THE DISAPPEARANCE OF AN ENDEMIC FOCUS OF VESICULAR STOMATITIS VIRUS, OSSABAW ISLAND, GEORGIA

by

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(Under the Direction of David Stallknecht)

ABSTRACT

Ossabaw Island, Georgia is the only known endemic focus of Vesicular Stomatitis Virus, New Jersey (VSNJV) in the United States. Decades of data indicate that VSNJV activity on the island may be declining. To investigate this decline, samples collected from 1291 white-tailed deer (*Odocoileus virginianus*) and 593 feral pigs (*Sus scrofa*) from 1990-2007, were tested for exposure to VSNJV. Results showed that all animals were negative for VSNJV antibodies from 2005 to the present. Attempts to isolate virus from the biological vector, sand flies (*Lutzomyia shannoni*), yielded no VSNJV isolates. Analysis of 56 blood fed sand flies showed a decrease in bloodmeals taken from feral swine from 16% to 3%, while bloodmeals taken from deer remained stable (81% and 86%) when compared to previous estimates. Evidence points to a drop in the feral pig population, a potential amplifying host, as the most likely cause of the disappearance of this endemic focus.

INDEX WORDS: vesicular stomatitis virus, feral swine, white-tailed deer, *Lutzomyia shannoni*, Ossabaw Island

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CHAPTER 1

INTRODUCTION

Purpose of Study

Vesicular stomatitis New Jersey virus (VSNJV) is endemic on Ossabaw Island, a barrier island off the coast of Georgia. Although wildlife, domestic livestock and arthropod species are diverse, Ossabaw is essentially a closed system that allows researchers an "outside laboratory" in which to conduct systematic epidemiological investigations with limited variables.

Previous surveillance for VSNJV on Ossabaw Island revealed the presence of antibodies in several wild and domestic animals [1-8], and identified a sand fly, *Lutzomyia shannoni,* as the vector [9]. Surveillance of wildlife demonstrated that white-tailed deer (*Odocoileus virginianus*) (83%) and feral swine (*Sus scrofa*) (60%) had the highest prevalence of VSNJV neutralizing antibodies [1]. Clinical disease in the form of vesicular lesions was detected in only a small proportion of sampled pigs [4, 9], and viremia has not been detected in any naturally infected wild or domestic animal to date [10-21]. The natural vertebrate reservoir of VSNJV on Ossabaw, if one exists, remains unknown. Feral swine [11] and white-tailed deer [12] were initially ruled out as amplifying hosts due to a lack of viremia. Despite these findings, feral swine are still thought to play a major role in the persistence of the virus in this endemic focus. Feral pigs are well established in areas where VSNJV antibodies have been detected in other species [2, 6, 22]. Swine from Ossabaw Island, and other parts of the southeast, have high prevalence

rates of VSNJV neutralizing antibodies [2-4, 6, 22], and are the only known wildlife species to develop clinical disease [4, 6]. In this capacity, feral swine are potentially the only source of VSNJV on Ossabaw outside the vector population. Their high reproductive rates ensure a large susceptible population of juveniles every year [23], and seroconversions in this naïve population coincide with the emergence of the vector, *L. shannoni* [7, 24]. Contact transmission from infected to uninfected pigs, and vector transmission of VSNJV by infected insects to swine have been demonstrated as has the ability of insects to acquire the virus by cofeeding on a non-viremic pig [25, 26]. Recent studies involving laboratory transmission of VSV by insect vectors in the absence of host viremia indicate that the traditional definitions for arboviruses may not apply and that feral swine should not yet be ruled out as amplifying hosts [26, 27].

Recent serologic data indicates that VSNJV neutralizing antibodies may be decreasing in the white-tailed deer population [2, 3, 5, 8]. Antibodies to VSNJV have dropped from 60% and 62% in 1956 [1] and 1983 [5], respectively, to 23% in 1989 [8]. Whether this decline is an artifact of sampling bias or whether it is due to an actual decrease in transmission is unknown. Age and location are known factors affecting prevalence of VSNJV neutralizing antibodies in both white-tailed deer [5, 8] and feral swine on the island [4, 7], and this apparent decrease could be a reflection of these biases in the animals sampled. A decline in antibody prevalence in white-tailed deer due to changing transmission patterns could also be related to population changes in the biological vector, *Lutzomyia shannoni*. Possibilities include a decline in VSNJV prevalence in the vector population, decrease in the number of sand flies on the island, or changes in host feeding preferences.

