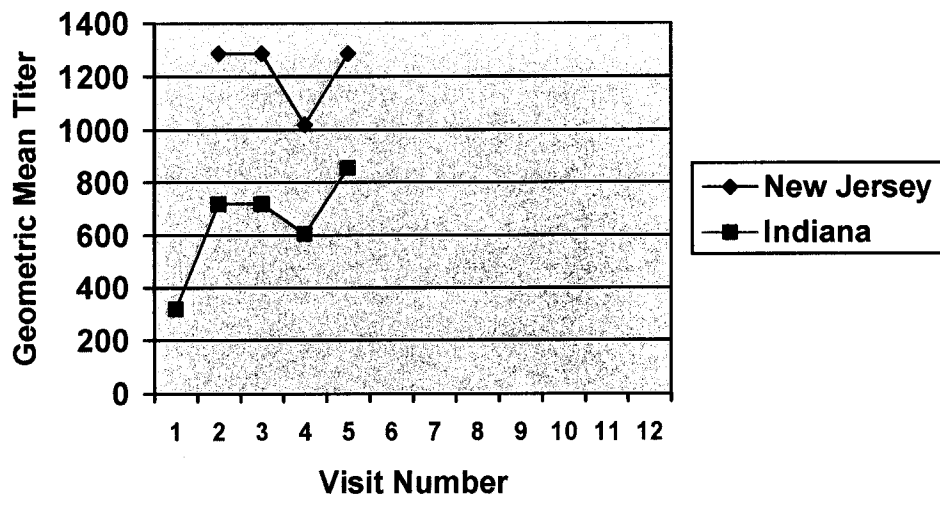
*Landcover:* Shrubland

*Soil:* Chipeta-Persayo-Badland

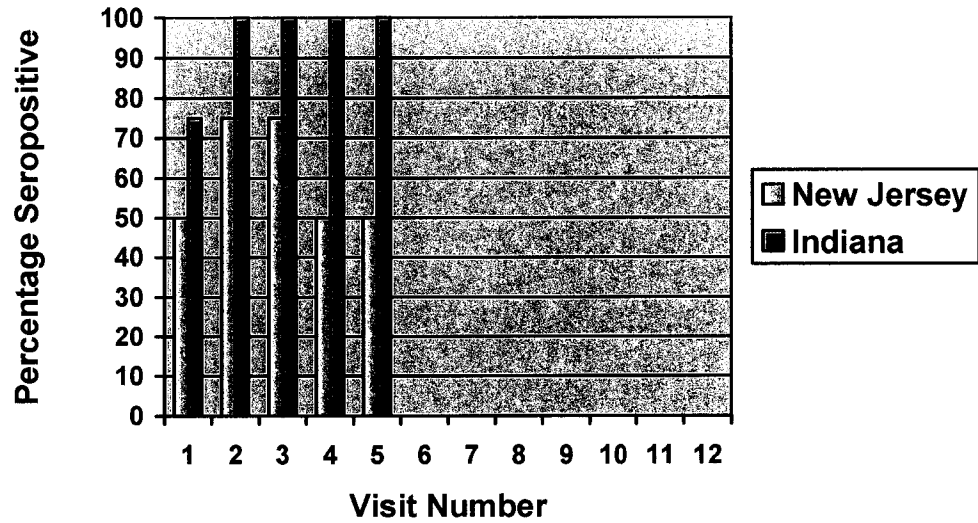
*Plant Hardiness Zone:* 6a



Premises 99CO0001



Premises 99CO0001



**NM-1**

*Initial Date:* 6/6/95

*Number of Visits:* 6

*County:* Valencia

*Coordinates:* 34.85007 N  
106.68886 W

*Slope:* 1.01275

*Aspect:* 225 SW

*Elevation:* 1489.6 m

*Domain:* Dry

*Division:* Tropical/Subtropical Steppe

*Province:* Colorado Plateau Semi-Desert

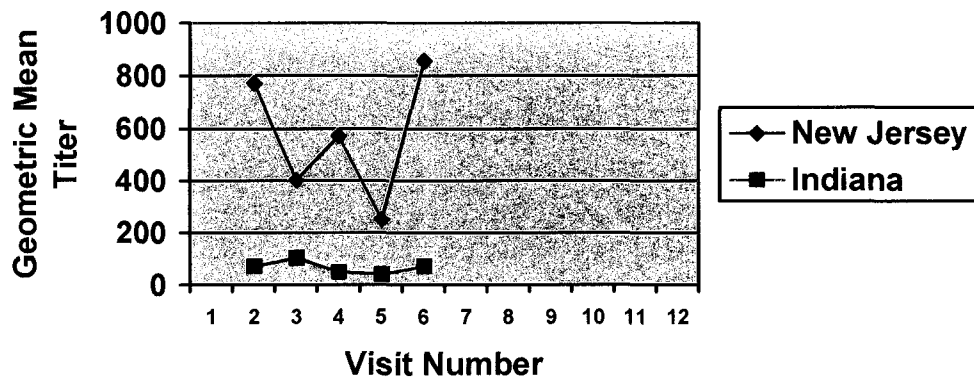
*Section:* Northern Rio Grande Intermountain

*Landcover:* Evergreen needleleaf forest

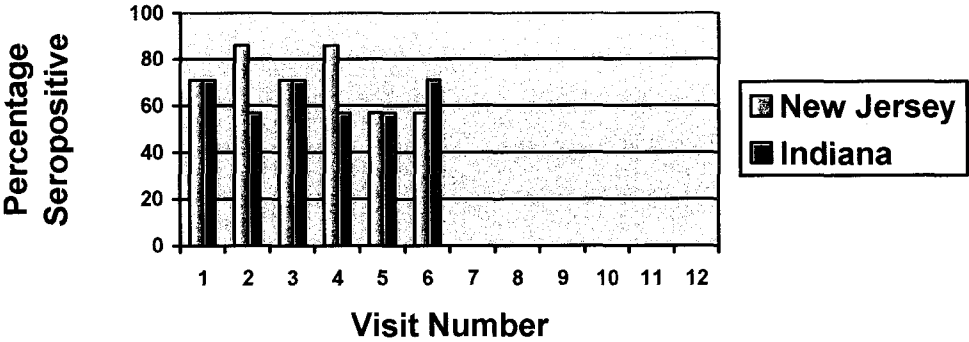
*Soil:* Gila-Bluepoint-Riverwash

*Plant Hardiness Zone:* 7a

**Premises 95NM0002**



Premises 95NM0002



**NM-2**

*Initial Date:* 6/17/95

*Number of Visits:* 7

*County:* Socorro

*Coordinates:* 34.22999 N  
106.91208 W

*Slope:* 2.263636

*Aspect:* 108.434952 E

*Elevation:* 1787 m

*Domain:* Dry

*Division:* Tropical/Subtropical Steppe

*Province:* Colorado Plateau Semi-Desert

*Section:* Northern Rio Grande Intermontane

*Landcover:* Grassland

*Soil:* Glendale-Armijo-Harkey

*Plant Hardiness Zone:* 7a

**NM-3**

*Initial Date:* 6/29/95

*Number of Visits:* 5

*County:* Sandoval

*Coordinates:* 35.27123 N  
106.60408 W

*Slope:* 3.199601

*Aspect:* 116.56 E

*Elevation:* 1532 m

*Domain:* Dry

*Division:* Tropical/Subtropical Steppe

*Province:* Colorado Plateau Semi-Desert

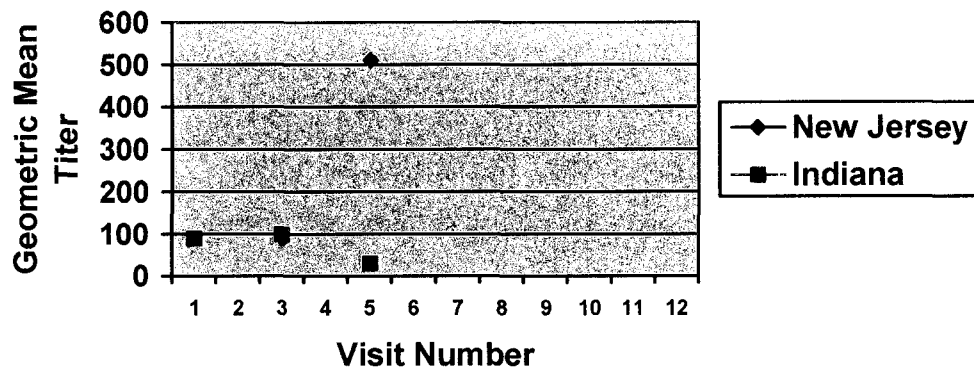
*Section:* Northern Rio Grande Intermontane

