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DISSERTATION

EVOLUTION AND ECOLOGY OF VESICULAR STOMATITIS VIRUS IN THE SOUTHWESTERN UNITED STATES: 1995 to 1998

Submitted by

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Department of Environmental Health

In partial fulfillment of the requirements

For the Degree of Doctor of Philosophy

Colorado State University

Fort Collins, Colorado

Summer 2002

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COLORADO STATE UNIVERSITY

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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY ZARA LLEWELLYN ENTITLED EVOLUTION AND ECOLOGY OF VESICULAR STOMATITIS VIRUS IN THE SOUTHWESTERN UNITED STATES: 1995 TO 1998 BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTORATE OF PHILOSOPHY.

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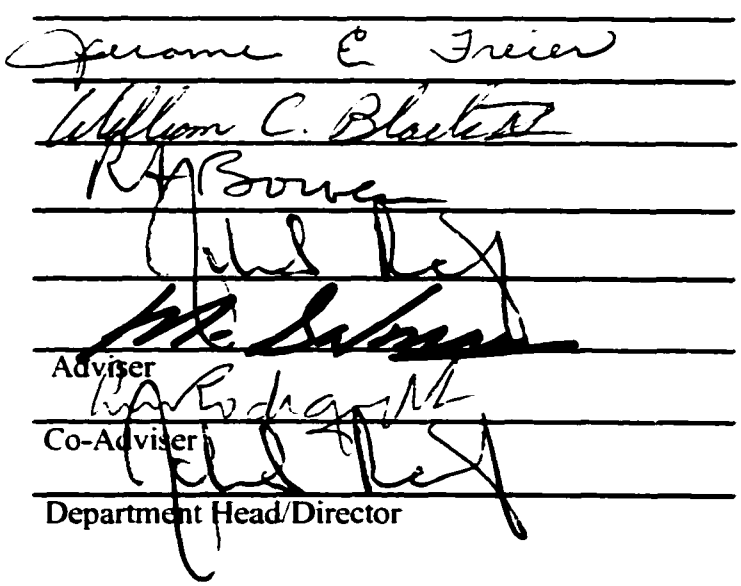

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ABSTRACT OF DISSERTATION

EVOLUTION AND ECOLOGY OF VESICULAR STOMATITIS VIRUS IN THE SOUTHWESTERN UNITED STATES: 1995 to 1998

Vesicular stomatitis (VS) is a disease of horses, pigs, and cattle that is clinically undistinguishable from foot and mouth disease that causes considerable economic loss during outbreaks in the United States (US). Cases of vesicular stomatitis virus (VSV) occur in the southwestern United States approximately every ten years and the complete epidemiological relationship among agent, host, environment and time remains unknown. This dissertation was a multi-phase approach to understanding specific essential unsolved components of the epidemiology of the disease. Both molecular and spatial analytical techniques are used in the study's approach.

The phylogenetic relationship of viruses circulating in the southwestern United States in 1995 with viruses in Mexico from 1984 to 1997 was investigated. A 750 base fragment of the glycoprotein gene (G gene) was amplified by RT-PCR and sequenced from eleven isolates of bovine origin from different regions of Mexico from 1984 to 1997. Several nucleotide changes were found with viruses from Mexico and viruses previously sequenced from the western United States. Viruses from the eastern US formed a different lineage and were not closely related to viruses causing outbreaks in the western US. Overall sequence analysis indicated relatively homogenous and distinct viral lineages for each documented outbreak in the western US, with viruses from Mexico in each lineage of the major US outbreaks from 1982/83 to 1995, suggesting a common

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source for these viruses. Viruses from Central America formed a diverse collection of lineages, with a marked correlation between position in the phylogenetic tree and their geographical origin.

VSV has been used as a laboratory model for viral evolution and *in vitro* studies due to its capacity to grow well in multiple cell lines. VSV is an RNA virus and despite its capability for rapid mutation, VSVs circulating within endemic areas remain genetically conserved over long periods of time. One possible selective factor could be the vector(s) involved in its natural cycle. In this study we compared the growth rate and rate of evolution of three genetically distinct VSV-NJ viruses from different ecological regions and different hosts in a natural (sand fly) versus non-natural (mosquito and mammalian) host cell lines. The genetic mutations and rate of evolution through serial passage of each virus was compared with viruses in nature. The virus originating from sand flies grew to higher titers in insect cells than did viruses of mammalian origin. Although all viruses readily established persistent infections both in mosquito and sand fly cells maintained in culture for up to 81 days, sustained virus yields were observed only in sand fly cells. Sequence analyses of the viruses after 0, 10 or up to 25 passages in each cell line showed no changes in the hypervariable region of the phosphoprotein (P) gene or the intergenic junction between the glycoprotein (G) and the polymerase (L). In contrast, the G gene demonstrated mutation rates between 1.39×10^{-4} and 6.95×10^{-5} in most cell-virus combinations. The majority of the mutations were non-synonymous, suggesting positive selection. These results showed that although insect cells can be a selective factor in VSV evolution *in vitro*, no significant differences between rates of

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evolution of VSV-NJ by passage in homologous (sand fly) versus heterologous (mosquito or mammalian) cells were detected.

To investigate possible ecological factors associated with regions of VSV activity, a study using geographical information systems (GIS) and subsequent spatial analysis were used to identify spatial-temporal clustering of VS premises from the 1995, 1997, and 1998 VS outbreaks. Ecological and climatic conditions were investigated in regions identified to have a high risk of VS in relation to possible vector associations. The SaTScan program was used for the spatial, temporal, and spatial-temporal analyses that identified clusters of higher risk for VSV in Arizona, Colorado, New Mexico, and Utah. Further analyses in Colorado and New Mexico indicated clusters in western and southwestern Colorado and central New Mexico along the Rio Grande River in 1995 and in north central Colorado in 1998. Descriptive statistics indicated that positive premises were located in close proximity to perennial streams and canals, at an elevation of 1377 meters to 3207 meters, and located within the dry domain ecological region in the southwestern United States. An increase in precipitation was observed from 1994 to 1999 from the 30-year average, but no substantial departures in temperature were identified from 1994 to 1999. Statistically significant associations with precipitation or temperature were not identified with VS positive premises for each month prior to the first or last month of the outbreak during 1995, 1997 or 1998. The results suggest that close proximity of VS positive premises to perennial water sources, the clustering of

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cases near major water sources and vegetative areas in dry ecological regions could be associated with nearby vector habitat.

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ACKNOWLEDGEMENTS

Many individuals and agencies helped in the areas of knowledge, equipment, and finances for this dissertation. I would specifically like to thank my committee members for their personal and professional guidance. I would like to thank my adviser Dr. Mo Salman and co-adviser Dr. Luis Rodriguez, for their financial assistance, professional help, and personal wisdom. I would also like to thank Dr. Jerry Freier for his assistance in geographical information systems and spatial analyses and I extend my thanks to Dr. John Reif, Dr. William Black IV, and Dr. Richard Bowen for their time and commitment on my dissertation committee.

I would like to thank Mr. Steve Pauszek at Plum Island Animal Disease Center, Agricultural Research Services (ARS), United States Department of Agriculture (USDA) for his technical and scientific support, Ms. Susan Maroney and Ms. Priscilla FitzMaurice for technical guidance and support in geographical information systems and spatial analyses at the Centers for Epidemiology and Animal Health (CEAH), Veterinary Services (VS), Animal Plant Health Inspection Services (APHIS), USDA.

I would also like to thank Dr. Joaquin Peral and Dr. Joaquin Cesar Villarreal of the Mexico -- USA Exotic Animal Disease Commission for providing information on the prevalence of VSV in Mexico, Dr. David Stallknecht at the University of Georgia for the 89GAS virus, Dr. Daniel Mead at Arizona State University for supplying the 95NME virus, Dr. Robert Tesh from the University of Texas, Galveston and Dr. Isabella Novella at the University of California, San Diego for providing the sand fly LL5 cells and Dr. Fraire at the Exotic Laboratory in Mexico City for providing the VSV isolates.

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On a personal level, I would like to thank my parents, Sandy and Klaus Meyer, for their support and advice throughout my entire education. I would also like to thank my family and friends for their support, kindness, time, and assistance. I would not have been able to complete this dissertation without them.

This dissertation was funded by the Center of Economically Important Infectious Animal Diseases with a grant from the USDA: CREES, USDA, ARS CRIS project 1940-32000-033, that is part of a Specific Collaborative Agreement between the ARS and Colorado State University. Additional funding was provided through a cooperative contract with the USDA:FAS and the USDA, APHIS, VS, CEAH.

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STATEMENT OF OBJECTIVES, AIM , IMPACT, AND DISSERTATION STRUCTURE

Overall Objectives

To understand the specific, essential, and unsolved epidemiological components of agent, host, and environment of vesicular stomatitis

Specific Objectives

1) To evaluate genetic variation in the vesicular stomatitis virus-New Jersey glycoprotein gene from viruses collected from 1985 to 1997 in Mexico with viruses in the United States
2) To correlate changes in viral sequence with epidemiological characteristics of host, date of isolate collection and geographical location
3) To compare growth rate and rate of evolution of vesicular stomatitis virus in a natural versus non-natural host cell
4) To compare genetic mutations under selective laboratory conditions to the evolution of viruses in nature
5) To identify spatial, temporal, and spatial-temporal clusters of vesicular stomatitis from outbreaks in the western United States
6) To investigate climatic and ecological factors in regions of high vesicular stomatitis activity in the western United States

Hypotheses

1) There is no genetic variation in the glycoprotein gene from viruses collected in Mexico with viruses circulating in the United States
2) The epidemiological characteristics of host, date of isolate collection, and geographical location do not correlate with genetic sequence
3) There are no differences in growth rate or rate of evolution of vesicular stomatitis viruses in a natural versus non-natural host cell
4) There are no genetic mutations under selective laboratory conditions to the evolution of viruses in nature
5) There are no spatial, temporal, or spatial-temporal clusters of vesicular stomatitis outbreaks in the western United States
6) There is no difference in climatic conditions during outbreaks of vesicular stomatitis
7) There are no specific ecological factors associated with regions of vesicular stomatitis activity in the western United States

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Aim and Impact of this Work

Vesicular stomatitis (VS) has been classified by the Office International des Epizooties (OIE) as a List A disease that during outbreak years causes barriers of trade for international and domestic transport on meat and/or livestock. These trade restrictions within North America or worldwide can cause great economic loss to individual states and to the United States. The complete epidemiological relationship among virus, host, environment and time remains unknown. This dissertation is a multi-factorial approach to understanding specific, essential, and unsolved components of the epidemiological triangle of the disease. Both molecular and spatial analytical techniques are used in the study's approaches.

The origin and natural cycle of the viruses causing VS outbreaks in the western United States and northern Mexico remain unknown. Molecular technology and genetic analyses of the vesicular stomatitis viruses (VSVs) can be performed to aid in the knowledge of viral change and evolution as well as the epidemiology of the disease. By correlating the molecular information with epidemiological data we can gain a better understanding of the VSV natural cycle and help in its diagnosis, prevention and control.

Knowledge about environmental and climatic conditions during VS outbreaks is limited. Constructing a model to understand environmental conditions that are associated with outbreaks will aid in surveillance and methods for prediction and control of VS. The control of outbreaks of VS in the United States would both enhance the competitiveness of the American livestock industry globally and reduce the risk to independent producers.

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Dissertation Structure

The structure of this dissertation provides the research and findings organized by individual research project. Each chapter is organized into a manuscript format, specifying each project's objectives, material and methods, results, conclusions, references, tables, and figures. Chapter two contains two separate studies and Chapter three has one.

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CHAPTER 1

LITERATURE REVIEW

1.1 Introduction

Vesicular stomatitis (VS) is an economically important disease in livestock and horses as it is clinically identical to foot and mouth disease, producing vesicles on the tongue, teats and coronary bands. VS has been classified by the Office International des Epizooties (OIE) as a List A disease and during outbreak years, causes barriers of trade for international and domestic transport on meat and/or livestock. These trade restrictions within North America and worldwide can cause great economic loss to each individual state and to the United States as a whole. Despite research efforts, knowledge about the agent, host, transmission and environmental conditions in endemic and non-endemic regions is limited.

1.2 Clinical Aspects, Host Susceptibility, and Transmission

VS is clinically identical to foot and mouth disease with clinical signs of vesicles on the tongue, snout, coronary bands and teats of infected animals (Dietzschold B et al., 1996; Reif JS, 1994). Unlike foot and mouth disease, horses and humans can become infected and develop clinical signs. Classical signs include the formation of vesicles on the oral cavity, feet and teats. Salivation, anorexia, weight loss and mastitis occur as secondary effects (Bridges VE et al., 1997; Monath TP et al., 1986). In animals, the

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disease can range from severe to mild with most natural infections remaining asymptomatic (Webb PA, Holbrook FR, 1988; Letchworth GJ et al., 1999).

Clinical disease is mainly reported for cattle, horses, swine and humans; however, a number of mammalian and insect hosts can also become infected (Nichol ST, 1994). Antibodies to VSV have been identified in deer, antelope, rodents, elk, wild turkeys, goats, sheep, ducks, coyote, dogs, feral swine, rabbits, bats, monkeys, skunks and raccoons (Aguirre AA et al., 1992; Webb PA, et al., 1987; Hanson RP, Karstad L, 1955; Stallknecht DE, et al., 1993). The disease in wildlife has mainly been documented from experimental infections, with vesicular lesions appearing on infected deer (Webb PA, Holbrook FR, 1988), however in Ossabaw Island, Georgia longitudinal studies have documented feral swine to seroconvert without clinical disease (Stallknecht DE et al., 1986). It has been speculated that cold-blooded animals could act as reservoirs for the virus, which would account for the disappearance of the virus in the winter and its reappearance in the spring (Saulmon EE, 1968). Bats are also thought to harbor the virus. They are strong fliers and hibernators, which would coincide with the patterns of VS occurrence in the United States and allow for transmission of the virus to a wide area depending on the bats flight patterns (Saulmon EE, 1968; Ubico SR, McLean RG, 1995).

The first report of a human infection with VSV was described in 1917 as an acute illness in persons who worked with horses affected by "stomatitis contagiosa" (Burton AC, 1917) and the first confirmed documented case was in a laboratory worker in 1950 (Hanson RP et al., 1950). Most of the recorded human cases of VS have occurred in laboratory workers (Johnson KM et al., 1969), but natural infection can occur in endemic regions and through contact with infected animals in non-endemic regions, especially in

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individuals with occupational risks (Reif JS, et al., 1987; Sherlokov et al., 1967; Brody JA, et al., 1967; Peralta PH et al., 1958; Johnson KM et al., 1969; Dietzschold B et al., 1996). Most human infections with VSV are asymptomatic or show mild febrile flu-like symptoms in adults after an incubation period of two to six days (Dietzschold B et al., 1996; Johnson KM et al., 1969; Reif JS, 1994). In symptomatic cases, about one-quarter of the patients develop herpes-like lesions in the mouth, lips, or nose (Dietzschold B et al., 1996). Headache, retro orbital pain, general malaise, pharyngitis, nausea, vomiting, and diarrhea can also occur (Dietzschold B et al., 1996). No fatalities in humans have been reported (Dietzschold B et al., 1996).

The mode of viral transmission is not completely understood. The incubation period for VSV is between one to five days post infection (Letchworth GJ et al., 1999). The virus has not been detected in the feces or urine of infected animals, but the vesicles contain high levels of virus that can be spread via direct contact with the vesicular fluid or via aerosol (Dietzschold B et al., 1996; Knight AP, Messer NT, 1983). Transmission studies between laboratory animals have demonstrated that contact transmission was lesion-dependent, with vesicular lesions being subtle to few or exhibiting no clinical signs of infection (Stallknecht DE et al., 2001). In humans, VSV can be transmitted through direct inoculation, viral contact with skin wounds, or inhalation of virus particles (Johnson KM et al., 1969). Insect vectors also play a role in transmission, and it is hypothesized that VSV is maintained by a complex cycle of agent, host, vector, and environmental interactions (Comer JA et al., 1991; Johnson KM et al., 1969; Monath TP et al., 1986; Walton TE, et al., 1987; Bridges VE et al., 1997).

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1.3 Diagnosis, Prevention and Treatment

VSV can be isolated from throat swabs, mouth swabs, vesicle fluids, or epithelial tissue from ruptured vesicles and identified by complement fixation, serum neutralization, or fluorescent antibody (Dietzschold B et al., 1996). Antibodies usually appear five to ten days post-infection with the optimum time of serum collection at 21 days post-infection (Meyer NL et al., 1960). Several tests have been developed for the diagnosis of VSV. To detect serum antibodies against VSV a variety of tests include complement fixation, neutralization, and enzyme-linked immunosorbent assay (ELISA) (Dietzschold B et al., 1996). The cELISA is a rapid, sensitive test used in screening. It measures serum primarily IgG and some IgM with a minimum exposure of five to six days and can remain positive for up to one to three years. The complement fixation test is used in recent exposure of VSV and measures IgM within six to eight days and up to two weeks of exposure. Viral isolation is the gold standard for diagnosis, but samples with reported exposure often yield no virus. Molecular tests are also available for diagnosis of VSV by PCR (Rodriguez LL et al., 1993; Rodriguez LL et al., 2000; Llewellyn ZN et al., 2000).

There are a number of methods for prevention and control. VSV can have up to a 95% attack rate in dairies and immediate control measures must be taken (Ellis EM, Kendall HE, 1964). Initially, any animal with clinical signs of VS should be isolated from the rest of the herd and any newly introduced animals should be quarantined, provided clean feed, and all saliva-contaminated materials should be destroyed (Reif JS, 1994). Animals within the region should receive insect control measures, a high quality diet to reduce oral trauma, proper milking procedures observed to minimize mammary

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trauma and limitations in animal movement to prevent transmission from affected to unaffected animals (Reif JS, 1994; Hurd HS et al., 1999).

Vaccination is not routinely practiced in the United States. However, a commercial vaccine is available in Colombia and vaccination is routinely practiced in endemic areas there (Arbelaez et al., 1988; Arbelaez et al., 1989). More recent studies have indicated that rearrangement of the VSV genes, specifically the nucleocapsid protein gene away from the single transcriptional promoter and relocating the glycoprotein gene eliminated the potential of the virus to cause disease (Flanagan EB et al., 2001). Rearrangement of the viral properties might be a new approach to generating live attenuated vaccines (Flanagan EB et al., 2001).

1.4 Prevalence of VS

VS is a disease of the Western Hemisphere. VS has occurred in Africa and Europe, but has not persisted. The first report was an outbreak in horses and mules in Transvaal, South Africa in the late 19th century, and the second was an outbreak in France during the First World War from a shipment of infected army horses from the United States (Nichol ST, 1994). There has been no additional evidence of VSV activity persisting for prolonged periods on either of these continents (Nichol ST, 1994).

VS occurs in endemic and non-endemic regions throughout the Americas. In the southwestern United States and northern Mexico, the disease is diagnosed approximately every five to ten years. In southern Mexico, Ossabaw Island, Georgia, regions of the Caribbean, Central America and South America, VS is endemic with annual reporting of infected animals (Vernon SD et al., 1990; Nichol ST, 1994).

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There are two main serotypes of VSV, VSV- New Jersey (VSV-NJ) and VSV-Indiana (VSV-IN). VSV-NJ is the most common serotype causing clinical disease and it has the widest distribution with viral isolations as far north as Canada and as far south as Peru (Nichol ST, 1994).

1.5 VS Occurrence in the United States

The first reported case of VS in the United States occurred in 1821, but it was not until the Civil War that the disease received considerable notice when reports of army horses with characteristics of VS were described in 1862 (Dietzschold B et al., 1996). For a listing of outbreaks in the United States from 1906 to 1998 see Table 1 (Rodriguez LL et al., 2000). The first large outbreak in the United States was documented during World War I in 1916 with cases first diagnosed in the Denver stockyards, spreading to ten states (Hanson RP, 1952). In the 1920s, two viral serotypes were discovered. In 1925 the first serotype was isolated from cattle in Richmond, Indiana, thereby giving the first serotype its name, Indiana (Nichol ST, 1994; Saulmon EE, 1968). A year later another prototype virus strain was discovered and isolated from cattle in New Jersey, hence that name (Nichol ST, 1994; Saulmon EE, 1968).

There seem to be two main patterns of occurrence of VS in the United States. A region of sporadic occurrence in the western United States with the majority of cases of the NJ serotype that occur in one to three year cycles approximately every ten years and an endemic-like cycle in the eastern United States, with cases almost every year from 1934 to 1977 (Rodriguez LL et al., 2000). After that, the only clinical reports of infection are in wildlife without clinical disease in domestic animals (Fletcher WO et al., 1991).

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Since November 1998 there have been no documented clinical cases of VS in the United States.

Ossabaw Island off of the coast of Georgia is the only documented endemic region in the United States where subclinical infections of wildlife with VSV-NJ are reported from May to September (Stallknecht DE, 2000). VSV-NJ transmission is seasonal with antibodies detected from feral swine, cattle, horses, deer, and raccoons (Stallknecht DE, 2000). Despite the high transmission rates, the clinical disease is rarely detected.

1.6 VS Occurrence in Mexico, Central and South America

Reports of VSV cases from 1981 to 1998 from the Exotic Animal Disease Commission of Mexico indicate two cycles of VSV, an endemic cycle in the southern states and a non-endemic cycle in the northern states (Rodriguez LL et al., 2000). A list of outbreaks in selected areas of Mexico from 1981 to 1998 are provided in Table 2 (Rodriguez LL et al., 2000). In northern Mexico, cases are sporadic within the states of Chihuahua, Sonora, and Nuevo Leon. In southern Mexico, cases occur approximately every year in Veracruz, Chiapas, and Tabasco (Rodriguez LL et al., 2000). Similar to the western United States, the majority of outbreaks are caused by the NJ serotype; the last VSV-IN case was reported in Colima, central Mexico in 1997 (Rodriguez LL et al., 2000).

Central and South America are endemic regions for VSV activity. In Colombia, VS was recognized in the 1920s in outbreaks that involved cattle, horses, and pigs (Hanson RP et al., 1968). In 1939, cattle and horses were infected with VSV in

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Argentina (Hanson RP, 1952). Two years later cows, horses and pigs were diagnosed in Venezuela (Hanson RP, 1952). That was the first time the virus had been isolated from swine.

In 1961, another Indiana-related virus was isolated from mites in Cocal, Trinidad and became the prototype strain of VSV Indiana serotype, Type 2 viruses (Nichol ST, 1994). In 1964, a mule from Alagoas, Brazil was found to have another strain of Indiana-related virus, which was designated Indiana Type 3 virus (Nichol ST, 1994). In the 1980s two other VSV Indiana-related viruses, Maraba and Carajas, were identified (Rodriguez LL et al., 1993).

1.7 Potential Vector

The role of insect vectors in the cycle of VSV is not completely understood. The cycles of VS epidemics are associated with high insect population levels. Cases are located throughout river valley systems and infected arthropods are closely associated with new animal infections (Nichol ST, 1994; Webb PA, Holbrook FR, 1988; Letchworth GJ et al., 1999). However, no vertebrate host with a suitable viremia has been identified, vesicles and lesions that appear to require inoculation in the oral mucosa are generally inaccessible to biting insects, there is a lack of success in virus isolation from biting arthropods, and during some outbreaks new cases are identified into the winter months (Webb PA, Holbrook FR, 1988).

The role of biological versus mechanical transmission of VSV is still undetermined. In mechanical transmission, the virus is transmitted physically from one vertebrate host to another without viral replication in the insect (Woodring et al., 1996).

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In biological transmission, the virus is ingested through a blood meal and replicates in the midgut and then is disseminated to infect secondary target organs (Woodring et al., 1996). Virions disperse in the circulating vector blood and once reached the salivary ducts can be transmitted to vertebrates through a blood meal (Woodring et al., 1996).

The virus has been isolated from *Lutzomyia* (sand flies), *Simulidae* (black flies), *Culicoides* (midges), *Culex nigripalpus* and *Aedes aegypti* (mosquitoes), *Hippilates* (eye gnats), *Musca domestica* (houseflies) and *Gigantolaelaps* sp. (mites) (Bergold GH et al., 1968; Corn JL et al., 1990; Comer JA et al., 1991; Herrero MV et al., 1994). Phlebotomine sand flies are suspected to play a role in the maintenance and transmission of VSV-NJ in Ossabaw Island, Georgia as sand flies are active and numerous during feral swine seroconversion and sentinel domestic swine (Stallknecht DE, et al., 1993; Comer JA et al., 1993). Sand flies from Ossabaw Island have been shown capable of replicating and transmitting VSV-NJ (Corn JL et al., 1990; Comer JA et al., 1992). Sand flies have been found to be a risk factor for VSV occurrence in Costa Rican dairy farms (Herrero MV et al., 1994), since farms with a forested land and the presence of sand flies had a higher risk of VSV infection (Vanleeuwen JA et al., 1995).

It has been hypothesized that black flies play a role in the transmission of VSV in the southwest United States (Walton TE, et al., 1987; Webb PA, Holbrook FR, 1988; Mead DG et al., 1999). VSV-NJ was isolated from black flies during the 1982 outbreak in the southwestern United States (Francy DB et al., 1988). These insects are known pests of livestock (Cupp EW, 1996), and could be the vector involved in transportation of VSV by wind from enzootic regions in Mexico northward through the United States (Sellers RF, Maarouf AR, 1990). Experimental evidence demonstrated that black flies

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are competent vectors of VSV-NJ as the virus replicates and is secreted in their saliva (Cupp EW et al., 1992), infected flies can transmit the virus to laboratory mice (Mead DG et al., 1999) and can transmit the virus to other black flies through co-feeding on a non-viremic host (Mead DG et al., 2000).

VSV has been isolated from mosquitoes in New Mexico during the 1960s (Sudia WD et al., 1967) and has been shown to be a competent vector in the laboratory (Bergold GH et al., 1968); however, the efficiency of transmission of VSV-NJ is so low that it could not play an important role as the main vector in nature (Bergold GH et al., 1968). Low amounts of VSV was isolated from mosquitoes during the 1982 outbreak in Colorado (Francy DB et al., 1988). Over fifty-one thousand insects were pooled and tested for VSV; despite isolation if other viruses, VSV was never recovered (Francy DB et al., 1988). It has been hypothesized that VSV is a plant virus infecting vegetation on which both male and female insects feed (Johnson KM et al., 1969). The virus is believed to replicate in the insect followed by the transmission onto flora and fauna (Johnson KM et al., 1969). VSV-NJ has been isolated from non-biting flies, *Anthomyidae*, *Musca autumnalis* and *Mansonia indubitans* (Monath TP et al., 1986). The non-biting flies feed on the mammalian oral, nasal and lachrymal secretions, or on lesions on the feet and teats, and are thought to transmit the virus mechanically from animal-to-animal or through contaminated food sources (Monath TP et al., 1986). However, no direct evidence has ever been found to support this hypothesis.

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1.8 Viral Ecology

1.8.1 Environment

Ecology is thought to play a role on the viral maintenance of VSV. Endemic regions of VS occur in forested tropics (Webb PA, Holbrook FR, 1988). Within the tropical regions, infections are likely to occur in the heavily grazed tropical dry forest, at higher altitudes further inland on the savannahs and higher pastures in the mountain valleys, (Webb PA, Holbrook FR, 1988) with cases being reported as high as 9000 feet in Mexico (Mason J et al., 1976).

In the southeastern United States, VS occurs by low-lying coastal swampland with sluggish streams (Webb PA, Holbrook FR, 1988). Vegetation is lush and the moisture level is high (Webb PA, Holbrook FR, 1988).

Regions with sporadic occurrence are located in temperate regions (Webb PA, Holbrook FR, 1988). It has been hypothesized that outbreaks in the southwestern United States originate in enzootic regions of Mexico and proceed in a northern migratory direction starting in the spring (Jonkers AH, 1967; Rodriguez LL et al., 2000; McCluskey BJ et al., 1999). VS cases are located along waterways, valleys in the Rocky Mountains or in the Upper Mississippi Valley, not along roads or trucking routes and often missing concentrated regions of livestock (Hanson RP, 1952; Nichol ST, 1994). In 1937 infected livestock were confined to wooded areas east of the Red River and along Lake Manitoba and Lake Winnipeg (Hanson RP, 1952). No livestock tested positive for VSV on farms located on the open plains (Hanson RP, 1952). In 1942, cases of VSV were located along the Platte River in Colorado and the next year along the Rio Grande and Gunnison Rivers (Hanson RP, 1952). Between 1982 and 1983, cases of VSV-NJ increased rapidly north

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along the Rio Grande in New Mexico and Colorado River valleys on the western slope of Colorado (Walton TE, et al., 1987). Clinically affected and non-affected premises can occur side by side (Jonkers AH, 1967). The same premises, and even on the same pasture, have known to be infected with VSV in multiple outbreak years (Jonkers AH, 1967). VSV infected premises have been associated with waterways, trees, brush, and weeds (Webb PA, Holbrook FR, 1988). Livestock located on adjacent premises with short dry grass pastures, open sandy pastures, or upland pastures and well-drained fields avoid or have a lower incidence of infection (Webb PA, Holbrook FR, 1988).

In South America, vegetation type, land use, and ground moisture were associated with the prevalence of VSV-NJ antibodies with cases of VSV starting in the end of the rainy season (Cline BL, 1976; Nichol ST, 1994).

1.8.2 Climate and Weather

In the endemic regions of Mexico, Panama, Colombia, and Venezuela, the tropical climate has high annual rainfall, high humidity, and a lack of a dry season (Webb PA, Holbrook FR, 1988). The annual temperature is 25°C with water surplus and cooler temperatures during the high season of VSV activity (Webb PA, Holbrook FR, 1988). In regions of epidemic activity in Mexico the mean annual temperature is between 15 to 21°C in the higher altitudes (Mason J et al., 1976).

In the endemic regions of the southeastern United States seroconversion occurs from May to October after rains and in the warmer weather (Webb PA, Holbrook FR, 1988). During periods of higher than average rainfall, VSV can spread to other regions (Webb PA, Holbrook FR, 1988).

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In temperate regions, outbreaks usually begin in late spring, peak in the late summer and cease with the first frosts of late fall and early winter (Jonkers AH, 1967; Nichol ST, 1994). VS is associated with warm weather during the summer months, heavy rainfall or water surplus prior to outbreaks (Meyer NL et al., 1960; Jonkers AH, 1967; Webb PA, Holbrook FR, 1988). Outbreaks of VS normally end during the first winter frost in the fall or after the cold and dry weather (Jonkers AH, 1967), however in 1949 cases were reported two weeks after the first winter frost (Brandly CA et al., 1951) and new cases were being reported during the winter of 1982 to 1983 (Thurmond MC, et al., 1987). Infected animal movement is thought to have played a role in animal-to-animal transmission. Trajectory winds have been proposed to play a role in the transmission and spread of VSV by transportation of infected insects (Sellers RF, Maarouf AR, 1990).

Rodriguez et. al., examined the relationship of genetic diversity between different ecological regions and demonstrated that different genetic lineages were maintained in distinct ecological zones over long periods of time (Rodriguez LL et al., 1996).

1.9 Viral Characteristics

1.9.1 Introduction

Vesicular stomatitis viruses are members of the *Rhabdoviridae* family, order *Mononegavirales* and genus *Vesiculovirus* (Nichol ST, 1994). Rhabdoviruses are enveloped viruses with an outer surface of projections 5 to 10 nm long and about 3nm in diameter (Dietzschold B et al., 1996). The nucleocapsid or ribonucleoprotein (RNP) core exhibits helical symmetry with a diameter of 30 to 70 nm that includes the

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nucleocapsid (N) protein, the polymerase associated phosphoprotein (P or NS) and the large (L) polymerase protein. The RNP core is encapsulated by the matrix (M) protein, which is enveloped by a lipid bilayer membrane derived from the host cell (Nichol ST, 1994). The glycoprotein (G protein) protrudes from the membrane to form the complete viral structure (Nichol ST, 1994).

The molecular weight of Rhabdoviruses is 300 to 1,000 x 10^6 daltons (Dietzschold B et al., 1996). Virus infectivity is stable at pH 5 to pH 10, but is inactivated at 50°C, by ultraviolet or x-ray irradiation or exposure to lipid solvents and oxidizing agents (Dietzschold B et al., 1996). Viral RNA accounts for 1 to 4% of the particle weight, and has an estimated molecular mass of 4.2 to 4.6 x 10^6 daltons (Dietzschold B et al., 1996). The RNA genome is single-stranded, non-segmented, negative-sense approximately 11kb in length (Rodriguez LL, Nichol ST, 1999). The genome has complementary terminal sequences with a 5' terminal triphosphate that is not polyadenylated (Dietzschold B et al., 1996). The naked RNA is not infectious (Rodriguez LL, Nichol ST, 1999).