Alternatively, changes in the feral swine population could be responsible for this phenomenon. Recently there has been a significant decrease in numbers of feral swine on the

island [personal communication, Georgia Department of Natural Resources (GADNR)]. Specifically, ecological management practices begun in 1990 have led to the removal of over 14,000 pigs. If feral swine are in fact amplifying hosts, this reduction of the population could result in a disruption of the transmission cycle.

Objectives

The goal of this study is to investigate the apparent decrease in the prevalence of VSNJV antibodies in white-tailed deer. Our central hypothesis is that this decline in white-tailed deer is a direct consequence of a break in the VSNJV transmission cycle due to a documented decrease in numbers of the feral swine population, a suspected amplifying host and the only species on the island known to develop clinical disease. We will investigate our central hypothesis by completing the following objectives. First, we will determine the status of VSNJV antibody prevalence in white-tailed deer and validate any trends by comparing data with VSNJV prevalence estimates in feral swine. To eliminate the potential for sampling bias in the data, age of the animals sampled and location of animals when they were sampled will be taken into account. To further our understanding of these trends, we will attempt isolation of VSNJV from field caught sand flies and perform analyses on blood fed females to identify the preferred bloodmeal sources. These data will then be compared to rates of virus isolation and host preferences observed in previous studies on the island. To ensure that the environment on Ossabaw is still capable of supporting the vector population, we will also conduct an analysis of

sand fly habitat through quantification and characterization of tree holes, the diurnal resting shelters and nesting sites of *L. shannoni*.

Significance

A comprehensive evaluation of the apparent trends in recent data from Ossabaw Island will provide additional information regarding the maintenance and transmission of an arbovirus, like VSNJV, that does not appear to require host viremia for vector acquisition. The role of wildlife in the epidemiology of vesicular stomatitis is still not completely understood, and this study will help to determine the extent of the roles played by white-tailed deer and feral swine in this maintenance cycle. The determination of which factors have led to this apparent decline in viral activity will help to further our understanding of what pieces are required to maintain VSNJV in an endemic focus.

References

1. Karstad, L.H., et al., *Evidence for the role of wildlife in epizootics of vesicular stomatitis.* J Am Vet Med Assoc, 1956. **129**(3): p. 95-6.
2. Jenney, E.W., F.A. Hayes, and C.L. Brown, *Survey for vesicular stomatitis virus neutralizing antibodies in serums of white-tailed deer Odocoileus virginianus of the southeastern United States.* J Wildl Dis, 1970. **6**(4): p. 488-93.
3. Fletcher, W.O., D.E. Stallknecht, and E.W. Jenney, *Serologic surveillance for vesicular stomatitis virus on Ossabaw Island, Georgia.* J Wildl Dis, 1985. **21**(2): p. 100-4.
4. Stallknecht, D.E., et al., *Enzootic vesicular stomatitis New Jersey type in an insular feral swine population.* Am J Epidemiol, 1985. **122**(5): p. 876-83.
5. Stallknecht, D.E. and G.A. Erickson, *Antibodies to vesicular stomatitis New Jersey type virus in a population of white-tailed deer.* J Wildl Dis, 1986. **22**(2): p. 250-4.
6. Stallknecht, D.E., et al., *Antibodies to vesicular stomatitis virus in populations of feral swine in the United States.* J Wildl Dis, 1986. **22**(3): p. 320-5.
7. Stallknecht, D.E., et al., *Antibodies to vesicular stomatitis New Jersey type virus in wild and domestic sentinel swine.* Am J Epidemiol, 1987. **125**(6): p. 1058-65.
8. Fletcher, W.O., et al., *Antibodies to vesicular stomatitis New Jersey type virus in white-tailed deer on Ossabaw Island, Georgia, 1985 to 1989.* J Wildl Dis, 1991. **27**(4): p. 675-80.
9. Stallknecht, D.E., et al., *Feral swine as a potential amplifying host for vesicular stomatitis virus New Jersey serotype on Ossabaw Island, Georgia.* J Wildl Dis, 1993. **29**(3): p. 377-83.
10. Clarke, G.R., D.E. Stallknecht, and E.W. Howerth, *Experimental infection of swine with a sandfly (Lutzomyia shannoni) isolate of vesicular stomatitis virus, New Jersey serotype.* J Vet Diagn Invest, 1996. **8**(1): p. 105-8.
11. Comer, J.A., D.E. Stallknecht, and V.F. Nettles, *Incompetence of domestic pigs as amplifying hosts of vesicular stomatitis virus for Lutzomyia shannoni (Diptera: Psychodidae).* J Med Entomol, 1995. **32**(5): p. 741-4.
12. Comer, J.A., D.E. Stallknecht, and V.F. Nettles, *Incompetence of white-tailed deer as amplifying hosts of vesicular stomatitis virus for Lutzomyia shannoni (Diptera: Psychodidae).* J Med Entomol, 1995. **32**(5): p. 738-40.
13. Cornish, T.E., et al., *Pathogenesis of experimental vesicular stomatitis virus (New Jersey serotype) infection in the deer mouse (Peromyscus maniculatus).* Vet Pathol, 2001. **38**(4): p. 396-406.
14. Howerth, E.W., et al., *Experimental vesicular stomatitis virus infection in horses: effect of route of inoculation and virus serotype.* Vet Pathol, 2006. **43**(6): p. 943-55.
15. Howerth, E.W., et al., *Experimental vesicular stomatitis in swine: effects of route of inoculation and steroid treatment.* J Vet Diagn Invest, 1997. **9**(2): p. 136-42.
16. Mead, D.G., C.J. Mare, and F.B. Ramberg, *Bite transmission of vesicular stomatitis virus (New Jersey serotype) to laboratory mice by Simulium vittatum (Diptera: Simuliidae).* J Med Entomol, 1999. **36**(4): p. 410-3.
17. Perez De Leon, A.A., D. O'Toole, and W.J. Tabachnick, *Infection of guinea pigs with vesicular stomatitis New Jersey virus Transmitted by Culicoides sonorensis (Diptera: Ceratopogonidae).* J Med Entomol, 2006. **43**(3): p. 568-73.