*Landcover:* Grassland

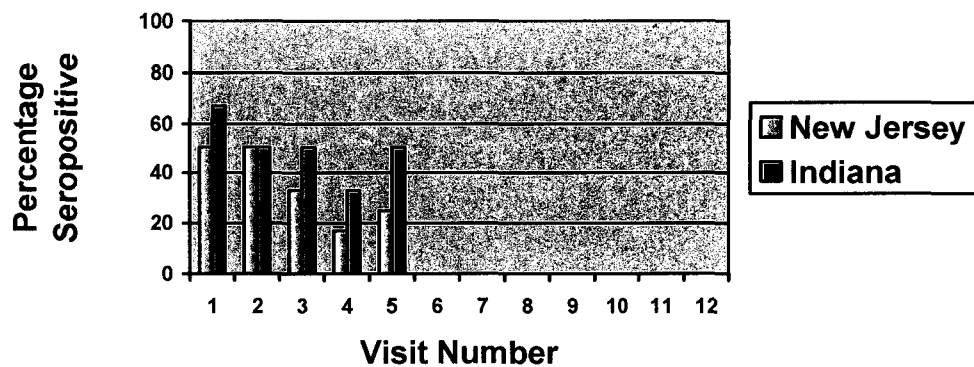
*Soil:* ND

*Plant Hardiness Zone:* 7a

**Premises 95NM0115**



**Premises 95NM0115**



**NM-4**

*Domain:* Dry

*Initial Date:* 7/11/95

*Division:* Temperate Steppe Regime Mountains

*Number of Visits:* 6

*County:* Sandoval

*Province:* Southern Rocky Mountain Steppe-Open Woodland-Coniferous Forest-Alpine Meadow

*Coordinates:* 35.60918 N  
106.34414 W

*Section:* South-Central Highlands

*Slope:* 13.3780465

*Landcover:* Grassland

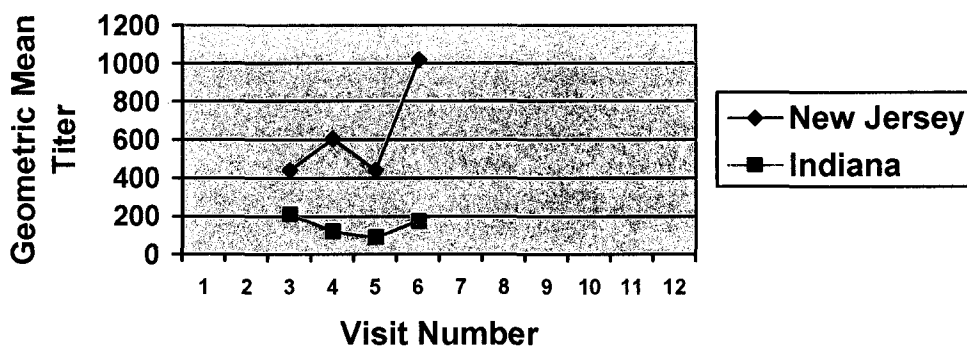
*Aspect:* 93.012787 E

*Soil:* Cochiti-Espiritu-Waijmac

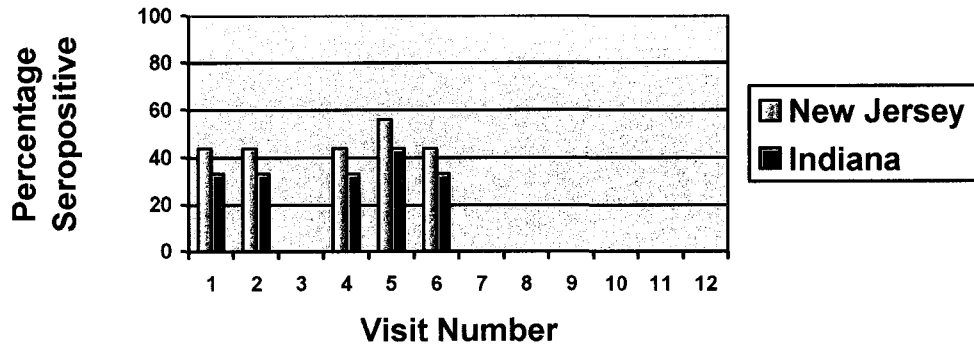
*Elevation:* 1601.9 m

*Plant Hardiness Zone:* 6

### Premises 95NM0216



### Premises 95NM0216



**NM-5**

*Initial Date:* 7/12/95

*Number of Visits:* 3

*County:* Sandoval

*Coordinates:* 35.25661 N  
106.60016 W

*Slope:* 0

*Aspect:* -1' flat

*Elevation:* 1532 m

*Domain:* Dry

*Division:* Tropical/Subtropical Steppe

*Province:* Colorado Plateau Semi-Desert

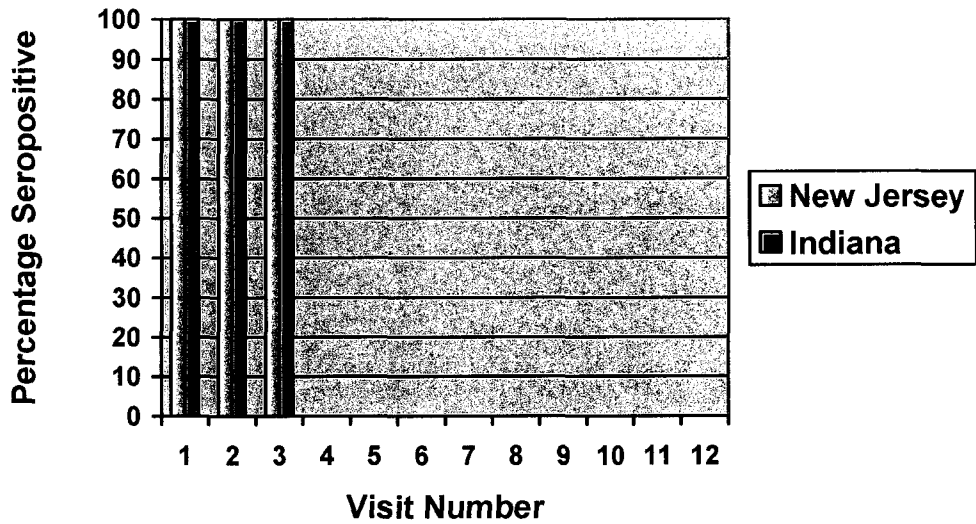
*Section:* Northern Rio Grande Intermontane

*Landcover:* Grassland

*Soil:* Gilco-Vintas-Aga

*Plant Hardiness Zone:* 7a

**Premises 95NM0255**



**NM-6**

*Initial Date:* 6/23/97

*Number of Visits:* 7

*County:* Valencia

*Coordinates:* 34.73671 N  
106.70966 W

*Slope:* 0

*Aspect:* -1' flat

*Elevation:* 1472.8 m

*Domain:* Dry

*Division:* Tropical/Subtropical Steppe

*Province:* Colorado Plateau Semi-Desert

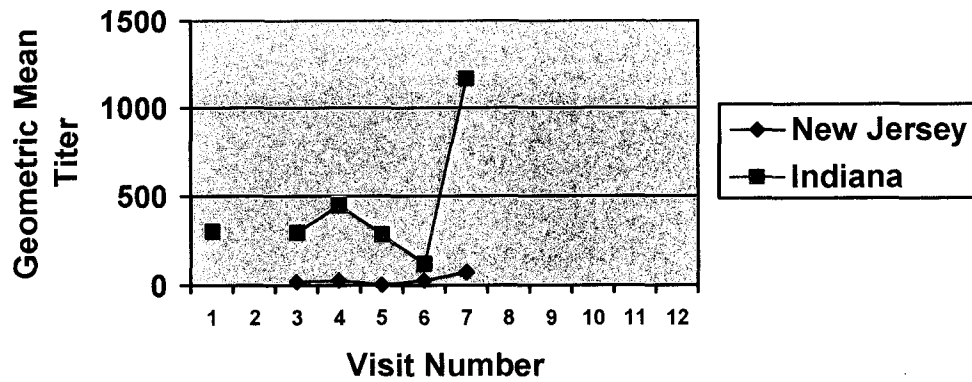
*Section:* Northern Rio Grande Intermontane