Rhabdoviruses share a common bullet- or rod-shaped morphology with common basic biochemical properties (Nichol ST, 1994). Vesiculoviruses exhibit various degrees of serological cross-reactivity (Dietzschold B et al., 1996), and are grouped together by the antigenic cross-reactivity with genetic and biochemical similarities (Nichol ST, 1994). The New Jersey and Indiana serotypes have been defined based on cross-neutralization properties with the Indiana serotype divided into Types 1, 2, 3 and within Indiana Type 1, there are four subtypes. The New Jersey serotype has been separated into three separate subtypes (Nichol ST, 1994).

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The vesiculoviruses contains shaped viral particles that are 100 to 430 nm long and 45 to 100nm in diameter. They are typically rodlike in appearance with one flat and one rounded end. Virions of defective-interfering (DI) particles have the same configuration, but are shorter by about 20% to 50% in length in comparison with the infectious virus that resemble viruses in their protein and lipid composition, yet are not infectious because they lack 50% to 80% of the genome (Dietzschold B et al., 1996).

1.9.2 Properties of VSV Proteins

The VSV genome codes for five proteins in the order of: nucleocapsid (N gene) - phosphoprotein (P gene) - matrix (M gene) - glycoprotein (G gene) - large polymerase (L gene) that are separated by nonoverlapping open reading frames (ORFs) and there are two small highly basic nonstructural proteins that are coded in a second ORF within the P gene (Letchworth GJ et al., 1999). The N (nucleocapsid) protein is 422 amino acids in length that encapsulates the virion RNA and is essential for transcribing or replicating viral cores and functions in close association with the P protein (Nichol ST, 1994). The N protein may also be the predominant cross-reactive antigen recognized by primary anti-VSV cytolytic T-lymphocytes and the major target antigen for secondary anti-VSV cytolytic T-lymphocytes response (Dietzschold B et al., 1996).

The P or NS (phosphoprotein) protein is a highly phosphorylated protein that has a length of 274 amino acids in VSV-NJ and 265 amino acids in VSV-IN (Dietzschold B et al., 1996). There is 68% similarity of the phosphoprotein at the amino acid level between serotypes Indiana and New Jersey (Nichol ST, 1994). This protein is associated with viral polymerase activity; its primary functions appear to be the mediation of

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binding of the L protein binding to the nucleocapsid core and facilitating access of the polymerase to the RNA template during transcription and replication (Nichol ST, 1994).

The M (matrix) protein is 229 amino acids in length and is believed to have two main functions. The protein plays a critical role during assembly of virion particles prior to budding from the cell surface membrane. It binds to the glycoprotein monomers and promotes their trimerization and is associated with the cellular lipid bilayer through the C-terminal domain and is believed to promote the condensation of viral RNP cores into tightly coiled helical structures subsequent to release within virion particles. The M protein, in association with RNPs, inhibits genome transcription and can play a role in the regulation of viral genome transcription (Nichol ST, 1994).

The VSV-NJ G protein is the surface glycoprotein comprised of 517 amino acids in length. It is a typical Class I membrane-associated glycoprotein, with the N-terminal projecting from the surface of the virion or infected cell and the C-terminal projecting into the interior of the cell or virion (Nichol ST, 1994). The first 29 amino acids of the C-terminus comprise the cytoplasmic domain and the next 20 hydrophobic residues form the transmembrane domain (Dietzschold B et al., 1996). The major segment of the G protein, extending from the transmembrane to the N-terminus, is the external antigen domain. The G protein forms trimers that are approximately 400 spikes on the virion envelope (Rodriguez LL, Nichol ST, 1999). The major role of the G protein is to attach and to penetrate the VSV into the susceptible cells and to bud virions from the infected cells (Rodriguez LL, Nichol ST, 1999). It is the major target of VSV-specific neutralizing antibodies and is capable of inducing cell membrane fusion at a low pH (Rodriguez LL, Nichol ST, 1999). There is 50% similarity at the amino acid level

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between the G proteins of the Indiana and New Jersey serotypes. In both VSV-NJ and VSV-IN, the position and size of the transmembrane domain, signal sequence, and glycosylation sites are identical, except that the G gene of the New Jersey serotype is not acetylated (Dietzschold B et al., 1996; Pal R et al., 1985). There are four major antigenic epitopes that are known to react with neutralizing monoclonal antibodies on the G protein. The monoclonal antibodies that recognize these epitopes do not cross-react between the G proteins of the two serotypes. There is a fifth neutralizing epitope that is shared by G proteins of the two serotypes. The glycoprotein spikes of VSV represent the only antigens that induce viral neutralizing antibodies (VNAs) and are able to confer immunity against a lethal challenge infection. These abilities are crucially dependent on the intact secondary and tertiary structure of the glycoprotein. Infection with VSV results in a generation of virus-specific CD8+ and CD4+ T-cells (Dietzschold B et al., 1996). The glycoprotein is also the major antigen of the virus that mediates cytolytic T-lymphocyte (CTL) responses (Dietzschold B et al., 1996).

The large L (RNA-dependent RNA polymerase) protein is 2109 amino acids in length and is a multifunctional enzyme in that performs most of the polymerase-associated functions of the virus. This can include RNA synthesis, capping, methylation, and polyadentilation. The L protein possesses the protein kinase activity and preferentially phosphorylates serine residues on the P protein. The inhibition of P protein phosphorylation prevents transcriptase activity (Nichol ST, 1994).

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1.9.3 Replication and Infection

The replication of VSV starts when the G protein interacts with the receptors of the host cell and the virion penetrates the host cell by receptor-mediated endocytosis. Once inside the cell, the G protein mediates fusion of the cell and viral membranes under the low-pH conditions in the endosomes. The viral ribonucleoprotein or nucleocapsid is released into the cytoplasm and the negative-sense viral RNA is transcribed by the virion-associated polymerase complex. Six viral transcripts are produced and the active transcribing complexes consisting of the RNA genome associated with the N protein, the polymerase, P and L proteins. The polymerase complex then starts transcribing at the single entry site at the 3' end of the genome and transcribes each gene in decreasing amounts as it moves away from the 3' entry site. The leader RNA, composed of the 47-nucleotide transcript, is an exact copy of the genome 3' end. This sequence is then followed by an untranscribed junctional sequence AAA in the Indiana serotype and AAAA in the New Jersey serotype. The leader transcript is transported to the nucleus where it inhibits the host cell transcription. The N mRNA is capped during synthesis by the virion polymerase complex and at the end of all five viral genes is a sequence 5'-AGUUUUUUUCAUA-3' which signals the termination and polyadenylation of the mRNA. Translation of the mRNAs and transcription reflects the protein amounts and their relative abundance of each mRNA. After translation of the viral proteins, a full-length positive-sense copy of the genome is made. Once the full-length positive strand is made, production of the negative-sense RNA genomes necessary for packaging into released virions are created. The viral mRNAs are transcribed in the cytoplasm of infected cells and their translation is directly connected to the transcription process. In

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order for the virions to be assembled and released, the glycoprotein forms a patch on the surface of the infected cell and the M protein forms a bridge between the G protein membrane and the virion RNP core. This promotes the tight condensation of the cores and assembly into mature virion particles. The virions are then released from the surface of the infected cell.

VSV can grow in a variety of vertebrate and insect tissue cell cultures. VSV grows to high titers of nine logs, is normally highly pathogenic and causes cell death less than 12 hours post-infection in mammalian cells (Rodriguez LL, Nichol ST, 1999). However, it does not produce CPE and grows to lower titers in insect cells (Nichol ST, 1994).

1.10 Genetics

1.10.1 Evolution

VSV belongs to the family *Rhabdoviridae* in the order Mononegavirales. Its genome consists of a negative-sense, 11kb single-stranded RNA molecule that encodes five proteins. Due to the lack of proofreading activity of its RNA polymerase and high rate of replication, VSV has the potential for rapid evolution with an error rate of 1×10^{-4} - 2×10^{-5} substitutions per nucleotide copied per round of replication (Holland JJ et al., 1982; Drake JW, Holland JJ, 1999; Steinhauer DA, Holland JJ, 1986). Provided the 11kb genome size, this means that each virion contains at least one mutation (Novella IS et al., 1995) and is approximately a million-fold more error prone than that of organismal DNA (Nichol ST et al., 1993). It has even been stated that the study of RNA virus evolution

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over a 50-year period is essentially equivalent to that of an organismal system over a 50-million year period (Nichol ST et al., 1993).

Due to the extremely high mutation frequencies of RNA viruses, in which complex genetically heterogeneous populations can evolve are referred to as quasispecies (Duarte EA et al., 1994). The quasispecies term describes the complex mixture of heterogeneous viral populations, which have related genomes differing from each other in one or more base sites. These viruses can adapt very rapidly to new conditions under selective factors (Drake JW, Holland JJ, 1999; Domingo E et al., 1996). There will always be members of the quasispecies that can grow better under certain conditions through competitive selection and these adaptations can revert rapidly by the same mechanism if the conditions are changed or reversed. Quasispecies are characteristics of RNA viruses, and despite the high amount of viral heterogeneity in the population, the viruses can maintain stable master sequences. They are still diverse quasispecies, but if the selective environment is not greatly altered, and if the RNA genome master sequences are highly fit, then the consensus sequence can be maintained for long periods (Domingo E et al., 1996).

1.10.2 Phylogenic Analysis

In 1987, Nichol published the T1 RNase fingerprint analyses of RNA genomes from 43 virus isolates from epidemic and enzootic areas of VSV activity (Nichol ST, 1987). His results indicated that VSV populations were relatively homogenous within the United States and that viruses isolated from feral swine on Ossabaw Island, Georgia were distinct from all other United States isolates (Nichol ST, 1987). Viruses from

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Mexico were related to viruses from the western United States and viruses circulating in enzootic regions of Central and South America had a higher degree of genetic heterogeneity (Nichol ST, 1987). Phylogenetic analyses of the glycoprotein gene of 34 VSV-NJ isolates that were fingerprinted in the prior study were sequenced to confirm the prior results and conclusions (Nichol ST et al., 1989). These results not only confirmed the phylogenetic relationship of viruses to their respective relatives, but also allowed for computations of the genetic divergence between viruses. Throughout the entire gene, up to a 19.8% sequence divergence between individual virus isolates was found between viruses from as far south as Ecuador to as far north as Idaho and from equine, bovine, porcine, and mosquito species (Nichol ST et al., 1989). Base changes were distributed randomly throughout the gene (Nichol ST et al., 1989). Most of the nucleotide substitutions occurred in the third position of the codon (Nichol ST et al., 1989). There were no insertions or deletions found, indicating that recombinational rearrangements do not play a major role in the VSV-NJ evolution (Nichol ST et al., 1989). Additional sequencing and phylogenetic analyses of the P and N genes demonstrated similar findings to the G gene analyses (Bilsel PA, Nichol ST, 1990). VSV-NJ isolates had a 17% and 19% sequence variation in the N and P genes, respectively and produced similar phylogenetic trees of the N and P genes (Bilsel PA et al., 1990).

In 1995, an outbreak of VSV-NJ occurred in the southwestern United States and phylogenetic analyses of twenty-three viral isolates of the G gene demonstrated that the 1995 VSV-New Jersey belong to a lineage distinct from that of the 1982 to 1985 viruses that caused previous outbreaks in the western United States and was distinct from that of strains from Central America and the Georgian Hazelhurst strain (Llewellyn ZN et al.,

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2000). Further sequencing and phylogenetic analyses of the P gene from viruses of the western United States in 1995, 1997, and 1998 outbreaks, indicated that the VSV-NJ viruses in 1997 were similar to viruses circulating in 1995 and that Indiana viruses circulating in 1997 and 1998 were identical (Rodriguez LL et al., 2000).

It is still unclear why there is such a clear separation between southwestern and southeastern genetic lineages. It has been suggested that this could be due to the differences in host restriction or ecological factors that could influence virus maintenance and transmission (Nichol ST et al., 1989).

1.11 GIS In Epidemiology

Geographical information systems (GIS) is a useful tool for integrating multiple types of data, managing, storing, analyzing, and presenting large volumes of spatial and non-spatial data. Although typically, results are presented as maps or images that summarize the data and analyses, it is not synonymous with mapping and has a vast capacity for application to epidemiological studies. GIS has expanded over the years from Snow's early work on cholera transmission in London to tracking cases during outbreaks, disease surveillance, risk assessment of infectious diseases, environmental impacts on disease, and assessing health surveys. GIS is an efficient system that can help store, manage, and query, model the process of generating data, and help make programmatic decisions.

GIS can be used for collecting and managing geographically referenced data for subsequent spatial analyses. There are many studies using GIS and spatial analysis that integrate infectious disease data with geographic and climatic data (Kitron U et al., 1997;

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Cross E et al., 1996; Colwell RR, 1996; Engelthaler DM et al., 1999; Parmenter RR et al., 1999). Some of the more common spatial techniques used in epidemiology include disease mapping, clustering techniques, and risk analysis using map comparisons and regression analysis (Moore DA, Carpenter TE 1999).

Clustering techniques are used to detect health events that are situated close together either in time, space, or time and space. These techniques were used to explore the time and space clustering of horses with *Corynebacterium pseudotuberculosis* infection in California (Doherr MG et al., 1999), the seasonal cyclicity of sheep scab outbreaks in the United Kingdom (French NP et al., 1999) and the occurrence of blowfly strike in Queensland, Australia (Ward MP, et al., 2000).

Risk factor analysis has used aerial patterns to identify potential risk factors for disease incidence or prevalence. These methods have been used extensively in the use of arthropod vector surveillance and control (Washino RK, Wood BL, 1994; Hay SI et al., 1997). Remote sensing using satellite imagery has increased the opportunities to explore infectious diseases and has been applied to studying many diseases including Lyme disease (Dister SW et al., 1997), malaria (Beck LR et al., 1997) and leishmaniasis (Cross E et al., 1996).

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1.12 Tables

Table 1. Vesicular stomatitis outbreaks in the United States 1906-1998 (+).

Year	States Reported	Species	Serotype	No. of premises affected	Reference
1906	**CO**	**N.D.**	**N.D.**	**>200**	**(Heiny E, 1945)**
1916	**CO, UT, MT, WY, MO, NE, KS, SD, IL, VA**	**B, E**	**N.D.**	**>200** **>500**	**(Eichhorn A, 1917; Hanson RP, 1952)**
1925	KS, IN	B, E	IN	N.D.	(Cotton, 1927)
1926	NJ	B, E	NJ	N.D.	(Cotton, 1927)
1926	**CO**	**B, E**	**N.D.**	**>200**	**(Heiny E, 1945)**
1934-35	VA, WV	B	N.D.	N.D.	(Hanson RP, 1952)
1937	WI, MN	B, E	N.D.	N.D.	(Brandly CA et al., 1951)
1941	TX, LA, AL	E	N.D.	N.D.	(Hanson RP, 1952)
1942	**CO**	**B, E**	**IN**	**>200**	**(Heiny E, 1945)**
1943	**CO**	**B,E**	**NJ**	**>200**	**(Heiny E, 1945)**
1943	MO	S	NJ	1	(Hanson RP, 1952)
1944	**CO, TX**	**B, E, S, H**	**NJ**	**>200**	**(Heiny E, 1945)**
1945	**CA**	**B, E**	**NJ**	**1**	**(Hanson RP, 1952)**
1947	**AZ**	**E**	**N.D.**	**50**	**(Hanson RP, 1952)**
1949	**AZ, TX, CO, UT WY, MT, MN, WI, CA**	**B, E**	**NJ**	**>5000**	**(Hanson RP, 1952)**
1949	AL, MS, GA,TN, FL	B	NJ	N.D.	(Hanson RP, 1952)
1952	GA	B, S	NJ	N.D.	(Meyer NL et al., 1960)
1953	GA, NC, VA, WV, MD, OK, NJ	B, S	NJ	N.D.	(Meyer NL et al., 1960)
1954	FL, GA, SC	B, S	NJ	N.D.	(Meyer NL et al., 1960)
1955	GA, NC, SC, LA	B, S	NJ	N.D.	(Meyer NL et al., 1960)
1956	GA, NC, SC, LA	S, E	NJ	N.D.	(Meyer NL et al., 1960)

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1956	**NM, CO**	**B, E**	**IN**	**N.D.**	**(Meyer NL et al., 1960)**
1957	GA, NC, SC, LA, AK, OK	B, S, E	NJ	372	(Meyer NL et al., 1960)
1958	GA, NC, SC, LA	B, S	NJ	N.D.	(Meyer NL et al., 1960)
1959	GA, NC, SC, VA WV, LA MS	B, S, E	NJ	N.D.	(Meyer NL et al., 1960)
1959	**TX, NM**	**B, E**	**NJ**	**115**	**(Meyer NL et al., 1960)**
1960	GA, NC, SC LA, TX	B, S	NJ	N.D.	(Meyer NL et al., 1960)
1961	AL	S	NJ	N.D.	(Jenney EW, 1967)
1962	AL	S	NJ	N.D.	(Jenney EW, 1967)
1963	GA, AL, SC, AR, FL	B, E	NJ	240	(Jenney EW, 1967)
1964	**TX, OK, AR, MO, CO**	**B, E, H**	**IN/NJ**	**>200**	**(Jenney EW, 1967)**
1965	**NM, CO, UT**	**B, E**	**IN**	**124**	**(Jenney EW, 1967)**
1966	TX, OK, AR, MO, CO	B, E	NJ	N.D.	(Jenney EW, 1967)
1966	**NM, CO, UT**	**B, E**	**IN/NJ**	**600**	**(Jenney EW, 1967)**
1967	LA	S	NJ	1	(Jenney EW, 1967)
1968	LA	B, E, S	NJ	N.D.	(Jenney EW, Brown CL, 1972)
1969	LA	B	NJ	N.D.	(Jenney EW, Brown CL, 1972)
1970	NC, SC	B	NJ	N.D.	(Jenney EW, Brown CL, 1972)
1972	**CO, NM**	**E**	**NJ**	**13**	**(Jenney EW et al., 1980)**
1972	LA	B	NJ	19	(Jenney EW et al., 1980)
1973	LA, MS	B	NJ	5	(Jenney EW et al., 1980)
1974	NC	B	NJ	1	(Jenney EW et al., 1980)
1977	SC	B	NJ	1	(Jenney EW et al., 1980)

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1982/83^	**AZ, NM, UT, CO, WY, ID, MT, SD, CA, NE, KS, MS, WA, OR**	**B, E, H**	**NJ**	**627**	**(Jenney EW et al., 1980)**
1984	TX	B	NJ	1	(Jenney EW et al., 1980)
1985	**NM, AZ, CO**	**B, E**	**NJ**	**256**	**(Hall AE, 1985)**
1995	**AZ, CO NM, TX, UT, WY**	**B, E**	**NJ**	**367**	**(Bridges VE et al., 1997)**
1997	**AZ, CO, NM, UT**	**B, E**	**IN/NJ**	**380**	**(McCluskey BJ et al., 1999)**
1998	**AZ, CO, NM, TX**	**E**	**IN**	**130**	**(McCluskey BJ et al., 1999)**

Note: (*) number of premises estimated by the authors based on the reference provided. Outbreaks involving the western United States are in bold. (^) indicates that outbreak continued through the winter of 1983. Species affected: B: bovine, E: equine, H: human, S: swine. (+) Table adapted from reference (Rodriguez LL et al., 2000).

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Table 2. Vesicular stomatitis outbreaks in selected areas of Mexico from 1981 – 1998 (+).

	Years of occurrence	Laboratory confirmed outbreaks
Northern States		
Sonora	1982, 1984, 1985	20
Chihuahua	1981, 1982, 1984, 1994	27
Cohauila	N/A	0
N. Leon	1985, 1996	4
Tamaulipas	N/A	0
Central and Southern States		
Veracruz	1981 to 1998	432
Chiapas	1981 to 1998	366
Tabasco	1981 to 1998	206
Jalisco	1981 to 1986, 1993 to 1994, 1996 to 1997	108
Guerrero	1982 to 1987, 1992, 1994 to 1996	98
Michoacan	1981, 1983, 1984, 1986, 1988, 1993 to 1996	96
Morelos	1981, 1982, 1984 to 1987, 1992 to 1996	76

(+) Table adapted from reference (Rodriguez LL et al., 2000).

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1.13 References

1. Aguirre AA, McLean RG, Cook RS, and Quan TJ (1992) Serological Survey for Selected Arboviruses and Other Potential Pathogens in Wildlife from Mexico. Journal of Wildlife Diseases 28: 435-442

2. Arbelaez G, Rocha J, Bernal C (1988) Vaccine Trial Against Vesicular Stomatitis III: Protective Response of a Serotype New Jersey Immunogen. Revista ACOVEZ 12:18-23

3. Arbelaez G, Valbuena R, Rocha J (1989) Protective Response to an Immunogen Against Vesicular Stomatitis (Indiana Serotype). Revista ICA 24:19-23

4. Beck LR, Rodriguez MH, Dister SW, Rodriguez AD, Washino RK, Roberts DR, Spanner MA (1997) Assessment of Remote Sensing-based Model for Predicting Malaria Transmission Risk in Villages of Chiapas, Mexico. American Journal of Tropical Medicine & Hygiene 56:99-106

5. Bergold GH, Suarez OM, Munz K (1968) Multiplication in and Transmission by *Aedes aegypti* of Vesicular Stomatitis Virus. Journal of Invertebrate Pathology 11: 406-428

6. Bilsel PA, Nichol ST (1990) Polymerase Error Accumulating During Natural Evolution of the Glycoprotein Gene of Vesicular Stomatitis Virus Indiana Serotype Isolates. Journal of Virology 64:4873-4883

7. Bilsel PA, Pamuk A, Row JE, Fitch WM, Nichol ST (1990) Phosphoprotein and Nucleocapsid Protein Evolution of Vesicular Stomatitis Virus New Jersey. Journal of Virology 64:2498-2504

8. Brandly CA, Hanson RP, Chow TL. (1951) Proceedings from the 88th Annual Meeting of the American Veterinary Medical Association. pp 61-69.

9. Bridges VE, McCluskey BJ, Salman MD, Scott HH (1997) Review of the 1995 Vesicular Stomatitis Outbreak in the Western United States. Journal of the American Veterinary Medical Association 211:556-560

10. Brody JA, Fischer GF, Peralta PH (1967) Vesicular Stomatitis Virus in Panama. Human Serologic Patterns in a Cattle Raising Area. American Journal of Epidemiology 86:158-161

11. Burton AC (1917) Stomatitis Contagiosa in Horses. Veterinary Journal 73:234-237

12. Cline BL (1976) Ecological Associations of Vesicular Stomatitis Virus in Rural Central America and Panama. American Journal of Tropical Medicine & Hygiene 25:875-883

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13. Colwell RR (1996) Global Climate and Infectious Disease: The Cholera Paradigm. Science 274:2025-2031

14. Comer JA, Kavanaugh DM, Stallknecht DE, Ware GO, Corn JL, Nettles VF (1993) Effect of Forest Type on the Distribution of *Lutzomyia shannoni* (Diptera: Psychodidae) and Vesicular Stomatitis Virus on Ossabaw Island, Georgia. Journal of Medical Entomology 30:555-560

15. Comer JA, Stallknecht DE, Corn JL, Landgraf JG, Nettles VF (1991) *Lutzomyia shannoni* (Diptera:Psychodidae): A Biological Vector of the New Jersey Serotype of Vesicular Stomatitis Virus on Ossabaw Island, GA. Parassitologia 33:151-158

16. Comer JA, Stallknecht DE, Corn JL, Landgraf JG, Nettles VF (1992) Titers of Vesicular Stomatitis Virus, New Jersey Serotype, in Male and Female *Lutzomyia shannoni* (Diptera:Psychodidae) in Georgia. Journal of Medical Entomology 29:368-370

17. Corn JL, Comer JA, Erickson GA, Nettles VF (1990) Isolation of Vesicular Stomatitis Virus New Jersey Serotype From Phlebotomine Sand Flies in Georgia. American Journal of Tropical Medicine & Hygiene 42:476-482

18. Cotton W (1927) Vesicular Stomatitis. Veterinary Medicine 22:169-175

19. Cross E, Newcomb W, Tucker CJ (1996) Use of Weather Data and Remote Sensing to Predict the Geographic and Season Distribution of Phlebotomus papatasi in Southwest Asia. American Journal of Tropical Medicine & Hygiene 54:530-536

20. Cupp EW (1996) Black Flies and the Agents They Transmit. In: Beaty BJ, Marquardt WC (eds) The Biology of Disease Vectors. University Press of Colorado, Niwot, pp 98-109

21. Cupp EW, Mare CJ, Cupp MS, Ramberg FB (1992) Biological Transmission of Vesicular Stomatitis Virus (New Jersey) by *Simulium vittatum* (Diptera:Simuliidae). Journal of Medical Entomology 29:137-140

22. Dietzschold B, Rupprecht CE, Fu ZF, Koprowski H (1996) Rhabdoviruses. In: Fields BN (ed) Fields Virology. Raven Publishers, Philadelphia, pp 1137-1152

23. Dister SW, Fish D, Bros SM, Frank DH, Banerjee AK, Wood BL (1997) Landscape Characterization of Peridomestic Risk For Lyme Disease Using Satellite Imagery. American Journal of Tropical Medicine & Hygiene 57(6): 687-692

24. Doherr MG, Carpenter TE, Wilson WD, Gardner IA (1999) Evaluation of Temporal and Spatial Clustering of Horses with *Corynebacterium*

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pseudotuberculosis Infection. American Journal of Veterinary Research 60:284-291

25. Domingo E, Escarmis C, Sevilla N, Moya A, Elena SF, Quer J, Novella IS, Holland JJ (1996) Basic Concepts in RNA Virus Evolution. FASEB 10:859-864

26. Drake JW and Holland JJ (1999) Mutation Rates Among RNA Viruses. Proceedings of the National Academy of Science U.S.A. 96:13910-13913

27. Duarte EA, Novella IS, Ledesma S, Clarke DK, Moya A, Elena SF, Domingo E, Holland JJ (1994) Subclonal Components of Consensus Fitness in an RNA Virus Clone. Journal of Virology 68:4295-4301

28. Eichhorn A (1917) Vesicular Stomatitis in Cattle. Journal of the American Veterinary Medical Association 12:162-162

29. Ellis EM, Kendall HE (1964) The Public Health and Economic Effects of Vesicular Stomatitis in a Herd of Dairy Cattle. Journal of the American Veterinary Medical Association 144:377-380

30. Engelthaler DM, Mosley DG, Cheek JE, Komatsu KK, Ettestad P, Davis T, Tanda DT, Miller L, Frampton JW, Porter R, Bryan RT (1999) Climatic and Environmental Patterns Associated with Hantavirus Pulmonary Syndrome, Four Corners Region, United States. Emerging Infectious Diseases 5:87-94

31. Flanagan EB, Zamparo JM, Ball LA, Rodriguez LL, Wertz GW (2001) Rearrangement of the Genes of Vesicular Stomatitis Virus Eliminates Clinical Disease in the Natural Host: New Strategy for Vaccine Development. Journal of Virology 75:6107-6114

32. Fletcher WO, Stallknecht DE, Kearney MT, Eernisse KA (1991) Antibodies to Vesicular Stomatitis New Jersey Type Virus in White-tailed Deer on Ossabaw Island, Georgia, 1985 to 1989. Journal of Wildlife Diseases 27:675-680

33. Francy DB, Moore CG, Smith GC, Jakob WL, Taylor SA, Calisher CH (1988) Epizootic Vesicular Stomatitis in Colorado, 1982: Isolation of Virus From Insects Collected Along the Northern Colorado Rocky Mountain Front Range. Journal of Medical Entomology 25:343-347

34. French NP, Berriatua E, Wall R, Smith K, Morgan KL (1999) Sheep Scab Outbreaks in Great Britain Between 1973 and 1992: Spatial and Temporal Patterns. Veterinary Parasitology 83:187-200

35. Hall AE (1995) Emergency Programs progress report. pp 229-232.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

36. Hanson RP (1952) The Natural History of Vesicular Stomatitis. Bacteriology Review 16:179-204

37. Hanson RP, Estupinan J, Casstaneda J (1968) Vesicular Stomatitis in the Americas. Bulletin of the Office of International Epizoology 70:37-47

38. Hanson RP and Karstad L (1955) Progress Report on Epizootiology of Vesicular Stomatitis of Swine. Wisconsin Agricultural Experimental Station, Madison, Wisconsin

39. Hanson RP, Rasmussen AF, Brandly CA, Brown JW (1950) Human Infection with the Virus of Vesicular Stomatitis. Journal of Laboratory Clinical Medicine 36:754-758.

40. Hay SI, Packer MJ, Rogers DJ (1997) The Impact of Remote Sensing on the Study and Control of Invertebrate Intermediate Hosts and Vectors for Disease. International Journal of Remote Sensing 18:2899-2930

41. Heiny E (1945) Vesicular Stomatitis in Cattle and Horses in Colorado. North American Veterinarian 26:726-730

42. Herrero MV, Jimenez AE, Rodriguez LL, Pereira R (1994) Phlebotomines (Diptera:Psychodidae) Collected at a Costa Rican Dairy Farm in a Vesicular Stomatitis Endemic Area. Journal of Medical Entomology 31:912-914

43. Holland JJ, Spindler K, Horodyski F, Grabau E, Nichol ST, Vanderpol S (1982) Rapid Evolution of RNA Genomes. Science 215:1577-1585

44. Hurd HS, McCluskey BJ, Mumford EL (1999) Management Factors Affecting the Risk for Vesicular Stomatitis in Livestock Operations in the Western United States. Journal of the American Veterinary Medical Association 215:1263-1268

45. Jenney EW (1967) Vesicular Stomatitis in the United States During the Last Five Years (1963-1967). Annual Meeting of the US Animal Health Association 71:371-385

46. Jenney EW and Brown CL (1972) Proceedings 76th Annual Meeting US Livestock Sanitary Association. pp 183-193

47. Jenney EW, Erickson GA, and Snyder BS (1980) Proceedings 23rd Annual Meeting American Association of Veterinary Diagnosticians. pp 337-349

48. Johnson KM, Tesh RB, Peralta PH (1969) Epidemiology of Vesicular Stomatitis Virus: Some New Data and a Hypothesis for Transmission of the Indiana Serotype. Journal of the American Veterinary Medical Association 155:2133-2140

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

49. Jonkers AH (1967) The Epizootiology of the Vesicular Stomatitis Viruses: A Reappraisal. American Journal of Epidemiology 86:286-290

50. Kitron U, Michael J, Swanson J, Haramis L (1997) Spatial Analysis of the Distribution of Lacrosse encephalitis in Illinois, Using a Geographic Information System and Local and Global Spatial Statistics. American Journal of Tropical Medicine & Hygiene 57:469-475

51. Knight AP, Messer NT (1983) Vesicular Stomatitis. Compend Continuing Education of Practicing Veterinarian 5:517-522

52. Letchworth GJ, Rodriguez LL, Del C.Barrera J (1999) Vesicular Stomatitis. The Veterinary Journal 157:239-260

53. Llewellyn ZN, Ou X, Chang GJ, Schmitt B, Salman MD, Akkina RA (2000) Genetic Analysis of Vesicular Stomatitis Virus-New Jersey from the 1995 Outbreak in the Western United States. American Journal of Veterinary Research 61:1358-1363

54. Mason J, Saldana AH, and Turner WJ (1976) Vesicular Stomatitis in Mexico. Animal Health Association. 80:234-253.

55. McCluskey BJ, Hurd HS, Mumford EL (1999) Review of the 1997 Outbreak of Vesicular Stomatitis in the Western United States. Journal of the American Veterinary Medical Association 215:1259-1262

56. Mead DG, Mare CJ, Ramberg FB (1999) Bite Transmission of Vesicular Stomatitis Virus (New Jersey serotype) to Laboratory Mice by *Simulium vittatum* (Diptera: Simuliidae). Journal of Medical Entomology 36:410-413

57. Mead DG, Ramberg FB, Besselsen DG, Mare CJ (2000) Transmission of Vesicular Stomatitis Virus from Infected to Noninfected Black Flies Co-Feeding on Nonviremic Deer Mice. Science 287:485-487

58. Meyer NL, Moulton WM, Jenney EW, Rodger RJ, Aguirre AA (1960) Proceedings 64th Annual Meeting US Livestock Sanitary Association. 64:324-332

59. Monath TP, Webb PA, Francy DB, Walton TE (1986) The Epidemiology of Vesicular Stomatitis-New Data, Old puzzles. St George TD, Kay BH, and Blok J. pp 193-199. Brisbane, CSIRO.