18. Perez de Leon, A.A. and W.J. Tabachnick, *Transmission of vesicular stomatitis New Jersey virus to cattle by the biting midge Culicoides sonorensis (Diptera: Ceratopogonidae).* J Med Entomol, 2006. **43**(2): p. 323-9.
19. Redelman, D., et al., *Experimental vesicular stomatitis virus infection of swine: extent of infection and immunological response.* Vet Immunol Immunopathol, 1989. **20**(4): p. 345-61.
20. Schnitzlein, W.M. and M.E. Reichmann, *Characterization of New Jersey vesicular stomatitis virus isolates from horses and black flies during the 1982 outbreak in Colorado.* Virology, 1985. **142**(2): p. 426-31.
21. Stallknecht, D.E., et al., *Effect of strain and serotype of vesicular stomatitis virus on viral shedding, vesicular lesion development, and contact transmission in pigs.* Am J Vet Res, 2004. **65**(9): p. 1233-9.
22. Hanson, R.P., *The natural history of vesicular stomatitis.* Bacteriol Rev, 1952. **16**(3): p. 179-204.
23. Taylor, R.B., et al., *Reproduction of feral pigs in southern Texas.* Journal of Mammalogy, 1998. **79**(4): p. 1325-1331.
24. Comer, J.A., et al., *Population dynamics of Lutzomyia shannoni (Diptera: Psychodidae) in relation to the epizootiology of vesicular stomatitis virus on Ossabaw Island, Georgia.* J Med Entomol, 1994. **31**(6): p. 850-4.
25. Mead, D.G., et al., *Biological transmission of vesicular stomatitis virus (New Jersey serotype) by Simulium vittatum (Diptera: Simuliidae) to domestic swine (Sus scrofa).* J Med Entomol, 2004. **41**(1): p. 78-82.
26. Mead, D.G., et al., *Black fly involvement in the epidemic transmission of vesicular stomatitis New Jersey virus (Rhabdoviridae: Vesiculovirus).* Vector Borne Zoonotic Dis, 2004. **4**(4): p. 351-9.
27. Mead, D.G., et al., *Transmission of vesicular stomatitis virus from infected to noninfected black flies co-feeding on nonviremic deer mice.* Science, 2000. **287**(5452): p. 485-7.