*Landcover:* Shrubland

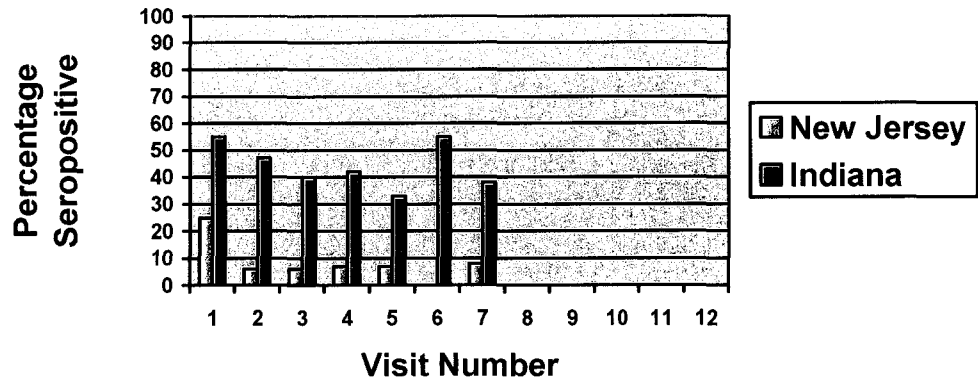
*Soil:* Gila-Bluepoint-Riverwash

*Plant Hardiness Zone:* 7a

**Premises 97NM0020**



### Premises 97NM0020



**NM-7**

*Initial Date:* 7/3/97

*Number of Visits:* 1

*County:* Valencia

*Coordinates:* 34.52035 N  
106.41781 W

*Slope:* 1.817522

*Aspect:* 203.19 SW

*Elevation:* 1958 m

*Domain:* Dry

*Division:* Tropical/Subtropical Steppe

*Province:* Colorado Plateau Semi-Desert

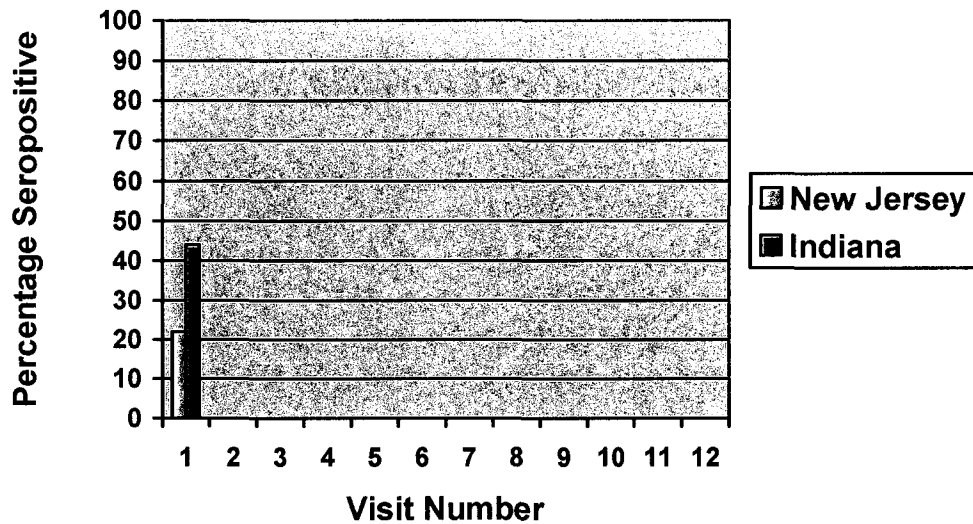
*Section:* Northern Rio Grande Intermontane

*Landcover:* Shrubland

*Soil:* Rock Outcrop-Deama-Ponciano

*Plant Hardiness Zone:* 6b

**Premises 97NM0036**



**NM-8**

*Initial Date:* 7/16/97

*Number of Visits:* 5

*County:* Santa Fe

*Coordinates:* 35.43945 N  
105.54648 W

*Slope:* 8.063246

*Aspect:* 298.072479 NW

*Elevation:* 1993 m

*Domain:* Dry

*Division:* Tropical/Subtropical Regime  
Mountains

*Province:* Arizona-New Mexico  
Mountains Semi-Desert-Open  
Woodland-Coniferous Forest-Alpine  
Meadow

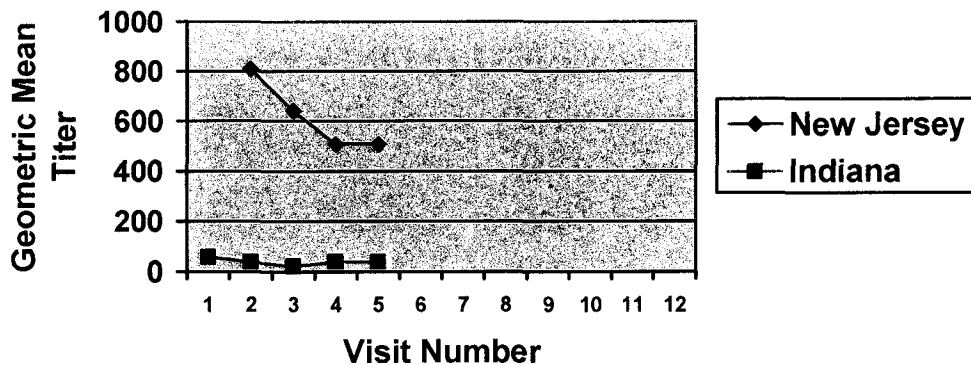
*Section:* Sacramento-Monzano  
Mountain

*Landcover:* Shrubland

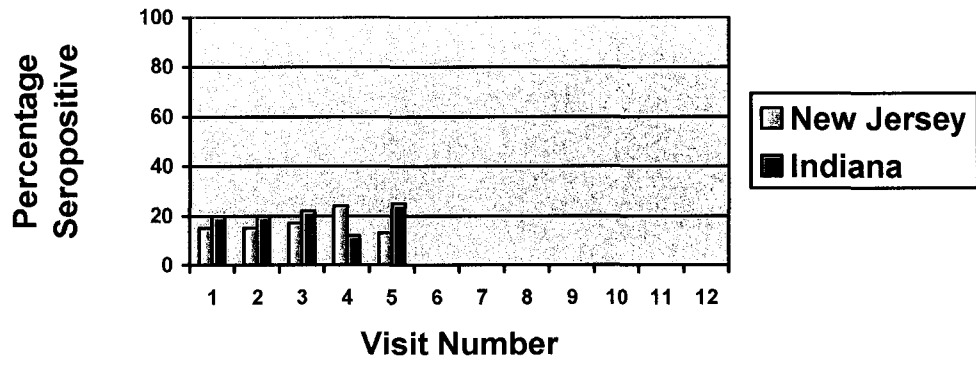
*Soil:* Typic Haplustalfs-eutric  
glossoboralfs-Rock Outcrop

*Plant Hardiness Zone:* 6a

**Premises 97NM0054**



### Premises 97NM0054



**NM-9**

*Elevation:* 1245 m

*Initial Date:* 7/22/97

*Domain:* Dry

*Number of Visits:* 6

*Division:* Tropical/Subtropical Desert

*County:* Dona Ana

*Province:* Chihuahuan Semi-Desert

*Coordinates:* 32.16218 N  
106.46119 W

*Section:* Basin and Range

*Slope:* 0

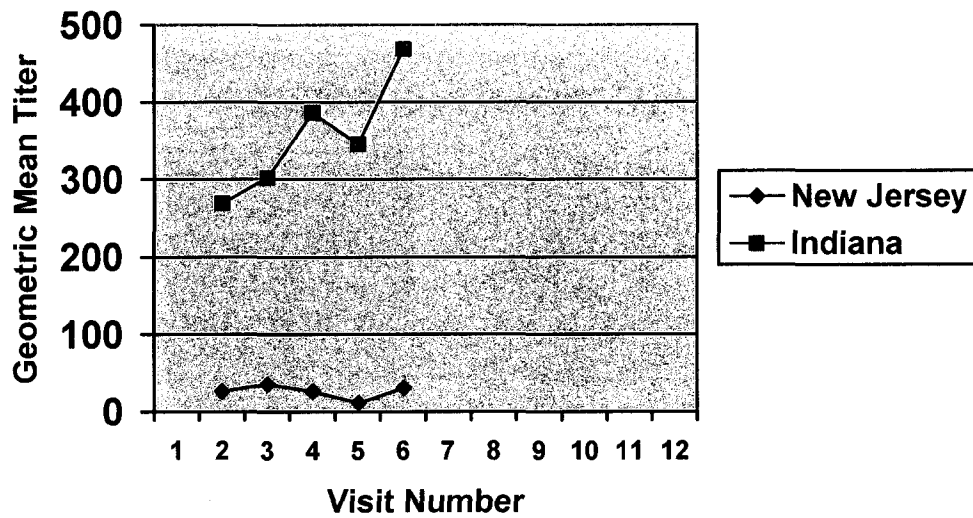
*Landcover:* Shrubland

*Aspect:* -1° flat

*Soil:* Sellar-Mimbres-Mohave

*Plant Hardiness Zone:* 8a

**Premises 97NM0059**



**NM-10**

*Initial Date:* 7/29/97

*Number of Visits:* 6

*County:* San Miguel

*Coordinates:* 35.46953 N  
105.63672 W

*Slope:* 21.743418

*Aspect:* 32.20 NE

*Elevation:* 2041.7 m

*Domain:* Dry

*Division:* Temperate Steppe Regime Mountains

*Province:* Southern Rocky Mountain Steppe-Open Woodland-Coniferous Forest-Alpine Meadow