60. Moore DA, Carpenter TA (1999). Spatial Analytical Methods and Geographic Information Systems: Use in Health Research and Epidemiology. Epidemiology Review 21:143-161

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

61. Nichol ST (1987) Molecular Epizootiology and Evolution of Vesicular Stomatitis Virus New Jersey. Journal of Virology 61:1029-1036

62. Nichol ST (1994) Vesicular Stomatitis Virus. In: Webster RG, Granoff A (eds) Encyclopedia of Virology. Saunders, London, pp 1547-1555

63. Nichol ST, Row JE, Fitch WM (1989) Glycoprotein Evolution of Vesicular Stomatitis Virus New Jersey. Virology 168:281-291

64. Nichol ST, Row JE, Fitch WM (1993) Punctuated Equilibrium and Positive Darwinian Evolution in Vesicular Stomatitis Virus. Proceedings of the National Academy of Science 90:10424-10428

65. Novella IS, Clarke DK, Quer J, Duarte EA, Lee CH, Weaver SC, Elena SF, Moya A, Domingo E, Holland JJ (1995) Extreme Fitness Differences in Mammalian and Insect Hosts after Continuous Replication of Vesicular Stomatitis Virus in Sand fly Cells. Journal of Virology 69:6805-6809

66. Pal R, Grinnell BW, Snyder RM, Wiener JR, Volk WA, Wagner RR (1985) Monoclonal Antibodies to the M Protein of Vesicular Stomatitis Virus (Indiana Serotype) and to a cDNA *M* Gene Expression Product. Journal of Virology 55:298-306

67. Parmenter RR, Yadav EP, Parmenter CA, Ettestad P, Gage KL (1999) Incidence of Plague Associated with Increased Winter-spring Precipitation in New Mexico. American Journal of Tropical Medicine & Hygiene 61:814-821

68. Peralta PH, Johnson KM (1958) Vesicular Stomatitis in Man. Journal of the American Veterinary Medical Association 133:57-62

69. Reif JS (1994) Vesicular Stomatitis. In: Handbook of Zoonoses. CRC Press, pp 171-181

70. Reif JS, Webb PA, Monath TP, Emerson JK, Poland JD, Kemp GE, Cholas G (1987) Epizootic vesicular stomatitis in Colorado, 1982: infection in occupational risk groups. American Journal of Tropical Medicine & Hygiene 36:177-182

71. Rodriguez LL, Bunch TA, Fraire M, and Llewellyn ZN (2000) Re-emergence of Vesicular Stomatitis in the Western United States in Associated with Distinct Viral Genetic Lineages. Virology 271:171-181

72. Rodriguez LL, Fitch WM, Nichol ST (1996) Ecological Factors Rather Than Temporal Factors Dominate the Evolution of Vesicular Stomatitis Virus. Proceedings of the National Academy of Science 93:13030-13035

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

73. Rodriguez LL, Letchworth GJ, Spiropoulou CF, Nichol ST (1993) Rapid Detection of Vesicular Stomatitis Virus New Jersey Serotype in Clinical Samples by Using Polymerase Chain Reaction. Journal of Clinical Microbiology 31:2016-2020

74. Rodriguez LL, Nichol ST (1999) Vesicular Stomatitis Viruses. In: Webster RG, Granoff A (eds) Encyclopedia of Virology. Academic Press, London, pp 1910-1919

75. Saulmon EE (1968) The Epidemiology of Vesicular Stomatitis Virus in the United States. Bulletin of the Office of International Epizoology 70:49-63

76. Sellers RF, Maarouf AR (1990) Trajectory Analysis of Winds and Vesicular Stomatitis in North America, 1982-5. Epidemiology of Infectious Diseases 104:313-328

77. Stallknecht DE (2000) VSV-NJ on Ossabaw Island, Georgia. The Truth is Out There. Annals of New York Academy of Sciences 916:413-436

78. Stallknecht DE, Kavanaugh DM, Corn JL, Eernisse KA, Comer JA, Nettles VF (1993) Feral Swine as a Potential Amplifying Host for Vesicular Stomatitis Virus New Jersey Serotype on Ossabaw Island, Georgia. Journal of Wildlife Diseases 29:377-383

79. Stallknecht DE, Nettles VF, Erickson GA, Jessup DA (1986) Antibodies to Vesicular Stomatitis Virus in Populations of Feral Swine in the United States. Journal of Wildlife Diseases 22:320-325

80. Stallknecht DE, Perzak DE, Bauer LD, Murphy MD, Howerth EW (2001) Contact Transmission of Vesicular Stomatitis Virus New Jersey in Pigs. American Journal of Veterinary Research 62:516-520

81. Steinhauer DA, Holland JJ (1986) Direct Method for Quantitation of Extreme Polymerase Error Frequencies at Selected Single Base Sites in Viral RNA. Journal of Virology 57:219-228

82. Sudia WD, Fields BN, Calisher CH (1967) The Isolation of Vesicular Stomatitis Virus (Indiana Strain) and Other Viruses from Mosquitoes in New Mexico, 1965. American Journal of Epidemiology 86:598-602

83. Thurmond MC, Ardans AA, Picanso JP, McDowell T, Reynolds B, Saito J (1987) Vesicular Stomatitis Virus (New Jersey Strain) Infection in Two California Dairy Herds: An Epidemiologic Study. Journal of the American Veterinary Medical Association 191:965-970

84. Ubico SR, McLean RG (1995) Serological Survey of Neotropical Bats in Guatemala for Virus Antibodies. Journal of Wildlife Diseases 31:1-9

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

85. Vanleeuwen JA, Rodriguez LL, Waltner-Toews D (1995) Cow, Farm, and Ecological Risk Factors of Clinical Vesicular Stomatitis on Costa Rican Dairy Farms. American Journal of Tropical Medicine & Hygiene 53:942-950

86. Vernon SD, Rodriguez LL, Letchworth GJ (1990) Vesicular Stomatitis New Jersey Virus Glycoprotein Gene Sequence and Neutralizing Epitope Stability in an Enzootic Focus. Virology 177:209-215

87. Walton TE, et al. (1987) Epizootic Vesicular Stomatitis in Colorado, 1982: Epidemiologic and Entomologic Studies. American Journal of Tropical Medicine & Hygiene 36:166-176

88. Ward MP, Armstrong RT (2000) Time-space clustering of reported blowfly strike in Queensland sheep flocks. Preventive Veterinary Medicine 43:195-202

89. Washino RK, Wood BL (1994) Application of Remote Sensing to Arthropod Vector Surveillance and Control. American Journal of Tropical Medicine & Hygiene 50:134-144

90. Walton TE, Webb PA, Kramer WL, Smith GC, Davis T, Holbrook FR, Moore CG, Schiefer TJ, Jones RH, Janney GC (1987) Epizootic Vesicular Stomatitis in Colorado, 1982: Some Observations on the Possible Role of Wildlife Populations in an Enzootic Maintenance Cycle. Journal of Wildlife Diseases 23:192-196

91. Webb PA, Holbrook FR (1988) Vesicular Stomatitis. In: Monath TP (ed) The Arboviruses: Epidemiology and Ecology. CRC Press, Inc., Boca Raton, pp 1-29

92. Woodring JL, Higgs S, Beaty BJ (1996) Natural Cycles of Vector-Borne Pathogens. In: The Biology of Disease Vectors. University Press of Colorado, pp 51-72

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CHAPTER 2

Evolution of VSV in Nature and *In vitro*

2a. Molecular Evolution of Natural Isolates of VSV in Mexico and the United States

2a.1 Introduction

Vesicular stomatitis virus (VSV) causes periodic outbreaks of disease every five to ten years in livestock and horses in the western United States. VS is classified by the Office of Epizootics (OIE) as a List A disease and therefore mandatory reporting of clinical cases results in trade restrictions to animal and animal products, with consequential economic loss. Despite research efforts, the origin and natural cycle of the viruses causing vesicular stomatitis (VS) outbreaks in northern Mexico and the western United States remain unknown.

VS is clinically identical to foot and mouth disease with signs of vesicles on the tongue, snout, coronary bands and teats of infected animals (Reif JS, 1994). Unlike foot and mouth disease, horses and humans can become infected and display clinical signs. VSV has a broad host range and antibodies to VSV have been identified in deer, antelope, rodents, elk, wild turkeys, goats, sheep, monkeys, bats, ducks, coyote, dogs, feral swine, rabbits, skunks and raccoons (Aguirre AA et al., 1992; Nichol ST, 1994; Webb PA, et al., 1987; Hanson RP, Karstad L, 1955; Stallknecht DE, et al., 1993; Zuluaga, Yuill TM, 1979). There are two main serotypes of VSV: VSV-Indiana (VSV-

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IN) and VSV-New Jersey (VSV-NJ). The later is the most prevalent serotype and is the most widely distributed through out the Americas (Nichol ST, 1994). There is a 50% similarity in the glycoprotein gene sequence between the two serotypes (Dietzschold B et al., 1996). Both VSV-IN and VSV-NJ are restricted to the New World, with only a few isolated cases outside of the Americas (Nichol ST, 1994).

VSV belongs to the family *Rhabdoviridae*, genus *Vesiculovirus* (Dietzschold B et al., 1996). Vesicular stomatitis virus is a negative-strand RNA virus with a genome size of approximately 11 kilobases. It contains five genes (in order from 3' to 5'): nucleocapsid (N), phosphoprotein, matrix, glycoprotein (G), and large (Wagner RR, 1990). The sequences of N and G genes have been extensively used for phylogenetic analysis (Bilsel PA, Nichol ST, 1990; Bilsel PA et al., 1990). The G gene of VSV-NJ encodes for 517 amino acids including a signal peptide of 16 amino acids (Pal R, Wagner RR, 1987). The VSV G protein is anchored in the virus envelope and forms spikes on the surface of the virion (Wagner RR, 1990). The G protein is the serotype-specific antigen that induces neutralizing antibodies (Dietzschold B et al., 1996; Pal R, Wagner RR, 1987).

Although researchers have established detailed molecular and biochemical aspects of VSV (Wagner RR, Rose JK, 1996), the origin and natural cycle of VSV in Mexico and the western United States remains an enigma. In this study, isolates from seven states in Mexico were selected from 1984 to 1997 for phylogenetic analysis to better understand the genetic relationship of viruses circulating in Mexico with viruses previously sequenced from the outbreaks in the United States, Mexico and Central and South America.

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2a.2 Materials and Methods

2a.2.1 Viruses

Out of eighty-one field epithelium samples from Mexico from 1985 to 1997, confirmed positive by CF test for VSV-NJ, eleven yielded PCR product for sequencing. The low recovery rate might be due to inadequate storing of samples over the 15 years. All isolates were of bovine origin, and ten were from the southern states of Chiapas, Tabasco, Veracruz, Morelos, Puebla, Guerrero. One virus was from the central state of Hidalgo (Table 1).

2a.2.2 RNA Extraction, RT-PCR, PCR, Automated Sequencing

Sequences were obtained for 750bp of the glycoprotein gene. The RNA was collected by the acid guanidine thiocyanate method using Trizol (Life Technologies, Grand Island, NY) as previously described (Rodriguez LL et al., 1997). RNA was resuspended in sterile water and stored at -70°C until tested. The RNA was used as a template for reverse transcriptase-polymerase chain reaction using the rTth RNA PCR kit (GeneAmp EZ rTth RNA PCR kit, Perkin-Elmer, Branchburg, NJ) as previously described (Rodriguez LL et al., 1997). PCR reactions were done for 35 cycles with a temperature profile as follows: 50°C (30 min) reverse transcription, 95°C (3 min) denaturation, and 35 cycles of 95°C (45s) denaturation, 50°C (120s) annealing, 72°C (120s) elongation, and a final elongation of 72°C for 5 min. The amplified DNA products were analyzed on a 1.0% agarose gel with ethidium bromide staining. Multiple internal and external primers were used. Primers combinations NJG-1F and NJG-850R were used in the RT-PCR and primers NJG-394F and NJG-368R were used for direct

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sequencing of RT-PCR products. The primer names indicate the serotype, target gene, position starting from the first nucleotide of the open reading frame and sense (forward or reverse).

2a.2.3 Sequencing and Phylogenetic Analysis

RT-PCR products were sequenced with the primers described above using a Big-Dye Terminator sequencing kit and a 370A automated sequencer (PE Applied Biosystems, Foster City, CA). The Sequencher software (Gene Codes Corporation, Ann Arbor, MI) was used to assemble nucleotide sequence fragments and to align sequences.

Sequences for an additional 57 VSV-NJ viruses from Mexico, United States, Central and South America that were previously sequenced were obtained from Genbank and used for phylogenetic analysis. Phylogenetic analysis was performed on a 742-nucleotide fragment comprising nucleotides 61-802 of the open reading frame of the glycoprotein by maximum parsimony using PAUP 4.0 beta version (Swofford DL, 1998). Maximum parsimony settings included a character weighting of 3:1 transition/transversion (ts/tv) ratio, branch-swapping algorithm was tree-bisection-reconnection (TBR), and bootstrap analysis was done by performing1000 replicates. Genetic distance was calculated by proportional distance using MEGA 1.02 version (Kumar S et al., 1993).

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2a.3 Results

2a.3.1. Molecular Epidemiology of VSV in the United States and Mexico

The phylogenetic tree obtained by maximum parsimony analysis of the partial glycoprotein gene sequences of 68 taxa from the United States, Mexico, Central and South America is shown in Figure 1. The overall topology showed a correlation between geographical origin and the position in the phylogenetic tree. Viruses originating in the United States and Mexico shared a common ancestor and formed a distinct group that was well supported by bootstrap analysis. Only the Utah 1949 virus was an exception to this and grouped with viruses originating in Central America. Viruses originating from Guatemala, El Salvador, and Honduras in northern Central America shared a common ancestry and formed a separate clade from the other Central and South American viruses with a bootstrap support of 100. Viruses from Honduras to Panama formed a separate lineage from the northern Central American, United States, and Mexico isolates and formed multiple lineages.

Viruses from the United States grouped into three distinct lineages. Viruses from Georgia between 1952 and 1983 form a separate lineage from viruses in the western United States. Viruses from the western United States formed two distinct lineages for each epidemic cycle from the 1982 to 1985 outbreak and 1995 outbreak containing viruses circulating in Mexico in each western United States clade. Viruses from 1982/1996/1997 Veracruz (VC), 1996 Chiapas (CP), 1994 Tabasco (TB), 1984 Sonora (SN), 1985/1986 Morelos (MR), 1987 Guerrero (GR) were closely related to viruses originating in the western United States from the 1982 to 1985 outbreak (Figure 2). Viruses from 1996 Guerrero (GR), 1990 Hidalgo (HG) and 1984 Oaxaca (OA) were

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closely related to viruses originating in the western United States from the 1995 outbreak (Figure 3). Viruses from 1995 Puebla (PB), 1995 Morelos (MR), and 1982 Veracruz (VC) form a separate lineage that are not associated with any western United States outbreaks. Viruses from 1996 Chiapas (CP) and 1997 Chiapas (CP) form distinct lineages, however the bootstrap support had values less than 50% (Figure 1).

2a.3.2 Genetic Distance

Viruses from the western United States and Mexico were genetically close with no more than 2.83% genetic nucleotide divergence among all viruses (Table 2). Viruses 1984 Sonora and 1985 Morelos are closely related to viruses from 1982 Idaho and 1985 New Mexico with a genetic distance of 0.7% or less. Viruses 1996 Guerrero, 1995 New Mexico and 1995 Colorado had a genetic distance of 0.54% or less. Virus 1995 Morelos had a longer genetic distance with a genetic diversity ranging between 2.4% to 2.7% with viruses 1995 Morelos, 1995 New Mexico and 1995 Colorado. Viruses circulating in Mexico and the western United States from 1982 to 1985 had a genetic divergence ranging from 1.8% and 2.8% with viruses from 1995 and 1996.

2a.3.3 VS Distribution in Mexico

Figure 4 shows the distribution of VSV cases by state reported by the Exotic Animal Disease Commission of Mexico from 1981 to 1995 (Anonymous, 1999; Rubi E, Guerrero L, 2000; Heneidi A, 1999). In northern Mexico, cases of VSV-NJ occur in sporadic cycles, with cases approximately every three to ten years. In 1981, 1982, 1984 and 1985 cases of VSV-NJ were identified within one or more of the northern states of

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Chihuahua, Sonora, and Nuevo Leon. No cases were identified in the northern states from 1986 to 1993, but in 1994 VSV-NJ cases were identified in Chihuahua. There was a higher incidence of VSV-NJ cases in central and southern Mexico, with cases identified each year from 1981 to 1995. The central states had a lower prevalence of the disease than in the southern states. In southern Mexico, cases of VSV-NJ were identified each year. For example, in the southern state of Chiapas, cases were identified each year from 1981 to 1995 except 1986, and the southern state of Veracruz had VSV-NJ cases from 1982 to 1995, expect 1994.

2a.4 Conclusion and Discussion

The phylogeny of VSV strains from United States and Mexico showed a correlation between geographical origin and position in the phylogenetic tree. Genetic lineages of viruses causing outbreaks in the western United States also contained viruses from Mexico, indicating common ancestry and possibly common origin as previously documented (Bilsel PA et al., 1990; Rodriguez LL et al. 2000; Nichol ST, 1987; Nichol ST et al., 1989).

VSV has not been proven to be an endemic disease in the western United States and phylogenetic analysis clearly separates the viruses circulating in 1982 to 1985 from the viruses in 1995. In addition, a close genetic relationship can be seen between the viruses from Mexico and the western United States virus isolates from 1982 to 1995. The sporadic occurrence of VSV in the western United States and in northern Mexico is similar. Cases are diagnosed approximately every three to ten years, with cases in northern Mexico preceding or occurring during outbreaks in the western United States

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from 1981 to 1995. For example, cases were identified in northern Mexico from 1981 to 1982, 1984 to 1985 and in 1994, with no cases reported between years. Cases of VSV-NJ in the southwestern United States occurred in 1982-1983, 1985, and 1995. Observations of VSV activity in north Mexico could reveal early warning for the southwestern United States. Virus originating in the northern state of Sonora in 1984 shared a common ancestor with viruses circulating in the western United States from 1982 to 1985. VSV-NJ cases were also identified in Chihuahua 1994, however no virus was obtained and the genetic relationship to those viruses in the western United States in 1995 remains unknown.

It has been suggested that viruses causing outbreaks in the southwestern United States and northern Mexico originate in central and southern Mexico (Nichol ST, 1987). Introduction of infected animals from Mexico to the United States is unlikely to be the cause, as there is no association between transportation of infected animals or clinical cases along trucking routes and major roads (Nichol ST, 1994). It has been hypothesized that the virus is introduced into the southwestern United States through airborne particles or infected insects originating in Mexico and under optimal environmental and climatic conditions are transported north (Sellers RF, Maarouf AR, 1990). In this work we showed that viruses from the western US are genetically closer to viruses from Mexico than to those circulating in endemic region in the southeastern states. Furthermore, the geographical and temporal distribution of VSV outbreaks in Mexico seems to support the hypothesis of a common origin for VSV strains.

In conclusion, the phylogenetic analysis indicated that viruses isolated between 1986 to 1997 from seven states in Mexico were located within each viral lineage of

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viruses from the southwestern United States 1982 to 1985 outbreak and 1995 outbreak, suggesting a common ancestor. The epidemiology of VSV-NJ in Mexico indicates two cycles, an endemic cycle in the southern and central states and a sporadic cycle similar to the southwestern United States in the northern states. However, ecological and active surveillance studies are needed to understand the different cycles of VSV-NJ between the two regions of VSV activity in Mexico.

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2a.5 Tables

Table 1. Description of vesicular stomatitis viruses in this study.

Virus	Collection Date	Species	State
NJ07/86MRB	7/11/86	Bovine	Morelos
NJ08/90HGB	8/24/90	Bovine	Hidalgo
NJ01/94TBB2	1/7/94	Bovine	Tabasco
NJ11/95PBB	11/22/95	Bovine	Puebla
NJ12/95MRB2	12/7/95	Bovine	Morelos
NJ08/96GOB	8/30/96	Bovine	Guerrero
NJ10/96VCB	10/21/96	Bovine	Veracruz
NJ10/96CPB2	10/26/96	Bovine	Chiapas
NJ12/96CPB	12/21/96	Bovine	Chiapas
NJ06/97CPB	6/16/97	Bovine	Chiapas
NJ09/97VCB	9/4/97	Bovine	Veracruz

Strain nomenclature includes serotype NJ, month/year of clinical case, state, host of bovine origin and individual case number.

Table 2. Comparison of genetic distance (% nucleotide divergence) in the glycoprotein gene of select genotypes of VSV-NJ originated in Mexico 1984/5 and 1995/6 and the western United States 1982/5 and 1995.

	9/82IDB	1/84SNP	1/85MRB	6/85NMOE	6/95NMB	9/95COE	12/95MRB	8/96GOB
9/82IDB	-							
1/84SNP	0.54	-						
1/85MRB	0.67	0.14	-					
6/85NME	0.67	0.14	0.27	-				
6/95NMB	2.29	2.02	2.16	2.02	-			
9/95COE	2.02	1.89	1.89	1.89	0.27	-		
12/95MRB	2.83	2.56	2.70	2.70	2.70	2.43	-	
8/96GOB	2.02	1.75	1.89	1.89	0.54	0.27	2.43	-

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2a.6 Figures

Figure 1. Phylogenetic tree obtained by maximum parsimony analysis of 68 partial glycoprotein sequences of VSV-NJ from North and Central America.

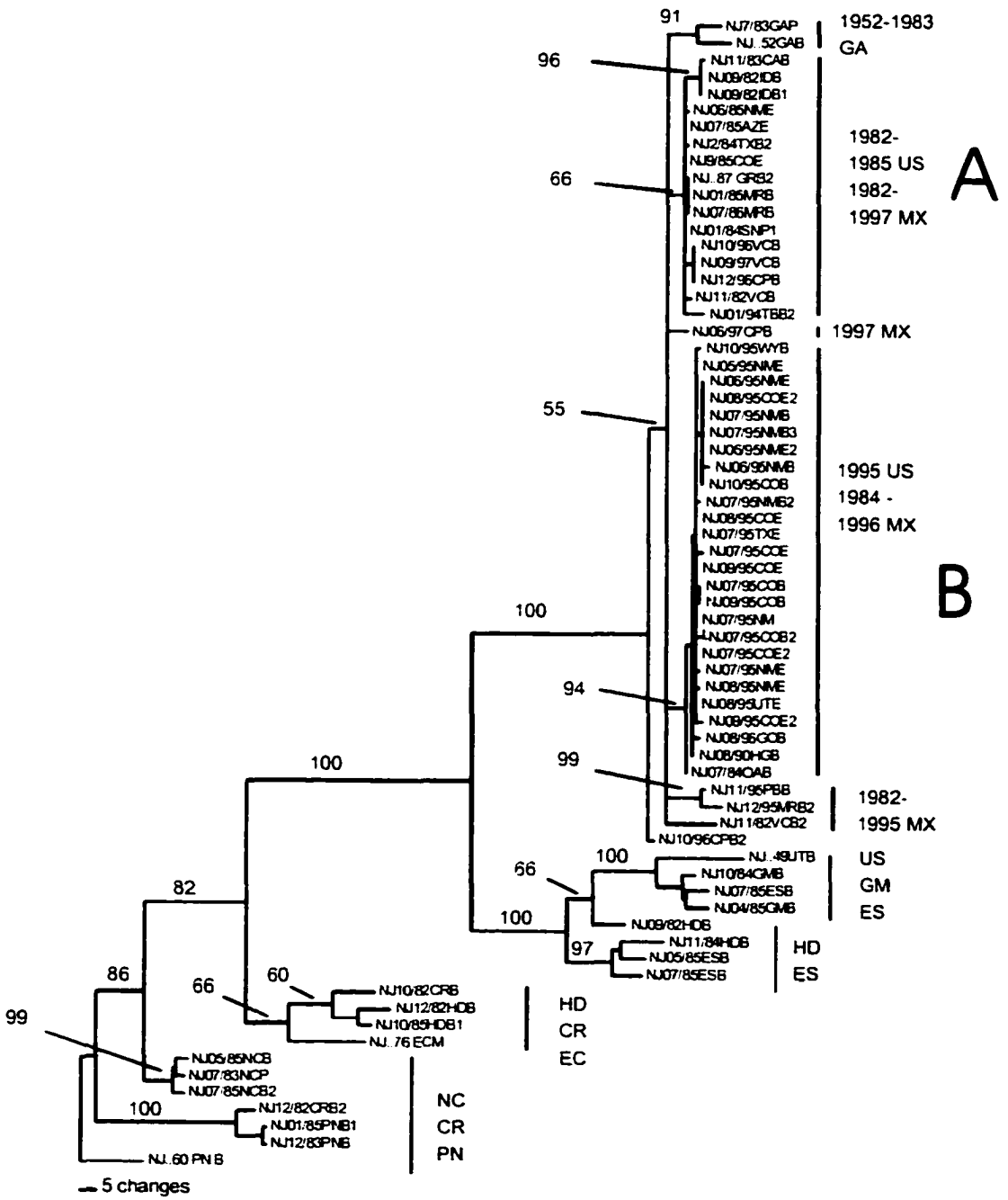

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Figure 2. Enlarged view of maximum parsimony tree in Figure 1.

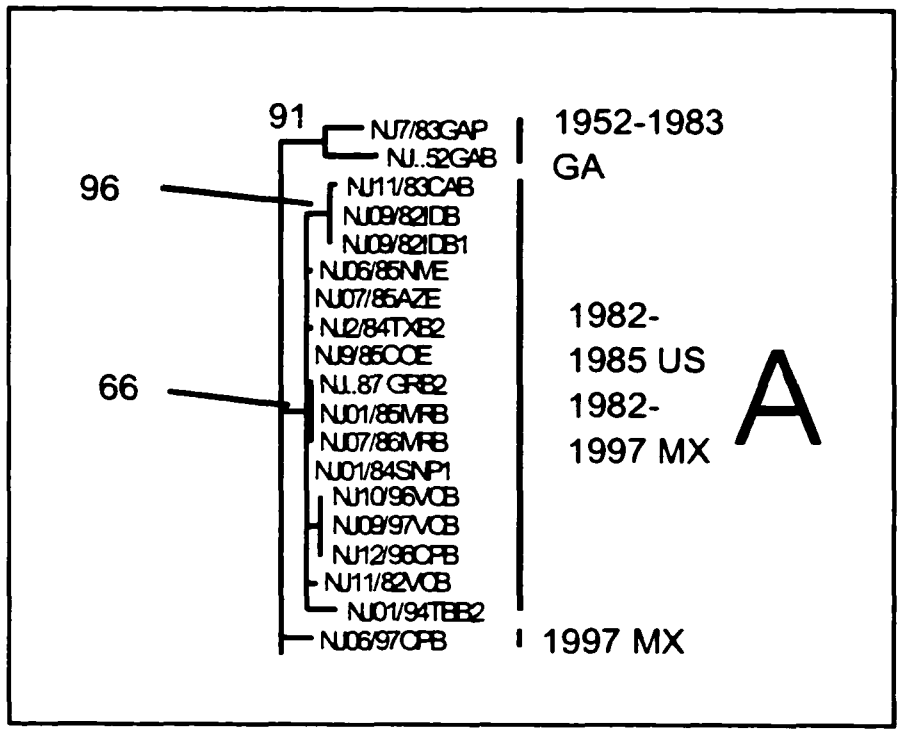

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Figure 3. Enlarged view of maximum parsimony tree in Figure 1.

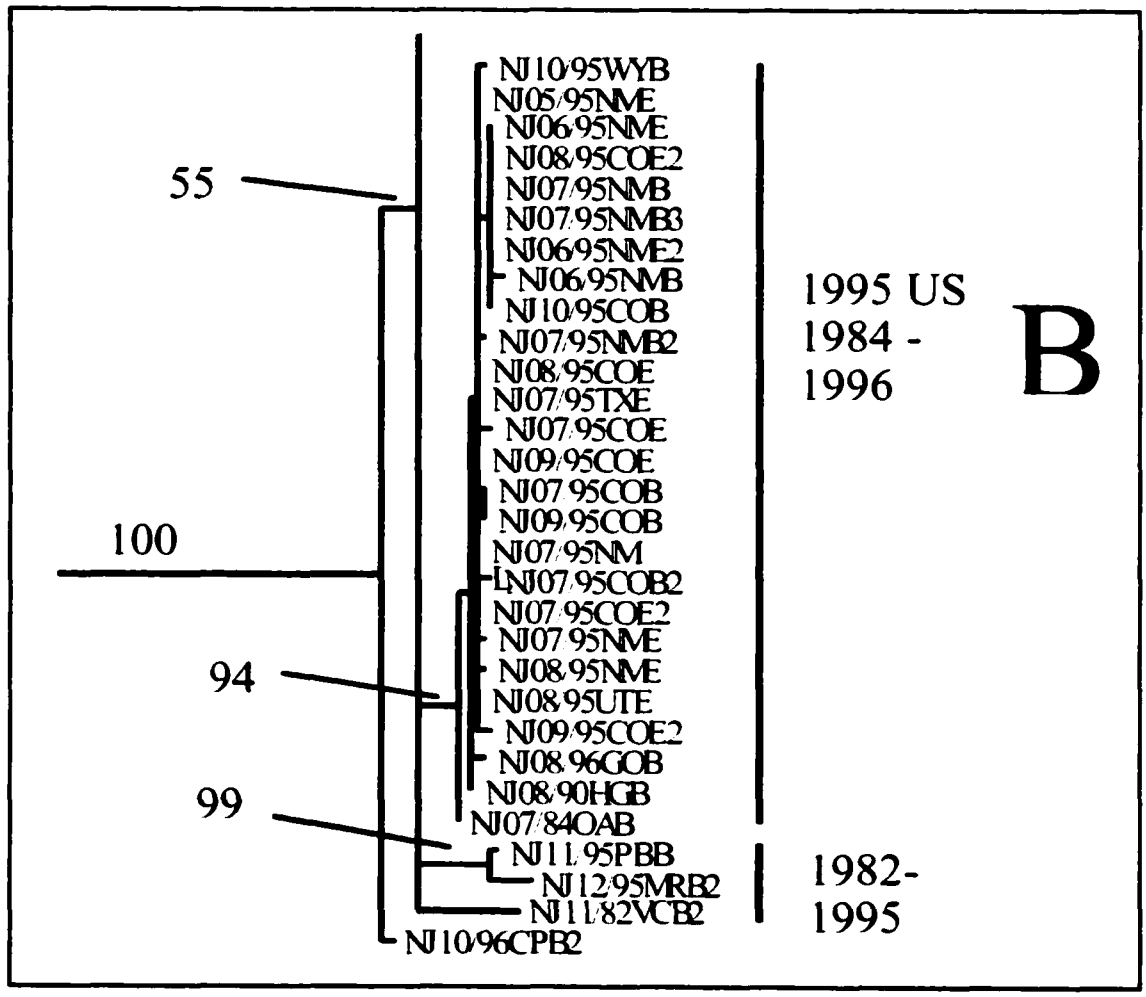

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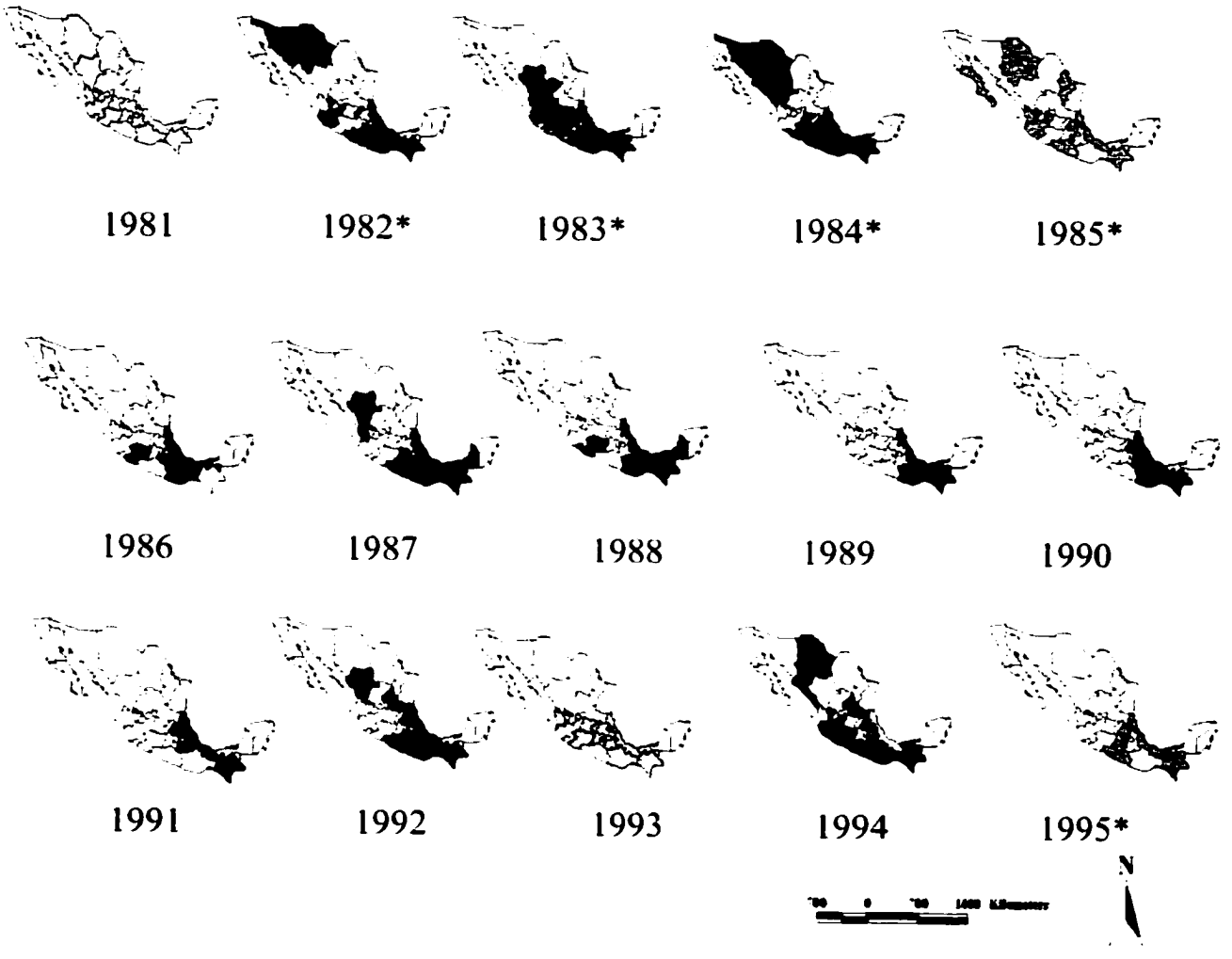
Figure 4. Distribution of vesicular stomatitis outbreaks in Mexico from 1981 to 1995.