CHAPTER 2

LITERATURE REVIEW

Causative Agent

Vesicular stomatitis is caused by related but distinct viruses in the Genus Vesiculovirus, Family Rhabdoviridae [1]. They have a single-stranded, negative sense RNA genome. The VSV genome codes for five proteins. The glycoprotein is responsible for cell binding and the release of the viral genome into the cytoplasm, and is also used to differentiate between serotypes. The large polymerase, nucleoprotein and the phosphoprotein are responsible for transcription and regulation, and the matrix protein plays roles in evading the host cell defenses, viral assembly, and budding. VSV binds to phosphotidylserine which is expressed on the surface of a wide range of cell types [2].

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History of VS in the United States

Circumstantial reports of VS in the United States date back to the 1800's when it was thought to have caused the lameness of thousands of Union horses during the Civil War [1]. It is suspected that possible VS outbreaks in South Africa around the same time occurred as a result of transport of the virus from the US in an unknown reservoir. In 1904, Mohler described

"mycotic stomatitis" of cattle with clinical signs identical to those of VS. The disease emerged during the summer and disappeared in the winter leading Mohler to believe the causative agent was a fungus dependant on certain climactic conditions. The first confirmed case did not occur until an outbreak in1916 [1, 2]. The infection started in horses at stockyards in Denver, Colorado, and eventually spread as far eastward as Virginia affecting both cattle and horses. The movement of remount horses through infected stockyards may account for the magnitude of this outbreak.

In 1925, a VS outbreak occurred in cattle in Richmond, Indiana. The cows appeared healthy during their transport from Kansas City, Missouri but developed oral lesions after delivery to individual farms [3]. The agent was isolated for the first time from horses that had become infected during the course of the 1925 outbreak and was maintained in laboratory animals for further study. The following year the agent was isolated during an epidemic involving cattle and horses in New Jersey [3]. Cotton reported that the viruses associated with the outbreaks in Indiana and New Jersey were antigenically distinct serotypes of the virus that were subsequently named vesicular stomatitis virus serotypes Indiana and New Jersey [4]. VS appeared in the upper mid-west and West Virginia late in the summer of 1937, but disappeared after the first frost. Infected animals were limited to wooded areas near water. It is not known which serotype was responsible since virus was not isolated. Vesicular stomatitis was again associated with military operations when an outbreak occurred in army horses and mules in Texas and Louisiana. The disease spread to other regions of Texas and then to Kansas by the return of horses to these posts [3].

Vesicular stomatitis was initially thought to be a disease only of horses and cattle, but in 1943 simultaneous outbreaks in swine the United States and Venezuela changed this perception

[5]. An outbreak in Missouri occurred in a hog cholera serum plant where half of the swine were affected and VSNJV was isolated from infected pigs [3, 5, 6]. It is not known how the virus was introduced into the facility, but investigations revealed that the disease was spread via inoculation with vaccine and contact between infected and uninfected animals.

In 1963, herds infected with the New Jersey serotype were found in South Carolina, Georgia, Florida, Alabama, Mississippi, Texas and Oklahoma, and although no large epidemic was reported, viral activity continued to be widespread in these areas in 1964 [7]. Also in 1964, for the first time in six years, the Indiana serotype was the cause of outbreaks in Texas, Oklahoma, Arkansas, Missouri and Colorado [7]. The following year several human cases were documented among workers who handled infected cattle in New Mexico and Colorado, which spurred an investigation by the State Health Department during which VSIV was isolated from *Aedes* mosquitoes [7]. During this same time period investigators at the National Animal Disease Laboratory were able to isolate the New Jersey serotype from eye gnats [7]. A subsequent lapse in reports of viral activity occurred from 1967 to 1972, except for a few scattered cases in cattle, horses and swine in North and South Carolina, Colorado, New Mexico and Louisiana [8].

Outbreaks of the New Jersey serotype have since occurred in 1982-3, 1995, 2004-2006 and most recently in 2009[9]. Outbreaks of the Indiana serotype occurred in 1942, 1956, and 1964-65. This serotype was not reported for over three decades until 1997 and 1998, and it has not been reported since [10]. Surveillance in the southeastern states throughout the late 70's showed no exposure to VSV among wild and domestic animals, with the exception of swine, cattle and white-tailed deer on Ossabaw Island, GA [11].