*Section:* Southern Parks and Ranges

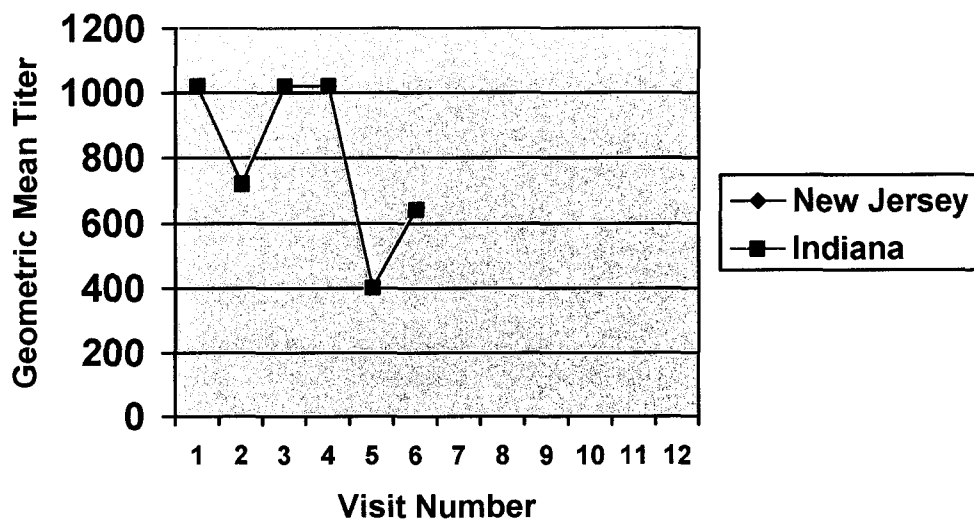
*Landcover:* Shrubland

*Soil:* Vibo-Rivera-Rock Outcrop

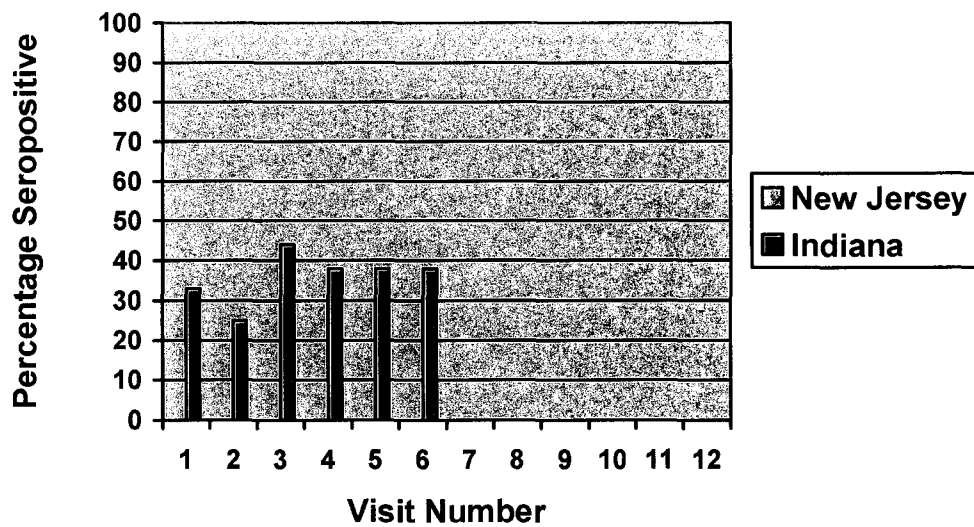
*Plant Hardiness Zone:* 5b



### Premises 97NM0065



### Premises 97NM0065



# NM-11

Initial Date: 7/30/97

Domain: Dry

Number of Visits: 7

Division: Temperate Steppe

County: Rio Arriba

Province: Great Plains-Palouse Dry Steppe

Coordinates: 35.94281 N  
106.08461 W

Section: Upper Rio Grande Basin

Slope: 1.01275

Landcover: Grassland

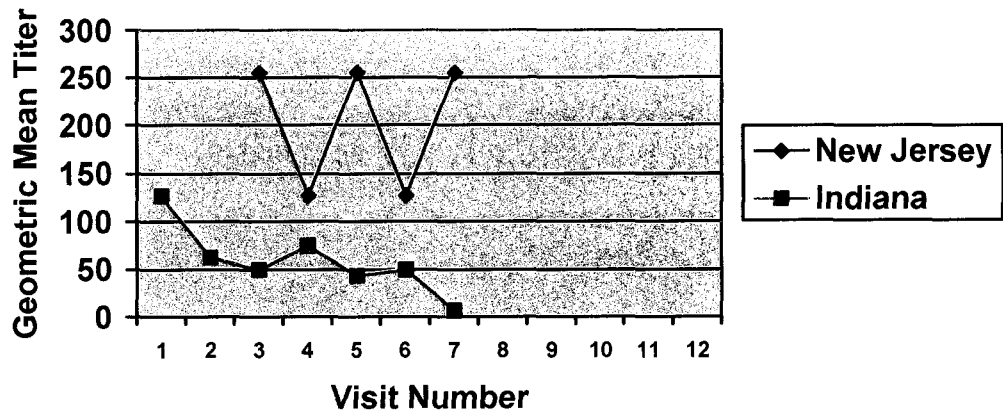
Aspect: 315 NW

Soil: Pojoaque-El Rancho-Fruitland

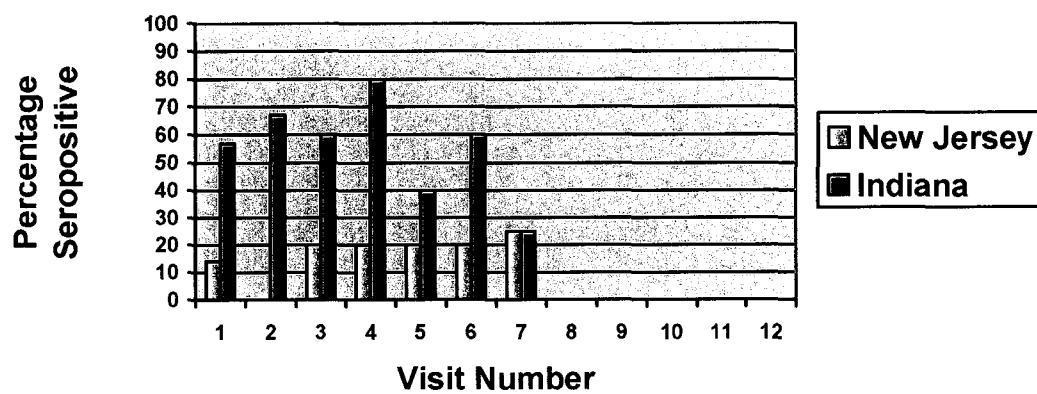
Elevation: 1693.7 m

Plant Hardiness Zone: 6b

## Premises 97NM0067



### Premises 97NM0067



**NM-12**

*Initial Date:* 8/26/97

*Number of Visits:* 6

*County:* San Juan

*Coordinates:* 36.91095 N  
108.20076

W

*Slope:* 0

*Aspect:* -1' flat

*Elevation:* 1767 m

*Domain:* Dry

*Division:* Tropical/Subtropical Steppe

*Province:* Colorado Plateau Semi-Desert

*Section:* Navajo Canyonlands

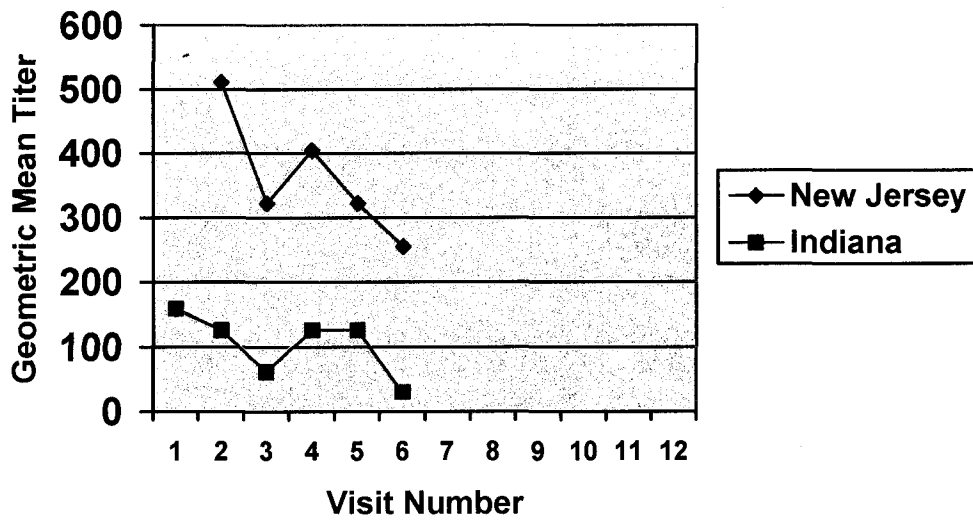
*Landcover:* Deciduous broadleaf forest

*Soil:* Badland-Persayo-Farb

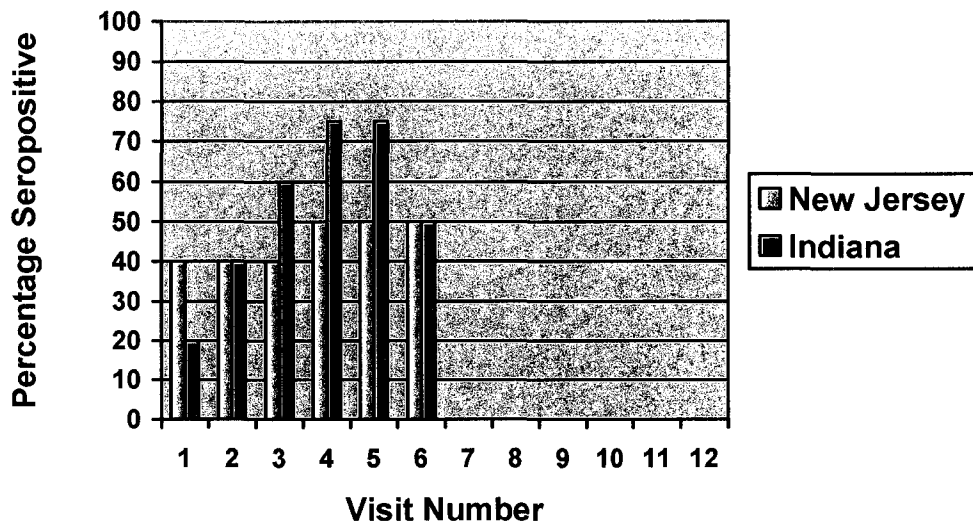
*Plant Hardiness Zone:* 6a



### Premises 97NM0093



### Premises 97NM0093



**NM-13**

*Initial Date:* 9/3/97