* = outbreak years of vesicular stomatitis in the western United States.

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2a.7 References

1. Aguirre AA, McLean RG, Cook RS, Quan TJ (1992) Serological Survey for Selected Arboviruses and Other Potential Pathogens in Wildlife from Mexico. Journal of Wildlife Diseases 28:435-442.

2. Anonymous (1999) Vesicular Stomatitis in Mexico: 16-year review (1981-1996). Sistema Nacional de Vigilancia Epizootiologica y Evaluacion de Riesgo, Comision Mexico-Estados Unidos para la Prevencion de la Fiebre Aftosa y otras enfermedades Exoticas de los Animales.

3. Bilsel PA and Nichol ST (1990) Polymerase Error Accumulating during Natural Evolution of the Glycoprotein gene of Vesicular Stomatitis Virus Indiana Serotype Isolates. Journal of Virology 64:4873-4883

4. Bilsel PA, Pamuk A, Row JE, Fitch WM and Nichol ST (1990) Phosphoprotein and Nucleocapsid Protein Evolution of Vesicular Stomatitis Virus New Jersey. Journal of Virology 64:2498-2504

5. Dietzschold B, Rupprecht CE, Fu ZF, Koprowski H (1996) Rhabdoviruses. In: Fields BN (ed) Fields Virology. Raven Publishers, Philadelphia, pp 1137-1152

6. Hanson RP and Karstad L (1955) Progress Report on Epizootiology of Vesicular Stomatitis of Swine. Wisconsin Agricultural Experimental Station, Madison, Wisconsin

7. Heneidi A (1999) Vigilancia Epidemiological de la Estomatitis Vesicular en Mexico. Departamento de Regionalizacion y Analisis de Riesgo, Comision Mexico-Estados Unidos para la Prevencion de la Fiebre Aftosa y otras enfermedades Exoticas de los Animales.

8. Kumar S, Tamura K, Nei M. MEGA Molecular Evolutionary Genetics Analysis. (1.01).1993. University Park, PA, 328 Mueller Laboratory.

9. Nichol ST (1987) Molecular Epizootiology and Evolution of Vesicular Stomatitis Virus New Jersey. Journal of Virology 61:1029-1036

10. Nichol ST (1994) Vesicular Stomatitis Virus. In: Webster RG, Granoff A (eds) Encyclopedia of Virology. Saunders, London, pp 1547-1555

11. Nichol ST, Row JE, Fitch WM (1989) Glycoprotein Evolution of Vesicular Stomatitis Virus New Jersey. Virology 168:281-291

12. Pal R, Wagner RR (1987) Rhabdovirus glycoprotein. In: Wagner RR (ed) *Rhabdoviruses*. Plenum Press, New York, pp 75-128

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13. Reif JS (1994) Vesicular Stomatitis. In: Handbook of Zoonoses. CRC Press, pp 171-181

14. Rodriguez LL, Maupin GO, Ksiazek TG, Rollin P, Khan AS, Schwarz TF, Lofts RS, Smith JF, Noor AM, Peters CJ, Nichol ST (1997) Molecular Investigation of a Multisource Outbreak of Crimean-Congo Hemorrhagic Fever in the United Arab Emirates. Proceedings of the National Academy of Science U.S.A. 93:13030-13035

15. Rubi E and Guerrero L. Situacion de la Estomatitis Vesicular en Mexico (2000) Departamento de Regionalizacion y Analisis de Riesgo, Comision Mexico-Estados Unidos para la Prevencion de la Fiebre Aftosa y otras enfermedades Exoticas de los Animales.

16. Sellers RF, Maarouf AR (1990) Trajectory Analysis of Winds and Vesicular Stomatitis in North America, 1982-5. Epidemiology of Infectious Diseases 104:313-328

17. Stallknecht DE, Kavanaugh DM, Corn JL, Eernisse KA, Comer JA, Nettles VF (1993) Feral Swine as a Potential Amplifying Host for Vesicular Stomatitis Virus New Jersey Serotype on Ossabaw Island, Georgia. Journal of Wildlife Diseases 29:377-383

18. Swofford DL. PAUP Phylogenetic Analysis Using Parsimony (and other methods). (4.0). 1998. Sunderland, MA, Sinauer Associates.

19. Wagner RR (1990) Rhabodviridae and Their Replication. In: Fields BN (ed) Virology. Raven Press, Ltd, New York, pp 867-881

20. Wagner RR, Rose JK (1996) *Rhabdoviridae*: The Viruses and Their Replication. In: Fields (ed) Fields Virology. Raven Publishers, Philadelphia.

21. Webb PA, McLean RG, Smith GC, Ellenberger JH, Francy DB, Walton TE, Monath TP (1987) Epizootic Vesicular Stomatitis in Colorado, 1982: Some Observations on the Possible Role of Wildlife Populations in an Enzootic Maintenance Cycle. Journal of Wildlife Diseases 23:192-196

22. Zuluaga F, Yuill TM (1979) Ecological Studies of Vesicular Stomatitis in Antioquia, Colombia. Bol Oficina Sanit Panam 87:377-388

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2b. Evolution of VSV-NJ in Cells from a Natural Insect Host

2b.1. Introduction

Vesicular stomatitis virus serotype New Jersey (VSV-NJ) is an arthropod-borne *Rhabdovirus* that causes disease in livestock. Sand flies (*Lutzomyia shannoni*) have been shown to be biological vectors of VSV-NJ in nature (Comer JA et al., 1991). VSV belongs to the family *Rhabdoviridae* in the order Mononegavirales. Its genome consists of a negative-sense, 11kb single-stranded RNA molecule that encodes five proteins. Due to the lack of proofreading activity of its RNA polymerase, VSV has the potential for rapid evolution with an error rate of 1×10^{-4} - 2×10^{-5} substitutions per nucleotide copied per round of replication (Drake JW, Holland JJ, 1999; Steinhauer DA, Holland JJ, 1986). This means that there is at least one mutation in each virion replication cycle (Novella IS et al., 1995). Despite its potential for rapid change, VSV can remain genetically conserved for long periods of time depending on the selective pressures exerted on the viral genome (Holland JJ et al., 1992; Steinhauer DA et al., 1989). Phylogenetic analyses based on the phosphoprotein, nucleocapsid, and glycoprotein genes of natural VSV isolates have demonstrated great genetic diversity among viruses from different geographical regions throughout the Americas (Nichol ST et al., 1989; Nichol ST et al., 1993; Bilsel PA et al., 1990). However, within specific endemic areas, viral lineages can be genetically conserved over long time periods (Nichol ST et al., 1993; Rodriguez LL et al., 1996). Ecological and environmental factors seem to be major selective forces driving VSV evolution in these endemic areas (Rodriguez LL et al., 1996).

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VSV has been used as a laboratory model for viral evolution and *in vitro* studies due to its capacity to grow well in multiple cell lines of mammalian, avian, piscine, and arthropod origins (Seganti L et al., 1986; Gillies S, Stollar V, 1980a; Mudd JA et al., 1973). In the laboratory, it has been shown that either continuous replication of VSV in sand fly cells or alternating environments between sand fly and baby hamster kidney (BHK-21) cells resulted in decreased viral fitness in BHK-21 cells and increased fitness in sand fly cells, suggesting that these cells could be a selective factor on VSV evolution (Novella IS et al., 1995; Novella IS et al., 1999).

The present study was conducted to test the hypothesis that passage in cells of homologous origin could influence VSV evolution *in vitro* differently than passage in cells of heterologous origin. We determined the growth rate and rate of evolution after repeated passage in cell lines derived from sand flies, mosquitoes or baby hamster kidneys for a VSV-NJ isolated from sand flies and compared it with two genetically distinct viruses collected from mammalian hosts in different ecological areas. We also compared the rates of evolution and the types of genetic mutations observed under laboratory conditions to those observed during viral evolution in nature.

2b.2 Materials and Methods

2b.2.1 Viruses

Three viruses from different geographical regions and of different host origins were selected for this study. A virus isolated in 1989 from a pool of sand flies (*Lutzomyia shannoni)* in an endemic focus in Georgia (89GAS), a 1995 equine isolate from New Mexico (95NME), and a virus isolated in 1992 from a bovine in an endemic

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area of Costa Rica (92CRB) were used. All viruses, obtained as first or second passages in baby-hamster kidney (BHK-21) cells were propagated once more in BHK-21 cells to produce viral stocks that were stored at –70°C. Infections of all cell lines were initiated with the same viral stock of each strain.

2b.2.2 Cell Lines

The sand fly cell line (LL-5) derived from *Lutzomyia longipalpis* was kindly provided by Dr. R. Tesh and Dr. I. Novella. The composition of this cell line was previously described as a mixture of epitheloid and fibroblastoid cells of various size and morphology (Tesh RB, Modi GB, 1983; Novella IS et al., 1995). The mosquito cell line C6/36 was obtained from the American Tissue Culture Collection (ATCC catalog # CRL-1660), these cells were derived from *Aedes albopictus* whole larvae. The BHK-21 cells, were obtained from ATCC (ATCC catalog #CRL-1632), and were used between passages 50-90.

2b.2.3 Virus Infection and Serial Passage

Flasks (T-25) of each cell line were infected with each virus at multiplicity of infection (MOI) of 8 for BHK-21, 222 LL-5 cells and 5 for CRL cells. After one-hour incubation at 28°C, the inoculums were removed and replaced with fresh growth media. The infected cells were incubated at 28°C for 48 hours. The flasks were then completely frozen and stored at –70°C. Cell supernatants were clarified by centrifugation at 1000 xg for five minutes and aliquots were frozen and kept at –70°C. In order to obtain an MOI between 1-10, cleared supernatants were diluted 1:10,000 for BHK-21 cells, 1:100 for

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C3/36 cells, and not diluted for sand fly LL-5 cells in each subsequent passage. The MOI of each subsequent infection depended on the titer of the previous passage. A change in morphology was observed in LL5 cells at passage 15. The cells went from a population of cells with a heterogeneous morphology to a more homogenous population with smaller cells predominating, thereby increasing the number of cells per flask and decreasing the MOI. The MOI varied between infections due to the change in viral yields obtained at each passage. Viral titers were calculated for each passage by tissue culture infectious dose 50% ($TCID_{50}$) in replicates of eight. Viruses were passed 25 times in mosquito and mammalian cells and 20 passages in sand fly cells.

2b.2.4 Persistent Virus Infection in Insect Cells

Persistent infections were established in mosquito and sand fly cells that were serially passed for 81 days. Confluent T-25 flasks of mosquito and sand fly cells were infected with each of the viruses that had been passed seven times in each cell line respectively. The infected cells were incubated at 28°C and supernatant was collected at various times after infection. Cells were split 1:4 every 5-14 days depending on cell growth with an average interval of seven to eight days in between cell passes.

2b.2.5 RNA Extraction, RT-PCR, PCR, Automated Sequencing

Sequences were obtained for the hypervariable region in the phosphoprotein gene (P gene), the glycoprotein gene (G gene), and glycoprotein-large polymerase intergenic junction (G-L junction). Viruses were sequenced from the first, tenth, and final passage. The RNA was collected by acid guanidine thiocyanate method using Trizol (Life

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Technologies, Grand Island, NY) as previously described (Rodriguez LL et al., 1997). RNA was resuspended in sterile water and stored at –70°C until tested. The RNA was used as a template for reverse transcriptase-polymerase chain reaction using the rTth RNA PCR kit (GeneAmp EZ rTth RNA PCR kit, Perkin-Elmer, Branchburg, NJ) as previously described (Rodriguez LL et al., 1997). PCR reactions were done for 35 cycles with a temperature profile as follows: 50°C (30 min) reverse transcription, 95°C (3 min) denaturation, and 35 cycles of 95°C (45s) denaturation, 50°C (120s) annealing, 72°C (120s) elongation, and a final elongation of 72°C for five min. The amplified DNA products were analyzed on a 1.0% agarose gel with ethidium bromide staining. Multiple internal and external primers were used for the different genes. For the P gene, primer combinations NJ-P102F/NJ-P831R were used for the RT-PCR. Primer combinations NJ-M658F/NJ-G1230R, NJ-G394F/NJ-G1230R, NJ-G1224F/L246R, were used for obtaining segments of the G gene and G-L junction. In addition, primers NJ-P426F, G394F, NJ-G623F, NJ-G837F, G911F, G1150F, G1354F, NJ-G519R, NJ-G580R, NJ-G820R, NJ-G850R, G15103R were used for nucleotide sequencing. The primer names indicate the serotype, target gene, and position starting from the first nucleotide of each gene. All primer sequences are available from the authors upon request.

Nucleotide sequencing for the G gene was obtained from nucleotide 112 to the terminal 5' end of the gene for all viruses, except for $92CRB_{10LL5}$ and $89GAS_{P25CRL}$; the former lacked the preceding 324 bases and base fragment position 660 to 1150 and the latter lacked 87 terminal bases.

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2b.2.6 Sequence Analyses

The amplified DNA products were sequenced by dideoxy sequencing with specific primers using a Big-Dye Terminator sequencing kit and a 373 XL automated sequencer (PE Applied Biosystems, Foster City, CA). The nucleotide sequences were cleaned and analyzed using the software Sequencher® (Gene Codes Corp., Ann Arbor, MI.) and exported for use into other molecular software programs. The nucleotide sequences were aligned using Clustal X Multiple Alignment Program (European Bioinformatics Institute, Cambridge, UK). The proportional distances and amino acid changes were identified and calculated in MEGA (version 1.01, Mueller Laboratory, University Park, PA).

2b.2.7 Single-step Growth Curve

A viral growth curve on each virus and cell lineage determined the rate and peak of viral infection. An MOI of one was used to infect individual six well plates of LL-5 and BHK-21 cells. Each infected plate was incubated for one hour at 28°C for absorption; the inoculum was removed and replaced with fresh media. The viral supernatant was removed, then 2 mL of fresh media was added to the cells, scraped and harvested at time intervals 0, 1, 6, 12, 24, 48, 72 hours. Each infection at the indicated time intervals was done in duplicates. Virus isolates were frozen at –70°C until used for viral titration using the $TCID_{50}$ method for the supernatant and cells.

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2b.2.8 Statistical Analyses

Correlations between viruses and passage were calculated by Spearman's correlation (Siegel S, Castellan Jr NJ, 1998) in Analyze-It (Excel, Microsoft Corporation, Redmond, Washington). A two-way analysis of variance (ANOVA) was performed to determine the significance of the differences in titer means observed between viruses, cell types, and the interaction between these two factors. The statistical software Minitab (Minitab, Minitab Incorporated, State College, Pennsylvania) was used to perform the two-way ANOVA. A p-value of ≤ 0.05 was considered statistically significant in this study.

2b.3 Results

The ability of the sand fly virus 89GAS to grow in each of two insect cells, C6/36 derived from mosquitoes and LL5 derived from sand flies; and mammalian cell line BHK-21 derived from baby hamster kidney was tested. Two viruses of mammalian origin (92CRB and 95NME) were used for comparison. All three viruses were capable of growing in all cell types, with BHK-21 cells producing the highest yields (Figure 1). The average titers for 89GAS, 95NME, and 92CRB viruses in BHK-21 cells ranged between 7.5 to 10.32 $\log_{10}$/ml with 95NME having the highest average titer (Figure 1). In sand fly cells, titers ranged between 4.6 to 7.8 $\log_{10}$/ml with 89GAS having the highest average titer (Figure 1). In mosquito cells the titers ranged from 5.2 to 8.4 $\log_{10}$ with 89GAS also having the highest average titer (Figure 1). Virus titers of 89GAS in C6/36 cells increased with each passage (correlation coefficient= 0.47). There was a difference in the interaction between viruses and cell types as indicated from a two-way ANOVA.

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One-step growth curves on each cell line demonstrated that 95NME virus grew to a higher titer in BHK-21 cells than did the 92CRB or 89GAS viruses (Figure 2). In these cells, virus titers peaked by 48 hours and similar titers were observed both in supernatant and cell associated virus (not shown). In sand fly cells virus growth peaked at 72 hours with 89GAS virus having the highest titer compared to 95NME or 92CRB viruses (Figure 2). One-step growth curves were not done on C6/36 cells.

In order to estimate the mutation rate after serial passage on each cell line, viruses were subjected to 25 passages in BHK-21 and C6-36 cells and 20 passages in LL-5 cells. Three areas of each virus's genome were sequenced for the first, the tenth, and final passage in each cell type: the hypervariable region of the P gene (238 nucleotides), the G-L intergenic junction (32 nucleotides), and most of the G gene (1438 nucleotides). There were no mutations, deletions, or insertions detected in any of the passages neither in P nor in the intergenic G-L region. Interestingly, only the G protein gene accumulated mutations during serial passages for all three viruses. In 89GAS, two mutations were identified, one on each the mosquito and sand fly cell lines at passage 10 and 20 respectively resulting in mutation rates (# changes/site/passage) of 6.95×10^{-5} and 3.48×10^{-5}, respectively (Table 1). Both of these substitutions were non-synonymous, but neither was located in previously identified functional domains or neutralizing epitopes. Virus 95NME accumulated mutations in each of the three cell lines, one synonymous mutation at passage 10 in BHK-21 cells, a non-synonymous change at passage 25 in mosquito cells and two non-synonymous mutations at passage ten in sand fly cells, resulting in a mutation rate of 1.39×10^{-4} (Table 1). Virus 92CRB accumulated one non-

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synonymous mutation in BHK-21 cells and 2 mutations (one non-synonymous) in LL5 cells (Table 1).

In order to compare the rate of evolution under laboratory conditions to that observed under natural conditions, two viruses (89GAS and 83GAP) isolated six years apart from the same endemic focus in Ossabaw Island, Georgia were used for this comparison. This is a very well-characterized closed endemic focus where a single lineage of VSV-NJ has been demonstrated to be maintained in the feral swine and sand fly populations (Stallknecht DE, et al., 1993; Comer JA et al., 1991; Comer JA et al., 1994). Previous studies showed that 89GAS is closely related to 83GAP as no nucleotide mutations were observed in the hypervariable region of the P gene (Rodriguez LL et al., 2000). In contrast, we observed three nucleotide differences (two of which were non-synonymous substitutions) in the glycoprotein between 83GAP and 89GAS, resulting in a mutation rate of 3.2×10^{-4} year. There was one change in the transmembrane domain, but no other mutations were located in previously described functional domains or near neutralizing epitopes.

It is believed that sand flies are a natural host for VSV, since the virus has been isolated from sand flies in endemic foci in Panama, Colombia and in Georgia, USA (Tesh RB et al., 1974; Comer JA, et al., 1990; Tesh RB et al., 1987). One possible indication of viral adaptation to its natural host could be the capacity of the virus to establish long-term persistent infections in cells derived from that host. We hypothesized 89GAS was capable of establishing and maintaining persistent infections in LL5 sand fly cells but not necessarily in C6/36 mosquito cells. We also wanted to determine the ability of viruses of mammalian origin to establish persistent infection in these insect cells. All viruses

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readily established persistent infections that were maintained for at least 81 days, both in mosquito and sand-fly cells. No significant differences were observed among the three viruses in their ability to persist in either insect cell line (Figure 3). However, each cell line showed a different pattern of persistence; while sand fly (LL5) cells yielded consistently high titers of VSV (5-6 $\log_{10}$) for up to 81 days, mosquito C6/36 cells produced higher virus bursts (7-8 $\log_{10}$) on the first passage, but virus yields were low or undetectable in later passages (Figure 3).

2b.4 Conclusion and Discussion

Adaptation to their natural hosts is thought to play an important role in the long-term persistence and maintenance of viruses in nature. Several viruses are capable of sustained growth and shedding in their natural hosts without causing any clinical signs, but can cause dramatically different symptoms and even death in non-natural hosts. Examples of such adaptation are hantaviruses and arenaviruses, which in their natural rodent hosts cause little or no clinical signs but in humans can cause fatal pulmonary or hemorrhagic syndromes (Peters CJ, 1996). In the cases of hantaviruses and arenaviruses, the evolutionary patterns of these agents represent a clear adaptation to their rodent hosts, the idea of each virus adapted to and being maintained in nature by a specific rodent has been well documented (Plyusnin A, Morzunov SP, 2001; Fulhorst CF et al., 2001; Mills JN et al., 1997). The present study tested the idea that a virus (89GAS) which has been maintained in an endemic focus by transmission between sand flies and feral swine, would be better adapted to sand fly cells than to mosquito cells. Therefore it would grow better, establish stable persistent infections and be genetically stable in cells derived from

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sand flies versus in cells from mosquito or mammalian origin. The results indicated that sand fly virus 89GAS, was better adapted to insect cells since it grew to higher average titers both in sand fly and mosquito cells than the two viruses of mammalian origin. There were significant differences in growth in different cell types among the three viruses tested. These interactions indicate that propagation of each virus is related to both the type of the cells as well as the virus source. There was a positive correlation between the titer of 89GAS and the number of passages in the mosquito cell line C6/36, perhaps as a reflection of adaptation of this virus to a new cell type.

We also evaluated the rate of evolution of each of the three viruses during repeated passage in each of the three cell types by performing direct sequencing of the RT-PCR products. We did not analyze the sequence composition of the quasi-species but rather the average population sequence. The P gene and the intergenic junction between the G and L genes (G-L junction) were selected for sequencing since these are the most genetically variable regions in the VSV genome (Bilsel PA et al., 1990; Gill DS et al., 1986; Masters PS, Banerjee AK, 1988; Luk D et al., 1987; Stillman EA, Whitt MA, 1997). Interestingly, no mutations were identified in the hypervariable region of the P gene or the G-L junction. The absence of mutations in two parts of the genome previously identified as highly variable suggest that the conditions in cell culture were not exerting a strong selective pressure on these regions. In contrast, we found evidence of positive selection in the glycoprotein gene of each of these viruses after passage in insect or mammalian cells. The glycoprotein G, the main membrane protein located on the surface of the virion, is the target of the neutralizing antibody responses and has been used to serologically classify VSV. The glycoprotein contains the antireceptor involved

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in attachment and membrane fusion with the cell surface receptors and mediates viral entry and exit from the host cell (Wagner RR, Rose JK, 1996). The fact that mutations were detected only in G and that most of them were non-synonymous substitutions indicates that this protein is under stronger selective pressure than the P gene or G-L junction in these *in vitro* conditions. This might be a reflection of differences in intracellular protein trafficking, or different mechanisms of virus budding between insect and mammalian cells (Bouamr F et al., 2000).

The mutation rate of VSV in nature was estimated by taking advantage of two viruses (89GAS and 83GAP) isolated six years apart from the same endemic focus in Ossabaw Island, Georgia. Mutations accumulating during *in vitro* cell passage were compared to those accumulating under natural conditions. In both cases no nucleotide changes were seen in the P gene. Three differences were observed in the glycoprotein gene between 83GAP and 89GAS, resulting in a mutation rate of 3.2×10^{-4} year which is ten-fold higher than the rate observed *in vitro* for 89GAS ($3.5\text{-}6.9 \times 10^{-5}$ changes passage). However, it is difficult to compare these rates since we do not know the number of replication cycles or host to host passages that 83GAP underwent in nature during a six-year period. Sequence analyses of natural VSV isolates from various geographical regions over a period of up to 20 years, showed that the G gene was more conserved than the P gene, with mutation rates between 10^{-4} to 10^{-5} mutations per base per year and most of the mutations being synonymous substitutions (Nichol ST et al., 1989). Furthermore, it has been shown that VSV circulating among cattle populations in endemic areas accumulate mostly synonymous changes in their glycoprotein gene despite the presence of high titers of VSV-neutralizing antibodies among the affected cattle

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(Vernon SD et al., 1990). This indicates that under natural conditions, changes on the G gene probably result from accumulation of mutations by genetic drift. This contrasts with our results *in vitro*, in which P was completely conserved and most of the changes observed in G were non-synonymous. Therefore, we can conclude that factors influencing VSV glycoprotein evolution in cell culture are different from those acting under natural conditions. It is important to point out that in this study we partially sequenced two genes, and an intergenic junction, which had been previously identified as an area of high genetic variability in the VSV genome. However, it is possible that mutations also accumulated in other areas of the VSV genome not analyzed in this study.

VSV causes cytopathic effect (CPE) in BHK-21 cells, as early as 24 hours post infection with almost complete CPE observed by 48 hours when grown at 28°C. In contrast, insect cells did not show signs of CPE and infected cells could be maintained in culture and passed as normal cells. This was previously documented with infections of VSV in *Aedes albopictus* cells, as CPE was either not detected or was greatly delayed at 28°C (Artsob H, Spence L, 1974; Gillies S, Stollar V, 1980a). Sand fly LL5 and mosquito C6/36 cells persistently infected with VSV-NJ demonstrated different patterns of viral persistence. While C6/36 cells yielded high titers (10^8TCID$_{50}$) of virus three days after the initial infection, viral yields decreased, became undetectable by day 12 and fluctuated at low titers in later passages. In contrast sand fly LL5 cells did not produce the large initial viral burst but consistently yielded relatively high titers of virus (10^5-10^6 TCID$_{50}$) for up to 81 days in culture. The fact that LL5 cells are capable of sustained production of VSV is consistent with the idea that LL5 cells were derived from a natural host for VSV-NJ (Lutzomyia sps) (Corn JL et al., 1990; Tesh RB et al., 1974; Tesh RB et

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al., 1972) whereas C6/36 were not (Walton TE, et al., 1987). The lower viral yields obtained in subsequent passages in C6/36 cells has been previously attributed to generation of defective interfering particles on these cells (Gillies S, Stollar V, 1980b).

In summary, our results showed that insect cells could be a selective factor on VSV evolution *in vitro*. Sand fly virus 89GAS seemed better adapted to sand fly cells than to mosquito or mammalian cells. However, we could not detect significant differences between rates of evolution of VSV-NJ by passage in homologous (sand fly) versus heterologous (mosquito or mammalian) cells. Although viral replication in cell culture does not mimic the complexity and the dynamics of natural evolution, it does allow us to observe how certain factors such as virus population size, genetic diversity, and host response might affect evolution.

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2b.5 Tables

Table 1. Mutations in the glycoprotein gene of VSV-NJ 89GAS, 95NME, and 92CRB viruses passed in sand fly, mosquito and mammalian cells. Mutation rate was calculated by number of substitutions per site per passage. Total fragment length was 1438 nucleotides (nt).

	Mammalian (BHK-21)	Mosquito (C6/36)	Sand fly (LL-5)
89GAS	No Mutations	P10 2nd pos change: 467nt: T to N Mutation Rate: 6.95×10^{-5}	P20 2nd pos change: 1388nt: G to D Mutation Rate: 3.48×10^{-5}
95NME	P10 3rd pos change: 252nt Mutation Rate: 6.95×10^{-5}	P25 2nd pos change: 584nt: Q to P Mutation Rate: 2.78×10^{-5}	P10 1st pos change: 304nt: E to K 367nt: P to S Mutation Rate: 1.39×10^{-4}
92CRB	P25 1st pos change: 362nt: F to L Mutation Rate: 2.78×10^{-5}	No mutations	P20 2nd pos change: 387nt: S to I 3rd pos change: 1078nt Mutation Rate: 6.95×10^{-5}

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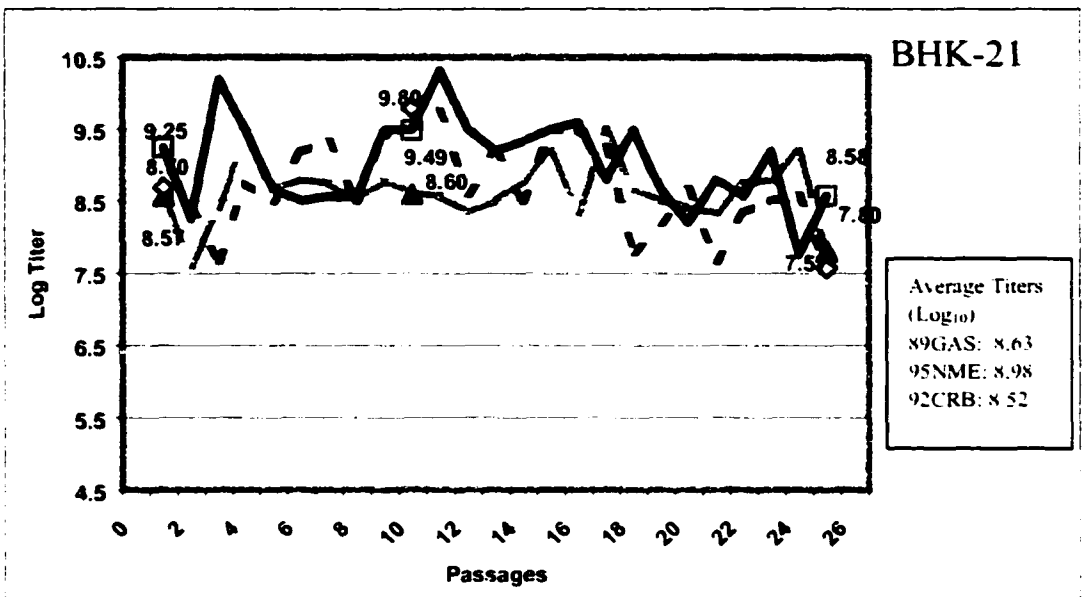
2b.6 Figures

Figure 1. Titers (log_{10}) for 89GAS, 95NME, and 92CRB VSV-NJ viruses passed 25 times in BHK-21 mammalian cells, or passed 20 times in sand fly (LL-5) cells, or 25 times in mosquito (C6/36) cells. Titer values at first, tenth, and last passages, and overall average titers are shown for each cell line.

A



B

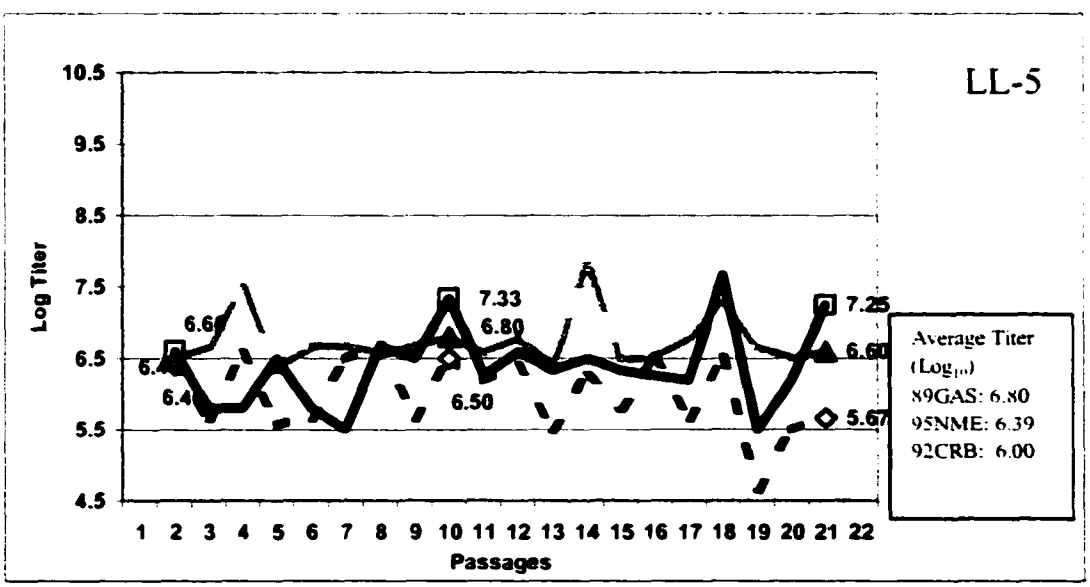


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Figure 1 continued.