VS in Humans and Domestic Animals

Human Infection

The first suspected case of VS in humans occurred in 1917 in a researcher and his assistant through contact with infected animals during the course of an investigation of "stomatitis contagiosa", a disease now believed to be VS [12]. In 1945, Heiny described three suspected cases of VS in men who had come into contact with lesions on the teats of cattle [13]. The first confirmed cases of infection with VSNJV occurred in three laboratory workers at the University of Wisconsin [12]. Two workers were exposed through contact with experimentally infected animals, and the third was exposed by fluid spatter while handling infected material. Diagnosis of VS was based on serum antibodies which neutralized 10^4 LD_{50} doses of VSNJV.

Onset of human infection typically occurs one to two days after exposure but can occur after as many as six days. In humans, VS presents as a flu-like illness sometimes accompanied by conjunctivitis. Generalized muscle pain and chest pains have been documented as well as fever, malaise and, in some instances, herpes-like ulcers around the mouth and lesions in the oral mucosa [14]. Encephalitis has been documented in children[15]. Symptoms are usually mild, last for three to six days and do not result in complications or death. Occasionally patients seem to recover but symptoms return after one or two days [12, 16]. Antibodies to VSV have been found in up to 80% of humans living in endemic areas [16], yet a survey of veterinarians and lab workers in Colorado, an epidemic area, showed seroprevalence of only 12% despite the high risk for VSV exposure in their professions [17].

Animal Infection

Vesicular stomatitis in domestic livestock is characterized by vesicular lesions of the oral mucosa, snout, teats, and coronary bands of infected animals. Excessive salivation due to characteristic lesions in the mouth is usually the first observed, and sometimes only observed, symptom. Pain associated with these clinical signs eventually leads to anorexia and weight loss [18]. Foot lesions and subsequent lameness are seen most frequently in swine, but do occur in cattle and horses as well. Teat lesions and mastitis are most often observed in dairy herds and appear to be associated with milking processes.

The incubation period of VS in animals ranges from 2-8 days but lesions have been observed after as few as 24 hours in experimentally infected animals [18, 19]. Fever, if it occurs, appears in the early stages of infection. Virus can be isolated from vesicular fluid, throat swabs, tonsils, saliva and epithelium. Viremia is extremely rare and has only been reported a handful of times. Cotton reported virus in the blood of experimentally infected cattle and horses after inoculating the blood of infected animals into guinea pigs that subsequently developed disease [20]. Viremia was also reported in experimentally infected deer mice (*Peromyscus maniculatus*) for a period of three days post-inoculation [21]. A naturally occurring viremia has never been reported. Mortality due to VS is low, but morbidity rates during outbreaks in epidemic areas can be as high as 90%. Vesicular stomatitis is a self-limiting disease and animals typically recover in 2-3 weeks if there are no complications from secondary bacterial infections [3].

Laboratory studies show that the development of clinical signs in domestic pigs is dependent upon on the dose of VSNJV administered. Inoculation of the snout of domestic pigs with VSNJV produces seroconversions and subclinical infection at $10^{0.7}$ median tissue culture infective doses ($TCID_{50}$) and clinical disease with doses of $10^{4.5}$ $TCID_{50}$ or more. Virus can be

recovered from lesions, tissues and saliva of animals inoculated with high dosages of virus, including those that seroconvert but do not develop clinical disease [19, 22, 23]. During laboratory trials it was also determined that route of inoculation was vital to the development of clinical signs in both swine and horses [24]. Inoculation of horses and pigs via the oral mucosa, snout, and coronary bands results in the development of vesicular lesions in the majority of animals [19, 25]. In contrast, inoculation of haired skin results only in seroconversion and subclinical disease. Development of clinical signs can be aided by cuts or abrasions in the skin or mucosal lining, with clinical disease resulting from inoculation with as little as 10^2 $TCID_{50}$. These methods of inoculation correlate to the natural modes of transmission such as vectors feeding on or around the snout and/or mouth, or routine contact between animals sharing stalls or feed troughs.

Importance of VSV Infection in Livestock

Despite its low mortality rate in affected herds, VS outbreaks can cause substantial economic impact. Financial losses can be in the tens of thousands of dollars per affected herd stemming from decreased milk production in dairy cattle, the value of food required to regain optimum production weight in livestock, the costs of veterinary services, drugs, extra labor, and monetary losses associated with the culling of infected animals [26].