*Number of Visits:* 7

*County:* San Juan

*Coordinates:* 36.82646 N  
108.22738 W

*Slope:* 1.432096

*Aspect:* 90.0 E

*Elevation:* 1672 m

*Domain:* Dry

*Division:*  
Tropical/Subtropical Steppe

*Province:* Colorado Plateau Semi-Desert

*Section:* Navajo Canyonlands

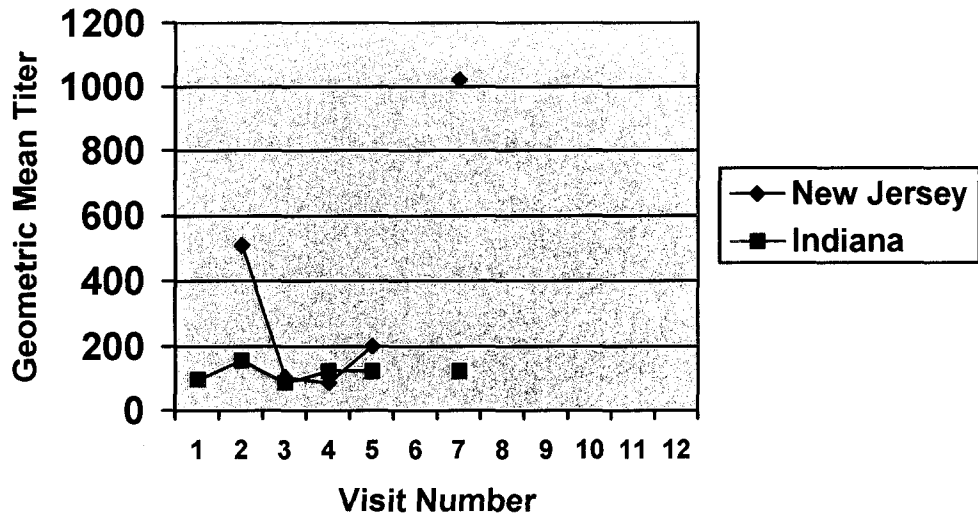
*Landcover:* Shrubland

*Soil:* Fruitland-Turley-Garland

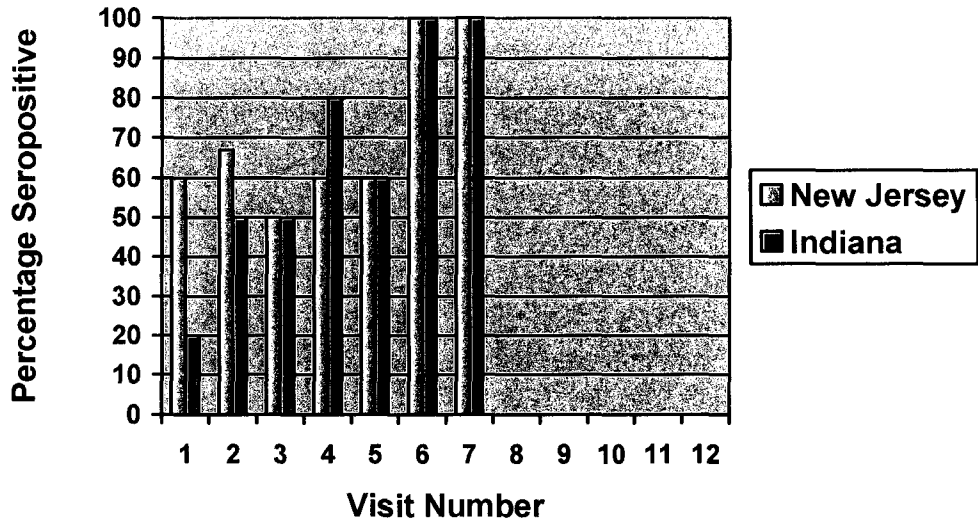
*Plant Hardiness Zone:* 6a



### Premises 97NM0095



### Premises 97NM0095



**NM-14**

*Initial Date:* 9/15/97

*Number of Visits:* 5

*County:* San Miguel

*Coordinates:* 35.80021 N  
104.98044 W

*Slope:* 0

*Aspect:* -1 flat

*Elevation:* 1951 m

*Domain:* Dry

*Division:* Tropical/Subtropical Steppe

*Province:* Southern Rocky Mountain  
Steppe-Open Woodland-Coniferous  
Forest-Alpine Meadow

*Section:* Southern Parks and Ranges

*Landcover:* Deciduous broadleaf forest

*Soil:* Patri-Carnero-Tricon

*Plant Hardiness Zone:* 5b

**NM-16**

*Initial Date:* 7/13/98

*Number of Visits:* 5

*County:* Valencia

*Coordinates:* 34.81625 N  
106.71591 W

*Slope:* 0

*Aspect:* -1 flat

*Elevation:* 1479.9 m

*Domain:* Dry

*Division:* Tropical/Subtropical Steppe

*Province:* Colorado Plateau Semi-Desert

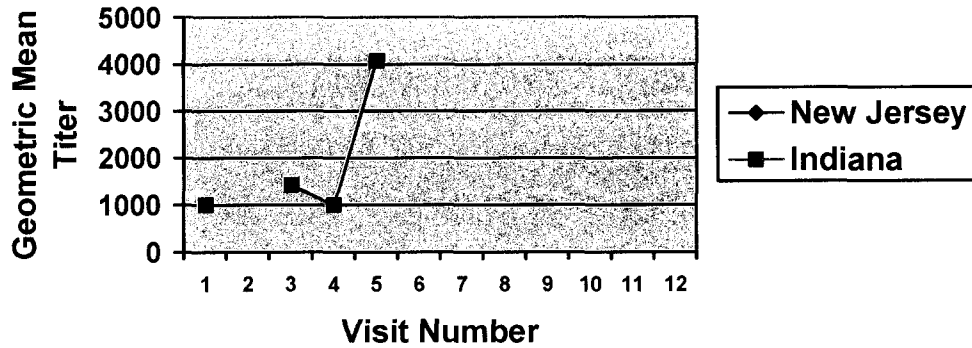
*Section:* Northern Rio Grand Intermontane