C

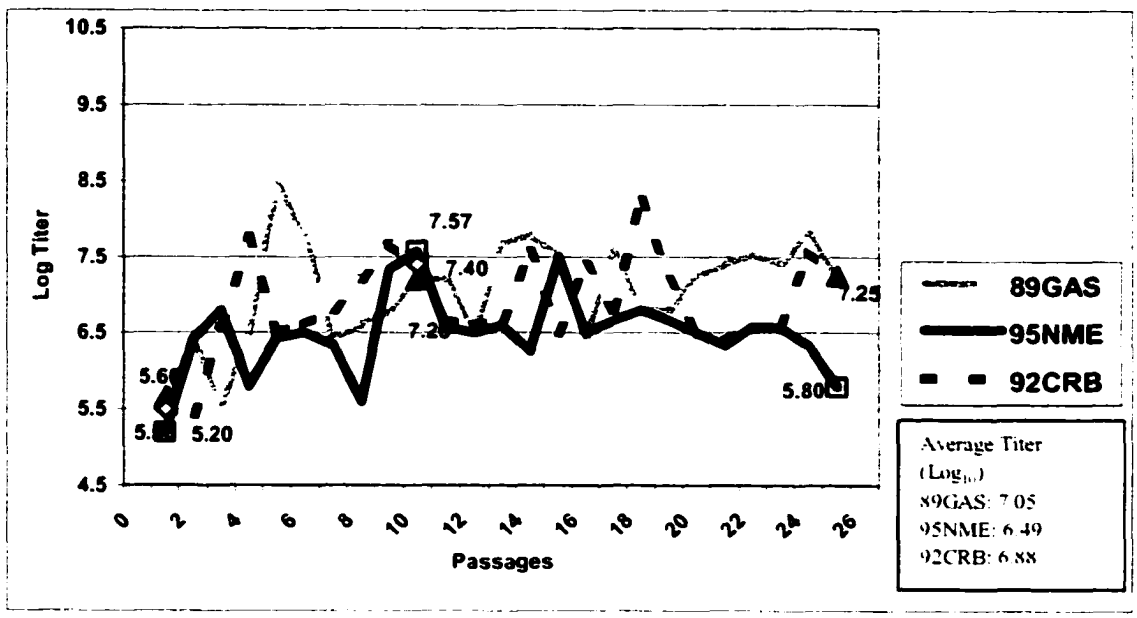

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Figure 2. Growth curves of VSV-NJ 89GAS, 95NME, and 92CRB viruses in BHK-21 and LL-5 cells. Initial infections were done at a multiplicity of infection of one. Titers in the supernatants at each time of collection were determined in BHK-21 cells and are indicated as $\log_{10}$.

BHK

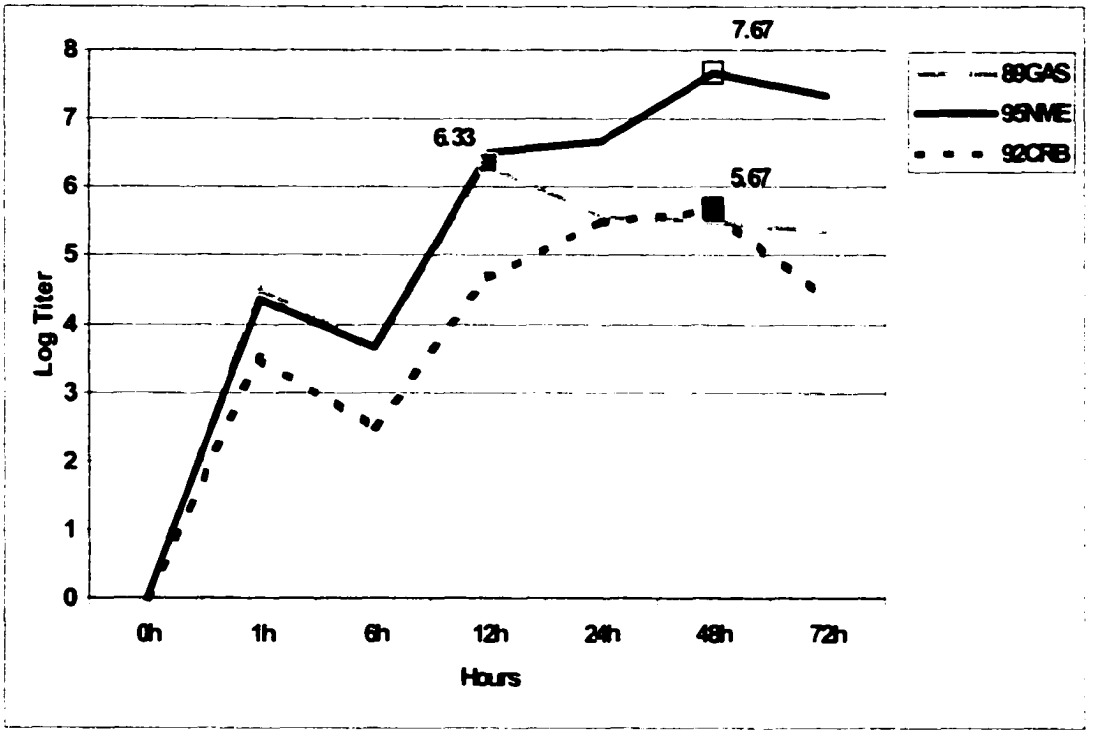

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Figure 2 continued.

LL-5

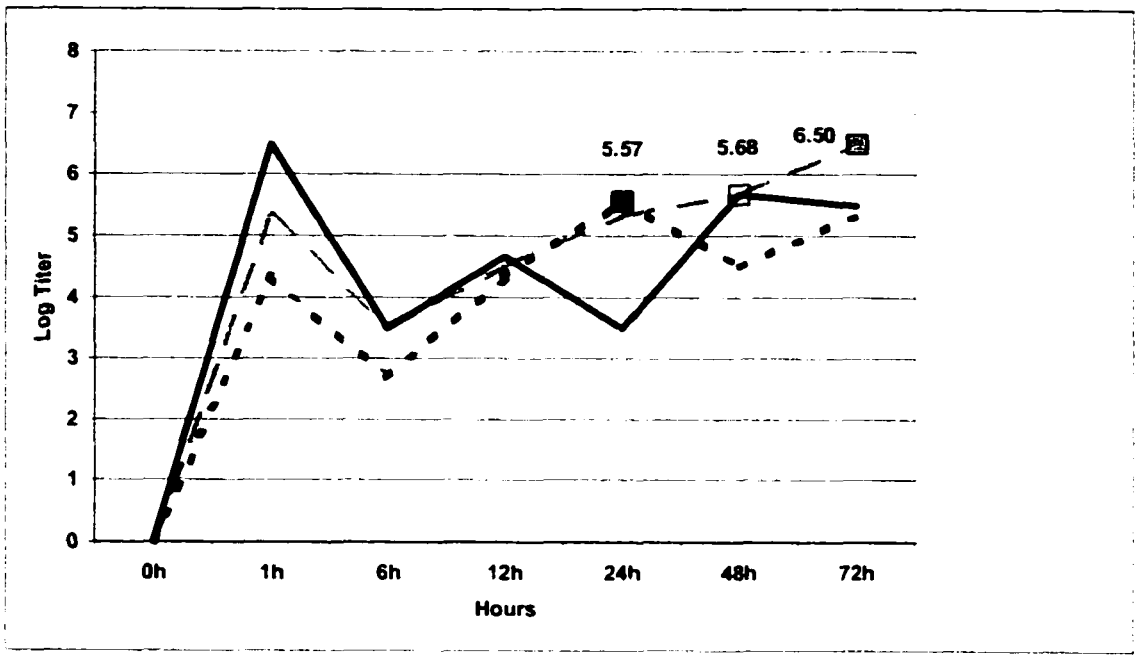

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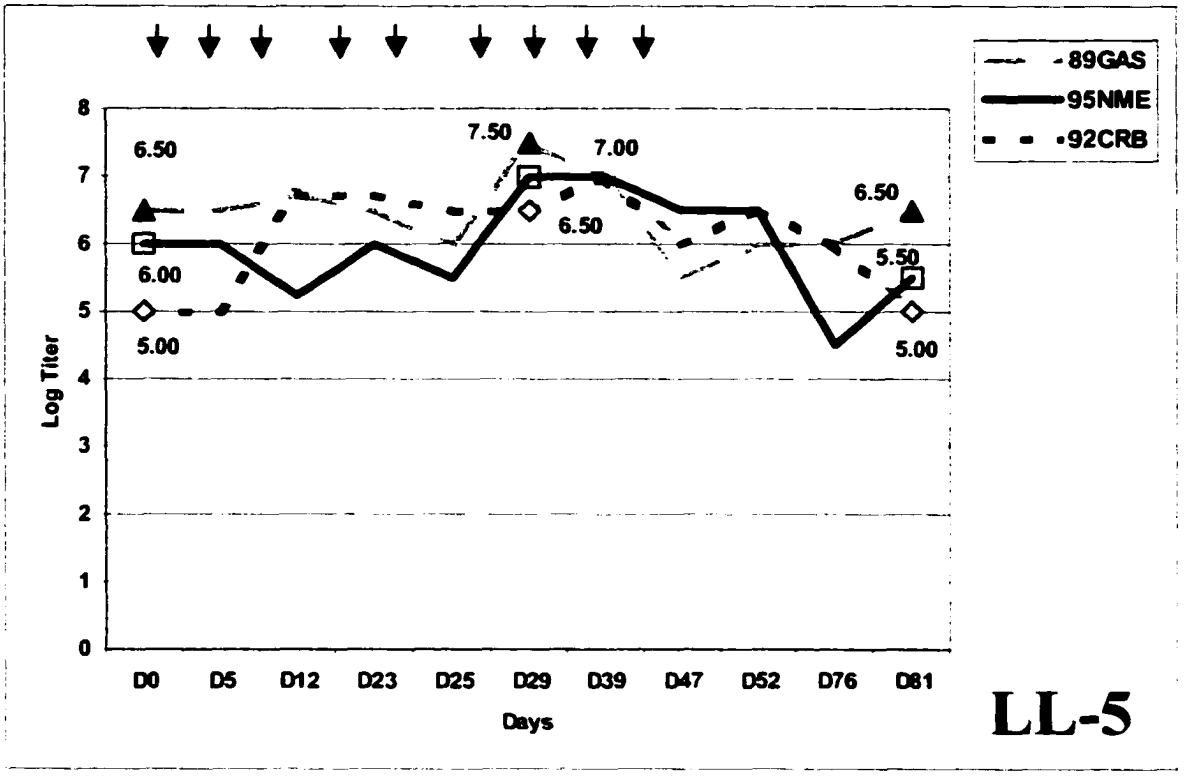
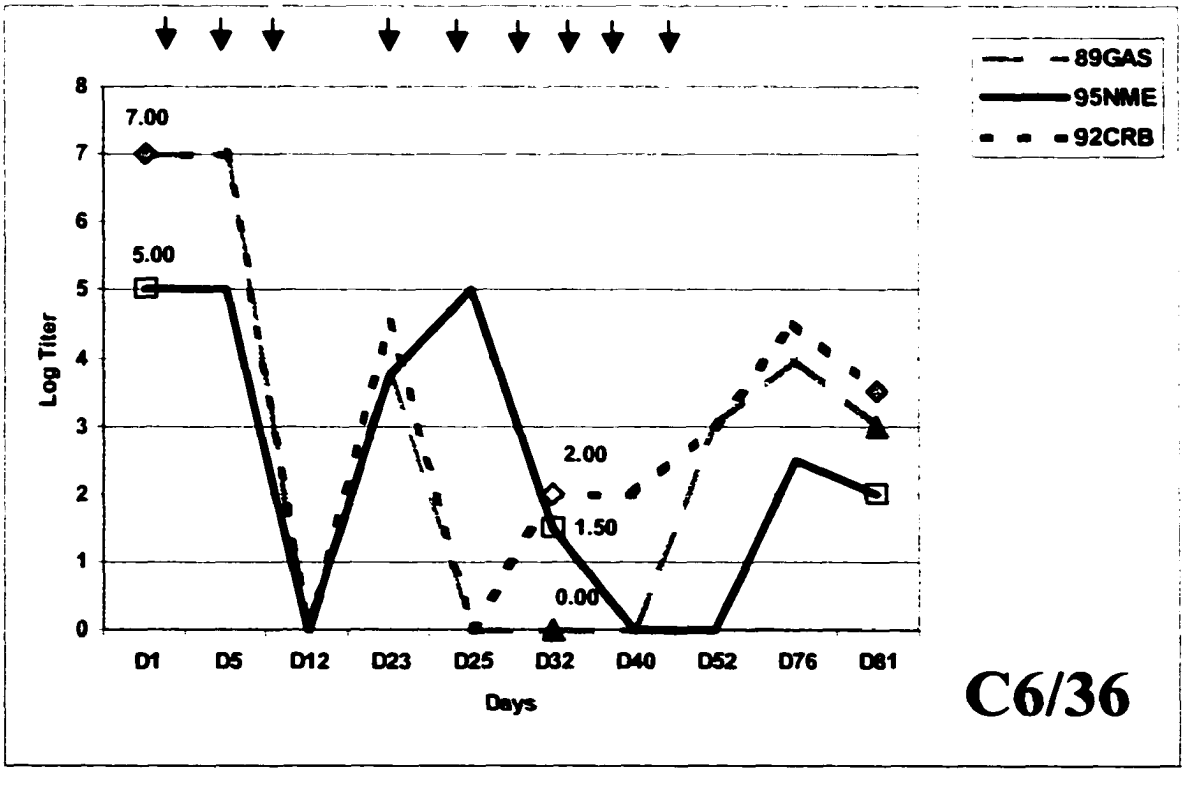
Figure 3. Persistence of VSV-NJ in sand fly (LL-5) or mosquito (C6/36) cells. Titers in cell culture supernatants were determined on BHK-21 cells and are indicated as $\log_{10}$. Arrows indicate days that cells were split.





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2b.7 References

1. Artsob H, Spence L (1974) Growth of Vesicular Stomatitis Virus in Mosquito Cell Lines. Canadian Journal of Microbiology 20:329-336

2. Bilsel PA, Pamuk A, Row JE, Fitch WM, Nichol ST (1990) Phosphoprotein and Nucleocapsid Protein Evolution of Vesicular Stomatitis Virus New Jersey. Journal of Virology 64:2498-2504

3. Bouamr F, Garnier L, Rayne F, Verna A, Rebeyrotte N, Cerutti M, Mare CJ (2000) Differential Budding Efficiencies of Human T-cell Leukemia Virus Type I (HTLV-I) Gag and Gag-Pro Polyproteins From Insect and Mammalian Cells. Virology 278:597-609

4. Comer JA, et al. (1990) Vesicular Stomatitis Virus, New Jersey Serotype:Replication in and Transmission by *Lutzomyia shannoni* (Diptera:Psychodidae). American Journal of Tropical Medicine & Hygiene 42:483-490

5. Comer JA, Kavanaugh DM, Stallknecht DE, Corn JL (1994) Population Dynamics of *Lutzomyia shannoni* (Diptera:Psychodidae) on Relation to the Epizootiology of Vesicular Stomatitis Virus on Ossabaw Island, Georgia. Journal of Medical Entomology 31:850-854

6. Comer JA, Stallknecht DE, Corn JL, Landgraf JG, Nettles VF (1991) *Lutzomyia shannoni* (Diptera:Psychodidae): A Biological Vector of the New Jersey Serotype of Vesicular Stomatitis Virus on Ossabaw Island, GA. Parassitologia 33:151-158

7. Corn JL, Comer JA, Erickson GA, Nettles VF (1990) Isolation of Vesicular Stomatitis Virus New Jersey Serotype From Phlebotomine Sand Flies in Georgia. American Journal of Tropical Medicine & Hygiene 42:476-482

8. Drake JW, Holland JJ (1999) Mutation Rates Among RNA Viruses. Proceedings of the National Academy of Science U.S.A. 96, 13910-13913.

9. Fulhorst CF, Charrel RN, Weaver SC, Ksiazek TG, Bradley RD, Milazzo ML, Tesh RB, Bowen MD (2001) Geographic Distribution and Genetic Diversity of Whitewater Arroyo Virus in the Southwestern United States. Emerging Infectious Diseases 7:403-407

10. Gill DS, Chattopadhyay D, Banerjee AK (1986) Identification of a Domain Within the Phosphoprotein of Vesicular Stomatitis Virus that is Essential for Transcription *in vitro*. Proceedings of the National Academy of Science U.S.A. 83:8873-8877

11. Gillies S, Stollar V (1980b) Generation of Defective Interfering Particles of Vesicular Stomatitis Virus in *Aedes albopictus* Cells. Virology 107:497-508

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12. Gillies S, Stollar V (1980a) The Production of High Yields of Infectious Vesicular Stomatitis Virus in *A. Albopictus* Cells and Comparisons with Replication in BHK-21 Cells. Virology 107:509-513

13. Holland JJ, Del La Torre JC, Steinhauer DA (1992) RNA Virus Populations as Quasispecies. Current Topics in Microbiology and Immunology 176:1-20

14. Luk D, Masters PS, Gill DS, Banerjee AK (1987) Intergenic Sequences of the Vesicular Stomatitis Virus Genome (New Jersey serotype): Evidence for Two Transcription Initiation Sites within the L Gene. Virology 160:88-94

15. Masters PS, Banerjee AK (1988) Complex Formation with Vesicular Stomatitis Virus Phosphoprotein NS Prevents Binding of Nucleocapsid Protein N to Nonspecific RNA. Journal of Virology 62:2658-2664

16. Mills JN, Ksiazek TG, Ellis BA, Rollin PE, Nichol ST, Yates TL, Gannon, WL, Levy CE, Engelthaler DM, Davis T, Tanda DT, Frampton JW, Nichols CR, Peters CJ, Childs JE (1997) Patterns of Association with Host and Habitat: Antibody Reactive with Sin Nombre Virus in Small Mammals in the Major Biotic Communities of the Southwestern United States. American Journal of Tropical Medicine & Hygiene 56:273-284

17. Mudd JA, Leavitt RW, Kingsbury DT, Holland JJ (1973) Natural Selection of Mutants of Vesicular Stomatitis Virus by Cultured Cells of *Drosophilia melanogaster*. Journal of General Virology 20:341-351

18. Nichol ST, Row JE, Fitch WM (1989) Glycoprotein Evolution of Vesicular Stomatitis Virus New Jersey. Virology 168:281-291

19. Nichol ST, Row JE, Fitch WM (1993) Punctuated Equilibrium and Positive Darwinian Evolution in Vesicular Stomatitis Virus. Proceedings of the National Academy of Science 90:10424-10428

20. Novella IS, Clarke DK, Quer J, Duarte EA, Lee CH, Weaver SC, Elena SF, Moya A, Domingo E, Holland JJ (1995) Extreme Fitness Differences in Mammalian and Insect Hosts after Continuous Replication of Vesicular Stomatitis Virus in Sand fly Cells. Journal of Virology 69:6805-6809

21. Novella IS, Duarte E, Elena SF, Moya A, Domingo E, Holland JJ (1995) Exponential Increases of RNA Virus Fitness During Large Population Transmissions. Proceedings of the National Academy of Science 92:5841-5844

22. Novella IS, Hershey CL, Escarmis C, Domingo E, Holland JJ (1999) Lack of Evolutionary Stasis During Alternating Replication of an Arbovirus in Insect and Mammalian Cells. Journal of Molecular Biology 287:459-465

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23. Peters CJ (1996) Viral hemorrhagic fevers. In: Nathanson E (ed) Viral Pathogenesis.

24. Plyusnin A, Morzunov SP (2001) Virus Evolution and Genetic Diversity of Hantaviruses and Their Rodent Hosts. Current Topics in Microbiology and Immunology 256:47-75

25. Rodriguez LL, Bunch TA, Fraire M, Llewellyn ZN (2000) Re-emergence of Vesicular Stomatitis in the Western United States in Associated with Distinct Viral Genetic Lineages. Virology 271:171-181

26. Rodriguez LL, Fitch WM, Nichol ST (1996) Ecological Factors Rather than Temporal Factors Dominate the Evolution of Vesicular Stomatitis Virus. Proceedings of the National Academy of Science 93:13030-13035

27. Rodriguez LL, Maupin GO, Ksiazek TG, Rollin P, Khan AS, Schwarz TF, Lofts RS, Smith JF, Noor AM, Peters CJ, Nichol ST (1997) Molecular Investigation of a Multisource Outbreak of Crimean-Congo Hemorrhagic Fever in the United Arab Emirates. Proceedings of the National Academy of Science U.S.A. 93:13030-13035.

28. Seganti L, Superti F, Girmenia C, Melucci L, Orsi N (1986) Study of Receptors for Vesicular Stomatitis Virus in Vertebreate and Invertebrate Cells. Microbiologica 9:259-267

29. Siegel S, Castellan Jr NJ (1998) Non-parametric Statistics for the Behavioral Sciences.

30. Stallknecht DE, Kavanaugh DM, Corn JL, Eernisse KA, Comer JA, Nettles VF (1993) Feral Swine as a Potential Amplifying Host for Vesicular Stomatitis Virus New Jersey Serotype on Ossabaw Island, Georgia. Journal of Wildlife Diseases 29:377-383

31. Steinhauer DA, Del La Torre JC, Meier E, Holland JJ (1989) Extreme Heterogeneity in Populations of Vesicular Stomatitis Virus. Journal of Virology 63:2072-2080

32. Steinhauer DA, Holland JJ (1986) Direct Method for Quantitation of Extreme Polymerase Error Frequencies at Selected Single Base Sites in Viral RNA. Journal of Virology 57:219-228

33. Stillman EA, Whitt MA (1997) Mutational Analyses of the Intergenic Dinucleotide and the Transcriptional Start Sequence of Vesicular Stomatitis Virus (VSV) Define Sequences Required for Efficient Termination and Initiation of VSV Transcripts. Journal of Virology 71:2127-2137

34. Tesh RB, Boshell J, Modi GB, Morales A, Young DG, Corredor A, Ferrero de Carrasquilla C, de Rodriguez C, Walters LL, Gaitan MO (1987) Natural

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Infection of Humans, Animals, and Phlebotomine Sand Flies with the Alagoas Serotype of Vesicular Stomatitis virus in Colombia. American Journal of Tropical Medicine & Hygiene 36:653-661

35. Tesh RB, Chaniotis BN, Johnson KM (1972) Vesicular Stomatitis Virus (Indiana Serotype): Transovarial Transmission by Phlebotomine Sandflies. Science 175:1477-1479

36. Tesh RB, Chaniotis BN, Peralta PH, Johnson KM (1974) Ecology of Viruses Isolated from Panamanian phlebotomine Sandflies. American Journal of Tropical Medicine & Hygiene 23:258-269

37. Tesh RB, Modi GB (1983) Development of a Continuous Cell Line from the Sand Fly *Lutzomyia longipalpis* (Diptera Psychodidae), and Its Susceptibility to Infection with Arboviruses. Journal of Medical Entomology 20:199-202.

38. Vernon SD, Rodriguez LL, Letchworth GJ (1990) Vesicular Stomatitis New Jersey Virus Glycoprotein Gene Sequence and Neutralizing Epitope Stability in an Enzootic Focus. Virology 177:209-215

39. Wagner RR, Rose JK (1996) *Rhabdoviridae*: The Viruses and Their Replication. In: Fields (ed) Fields Virology. Raven Publishers, Philadelphia

40. Walton TE, Webb PA, Kramer WL, Smith GC, Davis T, Holbrook FR, Moore CG, Schiefer TJ, Jones RH, Janney GC (1987) Epizootic Vesicular Stomatitis in Colorado, 1982: Epidemiologic and Entomologic Studies. American Journal of Tropical Medicine & Hygiene 36:166-176

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CHAPTER 3

Ecology of Vesicular Stomatitis Outbreaks in the Southwestern United States

3.1 Introduction

Vesicular stomatitis (VS) is a viral disease of horses, pigs and cattle that is clinically undistinguishable from foot and mouth disease with vesicular lesions forming on the mucous membranes, coronary bands, and teats of infected animals. VS causes considerable economic loss to the United States from trade restrictions domestically and internationally. This disease only occurs in the Western Hemisphere with a few isolated cases outside of the Americas from exported infected livestock (Webb PA, Holbrook FR, 1988).

There are two main serotypes of vesicular stomatitis virus (VSV), New Jersey (NJ) and Indiana (IN). Infections occur in endemic cycles in the southeastern United States, southern Mexico, Central, and South America and sporadic cycles in the southwestern United States and northern Mexico (Knight AP, Messer NT, 1983; Stallknecht DE et al., 1985; Rodriguez LL et al., 2000).

Transmission of the virus is thought to be by insect vector. VSV grows well in insect cells, replicates in insects, and can be transmitted to susceptible animals by hematophagous insect bites. It is hypothesized that black flies are a factor in the transmission and maintenance of VSV in the southwest United

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States (Mead DG et al., 1999) and sand flies (*Lutzomyia shannoni*) play a role in transmission of the virus in the southeastern United States (Corn JL et al., 1990; Comer JA et al., 1994). Although antibodies to VSV have been detected in many species of wildlife including deer, rodents, birds, and monkeys (Webb PA, et al., 1987; Tesh RB et al., 1969; Aguirre AA et al., 1992; Jimenez AE et al., 1996), no viremic host has been identified and the cycle between a reservoir, insect, and livestock still remains an enigma.

VS occurs sporadically in the southwestern United States approximate every five to ten years (Rodriguez LL et al., 2000). In 1995, 365 premises were identified as VSV-NJ positive in six western states. This was the first outbreak of VS in the southwestern United States since 1985 (Bridges VE et al., 1997). The first case was identified in May 1995 and the VSV spread northward with the last case identified in November. No cases were reported in 1996, however VSV-NJ and VSV-IN were isolated from cases in 1997 (McCluskey BJ et al., 1999). This was the first time in over 30 years that VSV-IN had been reported in the United States. In May 1998, VSV-IN was isolated from VS cases with the last case reported in November. No cases of VS have been reported in the southwestern United States since November 1998.

Despite research efforts, an understanding of the complex nature of the factors that could influence the maintenance of VSV is lacking. No techniques for disease clustering in space or time have been investigated and there are limited environmental studies on VS. One tool for evaluating natural environment associations is spatial analysis. Geographical information systems (GIS) can be used for collecting and managing geographically referenced data for subsequent spatial analyses. There are many studies using GIS to integrate infectious disease data with geographic and climatic

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data (Kitron and Kazmierczak, 1997; Cross et al., 1996; Colwell, 1996; Engelthaler, et al., 1999; Parameter et al., 1999). This is the first report of VS that includes spatial, climatological and ecological analyses.

The objectives of the study were to detect potential spatial, temporal, and spatial-temporal clusters of VS from the 1995, 1997 and 1998 outbreaks of VS in the southwestern United States, and to investigate ecological and climatic factors that could be associated with regions of VSV activity. This is a descriptive study that integrates GIS applications to assess regions of high risk for VS in the United States and in the states of Colorado and New Mexico where the predominant amount of cases occurred. This study is a preliminary investigation into ecological and climate factors that could be incorporated into a risk model for VS. A more thorough understanding of the clustering and environmental conditions prior and during outbreaks of VS may lead to improvement in disease surveillance, prediction, and control within these regions.

3.2 Materials and Methods

3.2.1 Data Collection and Management

Data from investigations conducted by the United States Department of Agriculture (USDA): Animal Plant Health Inspection Service (APHIS): Veterinary Services (VS) during 1995, 1997, and 1998 were obtained. A case was defined by veterinary services as a premise with an animal residing with vesicular lesions and positive by serology or virus isolation for VSV. The records from 1995 included 1162 investigations. There were 367 reported VS cases, of which 329 had geographic coordinates. Coordinates were obtained either by using a global positioning system

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(GPS) or by estimating the geographical location using Map Expert (version 2.0, DeLORME, Yarmouth, Maine). The 1997 dataset did not contain geographic coordinates. In 1998, geographic coordinates were collected on 118 premises out of a total 232 investigations. Only VS positive premises with geographic coordinates were used in the hydrological, descriptive environmental characteristics and climatic analysis. Because geographical coordinates were unavailable for some premises investigated, the cluster, census, wind, and ecoregion analyses were conducted using the coordinate of the 5-digit zip code centroid. The coordinate for the 5-digit zip code centroid was obtained by matching address zip codes with known coordinates of zip code centroids in ArcView. After the address matching process, unmatched zip codes were checked by the United States Postal Service address and zip code registry and/or by address and phone number using Street Atlas USA's address and phone search (version 6.0, DeLORME, Yarmouth, Maine), and updated addressed were re-matched in ArcView.

3.2.2 Cluster Analysis Methods

SaTScan software (version 2.1.3, National Cancer Institute 1998) was used in identification of temporal, spatial, and spatial-temporal clustering. The Bernoulli model (Kulldorf M, Nagarwalla N, 1995) using latitude and longitude coordinates of 5-digit zip code centroids was performed with 999 replications in the Monte Carlo procedure at high rates with a spatial cluster size-scanning window of 50. The spatial-temporal analyses were performed on a daily basis. Clusters were identified and information on the number of cases in the cluster, the expected number of cases, and an estimated relative risk for each individual cluster were given. This may be useful when examining a cluster area in

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more detail; however, the information is purely descriptive. Each type of cluster analysis was applied by individual year for all 42 state United States investigations, as well as at the state-level for Colorado and New Mexico.

3.2.3 Environmental Factors

Administrative boundaries were obtained from ESRI Data and Maps, (1999 edition, Environmental Systems Research Institute, Inc., Redlands, California). County level equine, cattle, and cow dairy population and farm data were obtained from the 1997 United States Census of Agriculture, Geographic Area Series (USDA: National Agriculture Statistics Service). Minitab, student version 1998 (Minitab Inc., State College, PA), was used for statistical analysis. Linear regression demonstrated the association between county census data and VS positive premises by county. The level of significance was $p \leq 0.05$. Data on ecological regions were compiled by Bailey (Bailey RG, 1995) and obtained from the United States Department of Agriculture Forest Service.

Digital elevation models (DEMs) 7.5 minute (1:24,000) scale were obtained from GIS Data Depot® (www.gisdatadepot.com) and from the United States Geological Survey (USGS). Geographic coordinates were obtained for VS positive premises in Colorado and New Mexico in 1995 and Colorado in 1998 and the elevations of individual VS positive premises were obtained using the point extraction function in the ArcView Grid Analyst extension. The slope (maximum rate of change from a cell to its neighbors) and aspect (steepest down-slope direction of the maximum rate of change from each cell

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to its neighbors) were derived from the surface command in ArcView Spatial Analyst and values were extracted using the same function described above.

Distance to the nearest hydrological feature from VS positive premises with latitude and longitude coordinates within the geographical clusters identified with the SaTScan program in western Colorado in 1995 (CO95), north central Colorado in 1998 (CO98) and central New Mexico in 1995 (NM95) were calculated. Hydrological features were categorized into five main classes: canals, shorelines, streams, and perennial and intermittent water bodies. Canals are man made waterways such as ditches and aqueducts. Streams are natural running bodies of water and shorelines are the periphery of lakes. Perennial include water sources that are lasting through the year and intermittent are seasonal features. The hydrological files were obtained from 1997 TIGER/Line™ files (US Department of Commerce, Bureau of Census) and imported into ArcView 3.2. The distance was calculated by the function "assign by location" in the ArcView Geoprocessing wizard extension. The polygon shape files were converted to centroids using the ArcView Xtools extension and the distance was calculated to each VS positive premises.

Landsat 5 TM satellite data were obtained from the USGS for central New Mexico (NM95) June 21st, 1995, path 033, row 036, and for north central Colorado (CO98) June 29th, 1998, path 033, row 032. Landsat 5 TM imagery was imported into TNTmips (version 6.5, Microimages, Lincoln, Nebraska). Digital Raster Graphics data (DRG) of 1:100,000, produced by USGS were obtained from GIS Data Depot®, and TIGER/Line files on roads were used for more accurate georeferencing satellite imagery. An unsupervised classification (Jensen JR, 1996) of the Landsat imagery was conducted

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using the Isodata classifier algorithm to determine specific classes for landscape characterization. The Isodata classifier used the non-thermal bands one through five and seven. Brightness values were grouped into ten spectral classes, a noise reducing 3x3 modal filter was applied and each class was assigned a different color.

3.2.4 Climate

3.2.4.1 Precipitation and Temperature

The total monthly average precipitation values, minimum temperature and maximum temperature 1.2 arc-minute raster grids, based on a 30-year average from 1961 to 1990 averages, were obtained from The Climate Source (The Climate Source, LLC, Corvallis, Oregon). Raster grids were produced using a climate elevation linear regression model called Parameter-elevation Regressions on Independent Slopes Model (PRISM). This model incorporates geographically referenced climate averages based on a 30-year average from 1961 to 1990 and Digital Elevation Models (DEMs). In addition, monthly values from 1994 to 1999 at weather stations in north central Colorado and central New Mexico on precipitation, minimum temperature, and maximum temperature were obtained from National Oceanic and Atmospheric Administration (NOAA).