Vesicular stomatitis in cattle and pigs is also clinically indistinguishable from foot and mouth disease, a devastating viral disease of livestock that is not known to occur in the United States[27]. Due to this similarity, and other factors, VS is classified by the World Organisation for Animal Health (OIE) as a multispecies notifiable disease. Outbreaks of VS lead to

quarantines and export restrictions adding to the substantial losses already incurred by livestock owners [3, 26].

Infection in Wildlife

Antibodies to vesicular stomatitis virus have been found in a wide range of wildlife throughout North and South America however infection is, with few exceptions, subclinical. Circumstantial reports from hunters indicate that lesions were once observed in white-tailed deer, but naturally occurring clinical disease has only been documented in feral swine. While white-tailed deer and feral swine are the two predominate wildlife species with evidence of exposure, VSNJV antibodies have also been found in raccoons (*Procyon lotor*), mule deer (*O. hemionus*), pronghorn (*Antilocapra americana*), wild donkeys (*Equus asinus*), turkeys (*Meleagris gallopavo*), collared peccaries (*Tayassu tajacu*), goats (*Capra hircus*), opossums (*Didelphis virginianus*), bobcats (*Felis rufus*), coyotes (*Canis latrans*), big horn sheep (*Ovis canadensis*), elk (*Cervus elaphus*), foxes (*Vulpes volex, V. macrotis*), jack rabbits (*Lepus californicus)*, and a variety of rodent species [28-37].

Epizootiology

Epidemic Vesicular Stomatitis

Historically, VS epidemics occurred across the US. However, since the late 1970s epidemics have been limited to the western US [20, 33, 39, 48, 56-62] where VS is considered to be sporadically epidemic [15, 38]. Recent epidemics associated with New Jersey virus have

occurred in 1982-83 [38, 39], 1995[40], and 2004-2006 [1, 41]. Epidemics associated with the Indiana virus occurred in 1965 [37, 63, 64] and 1997-98 [42].

Vesicular stomatitis epidemics typically begin in the late spring and the disease is usually first identified in livestock in states that border Mexico [41, 43]. It has been suggested that outbreaks occur as a result of point introductions or spillovers from endemic regions of Central America given that recent isolates of VSNJV in the US have been linked to almost identical sequences circulating in endemic areas of Mexico [41]. A northward progression of disease during the summer months has been described, and the virus seems to follow natural features such as mountain valleys, river basins, or defined ecological zones such as open woodlands or savannas [44, 45]. Pastured livestock are more frequently affected by VS than livestock in stables, barns, or on dry feed lots [46, 47].

Outbreaks of VS occur sporadically and involve rapid spread of the disease through large numbers of animals [48]. Epidemics reportedly spread irregularly, without infecting all susceptible groups of animals [42, 48, 49], and are usually terminated by the onset of killing frosts [50]. The observation that VS spreads sporadically, affecting some herds and skipping others, may be more of a perception than a reality. Observances of seropositive herds during a VSNJV outbreak indicate that the incidence of clinical disease in affected herds is only around 10 percent [15]. Laboratory studies demonstrated that clinical VS is dependent upon inoculation site[19, 24]; inoculation of virus on the snout produces vesicular lesions while inoculation haired skin induces seroconversion and subclinical infection [19, 24]. Contact transmission between animals also produces subclinical infection [51, 52]. Clinical disease can easily go unnoticed, even when present, due to small size of lesions. Excessive salivation is frequently the only

observed evidence of lesions in the oral mucosa[13, 19]. This suggests that VS may be present long before the disease is detected in a herd, leading to delayed response and containment.

Outbreaks begin in the spring, and reports indicate that the virus radiates outward in concentric circles from the area where it first appears, oftentimes following river systems and waterways [43]. Reports of VS continue through September or October and taper off after the first frost. These observations led to the hypothesis that VSV was transmitted by an arthropod vector. Radeleff (1949) suggested that VSV was spread by an insect vector and suggested the stable fly, *Stomoxys calcitrans*, as the most probable candidate since these flies were found in great numbers in areas where disease occurred [13]. Hanson (1952) considered several probable vectors including; horse flies, deer flies, mosquitoes, and black flies[3]. Since then, VSNJV has been isolated from several species of insects during epidemics in the US [10, 38, 53, 54]. Isolation of VSV from non-hemataphagous insects such as eye gnats and house flies suggests the possibility for mechanical transmission [53-55].