*Landcover:* Grassland

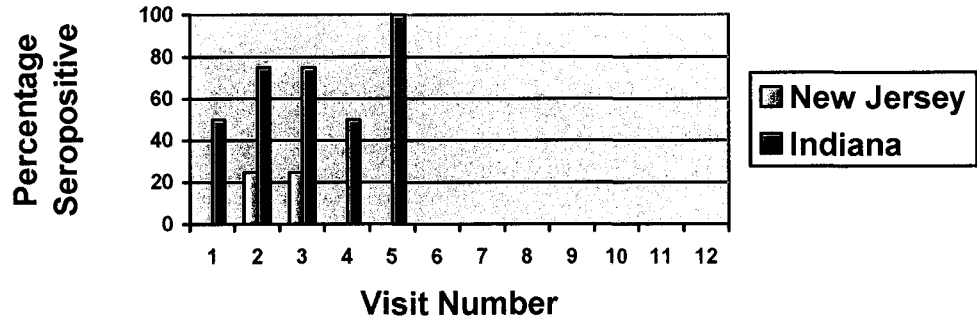
*Soil:* ND

*Plant Hardiness Zone:* 7a

**Premises 98NM0033**



### Premises 98NM0033



**NM-17**

*Initial Date:* 9/28/98

*Number of Visits:* 6

*County:* Bernalillo

*Coordinates:* 35.08797 N  
106.39044 W

*Slope:* 118.07 SE

*Aspect:* 8.063246

*Elevation:* 1995 m

*Domain:* Dry

*Division:* Tropical/Subtropical Steppe

*Province:* Colorado Plateau Semi-  
Desert

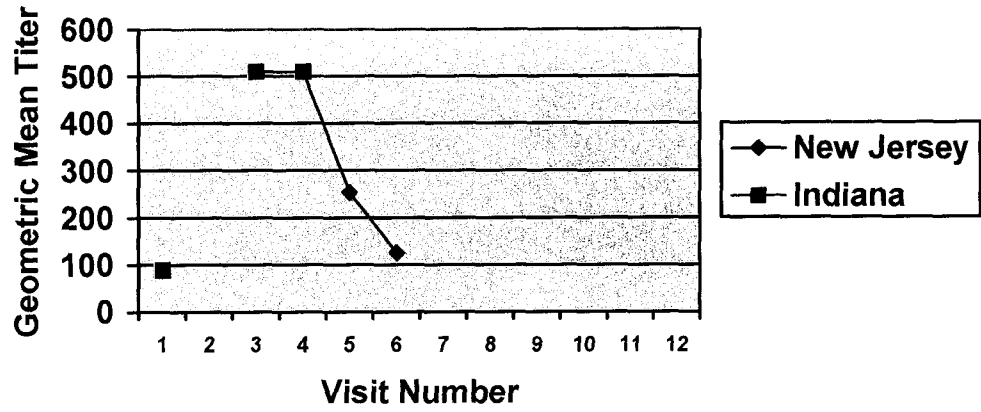
*Section:* Northern Rio Grande  
Intermontane

*Landcover:* Shrubland

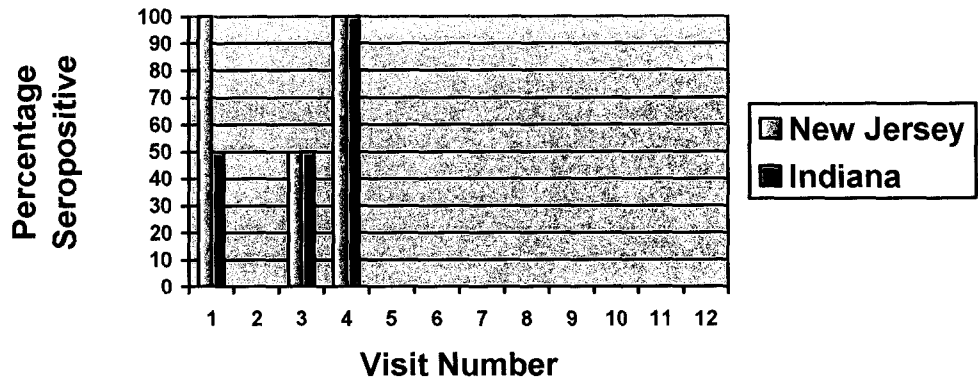
*Soil:* Typic Utechrepts lithis ustochrepts