Climate raster files containing 30-year temperature and precipitation averages were imported into ArcView and raster values of each of VS case in CO98 and NM95 were extracted. The 30-year average monthly value for each region was calculated.

Total average precipitation, average minimum temperature and average maximum temperature were calculated for each month from January 1994 to December 1999 from weather stations in CO98 and NM95 and a surrounding radius of 24 km. Sixteen stations

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were selected in New Mexico and 30 stations in Colorado. Climate values from each weather station were imported into ArcView and interpolated by the nearest neighbor function with the same spatial resolution as the raster grids produced by Climate Source. The average monthly value was calculated for each cluster using the methods described above.

The departure from normal for all monthly average climate values from 1994 to 1999 was calculated by subtracting the average monthly value from the January to December 1961 to 1990 Climate Source average values. Statistical analyses using the Spearman's correlation test (Siegel S, Castellan Jr NJ, 1998) and Wilcoxon test (Siegel S, Castellan Jr NJ, 1998) were calculated in Analyze-It (version 1.62, Analyse-It software, Ltd., Excel, Microsoft Corporation, Redmond, Washington). Spearman's correlation (Siegel S, Castellan Jr NJ, 1998) was used to determine the relationship between the climatic values up to 12 months prior to the first month of the outbreak and 12 months prior to the last case month of each outbreak year. The Wilcoxon test (Siegel S, Castellan Jr NJ, 1998) was used to compare variations in climate between years prior, during, and post outbreak. A p-value of ≤ 0.05 was considered statistically significant.

3.2.4.2 Wind

The daily wind direction values were obtained from five NOAA stations in Colorado (Colorado Springs, Denver, Pueblo, Grand Junction, Alamosa) and three NOAA stations in New Mexico (Roswell, Gallup, Albuquerque) from January 1995 to December 1998. A linear regression model was applied to identify associations in wind direction (NE, NW, SW, SE) for the month, and year with the absence or presence of VS

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within 80.5km radius of the station. A p-value of ≤ 0.05 was considered statistically significant.

3.3 Results

The descriptive statistics on investigations and the number of investigations used in the cluster analyses are listed in Table 1. More investigations were conducted in 1995 than in 1997 and 1998. The number of VS positive reported premises during the 1997 outbreak increased by 27% in Colorado and declined by 33% from 1995 in New Mexico. The number of VS positive reported premises increased by another 6% in Colorado and decreased by another 9% in New Mexico in 1998.

Separate analyses of spatial and temporal clustering by day led to the same findings as analyses including space and time together. Therefore, the results of spatial-temporal analyses are presented below, unless differences between the analyses were obtained. The cluster analyses were performed for all 42 state investigations and separately for states, Colorado, and New Mexico.

The 1995 spatial-temporal analysis identified one cluster with 92% (n= 336) of VS positive premises and 176 negative premises in the southwest (Figure 1a). The cluster analyses included non-positive premises within a region of no VS positive premises. The Colorado state spatial-temporal analysis produced a cluster containing 73% (n= 121) of Colorado cases and 30 negative premises in western and southwestern Colorado. The spatial analysis by itself identified a secondary cluster in south central Colorado along the Rio Grande River (Figure 1b). This cluster incorporated 9% (n=15) VS positive premises. In the New Mexico state-level analyses, one cluster along the Rio

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Grande River incorporated 84.4% of cases (Figure 1c). Unlike 1995, the 1997 outbreak had one cluster in Colorado with a few cases in eastern Utah and northern New Mexico (Figure 2a). In the Colorado state-level analyses two clusters were identified, the primary cluster located in the Grand Junction area with 36% (n=99) of Colorado VS positive premises and a secondary cluster in northern Colorado with 13% (n=36) of Colorado VS positive premises (Figure 2b). There were no statistically significant clusters identified in the New Mexico analyses for 1997. In 1998, the cluster analyses were located in north central Colorado for the national and state-level analyses (Figure 3).

The spatial-temporal analyses indicated dates with an increase risk of VSV infection for geographical clusters. Each year produced clusters of higher risk for VSV starting in the spring and ending during the first winter months. The state-level analyses had a shorter time period from June 10th to July 17th in New Mexico, and between July 1st and November 6th in Colorado in 1995. In 1997, the Colorado state-level analysis was shorter, from September 17th to October 30th and in 1998, longer, from March 13th to October 9th.

Horses, cattle, and dairy census data on populations and farms per county were used to investigate the association between animal density and occurrence of VS outbreaks. Simple linear regression comparisons of cases and county estimates of horse, cattle, and dairy population and farms were calculated for each outbreak year. The relationship between horse density for each county and VS positive premises estimates are displayed by the fifth quintile with the zip code centroid location of VS positive premises from 1995, 1997, and 1998 outbreaks in Figure 4, for dairy in Figure 5, and cattle in Figure 6. VS positive premises from the 1995 outbreak were associated with the

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density of equine farms and dairy population; 1997 cases with density of cattle farms; and 1998 cases with density of horse population.

The geographic distribution of VS positive premises by ecological region was determined for identification of particular ecological characteristics that might be associated with VS activity. The distribution of cases for ecoregions, described by Bailey (USDA: Forest Service) is shown in Figure 7. Table 2 describes the province and lists the twenty-two distinct ecoregion sections identified to have VS positive premises during one of the outbreak years between 1995 and 1998. All VS positive premises from 1995, 1997, and 1998 were located in the dry domain, with the highest number of premises located within the northern canyon lands section. There was variability between outbreak years with most VS positive premises located within the northern Rio Grande section in 1995, northern canyonlands section in 1997, and the northern parks and ranges section in 1998 with 34.8%, 23.9%, and 46.2% of VS positive premises respectively.

The case clusters in NM95, CO95, and CO98 were selected for further investigation regarding their environmental characteristics. Table 3 shows the elevation, slope, and aspect of the premises with geographic coordinate estimates within the clusters. The elevation range for all VS positive premises from 1995 to 1998 were 1377 m to 3207 m with the widest range of elevation in Colorado during the 1995 outbreak. Slopes ranged from 0^{o}-25^{o} with a mean 3^{o}, median 1^{o}, and the greatest numbers of premises faced the south (Colorado 1995), were non-directional (New Mexico 1995), and faced the southeast (Colorado 1998) direction.

VS positive premises identified in 1995, 1997, and 1998 had an average distance to a water source of 523 m with 93.9% of premises closest to canals and streams than

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bodies of water (Table 4). New Mexico 1995 VS positive premises had shorter median distances to hydrological features than Colorado 1995 and 1998 VS positive premises.

The spatial pattern of vegetation types was examined using TM Landsat 5 satellite imagery to classify vegetation types relative to VSV activity. An unsupervised classification was used to aggregate spectral classes with similar properties. Although this method does not identify vegetation, it does provide a means to analyze patterns of vegetation distribution using statistical methods of the ISODATA classifier. The classification of NM95 is shown in Figure 5. Using grid analysis methods with estimated geographic coordinates of cases indicated at least one VS positive premise was located within each classification. Most VS positive premises were located within classification 2, 9, and 10 that are shown in Table 5. The ISODATA classification for CO98 with geographical location of VS positive premises is shown in Figure 7. Each classification had at least one case, with most premises located within classifications 2, and 4 through 6 (Table 6). Because the ISODATA is an unsupervised method, predominate vegetation types in each class are presently unknown.

To determine climatic conditions before, during, and after VS outbreaks, precipitation and temperature were compared for each of the years when cases of VS occurred. Average total monthly precipitation for 95NM and 98CO cases with estimated geographical coordinates were extracted from the 30-year Climate Source average. The 30-year average for 95NM cases peaked in August at 44.4 mm and CO98 in May at 64.7 mm. There are different climatic seasons between the two regions with Colorado precipitation peaking in the spring and New Mexico in the summer (Figure 10). Precipitation data from weather stations in north central Colorado and central New

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Mexico from January 1994 to December 1999 was analyzed for trends over the five year period. The total precipitation for CO98 and NM95 is shown in Table 7, with Colorado and New Mexico having higher than normal average precipitation in the five-year period than the 30-year average. Precipitation by month in New Mexico fluctuated from the norm for each year with the highest average precipitation peaking in the summer (Figure 11a). A different trend was noticed in north central Colorado with higher levels of annual precipitation than New Mexico with a burst of rainfall occurring in May 1995 (Figure 11b). The departure from the average 1961 to 1990 monthly precipitation was calculated from January 1994 to December 1999 and graphed with the monthly distribution of cases within these clustered regions (Figure 12). Increasing precipitation values of at least 50mm above average in Colorado were observed prior to and during months of reported VS cases in 1995, and 1997 (Figure 12). However, this trend was not statistically significant from year to year. Statistical analyses identified no substantial departures from consecutive years 1994 to 1999. There was no correlation between months of VSV activity 12 months prior to the first month of the outbreak and last month of outbreaks during 1995, 1997, and 1998.

The annual mean temperature in the southwest is between -5°C to 25°C, peaking in the months of July and August. No substantial departures from normal temperature patterns were identified from 1994 to 1999 within north central Colorado for minimum or maximum temperatures. Both regions produced similar temperature trends and therefore only the minimum temperature graph is shown in Figure 13a and Figure 13b for Colorado and New Mexico, respectively. There were no significant correlation coefficients between number of VS positive premises and minimum and maximum temperatures

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starting 12 months prior to the first VS positive premises and ending with the last VS positive premises. No significant differences in the median minimum and maximum temperatures from each consecutive year were identified.

Predominant wind direction was compared with the presence or absence of VS from January 1995 to December 1998. There was no statistical association with predominant wind direction and VSV activity during this time, however predominant wind direction for each station was tabulated from 1995 to 1998 with all stations, except Pueblo, having predominant wind direction from the south (Table 8).

3.4 Conclusion & Discussion

In 1995, Arizona, Colorado, New Mexico, and Utah were states with higher risk for VSV infection, based on the cluster analyses. The southwestern United States is known to be a "hotspot" for VS with periodic outbreaks occurring periodically followed by years absent of detectable virus activity. The 1995 analyses contained negative VS premises with no surrounding VS positive premises within the cluster, which is not likely to be a region of higher risk and is a limitation of the SaTScan program. The SaTScan program uses a scan statistic that imposes a circular window around centroids positioned throughout the study region that can vary in size, in which a window including 50% of the investigations can enlarge the number of potential clusters, including negative premises (Kulldorf M, Nagarwalla N, 1995). It would be more appropriate to examine the clusters by an elliptical clustering mechanism to lower the measurement of error produced by the circular clustering mechanism; however, given the absence of such a test, one is limited to the techniques currently available. Separate analyses at the state-

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level for Colorado and New Mexico helped define the clusters within the region, identifying clusters on the western slopes and southwest Colorado and within the vegetation surrounding the Rio Grande River. Similar spatial distributions of cases were described in 1982 and in previous outbreaks throughout the last 50 years (Saulmon EE, 1968; Walton TE, et al., 1987). In 1997, few VS positive premises were located in south and central New Mexico along the Rio Grande River, with a greater number of VS positive premises located in Colorado. Case clusters were identified in Colorado, eastern Utah, and northern New Mexico and each cluster was in the same region as in 1995. The Colorado state-level analyses indicated two separate clusters; a case cluster along Colorado's western slope in the vicinity of the Colorado River and a new cluster identified along the Front Range in central Colorado. In 1998, VS positive premises were clustered in north central Colorado, similar to the secondary cluster from 1997.

In 1995, only clinical cases of VSV-New Jersey (VSV-NJ) were identified. In 1997, VSV-NJ and VSV-Indiana (VSV-IN) were both reported. This was the first United States reported case of VSV-IN in over 30 years. In 1998, only VSV-IN cases were identified. The lack of serotype records from each premises prevented the distinction between spatial-temporal analyses and serotype. Genetic typing of the viruses from 1995 and 1997 identified VS-NJ viruses to be closely related and VS-IN viruses from 1997 and 1998 were identical (Rodriguez LL et al., 2000). Either the virus was capable of persisting for up to two years or the virus was re-introduced into the southwestern United States. The temporal trend in 1995, 1997, and 1998 was typical to previous outbreaks with cases beginning in the south in spring and moving northward over time, usually ending with the onset of the first frost (Webb PA, Holbrook FR, 1988).

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Associations between VS positive premises and animal census data from the United States Census of Agriculture were compared for relationships between animal and host animal population densities per county. The census data are based on estimated animal numbers, population density and number of farms with livestock and horses. Farm population density does not always reflect the same proportion of animal population, as a county with low numbers of farms might have a high animal population and visa versa. The results suggest that during the 1995 outbreak counties with a higher number of equine and dairy farms, in 1997 cattle farms and in 1998 equine and cattle farms have a greater chance of VSV infection. Stratification of each yearly outbreak by species was lacking from the linear regression model, as multiple species were sampled on the same farms in 1995, species identification records were lacking for 1997 investigations and all VS positive premises for 1998 included equids. Errors in geographical distribution of animal populations and farm numbers within each county and distance to VS positive premises are not taken into consideration in this model. While accuracy of the U.S. Census of Agriculture demographic data is unknown, the number listed may reflect an underestimate of the actual susceptible population and number of farms. The location and density of susceptible populations during an outbreak is crucial for disease control and prevention.

The role of insect vectors in the cycle of VS is not completely understood; therefore, environmental characteristics, including distance to hydrological features, were calculated to identify the environment and the proximity to water source in potential vector habitats. Phlebotomine sand flies are known to play a role in the maintenance and transmission of VSV in Ossabaw Island, Georgia (Comer JA et al., 1994; Corn JL et al.,

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1990) and it has been hypothesized that black flies may play a role in the transmission of VSV in the southwest (Walton TE, et al., 1987; Webb PA, Holbrook FR, 1988; Mead DG et al., 1999). Black fly larvae develop in moving water, with shallow mountain torrents being favored breeding places (Cupp EW, 1996). Phlebotomine sand flies breed in places of darkness and humidity with a supply of organic matter, such as rodent burrows or hollow trees. The distribution within ecoregions of the United States demonstrated that all VS positive premises were located within a dry climate, with hot summers and cold winters. Streams in the dry region contain water only seasonally and perennial water sources were large rivers or ditches with water supplies regulated for irrigation. During the VS outbreaks, most VS positive premises were located within the dry regions, located in the lower elevations of the region, and along major riverbeds or waterways. Regions along riverbeds and permanent water systems are the only regions for vector habitats. Clustered cases in New Mexico and Colorado were located in regions with high agricultural productivity with many irrigation ditches and canals that were perfect for black fly breeding sites. The lack of controls makes it difficult to distinguish the environmental factors that indicate virus susceptibility from those that are associated with all livestock in the area. More detailed information on the ecology of potential vector breeding habitat combined with data on hydrological flow rates, organic content, and water temperature near VS positive premises would be helpful.

Satellite imagery was used to identify spectral classes of vegetation with similar properties in each region of central New Mexico and north central Colorado. These regions were examined individually with their proximity to VS positive premises. The unsupervised method performed allowed for identification of vegetation with similar

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properties, but did not identify the type of vegetation. Certain types of vegetation included more cases of VS than others and could be related to certain vector habitat within the region. Future information and data on the vegetation classifications and vector ecology within the region would be helpful to determine the relationship of vegetation and VS positive premises.

Changes in climate conditions, especially precipitation, can play an important role in outbreaks of diseases. An increase in the prevalence of hantavirus and plague has been correlated with an increase in precipitation (Parmenter RR et al., 1999; Engelthaler DM et al., 1999). Meterological data from 1994 to 1999 showed sufficient rainfall to support development of aquatic stages of insect growth. Low temperature starting in the late fall may have reduced insect biting activity to bring the outbreak in each year to an end and over-wintering of the virus might be present in certain insect species that could allow for transmission of the virus the following years from 1995 to 1997 and 1997 to 1998. No significant statistical changes in temperature or precipitation within each clustered region were identified with cases of VSV; however general comparisons between the regions were observed. Warmer temperatures are present earlier in the year in New Mexico and last longer through the year than in Colorado. This could be a cause for viral persistence within the Rio Grande River belt during each outbreak year. Wind factors might also be involved in transporting infected insects over relatively long distances, as previous investigated from 1982 to 1985 southwestern outbreaks (Sellers RF, Maarouf AR, 1990). Preliminary descriptive study of wind direction in New Mexico and Colorado indicate winds coming from the south, which could possibly be a route for viral transportation from Mexico. Wind direction alone cannot support the vector transport, as a complex

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model of wind speed, humidity, cloud cover, temperature, and rainfall is needed for insect flight.

The study only incorporates premises reported by livestock owners and visited by veterinary medical officers. Many animals seroconvert from VSV and remain asymptomatic (Letchworth GJ et al., 1999), and it is likely that many more animals, wildlife and domestic, were infected with VSV. The lack of geographic coordinates and negative controls hindered the study comparisons and statistical analyses. The geographic coordinates utilized in the study also contain a range of error, due to some variability in the location where the data were collected. Future investigations should incorporate sufficiently high standards of accuracy to enable more detailed farm-level studies. Lack of accuracy may change the information obtained on the land characteristics and distance to hydrological features.

Despite the study's limitations, this descriptive study was used as a preliminary investigation of ecological factors that could be incorporated into a risk model for VS to aid in future disease surveillance, control, and direction.

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3.5 Tables

Table 1. Descriptive statistics on the number of investigations and VS positive premises used in the spatial, temporal, and spatial-temporal cluster analyses. The number of VS positive premises by state-level (Colorado and New Mexico) is shown.

Outbreak Year	Investigations	Investigations Used in Study	United States Positive premises	Colorado Positive premises (%)	New Mexico Positive premises (%)
1995	1162	1153	367	165 (45%)	186 (51%)
1997	706	702	380	273 (72%)	67 (18%)
1998	232	231	130	101 (78%)	12 (9%)

( ) = number of VS positive premises

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Table 2. Bailey's ecoregion characteristics and the distribution of VS positive premises for outbreaks 1995, 1997, 1998 and for all VS positive premises by ecoregion section.

Ecological Division	Ecoregion Province	Province Description	Ecoregion Section	All (+) Premises (%)	1995 (+) Premises (%)	1997 (+) Premises (%)	1998 (+) Premises (%)
Tropical Subtropical Desert	American Semi-Desert and Desert	Plains, valleys, and basins. Summers are hot and long, winters are moderate. Vegetation is sparse with some cacti and shrubs. Soils are mainly gravel or bare rocks.	Mojave Desert	1 (0.11)	0	0	1 (0.77)
Tropical Subtropical Desert	American Semi-Desert and Desert	As Above	Sonoran Mojave Desert	8 (0.92)	0	1 (0.27)	7 (5.38)
Tropical Subtropical Regime Mountains	Arizona-New Mexico Mountains Semi-Desert-Open Woodland-Coniferous Forest-Alpine Meadow	Steep foothills and mountains with high plateaus. Climate varies with altitude. Mixed grass, brush, and forest. Soils are characteristically Entisols, Alfisols, Inceptisols.	Sacramento-Monzano Mountain	4 (0.46)	2 (0.55)	1 (0.27)	1 (0.77)
Tropical Subtropical Regime Mountains	Arizona-New Mexico Mountains Semi-Desert-Open Woodland-Coniferous Forest-Alpine Meadow	As Above	White Mountain-San Francisco Peaks	16 (1.84)	11 (3.01)	3 (0.80)	2 (1.54)
Tropical Subtropical Desert	Chihuahuan Semi-Desert	Mostly desert. Rio Grande and Pecos Rivers are the only main rivers. Summers are hot and long, winters are short. Vegetation is mainly shrubs and cacti. Soils are primarily Aridisols.	Basin and Range	37 (4.25)	16 (4.38)	10 (2.66)	11 (8.46)
Tropical Subtropical Steppe	Colorado Plateau Semi-Desert	Elevation varies between 150 to 2100m. Hot summers and cold winters. Vegetation varies with grassland, woodland, montane, and subalpine zones. Entisols soils located along major streams and Aridsols on plateau tops.	Grand Canyon Lands	24 (2.76)	17 (4.66)	7 (1.86)	0
Tropical Subtropical Steppe	Colorado Plateau Semi-Desert	As Above	Navajo Canyonlands	16 (1.84)	6 (1.64)	10 (2.66)	0
Tropical Subtropical Steppe	Colorado Plateau Semi-Desert	As Above	Northern Rio Grande Intermontane	151 (17.34)	127 (34.79)	19 (5.05)	5 (3.85)
Tropical Subtropical Steppe	Colorado Plateau Semi-Desert	As Above	Tonto Transition	1 (0.11)	0	1 (0.27)	0
Temperate Steppe	Great Plains-Palouse Dry Steppe	Rolling plains and tablelands near the foot of the Rocky Mountains. Climate is semi-arid with cold dry winters and hot summers. Vegetation is steppe, shortgrass prairie and scattered trees. The dominant soil is Mollisols.	Arkansas Tablelands	48 (5.51)	2 (0.55)	45 (11.97)	1 (0.77)

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Table 2 continued.

Temperate Steppe	Great Plains-Palouse Dry Steppe	As Above	Central High Plains	83 (9.53)	0	43 (11.44)	40 (30.77)
Temperate Steppe	Great Plains-Palouse Dry Steppe	As Above	Upper Rio Grande Basin	41 (4.71)	25 (6.85)	14 (3.72)	2 (1.54)
Temperate Desert	Intermountain Semi-Desert	Plains and tablelands. Climate is semiarid and cool. Hot summers, cold winters. Vegetation is sagebrush steppe and short grasses. The characteristic soils are Aridisols in the basins and lowland areas and Mollisols at the higher elevations.	Bighorn Basin	8 (0.92)	8 (2.19)	0	0
Temperate Desert	Intermountain Semi-Desert and Desert	As Above	Bonneville Basin	1 (0.11)	1 (0.27)	0	0
Temperate Desert	Intermountain Semi-Desert and Desert	As Above	Northern Canyon Lands	155 (17.80)	65 (17.81)	90 (23.94)	0
Temperate Desert	Intermountain Semi-Desert and Desert	As Above	Uinta Basin	26 (2.99)	0	26 (6.91)	0
Temperate Desert Regime Mountains	Nevada-Utah Mountains-Semi-Desert-Coniferous Forest-Alpine Meadow	Great Basin and Colorado Plateau. Streams are rare. Climate is temperate desert climate with high drought and short humid season. Precipitation is mainly in winter. Vegetation is characteristically brush and shrubs. Soil types Aridisols dominate basin and lowland area and Mollisols and Alfisols at higher elevations.	Tavaputs Plateau	6 (0.69)	3 (0.82)	3 (0.80)	0
Temperate Steppe Regime Mountains	Southern Rocky Mountain Steppe-Open Woodland-Coniferous Forest-Alpine Meadow	Rugged mountains. Climate is semiarid steppe influenced by prevailing west winds and north-south orientation of the mountain ranges. Vegetation varies by elevation. Soils are Mollisols and Alfisols.	North-Central Highlands	19 (2.18)	13 (3.56)	6 (1.60)	0
Temperate Steppe Regime Mountains	Southern Rocky Mountain Steppe-Open Woodland-Coniferous Forest-Alpine Meadow	As Above	Northern Parks and Ranges	89 (10.22)	1 (0.27)	28 (7.45)	60 (46.15)
Temperate Steppe Regime Mountains	Southern Rocky Mountain Steppe-Open Woodland-Coniferous Forest-Alpine Meadow	As Above	Overthrust Mountains	1 (0.11)	0	1 (0.27)	0
Temperate Steppe Regime Mountains	Southern Rocky Mountain Steppe-Open Woodland-Coniferous Forest-Alpine Meadow	As Above	South-Central Highlands	122 (14.01)	66 (18.08)	56 (14.89)	0

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Table 2 continued.

Temperate Steppe Regime Mountains	Southern Rocky Mountain Steppe-Open Woodland-Coniferous Forest-Alpine Meadow	As Above	Southern Parks and Ranges	11 (1.26)	1 (0.27)	10 (2.66)	0
Temperate Steppe Regime Mountains	Southern Rocky Mountain Steppe-Open Woodland-Coniferous Forest-Alpine Meadow	As Above	Uinta Mountains	2 (0.23)	0	2 (0.53)	0
Tropical Sub tropical Steppe	Southwest Plateau and Plains Dry Steppe and Shrub	Flat to rolling plains. Climate is semiarid with long, hot summers and mild short winters. Vegetation is arid grassland shrubs and low trees. Soils vary	Rolling Plains	1 (0.11)	1 (0.27)	0	0

Soils classes (Donahue RL et al., 1990)
Alfisols – High clay content soil that is not too highly leached, found in forested areas.
Aridisols – Soils of dry regions.
Entisols – Very young soils with little or no horizon development.
Inceptisols – Young soils that are in early stages of development. Only the more rapidly formed horizons present.
Mollisols – Wet and high clay content soils, found in grasslands.

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Table 3. Descriptive statistics on environmental characteristics on VS positive premises with geographic coordinates in the 1995 New Mexico Cluster and 1998 Colorado Cluster.

	Elevation (m)	Slope (°)	Aspect / Number of Premises	
Colorado 1995 (141 Premises)	$\bar{x}$ 1712.12 $\underline{x}$ 1497 SD 383.96 (1377 - 3207)	$\bar{x}$ 3.35 $\underline{x}$ 1.82 SD 4.225 (0 - 25.135)	Flat N NE E SE S SW W NW	7 21 7 16 20 29 14 12 15
New Mexico 1995 (153 Premises)	$\bar{x}$ 1586.47 $\underline{x}$ 1502.66 SD 234.21 (1402-2640)	$\bar{x}$ 1.76 $\underline{x}$ 0.58 SD 3.57 (0-24.45)	Flat N NE E SE S SW W NW	47 11 5 16 12 27 15 14 6
Colorado 1998 (94 premises)	$\bar{x}$ 1622.22 $\underline{x}$ 1551.5 SD 284.63 (1396-2756)	$\bar{x}$ 2.93 $\underline{x}$ 1.86 SD 3.11 (0-18)	Flat N NE E SE S SW W NW	6 8 17 13 18 5 6 9 12

$\bar{x}$ = mean
$\underline{x}$ = median
SD = standard deviation
( )= range of values

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Table 4. Descriptive statistics on hydrological characteristics 1995 New Mexico Cluster and 1998 Colorado Cluster.

	Distance Canal (m)	Distance Stream (m)	Distance Shoreline (m)	Distance Perennial (m)	Distance Intermittent (m)
Colorado 1995 (138 Premises)	$\bar{x}$ 234.39 $\underline{x}$ 230.20 (0.004-1664.04) 33.34%* n = 46	$\bar{x}$ 281.63 $\underline{x}$ 239.83 (8.59-927.661) 58.69%* n = 81	$\bar{x}$ 354.93 $\underline{x}$ 314.08 (5.296-851.94) 7.97%* n = 11	$\bar{x}$ 264.67 $\underline{x}$ 230.20 (5.296-1664.04) 59.4%* n = 82	$\bar{x}$ 282.05 $\underline{x}$ 244.27 (0.004-927.66) 40.58%* n = 56
New Mexico 1995 (157 Premises)	$\bar{x}$ 147.30 $\underline{x}$ 91.48 (1.44-594.91) 53.50%* n = 84	$\bar{x}$ 307.98 $\underline{x}$ 165.81 (5.712-4004.212) 45.22%* n = 71	$\bar{x}$ 353.25 $\underline{x}$ 353.25 (24.38-682.12) 1.27%* n = 2	$\bar{x}$ 209.33 $\underline{x}$ 110.10 (5.712-4004.2) 78.34%* n = 123	$\bar{x}$ 270.55 $\underline{x}$ 135.56 (1.44-1268.18) 21.66%* n = 34
Colorado 1998 (97 Premises)	$\bar{x}$ 382.28 $\underline{x}$ 168.92 (7.48-3539.57) 37.11%* n = 36	$\bar{x}$ 614.98 $\underline{x}$ 290.65 (63.52-1005.36) 51.55%* n = 50	$\bar{x}$ 568.45 $\underline{x}$ 564.73 (4.44-1005.36) 11.34%* n = 11	$\bar{x}$ 523.20 $\underline{x}$ 281.83 (4.44-6955.10) 64.95%* n = 63	$\bar{x}$ 521.80 $\underline{x}$ 290.65 (7.48-5611.8) 35.05%* n = 34
All Premises (Colorado and New Mexico 1995 & Colorado 1998)	n=166 42%*	n=202 52%*	n=24 6%*	n=268 68%*	n=124 32%*

$\bar{x}$ = mean, $\underline{x}$ = median, SD = standard deviation, ( )= range of values, * = percentage of premises closest to each hydrological feature, n = number of premises closest to each hydrological feature

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Table 5. Distribution of VS positive premises within Landsat 5 TM satellite imagery in Central New Mexico and ISODATA classification.

ISODATA Classification	Number of VS Positive Premises
1	5
2	24
3	6
4	3
5	9
6	1
7	1
8	1
9	25
10	28

Table 6. Distribution of VS positive premises within Landsat 5 TM satellite imagery in north central Colorado and ISODATA classification.

ISODATA Classification	Number of VS Positive Premises
1	2
2	15
3	3
4	11
5	11
6	14
7	8
8	7
9	1
10	8

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Table 7. Total yearly precipitation for VS positive premises in north central Colorado and central New Mexico.

Year	Colorado VS Positive Premises Total Yearly Precipitation (mm)	New Mexico VS Positive Premises Total Yearly Precipitation (mm)
1994	326.57	348.73*
1995	518.03*	186.45
1996	401.87*	286.71*
1997	576.68*	362.63*
1998	415.58*	321.88*
1999	411.12*	263.61*

* Years with yearly average values greater than the 30-year average

Table 8. The most days of wind direction within the 48 months from January 1995 to December 1998 for weather stations in Colorado and New Mexico

Station	State	Wind direction
Albuquerque	New Mexico	SW
Gallup	New Mexico	SW
Roswell	New Mexico	SE
Alamosa	Colorado	SW
Colorado Springs	Colorado	SE
Denver	Colorado	SW
Grand Junction	Colorado	SE
Pueblo	Colorado	NE

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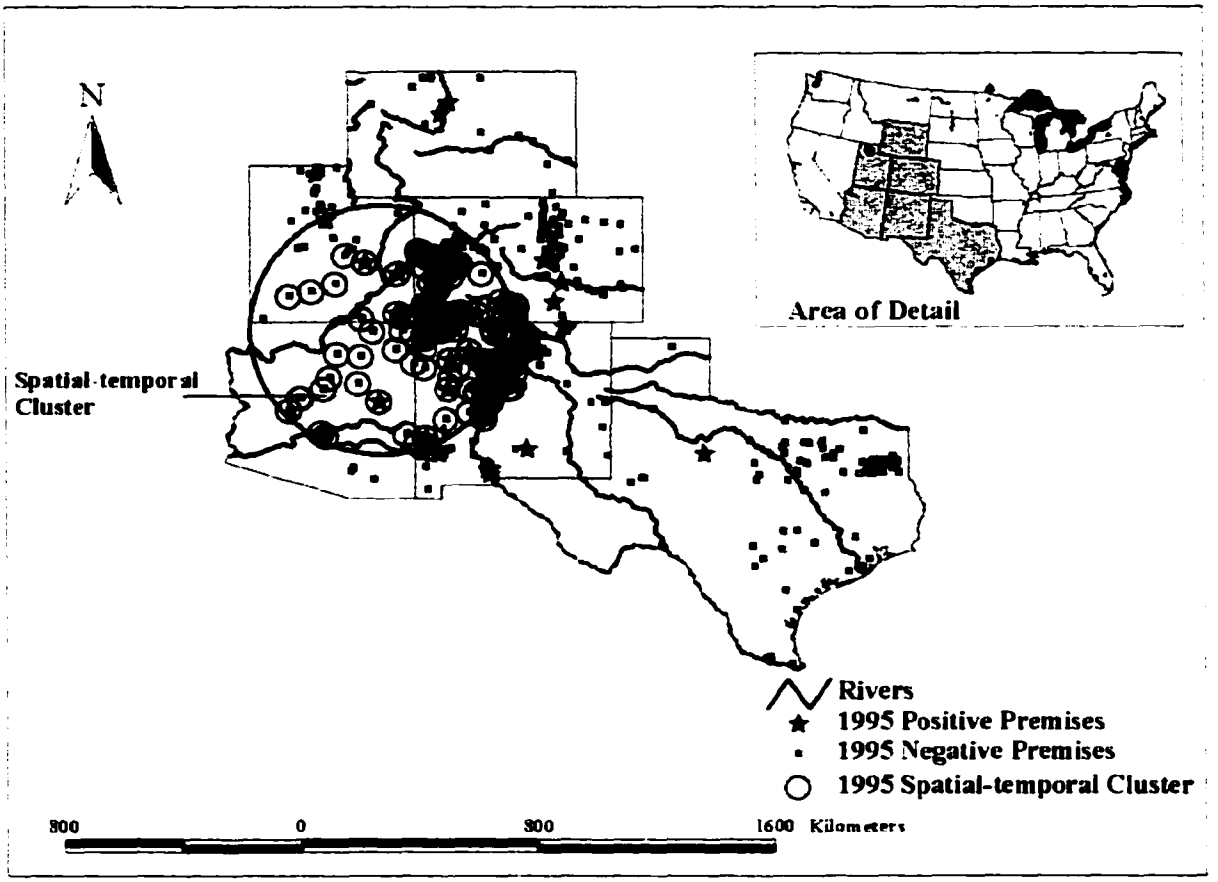
3.6 Figures

Figure 1a. Spatial-temporal analysis of 1995 United States VS outbreak cluster with a level of significance $p \leq 0.05$. Cluster was identified by 5-digit zip geocoded VS investigations of all reported 42 states. A base map with the area of interest is shown along with the detailed region of VS investigations within the southwestern United States.