The theory of vector transmission remained controversial despite the isolation of VSV from insects, due to the absence of a vertebrate host capable of producing a sustainable viremia, a qualification of the World Health Organization's definition of an arbovirus [22]. Laboratory studies have since demonstrated that black flies (*Simulium vittatum, Simulium notatum*) can become infected by feeding on or near lesions of hosts infected with VSNJV and that uninfected black flies can acquire VSNJV by cofeeding with infected black flies on an uninfected host. Infection rates of flies ranged from 16-18% in flies feeding on or near lesions and between 6-16% in cofeeding flies [56, 57].

During the 1982-83 outbreak, clinical cases were observed in animals after the first frosts and in the absence of vectors [15]. Laboratory studies and observations during epidemics have

shown that once VS was been introduced into a population, it is efficiently transmitted via contact between animals. As stated previously, contact transmission can be aided by cuts or abrasions on the snout or hooves [19, 24, 25, 51, 52, 58], and the demonstration of viral shedding in saliva indicates that feed troughs could also serve as a source of virus. [19]. These modes of transmission offer a mechanism by which VSNJV can overwinter and be maintained in between outbreaks.

Endemic Vesicular Stomatitis

Vesicular stomatitis is endemic in Central America (Mexico[35, 59], Costa Rica [36, 60-64] and Panama [65, 66] and on Ossabaw Island, Georgia in the United States [8, 11, 28, 33, 67, 68]. According to Hanson, there are three criteria that must be met for an area to be considered endemic for VS. Fifty percent or more of cattle and (domestic) swine, have VSV neutralizing antibodies, antibodies are found in wildlife, and VSV has been diagnosed either by serology or virus isolation for a period of several successive years [48]. Clinical cases of VS are even more rare than is seen in epidemic cycles, and tend to appear early in the summer. Seroprevalence in humans is widespread and has been recorded in 80-90 percent of the population [69-71]. Two enzootic areas have been studied in detail.

In Costa Rica, the prevalence of antibodies in cattle to the Indiana (type 1) and New Jersey serotypes are 21% and 46%, respectively [60], and 28.5% and 70.6%, respectively, in horses[61]. Vesicular stomatitis is diagnosed in livestock regularly at the end of the rainy season and transmission continues into the dry season. Older, lactating cows are at higher risk of disease than nonlactating old cows or calves [34, 60, 72]. Farm factors associated with an increased risk of VS include the presence of poultry and a long calving interval [72]. Variables

related to breeding sites for sand flies and black flies were found to be associated with occurrence of VS in livestock. In addition, risk of disease caused by the New Jersey serotype was found to be greater for cattle pastured in or near lower montane moist forests (500 to 1500 meters above sea-level) and tropical dry forests (< 2 meters of annual rainfall and from sea-level to 500 meters above sea-level) than for cattle pastured at other regions [60]. It has been suggested that these two ecological zones represent two separate transmission cycles, one associated with higher elevations and one associated with lower elevations that experience lower rainfall [60]. No environmental factors were found to be associated with the Indiana serotype and this suggests that independent transmission and maintenance cycles exist for these serotypes in Costa Rica [60].

The only known confirmed VSV enzootic area in the U.S. is on Ossabaw Island, Georgia [67, 68]. Ossabaw is approximately 26,000 acres consisting of 11,000 acres of upland habitat and 15,000 acres of salt marsh. The upland habitat is divided into three main forest types: pine stands, mixed hardwood areas, and the live oak/maritime forest. The maritime forest dominates the southern end of the island while the northern end is separated into pine stands to the west and mixed hardwoods to the east. Figure 1. [73].

These delineations are the result of agricultural practices that were in place until the end of the 1800's. Infertile soil on the southern end of the island prevented agricultural development, resulting in the expansion of old growth forest [73]. On the north and eastern portions of the islands, pine stands occur in the areas most recently cultivated yielding to older mixed hardwood forest. Agricultural practices are also responsible for the introduction of several non-native species, including feral swine, donkeys, horses and cows, all of which, with the exception of cattle, roam the island freely today.