*Plant Hardiness Zone:* 6b

### Premises 98NM0045



### Premises 98NM0045



**Appendix 2.**

**SENTINEL DAIRY OPERATIONS IN EL SALVADOR**

**Premises 1**

*Operation Type:* Dairy  
only

*Coordinates:* 13.72755 N  
89.64477 W

*Slope:* 89.98

*Aspect:* 219.08 SW

*Elevation:* 404 m



## Premises 2

*Operation Type:* Dairy only

*Coordinates:* 13.71395 N  
89.66186 W

*Slope:* 89.98

*Aspect:* 241.62 SW

*Elevation:* 336 m



### Premises 3

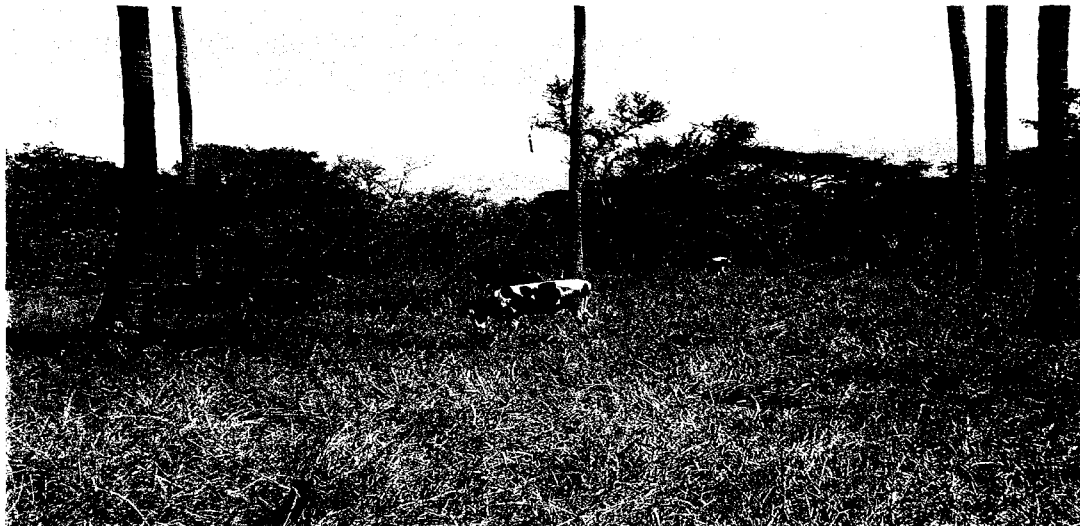
*Operation Type:* Dairy and Beef

*Coordinates:* 13.68600 N  
89.74082 W

*Slope:* 89.97

*Aspect:* 213.21 SW

*Elevation:* 172 m



## Premises 4

*Operation Type:* Dairy and Beef

*Coordinates:* 13.60449 N  
89.74495 W

*Slope:* 89.95

*Aspect:* 176.85 S

*Elevation:* 72 m



## Premises 5

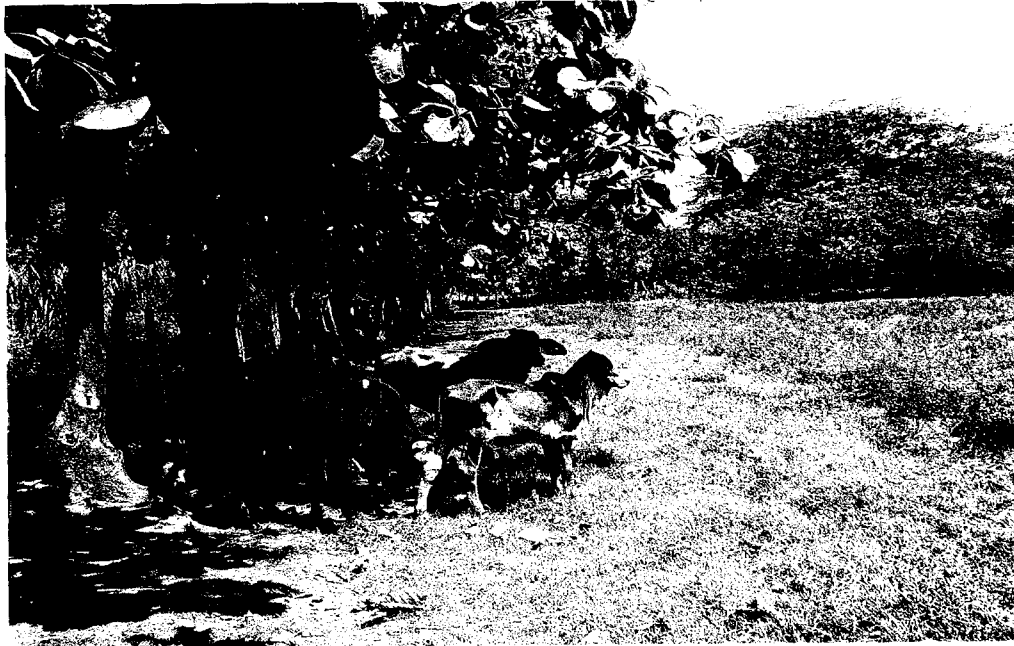
*Operation Type:* Dairy and  
Beef

*Coordinates:* 14.10749 N  
89.16961 W

*Slope:* 89.98

*Aspect:* 154.04 SE

*Elevation:* 301 m



## Premises 6

*Operation Type:* Dairy and Beef

*Coordinates:* 14.09747 N  
89.05104 W

*Slope:* 89.94

*Aspect:* 171.68 S

*Elevation:* 254 m



## **Premises 7**

*Operation Type:* Dairy and Beef

*Coordinates:* 14.11545 N  
89.05034 W

*Slope:* 89.96

*Aspect:* 200.43

*Elevation:* 279 m

## Premises 8

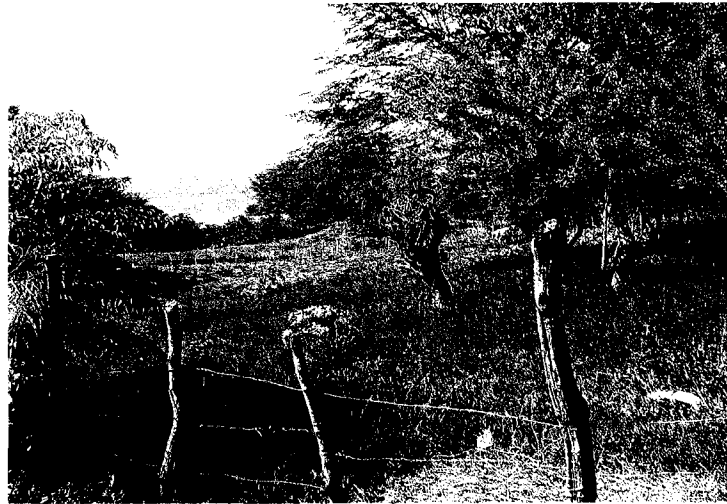
*Operation Type:* Dairy and  
Beef

*Coordinates:* 13.99303 N  
89.00744 W

*Slope:* 89.99

*Aspect:* 282.10 W

*Elevation:* 277 m



## Premises 9

*Operation Type:* Dairy only

*Coordinates:* 13.34939 N  
88.07878 W

*Slope:* 89.72

*Aspect:* 255.00 SW

*Elevation:* 57 m



## Premises 10

*Operation Type:* Dairy and beef

*Coordinates:* 13.43745 N  
88.15704 W

*Slope:* 89.95

*Aspect:* 116.56 SE

*Elevation:* 72 m



## **Premises 11**

*Operation Type:* Dairy and beef

*Coordinates:* 13.63262 N  
87.88567 W

*Slope:* 89.95

*Aspect:* 122.66 SE

*Elevation:* 85 m

**Premises 12**

*Operation Type:* Dairy and  
beef

*Coordinates:* 13.61035 N  
87.94179 W

*Slope:* 89.98

*Aspect:* 3.03 N

*Elevation:* 195 m



**Appendix 3.**

**QUESTIONNAIRES**

## Initial Visit Questionnaire

Premises Number \_\_\_\_\_ Date of Visit \_\_\_\_\_

Interviewer \_\_\_\_\_ Visit Number \_\_\_\_\_

### Section A - Background

1. Approximate altitude of premises..... \_\_\_\_\_
2. Prevailing winds are from which direction..... \_\_\_\_\_
3. To your knowledge has vesicular stomatitis ever been present on these premises before?..... YES NO
4. Are there any horses, not including the ones on this premises in an approximate one mile radius of this premises?..... YES NO  
IF YES  
A. Approximately how many horses?..... \_\_\_\_\_
5. Are there any cattle, not including the ones on this premises in an approximate one mile radius of this premises?..... YES NO  
IF YES  
A. Approximately how many cattle?..... \_\_\_\_\_
6. Does the rodent population currently appear above normal for this time of year?..... YES NO
7. Have any measures been taken to decrease the rodent populations?..... YES NO
8. Does the insect population currently appear above normal for this time of year?..... YES NO
9. Have any measures been taken to decrease the insect population in the animals or on the premises?..... YES NO

**Section B - Management**

1. Approximately how many hours per day to horses spend in each of the following locations?

- A. Pasture - ground with vegetation that supplies feed to horses..... \_\_\_\_\_
- B. Barn/shelter - a manmade structure with at least a roof..... \_\_\_\_\_
- C. Drylot - an outdoor area with little or no vegetation..... \_\_\_\_\_

2. What is the approximate distance, in feet, from where the horses are kept to a source of running water (stream,ditch,canal,etc)?..... \_\_\_\_\_

3. What is the approximate distance, in feet, from where the horses are kept to a source of standing water (pond.lake,etc)?..... \_\_\_\_\_

4. What is the approximate distance, in feet , from where horses are kept from a public access road?..... \_\_\_\_\_

5. Do horses have access to some shelter or barn?..... YES NO

***IF #5 is NO, skip to question 6***

A. Average number of days per week that horses use shelter..... \_\_\_\_\_

B. What type of barn or shelter is it?.....

- 1) Roof only (no walls) \_\_\_\_\_
- 2) Partially enclosed (2 or 3 walls) \_\_\_\_\_
- 2) All enclosed (4 walls) \_\_\_\_\_
- 3) All enclosed with screens \_\_\_\_\_

6. Do horses routinely spend time on pasture?..... YES NO

***IF #6 is NO, skip to question 7***

A. When are horses usually on pasture?(Check all that apply)

- 1) Morning (dawn to 9am)
- 2) Midday (9am to 5pm)
- 3) Evening (5pm to dark)
- 4) Night (dark to dawn)

7. Do you keep any of the horses on drylots?..... YES NO

***IF #7 is NO, skip to question 8***

A. While the animals are on drylots do they still have

access to pasture?..... YES NO

B. When are horses usually on the drylot?(Check all that apply)

- 1) Morning (dawn to 9am)
- 2) Midday (9am to 5pm)
- 3) Evening (5pm to dark)
- 4) Night (dark to dawn)

8. What are the sources of water for horses?

Water Source(municipal,well, ditch,pond,etc)

Shared with which species

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

**Section C - Exposure**

1. Have any new animals been introduced to the premises  
in the last 3 months?..... YES NO

IF YES, complete the following:

<u>Animal type</u>	<u>Number</u>	<u>From:County</u>	<u>State</u>
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____

2. In the past 3 months, were there any wild animal and horse  
interactions on your premises?..... YES NO

IF YES, how much interaction was there?

Deer/elk	Extensive___	Moderate___	Occasional___	Rare___	None___
Antelope	Extensive___	Moderate___	Occasional___	Rare___	None___
Rabbit/skunk/raccoon	Extensive___	Moderate___	Occasional___	Rare___	None___
Coyote/fox	Extensive___	Moderate___	Occasional___	Rare___	None___
Mice/rats	Extensive___	Moderate___	Occasional___	Rare___	None___
Prairie dogs	Extensive___	Moderate___	Occasional___	Rare___	None___
Other(_____)	Extensive___	Moderate___	Occasional___	Rare___	None___

## **Section D - Mapping**

Please map the premises below:

- a) All buildings, corrals, pens and areas that house horses and other animals.
- b) Naturally occurring waterways, standing water, irrigation canals, etc.
- c) Trees and forested areas.
- d) Fence lines and borders with neighbors. Indicate which species neighbors may have that contact sentinel animals.
- e) Public road locations.