The cluster includes 514 investigations, 336 positive premises, from April 15th to November 16th, with a relative risk of 2.1.

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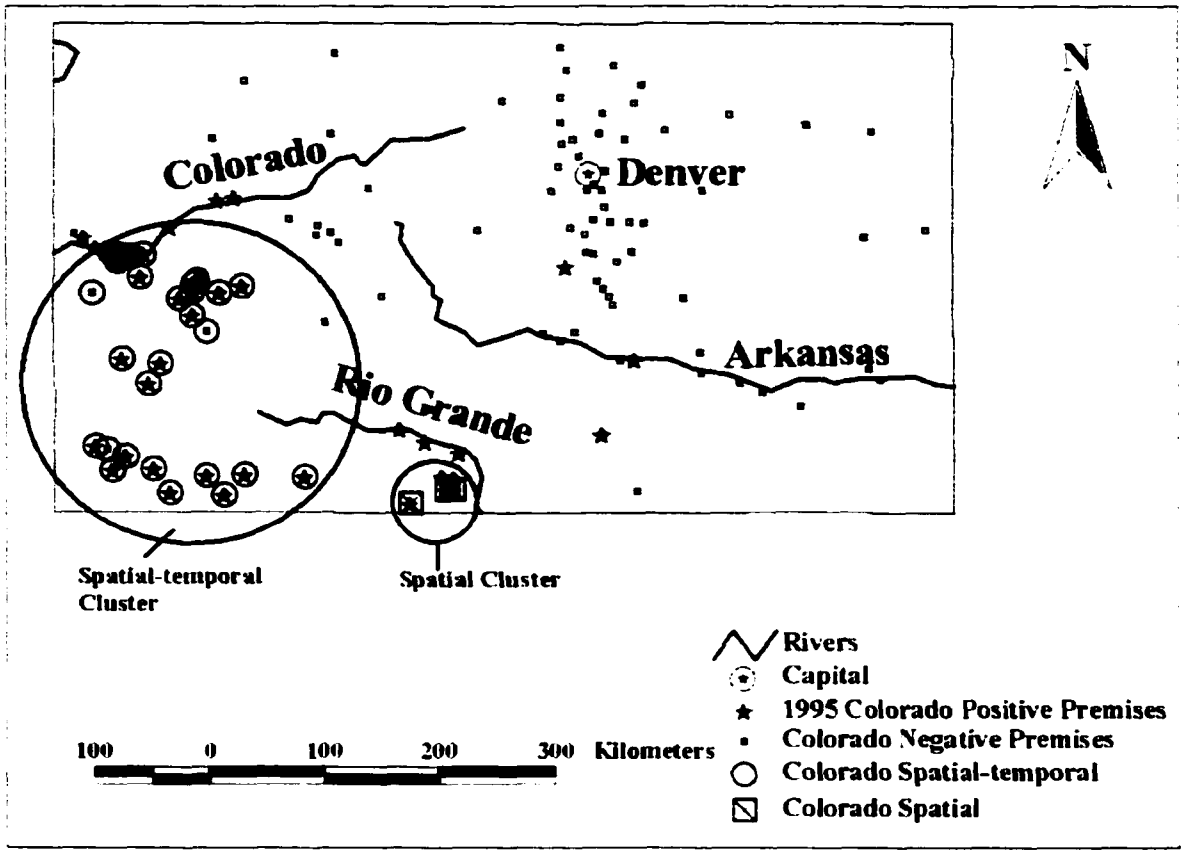
Figure 1b. Spatial-temporal analysis of 1995 Colorado VS outbreak with a level of significance $p \leq 0.05$. Clusters were identified by 5-digit zip geocoded VS investigations of all reported in Colorado.



The cluster includes 151 investigations, 121 positive premises, from July 1st to November 16th, with a relative risk of 1.9.

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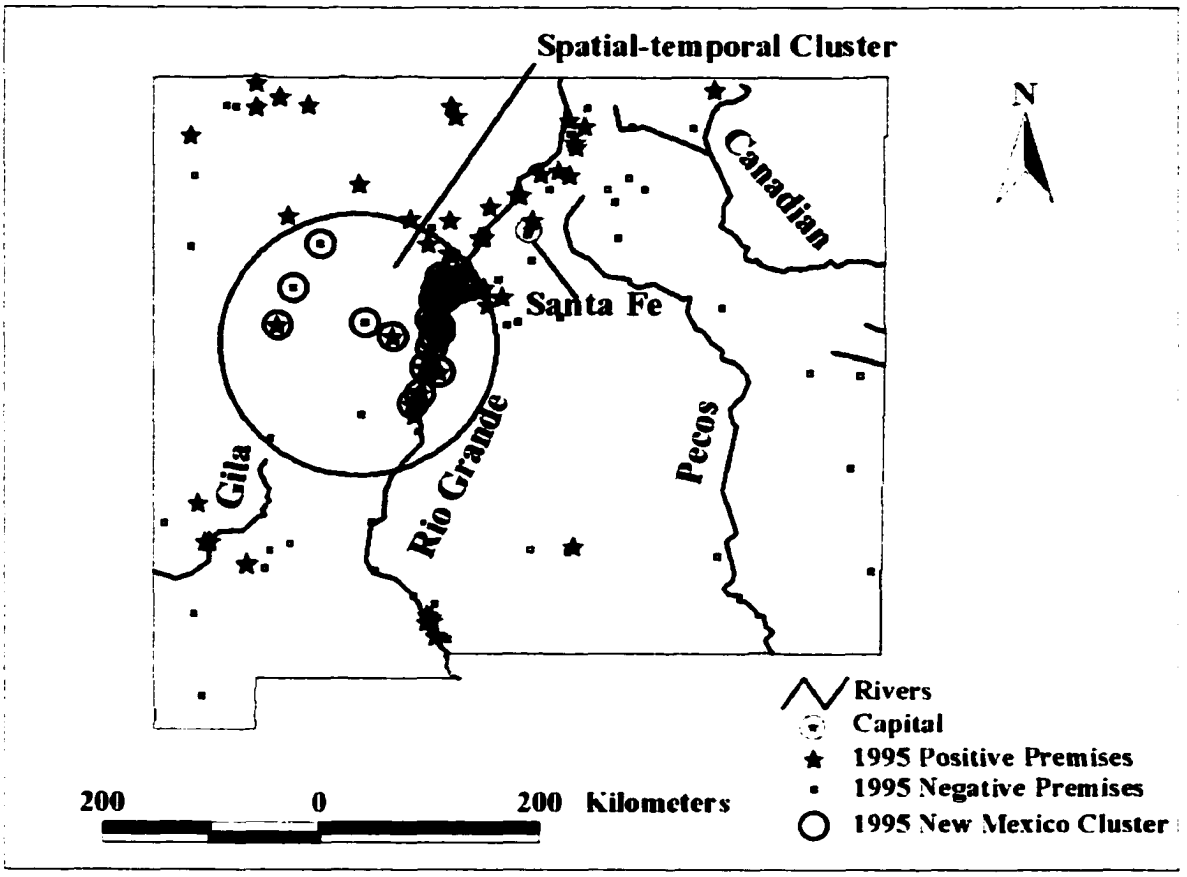
Figure 1c. Spatial-temporal analysis of 1995 New Mexico VS outbreak with a level of significance $p \leq 0.05$. Clusters were identified by 5-digit zip geocoded VS investigations of all reported in New Mexico.



The cluster includes 157 investigations, 122 positive premises, from June 10th to July 17th, with a relative risk of 1.4.

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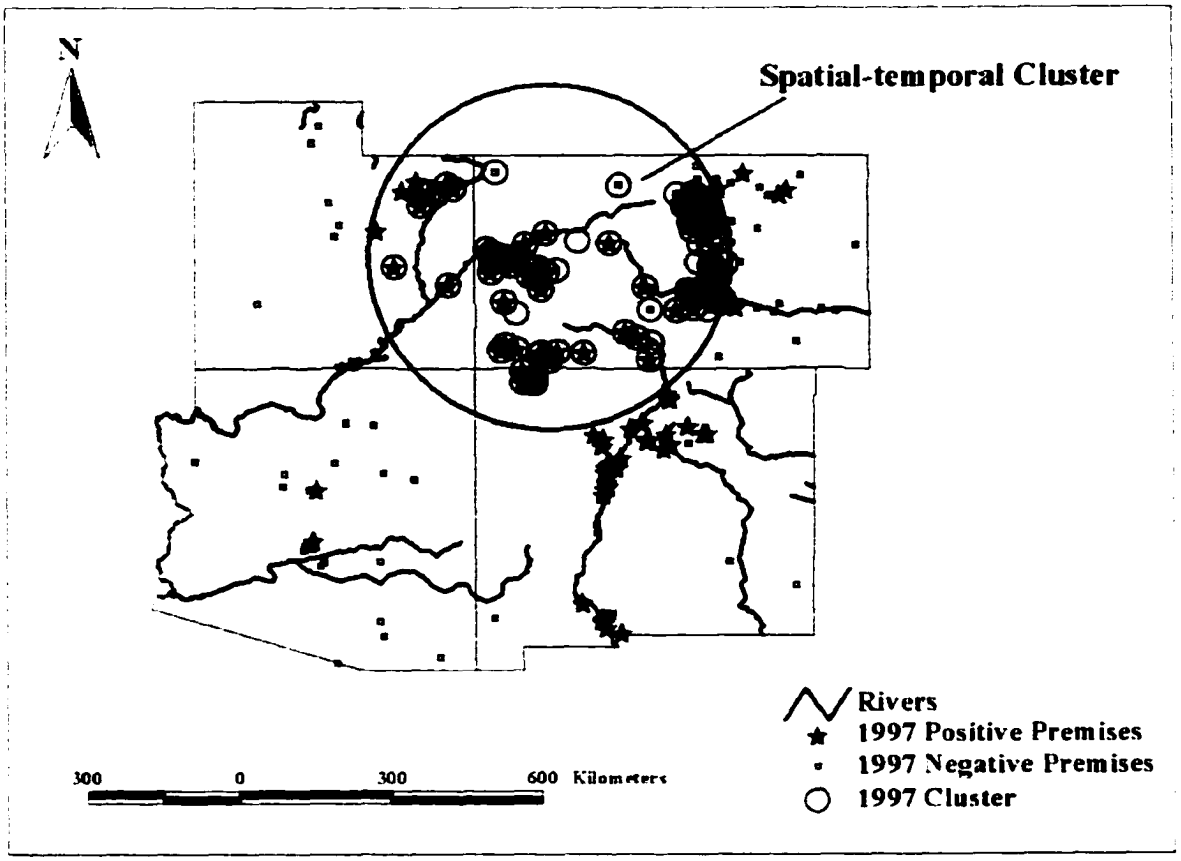
Figure 2a. Spatial-temporal analysis of 1997 United States VS outbreak cluster with a level of significance $p \leq 0.05$. Clusters were identified by 5-digit zip geocoded VS investigations of all reported 41 states.



The cluster includes 329 investigations, 272 positive premises, from July 8th to November 13th, with a relative risk of 1.5.

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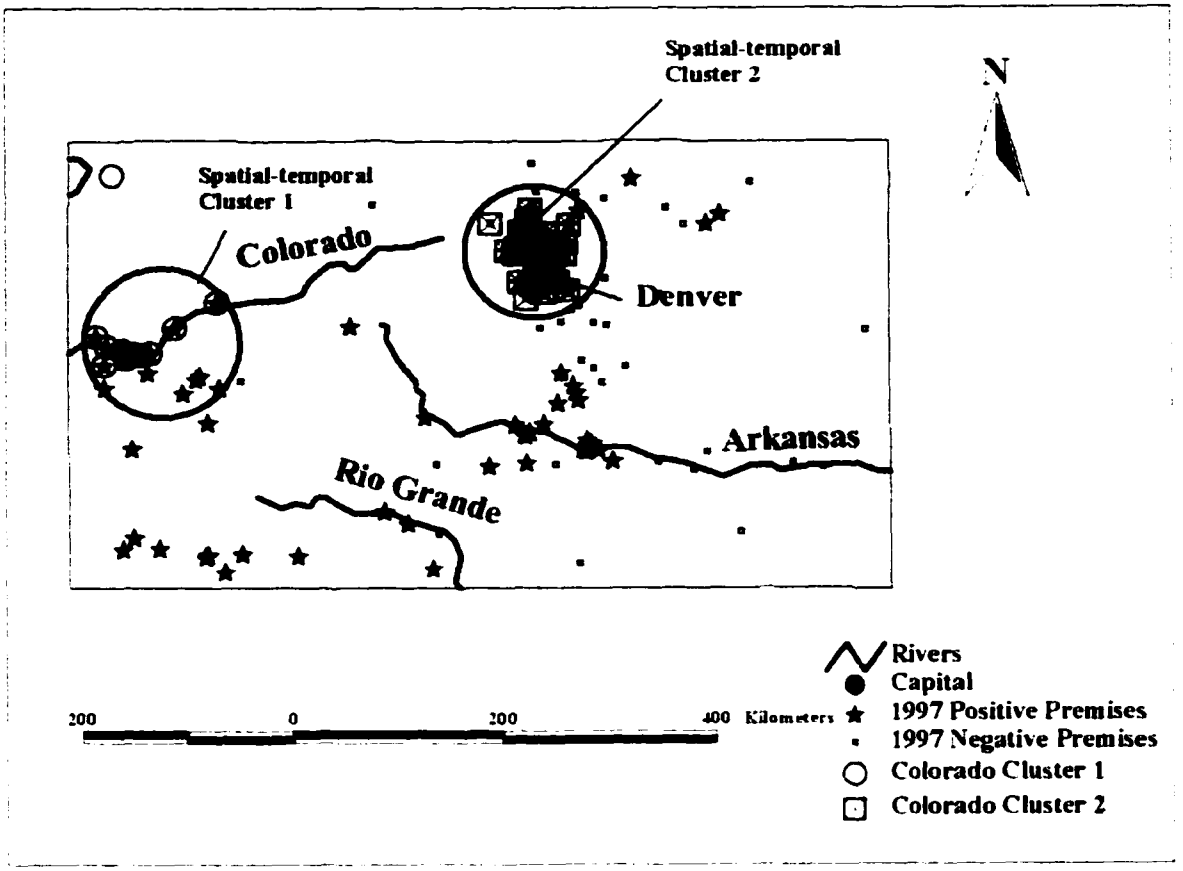
Figure 2b. Spatial-temporal analysis of 1997 Colorado VS outbreak with a level of significance $p \leq 0.05$. Clusters were identified by 5-digit zip geocoded VS investigations of all reported in Colorado.



The number of total VS investigations, VS positive premises, the relative risk (RR), and the dates of risk within the spatial-temporal cluster are given:
Cluster 1: 107 investigations, 99 positive premises, RR 1.26, September 22nd to October 30th.
Cluster 2: 36 investigations, 36 positive premises, RR 1.36, September 17th to October 31st.

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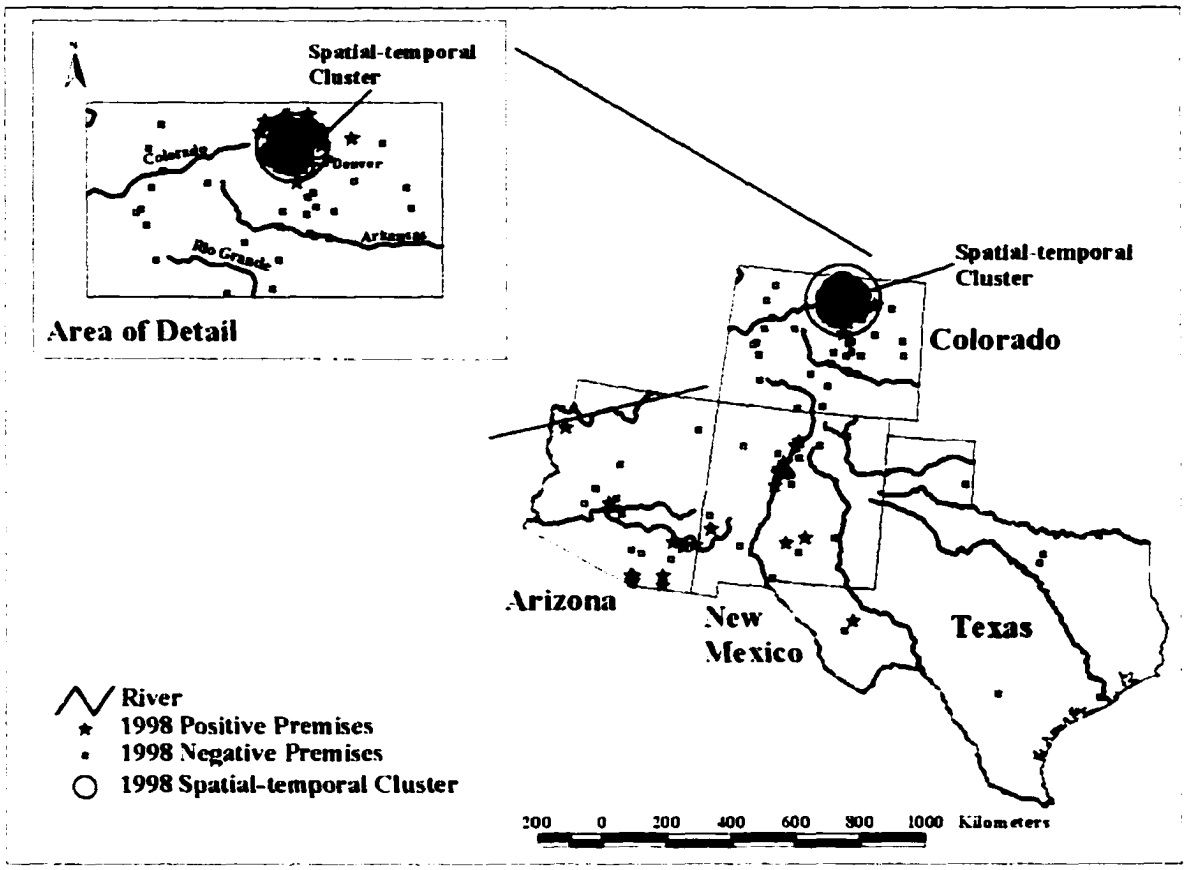
Figure 3. Spatial-temporal analysis of 1998 United States VS outbreak cluster with a level of significance $p \leq 0.05$. Clusters were identified by 5-digit zip geocoded VS investigations of all reported 5 states.



The number of total VS investigations, VS positive premises, the relative risk (RR), and the dates of risk within the spatial-temporal cluster are given:
All states cluster: 109 investigations, 91 positive premises, RR 1.46, March 12th to October 9th
Colorado cluster: 74 investigations, 65 positive premises, RR 1.39, March 12th to October 9th.

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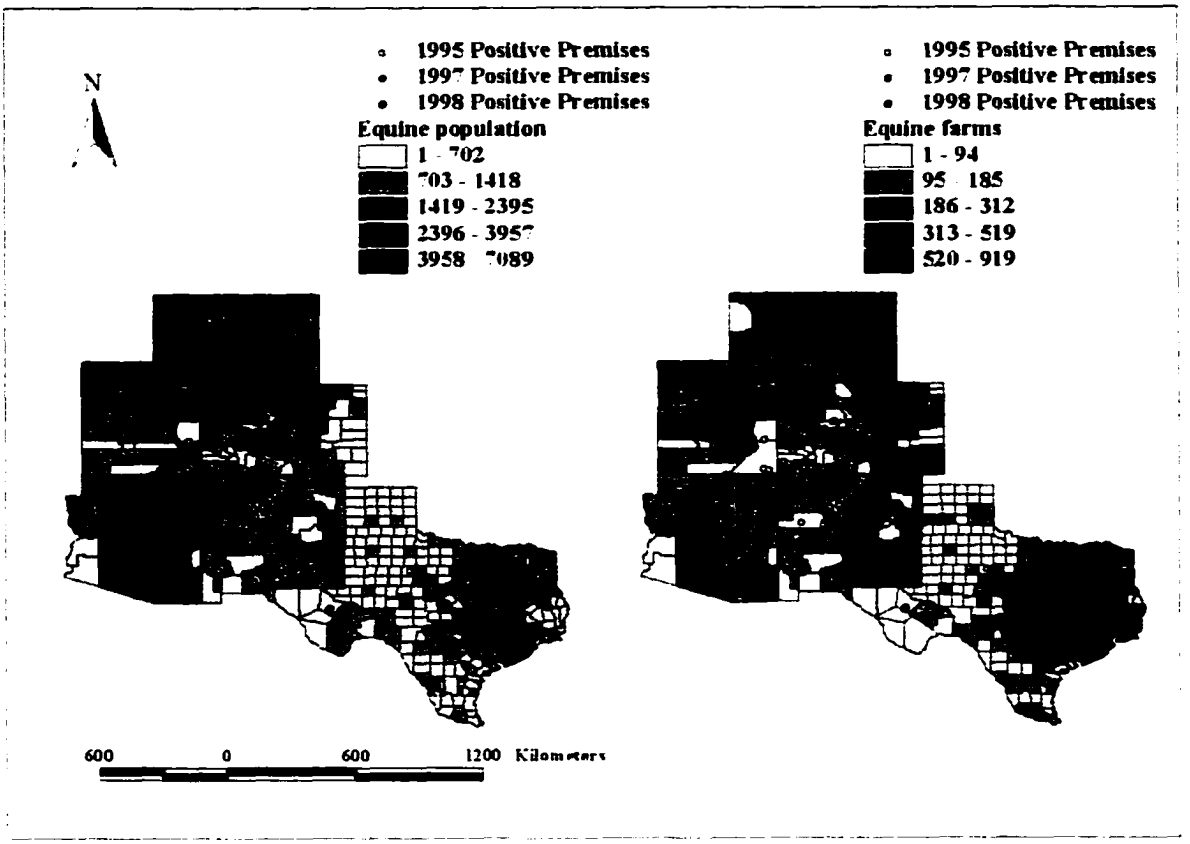
Figure 4. Estimated number of equids per county and state from the USDA's Census of Agriculture and the location by 5-digit zip code of the 1995, 1997, and 1998 VS positive premises.



1995 VS positive premises and farms per county were significant at $p \leq 0.05$.
1998 VS positive premises and population per county were significant at $p \leq 0.05$.

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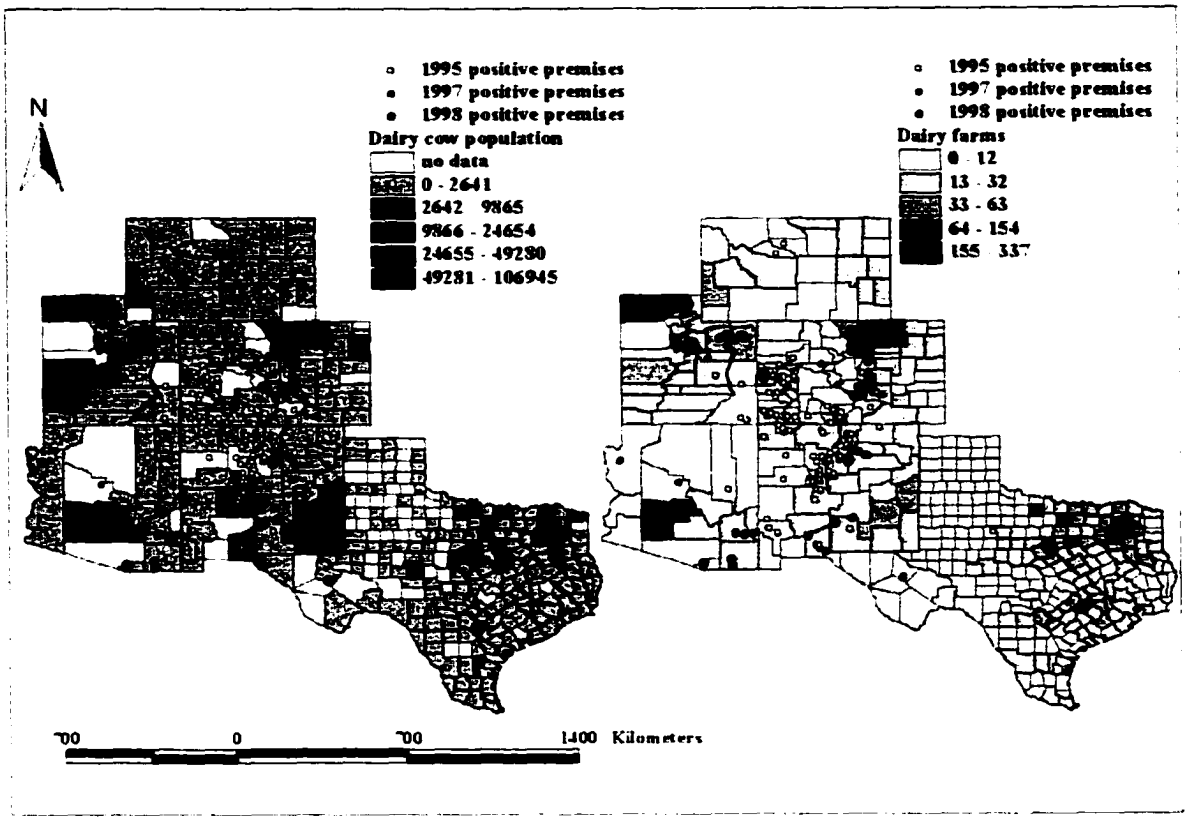
Figure 5. Estimated number of dairy cows per county and state from the USDA's Census of Agriculture and the location by 5-digit zip code of the 1995, 1997, and 1998 VS positive premises.



1995 VS positive premises and farms per county were significant at $p \leq 0.05$.

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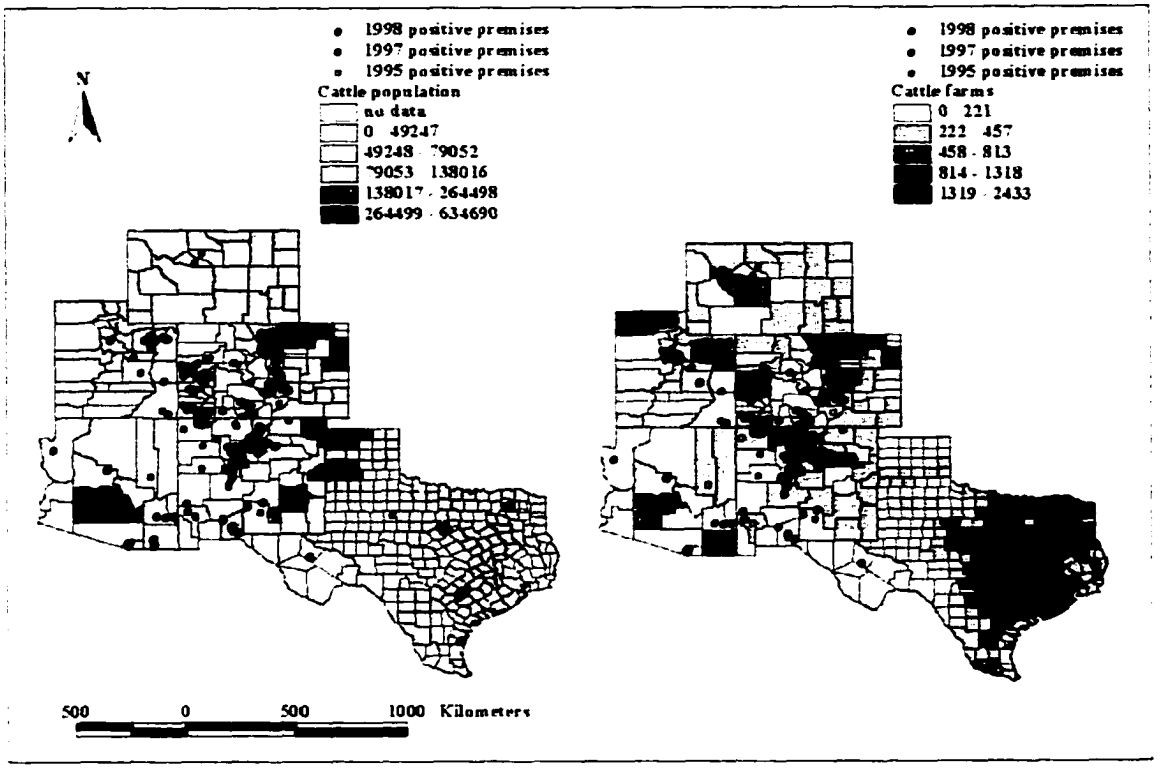
Figure 6. Estimated number of cattle per county and state from the USDA's Census of Agriculture and the location by 5-digit zip code of the 1995, 1997, and 1998 VS positive premises.



1997 & 1998 VS positive premises and farms per county were significant at $p \leq 0.05$.

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Figure 7. Bailey's ecoregion sections in six western US states reporting cases in 1995, 1997, and 1998. The geographical distribution of VS positive premises by year, are located by 5-digit zip code centroid.

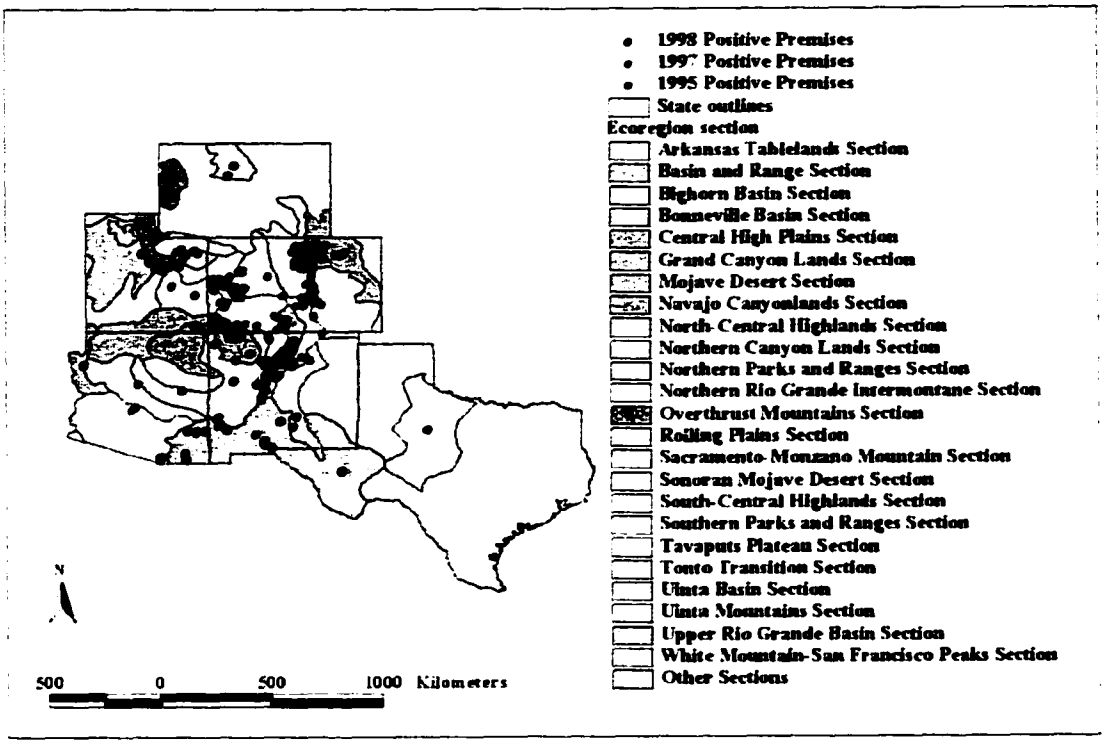

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Figure 8. Isodata classified Landsat 5 TM values from and compared with the location of VS positive premises. Inset shows class relationship and separability. VS positive premises are shown by black circles.