## Quarterly Visit Questionnaire

Premises Number \_\_\_\_\_ Date of Visit \_\_\_\_\_

Interviewer \_\_\_\_\_ Visit Number \_\_\_\_\_

### **Section A - Background**

4. In the past 3 months have the number of horses within a one mile radius of this premises changed?..... YES NO

IF YES

A. Approximately how many horses?..... \_\_\_\_\_

5. In the past 3 months have the number of cattle within a one mile radius of this premises changed?..... YES NO

IF YES

A. Approximately how many cattle?..... \_\_\_\_\_

6. Does the rodent population currently appear above normal for this time of year?..... YES NO

7. Have any measures been taken to decrease the rodent populations?..... YES NO

8. Does the insect population currently appear above normal for this time of year?..... YES NO

9. Have any measures been taken to decrease the insect population in the animals or on the premises?..... YES NO

### **Section B - Management**

1. Approximately how many hours per day to horses spend in each of the following locations?

A. Pasture - ground with vegetation that supplies feed to horses..... \_\_\_\_\_

B. Barn/shelter - a manmade structure with at least a roof..... \_\_\_\_\_

C. Drylot - an outdoor area with little or no vegetation..... \_\_\_\_\_

2. What is the approximate distance, in feet, from where the horses are kept to a source of running water (stream,ditch,canal,etc)?..... \_\_\_\_\_

3. What is the approximate distance, in feet, from where the horses are kept to a source of standing water (pond.lake,etc)?..... \_\_\_\_\_

4. What is the approximate distance, in feet , from where horses are kept from a public access road?..... \_\_\_\_\_

5. Do horses have access to some shelter or barn?..... YES NO  
**IF #5 is NO, skip to question 6**

A. Average number of days per week that horses use shelter..... \_\_\_\_\_

B. What type of barn or shelter is it?.....

- 1) Roof only (no walls) \_\_\_\_\_ 2) Partially enclosed (2 or 3 walls) \_\_\_\_\_  
2) All enclosed (4 walls) \_\_\_\_\_ 3) All enclosed with screens \_\_\_\_\_

6. Do horses routinely spend time on pasture?..... YES NO  
**IF #6 is NO, skip to question 7**

A. When are horses usually on pasture?(Check all that apply)

- 1) Morning (dawn to 9am) 2) Midday (9am to 5pm)  
3) Evening (5pm to dark) 4) Night (dark to dawn)

7. Do you keep any of the horses on drylots?..... YES NO  
**IF #7 is NO, skip to question 8**

A. While the animals are on drylots do they still have access to pasture?..... YES NO

B. When are horses usually on the drylot?(Check all that apply)

- 1) Morning (dawn to 9am) 2) Midday (9am to 5pm)  
3) Evening (5pm to dark) 4) Night (dark to dawn)

8. What are the sources of water for horses?

Water Source(municipal,well, ditch,pond,etc)

Shared with which species

\_\_\_\_\_  
\_\_\_\_\_

A. Is this a change from the previous visit?..... YES NO

**Section C - Exposure**

1. Have any new animals been introduced to the premises  
in the last 3 months?..... YES NO

IF YES, complete the following:

<u>Animal type</u>	<u>Number</u>	<u>From:County</u>	<u>State</u>
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____

2. In the past 3 months, were there any wild animal and horse  
interactions on your premises?..... YES NO  
IF YES, how much interaction was there?

Deer/elk	Extensive___	Moderate___	Occasional___	Rare ___	None ___
Antelope	Extensive___	Moderate___	Occasional___	Rare ___	None ___
Rabbit/skunk/raccoon	Extensive___	Moderate___	Occasional___	Rare ___	None ___
Coyote/fox	Extensive___	Moderate___	Occasional___	Rare ___	None ___
Mice/rats	Extensive___	Moderate___	Occasional___	Rare ___	None ___
Prairie dogs	Extensive___	Moderate___	Occasional___	Rare ___	None ___
Other(_____)	Extensive___	Moderate___	Occasional___	Rare ___	None ___

**Caracterización de las Fincas**  
**Estudio de Estomatitis Vesicular en el Hato Centinela**

Numero de Finca \_\_\_\_\_ Fecha de la Visita \_\_\_\_\_  
 Nombre del dueño \_\_\_\_\_ Número de Teléfono \_\_\_\_\_  
 Nombre del Encuestador \_\_\_\_\_ Número di Visita \_\_\_\_\_

**Sección A – Antecedentes**

1. Altura aproximada de la Finca. \_\_\_\_\_
2. Dirección do los vientos prevalentes. \_\_\_\_\_
3. Según sus conocimientos ¿Ha habido estomatitis vesicular en esta finca antes? .....SI NO  
 Si, SI:  
 A. ¿En que ano ocurrió la ultima infección de estomatitis vesicular? \_\_\_\_\_
4. ¿Hay caballos, sin contar los de la finca, en un radio proximado de 1 milla (1.6 Km) de la finca.....SI NO  
 Si, SI:  
 A. ¿Aproximado cuantos caballos? \_\_\_\_\_
5. ¿Hay ganado, sin contar los di la finca, en un radio aproximado di una milla (1.6 Km) de la finca.....SI NO  
 Si, SI:  
 A. ¿Aproximadamente cuanto ganado? \_\_\_\_\_
6. ¿Hay presencia de roedores por arriba de lo normal para esta época del ano? .....SI NO
7. ¿Se han tomado medidas pare controlar la población de roedores?.....SI NO
8. ¿Hay presencia de ínstelos por arriba de lo normal para esta época del ano?.....SI NO
9. ¿Se han tomado medidas para controlar la población e insectos?.....SI NO

**Sección B –Administración**

1. ¿Aproximadamente cuantas horas al día pasa el ganado en cada un de los siguientes lugares?

A. Pastura- tierra con vegetación que provee algo de alimento al ganado.

\_\_\_\_\_

B. Establo/refugio- estructura hecha por el hombre con por lo menos un techo.

\_\_\_\_\_

C. Lote seco- intemperie con poca o ninguna vegetación.

\_\_\_\_\_

2. Cual es la distancia aproximada, en metros, del lugar en que se mantiene el ganado a lo tiente de agua corriente? (río, canal, etc.) \_\_\_\_\_ Ft

3. Cual es la distancia aproximada, en metros, del lugar en que se mantiene el ganado a la fuente de agua estancada (poza, lago, etc.).

\_\_\_\_\_ Ft

4. Cual es la distancia aproximada, en metros, del lugar en que se mantiene el ganado al camino de acceso más cercano?

\_\_\_\_\_ Ft

5. ¿Tiene acceso su ganado a algún refugio o establo? .....SI NO

Si la respuesta a la pregunta No. 5 es NO pase a la pregunta No. 6

A. Numero de días en promedio que el ganado pasa en el ganado pasa en el refugio. \_ -

\_\_\_\_\_ días.

B. ¿Que tipo di refugio o establo es? \_\_\_\_\_ días.

1. Solo techo (sin paredes) \_\_\_\_\_ 2. Parcialmente cerrado (2 o 3 paredes)

\_\_\_\_\_ Corral sin techo SI NO Utilizan jaulas SI NO

6. ¿Pasa habitualmente el ganado algún tiempo pastando?

Si la respuesta la pregunta No. 6 es NO, pase a la pregunta No 7.

C. ¿Normalmente, a que hora pasta el ganado? (marque todas las que se apliquen)

1. Mariana (amanecer a 9 am) \_\_\_\_\_ 2. Medio día (9ama 5 pm)  
 3. Tarde (5pm a oscurecer) \_\_\_\_\_ 4. Noche (oscurecer a amanecer)  
 \_\_\_\_\_

7. ¿Tiene algo del ganado en lotes secos? .....SI NO

Si la respuesta a la pregunta No 7 es NO, pase a la pregunta No.8.

- A. ¿Tienen acceso a pasto aunque estén en el lote seco?.....SI NO  
 B. ¿Normalmente, a que hora esta el ganado en el lote seco? (marque todas las que se apliquen)

1. Mariana (amanecer a 9 am) \_\_\_\_\_ 2. Medio día (9ama 5 pm)  
 3. Tarde (5pm a oscurecer) \_\_\_\_\_ 4. Noche (oscurecer a amanecer)  
 \_\_\_\_\_

8. ¿Cuales son las fuentes de agua para el ganado?  
 Fuente di agua (municipal, pozo, arrollo, poza,  
 etc.) \_\_\_\_\_

Compartida con otras especies

9. ¿Durante que periodo de tiempo esta el ganado bajo techo?  
 de 05:00 h a 08:00 h \_\_\_\_\_  
 de 08:00 h a 12:00 h \_\_\_\_\_  
 de 12:00 h a 16:00 h \_\_\_\_\_  
 las 24 horas \_\_\_\_\_

### Sección C- Exposición

1. ¿Se han introducido animales nuevos a la finca en los últimos 3 meses? .....SI NO

Si, SI, complete la tabla siguiente:

Tipo de animal	Numero	De, Municipio	Departamento	Cantón

2. ¿En los últimos tres meses, ha habido interacción entre el ganado y otras especies de animales?

Si, SI ¿Que tanta interacción?

Extensiva  
Ninguna

Moderada

Ocasional

Rara

### **Sección D- Mapeo**

Por favor haga un mapa de lo siguiente:

1. todos los edificios, corrales y áreas que alberguen ganado y otros animales.
2. Contentes de agua naturales, aguas estancadas, canales de riego, etc.
3. Árboles y zonas boscosas.
4. Cercas y limites con los vecinos. Indique con que especies de animales de los vecinos pueden tener contacto los animales centinelas.
5. Localización de los caminos públicos.