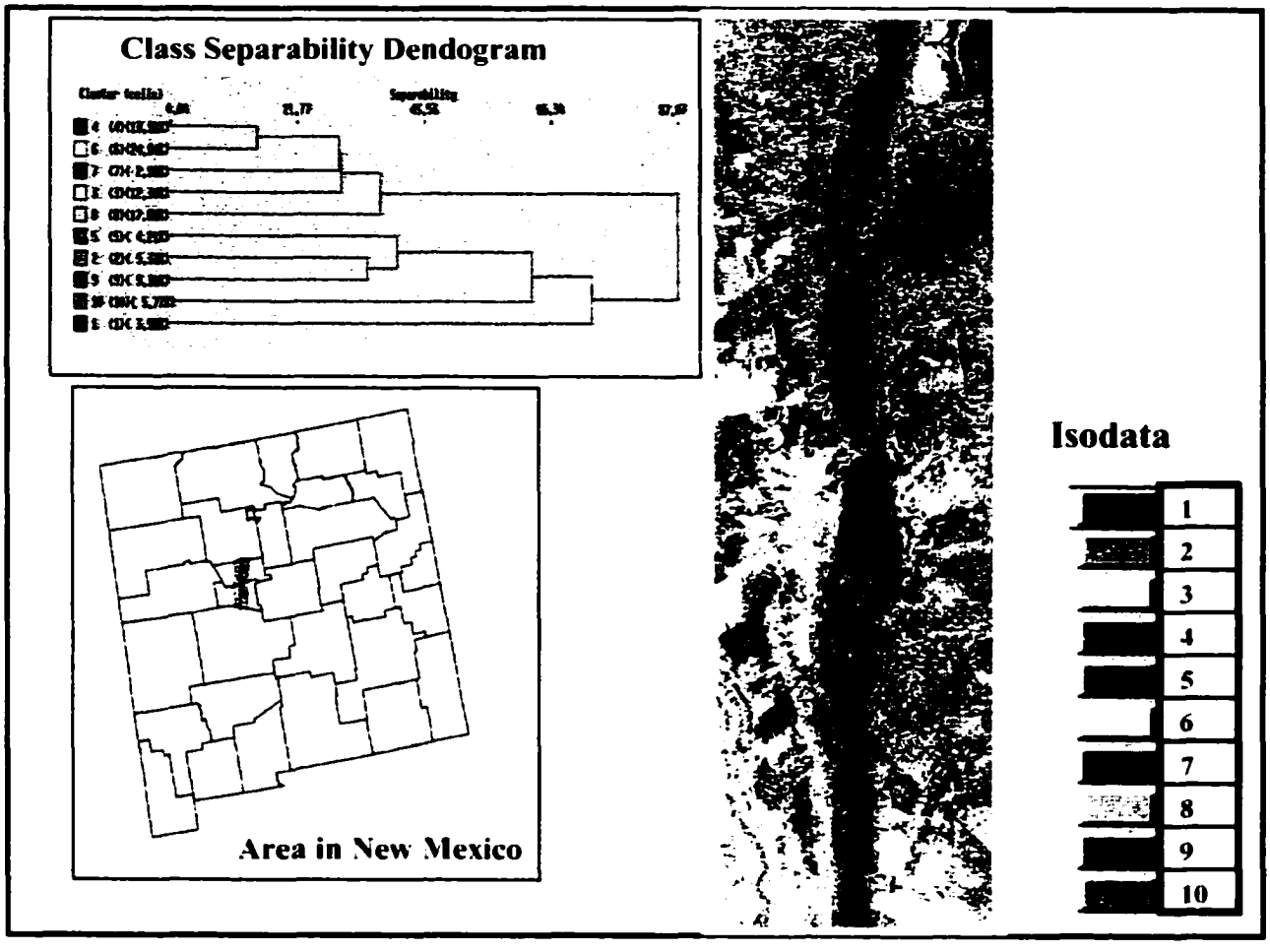

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Figure 9. Isodata classified Landsat 5 TM values from and compared with the location of VS positive premises. Inset shows class relationship and separability. VS positive premises are shown by black circles.

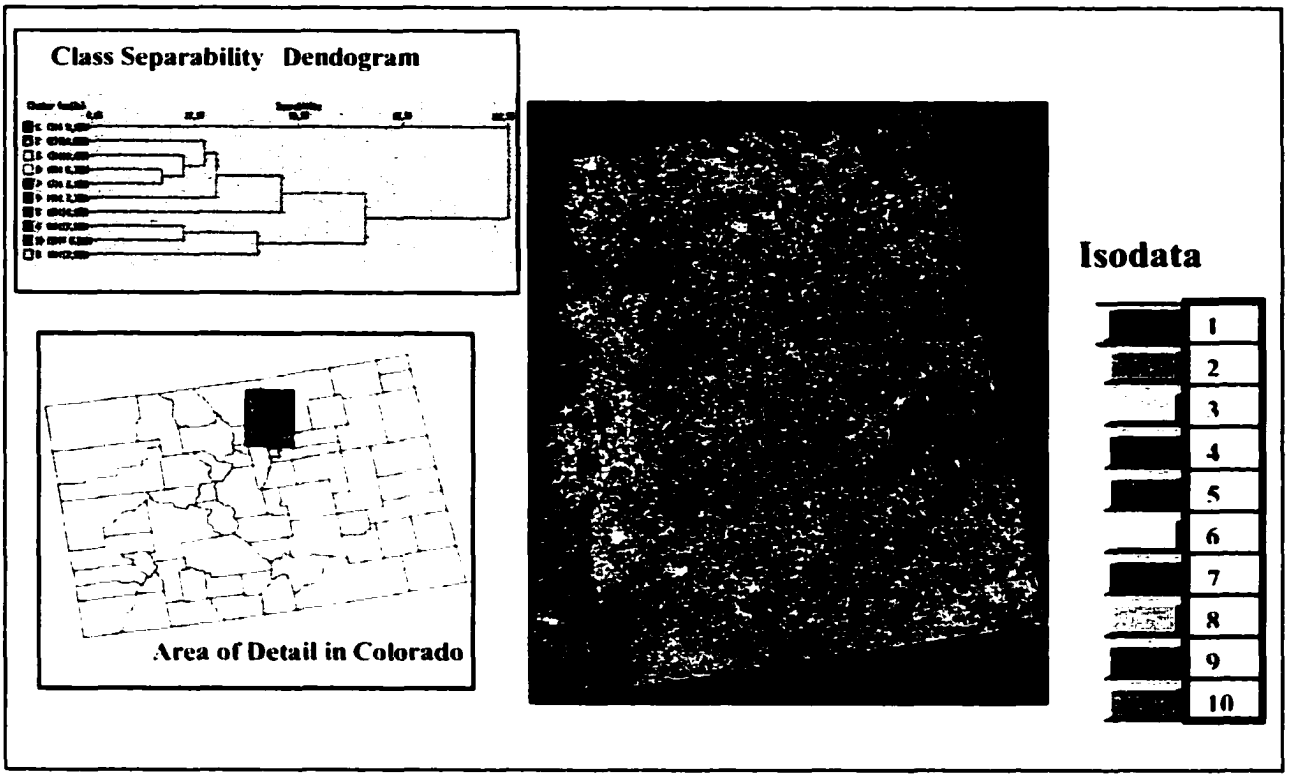

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Figure 10. Thirty-year total average precipitation from 1961-1990 of VS positive premises within central New Mexico and north central Colorado. Precipitation values are plotted by total average monthly values.

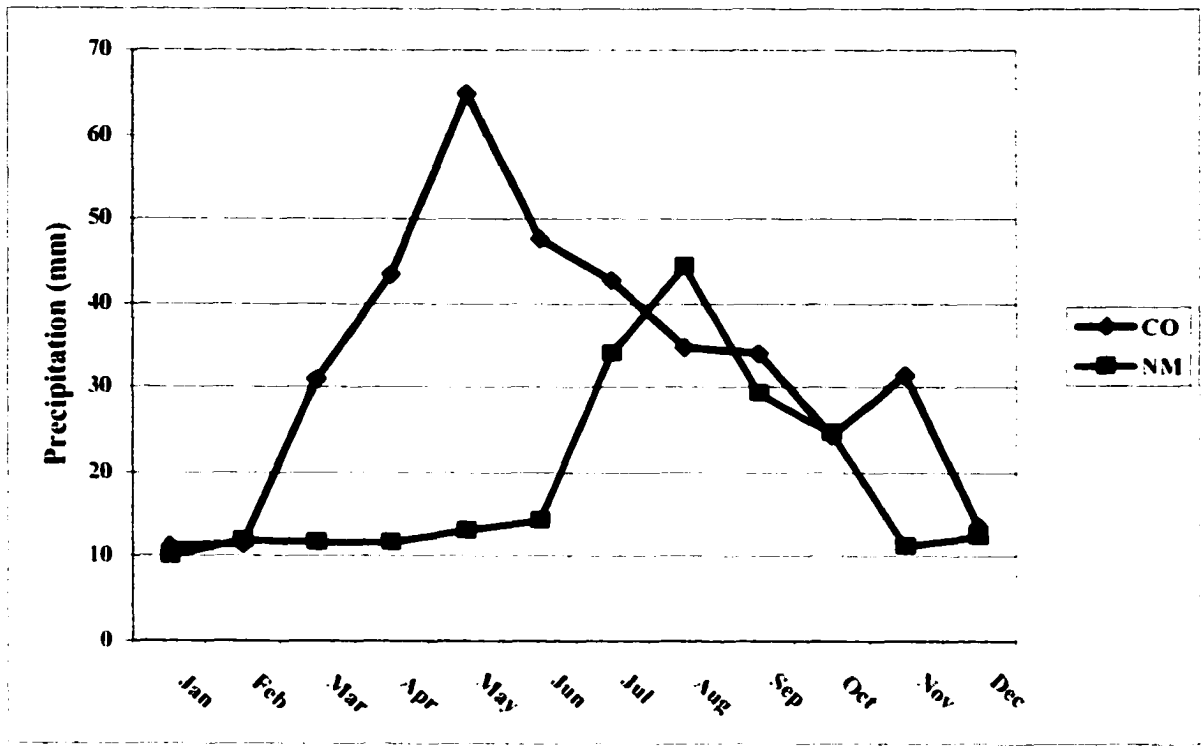

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Figure 11a. Average precipitation in central New Mexico from 1994 to 1999 for each month. Thirty-year average from 1961 to 1990 for each month are shown by the red boxes.

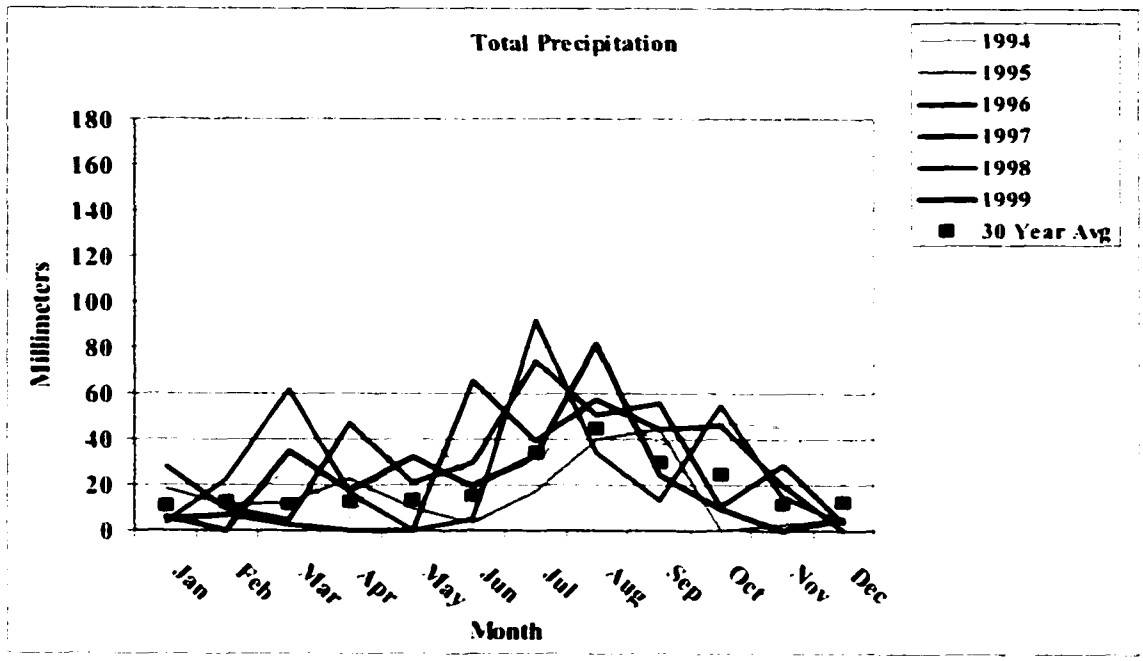

Figure 11b. Average precipitation in north central Colorado from 1994 to 1999 for each month. Thirty-year average from 1961 to 1990 for each month are shown by the red boxes.

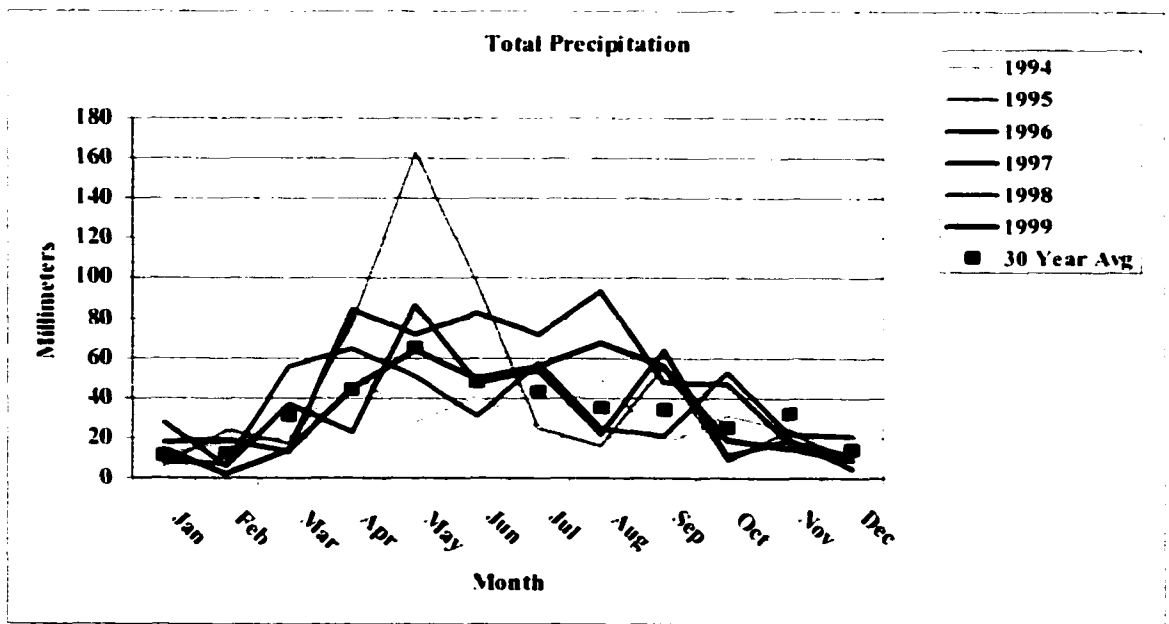

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Figure 12. Divergence of mean precipitation from 1994 to 1999 from the 30-year average precipitation with number of VS positive premises investigated during each month within clusters in central New Mexico and north central Colorado.

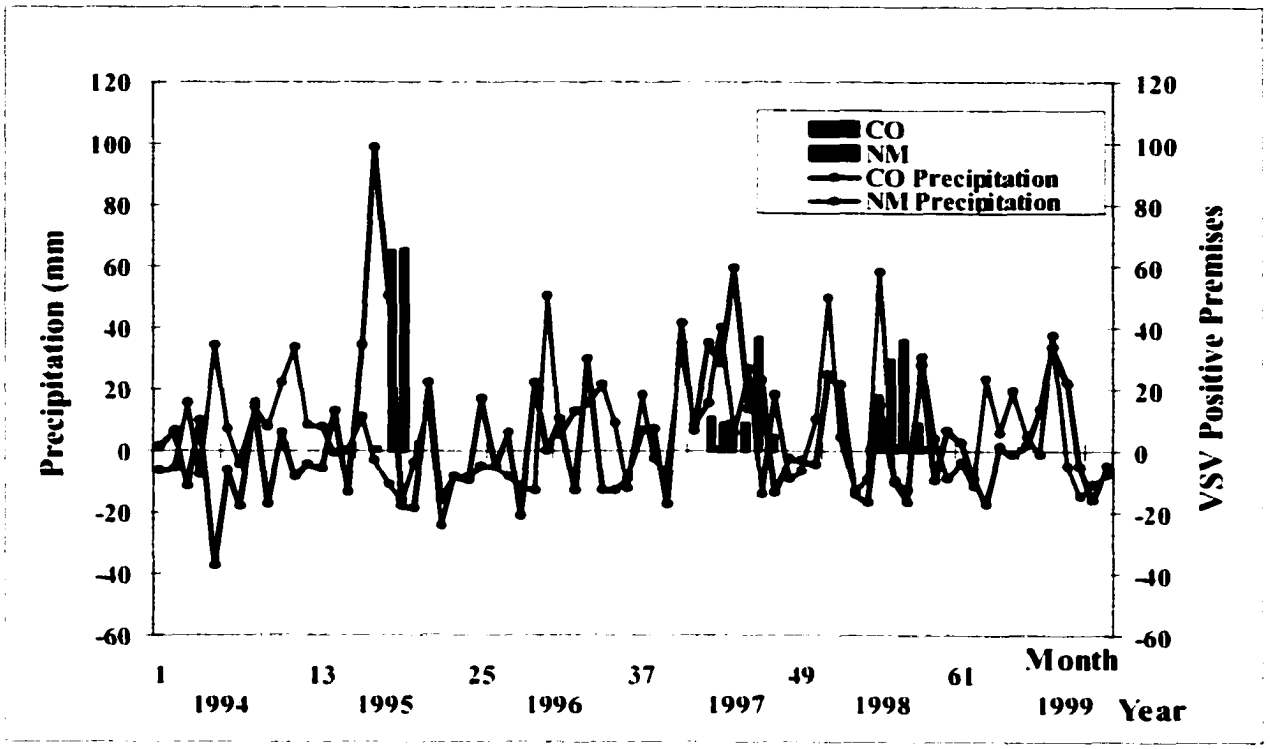

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Figure 13a. Minimum temperature in north central Colorado from 1994 to 1999 for each month. Thirty-year average from 1961 to 1990 are shown by the red boxes.

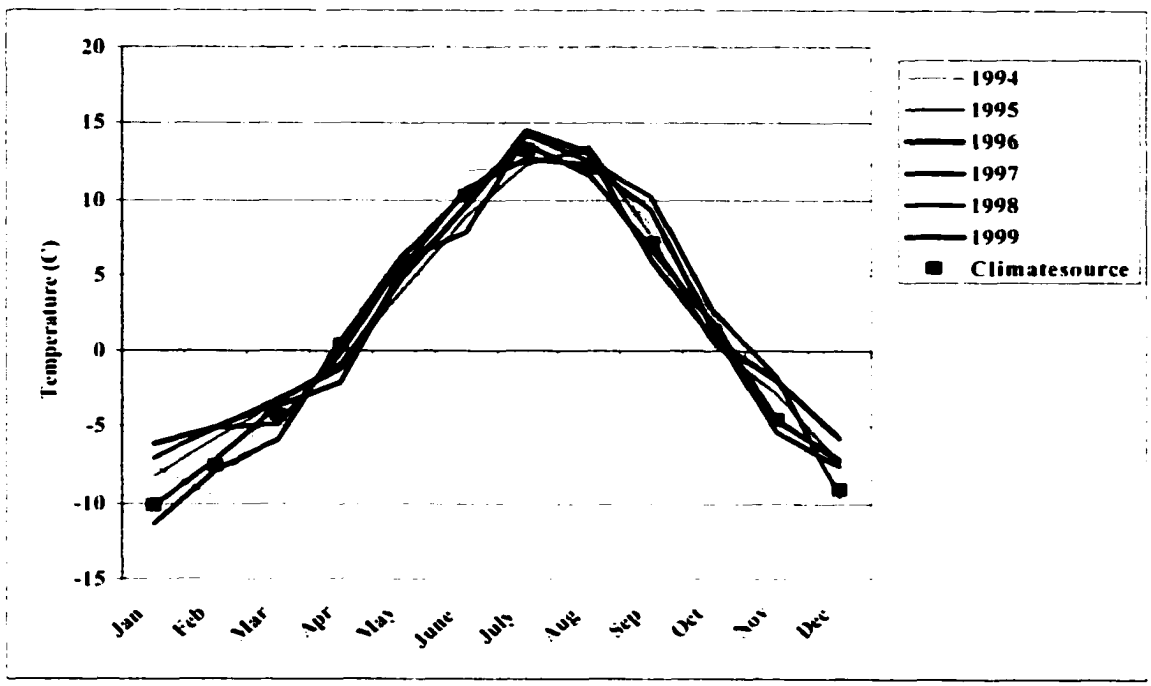
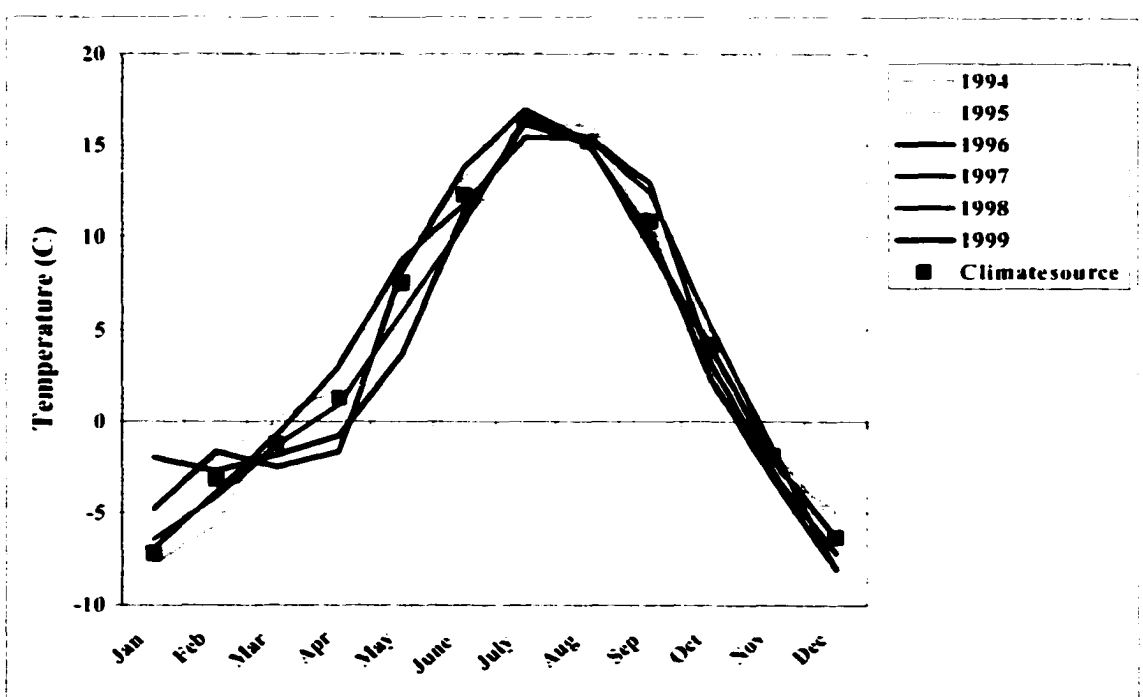


Figure 13b. Minimum temperature in central New Mexico from 1994 to 1999 for each month. Thirty-year average from 1961 to 1990 are shown by the red boxes.



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3.7 References

1. Aguirre AA, McLean RG, Cook RS, Quan TJ (1992) Serological Survey for Selected Arboviruses and Other Potential Pathogens in Wildlife from Mexico. Journal of Wildlife Diseases 28, 435-442

2. Bailey RG (1995) Description of the Ecoregions of the United States. USDA Forest Service, Washington, DC

3. Bridges VE, McCluskey BJ, Salman MD, Scott HH (1997) Review of the 1995 Vesicular Stomatitis Outbreak in the Western United States. Journal of the American Veterinary Medical Association 211:556-560

4. Comer JA, Kavanaugh DM, Stallknecht DE, Corn JL (1994) Population Dynamics of *Lutzomyia shannoni* (Diptera:Psychodidae) on Relation to the Epizootiology of Vesicular Stomatitis Virus on Ossabaw Island, Georgia. Journal of Medical Entomology 31:850-854

5. Corn JL, Comer JA, Erickson GA, Nettles VF (1990) Isolation of Vesicular Stomatitis Virus New Jersey Serotype From Phlebotomine Sand Flies in Georgia. American Journal of Tropical Medicine & Hygiene 42:476-482

6. Cupp EW (1996) Black flies and the agents they transmit. In: Beaty BJ, Marquardt WC (eds) The Biology of Disease Vectors. University Press of Colorado, Niwot, pp 98-109

7. Donahue RL, Follet RH, Tullloch RW (1990) Our soils and their management: increasing production through environmental soil and water conservation and fertility management. Interstate Publishers, Inc., Danville

8. Engelthaler DM, Mosley DG, Cheek JE, Komatsu KK, Ettestad P, Davis T, Tanda DT, Miller L, Frampton JW, Porter R, Bryan RT (1999) Climatic and Environmental Patterns Associated with Hantavirus Pulmonary Syndrome, Four Corners Region, United States. Emerging Infectious Diseases 5:87-94

9. Jensen JR (1996) Introductory Digital Image Processing: A Remote Sensing Perspective. Prentice Hall, Upper Saddle River

10. Jimenez AE, Jimenez C, Castro L, Rodriguez LL (1996) Serological Survey of Small Mammals in a Vesicular Stomatitis Virus Enzootic Area. Journal of Wildlife Diseases 32:274-279

11. Knight AP, Messer NT (1983) Vesicular Stomatitis. Compend Continuing Education of Practicing Veterinarian 5:517-522

12. Kulldorf M, Nagarwalla N (1995) Spatial Disease Clusters: Detection and Inference. In: Statistics in Medicine. pp 799-810

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13. Letchworth GJ, Rodriguez LL, Del C.Barrera J (1999) Vesicular Stomatitis. The Veterinary Journal 157:239-260

14. McCluskey BJ, Hurd HS, Mumford EL (1999) Review of the 1997 Outbreak of Vesicular Stomatitis in the Western United States. Journal of the American Veterinary Medical Association 215:1259-1262

15. Mead DG, Mare CJ, Ramberg FB (1999) Bite Transmission of Vesicular Stomatitis Virus (New Jersey serotype) to Laboratory Mice by *Simulium vittatum* (Diptera: Simuliidae). Journal of Medical Entomology 36:410-413

16. Parmenter RR, Yadav EP, Parmenter CA, Ettestad P, Gage KL (1999) Incidence of Plague Associated with Increased Winter-Spring Precipitation in New Mexico. American Journal of Tropical Medicine & Hygiene 61:814-821

17. Rodriguez LL, Bunch TA, Fraire M, Llewellyn ZN (2000) Re-emergence of Vesicular Stomatitis in the Western United States in Associated with Distinct Viral Genetic Lineages. Virology 271, 171-181

18. Saulmon EE (1968) The Epidemiology of Vesicular Stomatitis Virus in the United States. Bulletin of the Office of International Epizoology 70:49-63

19. Sellers RF, Maarouf AR (1990) Trajectory Analysis of Winds and Vesicular Stomatitis in North America, 1982-5. Epidemiology of Infectious Diseases 104:313-328

20. Siegel S, Castellan Jr NJ (1998) Non-parametric Statistics for the Behavioral Sciences.

21. Stallknecht DE, Nettles VF, Fletcher WO, Erickson GA (1985) Enzootic Vesicular Stomatitis New Jersey Type in an Insular Feral Swine Population. American Journal of Epidemiology 122:876-883

22. Tesh RB, Peralta PH, Johnson KM (1969) Ecological Studies of Vesicular Stomatitis Virus. I. Prevalence of Infection Among Animals and Humans Living in an Area of Endemic VSV Activity. American Journal of Epidemiology 90:255-266

23. Walton TE, Webb PA, Kramer WL, Smith GC, Davis T, Holbrook FR, Moore CG, Schiefer TJ, Jones RH, Janney GC (1987) Epizootic Vesicular Stomatitis in Colorado, 1982: Epidemiologic and Entomologic Studies. American Journal of Tropical Medicine & Hygiene 36:166-176

24. Webb PA, Monath TP, Reif JS, Smith GC, Kemp GE, Lazuick JS, Walton TE. (1987) Epizootic Vesicular Stomatitis in Colorado, 1982: Epidemiologic Studies Along the Northern Colorado Front Range. American Journal of Tropical Medicine & Hygiene 36:183-188

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25. Webb PA, Holbrook FR (1988) Vesicular Stomatitis. In: Monath TP (ed) The Arboviruses: Epidemiology and Ecology. CRC Press, Inc., Boca Raton, pp 1-29

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FINAL CONCLUSIONS AND SYNOPSIS

Vesicular stomatitis (VS) is a disease of horses, pigs, and cattle that is clinically undistinguishable from foot and mouth disease that causes considerable economic loss during outbreaks in the United States. Cases of vesicular stomatitis virus (VSV) occur in the southwestern United States approximately every ten years and the complete epidemiological relationship among agent, host, environment and time remain unknown. This dissertation was a multi-phase approach to understanding specific essential components on the epidemiology of the disease that are unsolved. Both molecular and spatial analytical techniques were used in the study's approach.

The phylogenetic relationship of viruses circulating in the southwestern United States in 1995 to viruses from 1984 to 1997 in Mexico showed a correlation between position in the phylogenetic tree and their genetic origin. Viruses from the western United States and Mexico were not closely related to viruses in the eastern United States and formed a separate lineage. Sequence analysis indicated relatively homogenous and distinct viral lineages for each documented outbreak in the western United States, with viruses from Mexico in each lineage of the major United States outbreaks from 1982/83 to 1995, suggesting a common source for these viruses. The phylogenetic analysis of VSV from previous outbreaks in the western United States indicates that the spatial, temporal, and genetic patterns are not consistent with an endemic cycle. Viruses from

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Central America formed a diverse collection of lineages, with a marked correlation between position in the phylogenetic tree and their geographical origin.

Comparison of growth rate and rate of evolution of three genetically distinct VSV-NJ viruses from different ecological regions and different hosts in a natural (sand fly) versus non-natural (mosquito and mammalian) host cells demonstrated that a virus originating from sand flies grew to higher titers in insect cells than did viruses of mammalian origin; indicating selective adaptation. Sequence analyses of the viruses after 0, 10 or up to 25 passages in each cell line showed no changes in hypervariable region of the phosphoprotein (P) gene or the intergenic junction between the glycoprotein (G) and the polymerase (L), suggests that the conditions in cell culture were not exerting a strong selective pressure on these regions. There was however, a low rate of evolution with non-synonymous mutations in the G gene. The G protein is the main membrane protein located on the surface of the virion that contains the antireceptor involved in attachment and membrane fusion with the cell surface receptors that mediate viral entry and exit from the host cell. Mutations in this protein could be a selective factor on VSV evolution *in vitro*. Persistent infections of VSV in sand fly and mosquito cells lines were maintained in culture for up to 81 days with sustained virus yields only in sand fly cells. Differences in viral replication and intracellular forces within the two different cell lines might be reasons for this effect. Sand flies are known vectors of VSV, whereas mosquitoes are not believed to play a role in the natural cycle.

Spatial-temporal clustering of VS premises from the 1995, 1997, and 1998 VS outbreaks identified clusters of up to twice the risk for VSV in Arizona, Colorado, New Mexico and Utah. Further analyses of VS premises in Colorado and New Mexico

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indicated clusters in the western and southwestern Colorado and central New Mexico along the Rio Grande river in 1995 and in north central Colorado in 1998, which are regions that have previously been documented with VS cases. VS positive premises were located within dry ecological regions with a close proximity to hydrological features in this area. Clustering of cases located near major water sources and surrounding vegetation could be associated with nearby vector habitat. Meteorological data from 1994 to 1999 showed sufficient rainfall to support development of aquatic stages of insect growth, with more than average precipitation from 1994 to 1999.

The lack of viruses from Mexico and partial sequencing of one gene is a limitation to the complete understanding of natural evolution of VSV in Mexico and how it relates to outbreaks in the western United States. The use of an *in vitro* system for understanding viral growth and evolution is a mere simulation of the natural host and does not take into account the complexity of viral dose, viral quasispecies, viral replication in multiple cell types, and host immunity. Limitations also occur with selective datasets for spatial analysis, for example; the lack of suitable controls and USDA case investigations makes it difficult for complete measurement of risk, but are helpful in generating new theories. Each study contains limitations, however we hope that these analyses will be good models for hypotheses generating and future research to understand the complex cycle of virus, host, environment, and time.

Genetic analysis and epidemiological data of natural and laboratory strains of VSV has aided in the knowledge of viral change and evolution in nature. Additional research on the environmental and climatic conditions associated with VS outbreaks has allowed for a better understanding of the natural cycle of VSV and the interactions

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among environment, host, and agent. This relationship among vector, ecology, and virus will aid in future disease surveillance, control, and direction for epidemiological research. The control of outbreaks of VS in the United States would enhance both the competitiveness of the American livestock industry globally and reduce the risk to independent producers.

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