The endemic focus on Ossabaw Island was discovered when antibodies to VSNJV were detected in 30% of cattle and 50% of feral swine several years after the last reported cases of VS in livestock [74]. Cattle, domestic swine, feral swine, horses, mule deer and raccoons that were tested during this time from other areas of Georgia and the southeastern states and all were negative for VSNJV antibodies. Since that time, vesicular stomatitis New Jersey virus neutralizing antibodies have been detected in feral swine white-tailed deer, raccoons, cattle, horses, feral donkeys and one goat [8, 33]. Several other species have been sampled including bats, rodents, dogs and birds but none have evidence of exposure [33].

Antibodies were detected annually in 23 to 62% of white-tailed deer from 1981 to 1989 [75, 76], and 26 to 32% of the feral swine on the island seroconvert each summer [77]. Age data obtained from hunter-killed white-tailed deer established a strong association between older animals and the presence of VSNJV serum neutralizing antibodies, and demonstrated that deer are exposed in an annual transmission cycle [75, 76]. Clinical VS has only been observed in feral swine, thirteen pigs on three separate occasions [67, 78], and VSNJV was isolated from swabs and tissues of the vesicular lesions [78]. The last documented occurrence of clinical VS was in 1990.

Sentinel studies with feral and domestic pigs indicate that antibodies to VSNJV appear earlier and with a higher incidence rate in feral swine from the live oak/maritime forest at the southern end of the island. Migration of VSNJV to the northern end of the island occurs later in the summer [67]. This geographic association correlates with established habitat of sand flies (*Lutzomyia shannoni*), the biological vector of VSNJV on the island [73, 77]. Attempts to isolate VSNJV from other insect species on the island were unsuccessful [77]. The seasonal emergence of sand flies in the spring corresponds with seroconversions in sentinel feral and domestic swine,

which begin in June and continue through September, and peaks in sand fly abundance mirror increased rates of seroconversions in pigs [77, 79, 80]. Virus isolations from wild caught male sand flies demonstrates transovarial transmission in these vectors [81].

Analysis of blood-fed female sand flies indicates that white-tailed deer and feral swine account for 97% of bloodmeals taken by the biological vector [82]. Also, the potential for proliferating VSV through contact transmission among feral swine in endemic regions has been demonstrated by laboratory studies [25, 51]. Animals exhibiting subclinical disease are capable of shedding virus, but contact transmission is lesion dependant, and due to the low rate of clinical infection and high rate of seroprevalence in endemic regions this may not result in long-term maintenance of the virus in natural cycles [25, 51]. Despite accumulating evidence, experimental studies suggest that wild swine [83] and white-tailed deer [84] are dead-end hosts for the virus and the natural reservoir remains unknown.

Summary

In summary, VS is a disease caused by several vesiculoviruses in the Rhabdovirus family. It affects livestock, wildlife and humans, and has an extensive history in the United States and other regions of the Americas. Clinical disease is rare but mimics the more notorious Foot and Mouth Disease. High morbidity rates can lead to devastating financial losses for livestock owners. Vesicular stomatitis virus is an arbovirus that relies on unconventional methods of acquisition and transmission that do not require the presence of a host viremia. Vesicular stomatitis can also be transmitted through contact, but it appears clinical disease is a

requisite. It occurs in both endemic and epidemic cycles, but questions remain as to how the two interact to perpetuate the disease, and why some regions are endemic and others are not.

Figure 2.1 Distribution of Forest Types on Ossabaw Island, GA [73].

References

1. *OIE Listed diseases*. 2009; Available from: http://www.oie.int/eng/maladies/fiches/a_A020.htm.
2. Lichty, B.D., et al., *Vesicular stomatitis virus: re-inventing the bullet.* Trends Mol Med, 2004. **10**(5): p. 210-6.
3. Hanson, R.P., *The natural history of vesicular stomatitis.* Bacteriol Rev, 1952. **16**(3): p. 179-204.
4. Cotton, W.E., *Vesicular Stomatitis.* Vet Med, 1927. **22**: p. 169-175.
5. Shahan M. S., A.H. Frank and L.O. Mott, *Studies of Vesicular Stomatitis with Special Reference to a Virus of Swine Origin.* J Am Vet Med Assoc, 1946. **108**: p. 5-19.
6. Hanson, R.P. and L. Karstad, *Vesicular stomatitis in deer.* Am J Vet Res, 1957. **18**(66): p. 162-6.
7. Jenney, E.W., *Vesicular stomatitis in the United States during the last five years (1963-1967).* Proc Annu Meet U S Anim Health Assoc, 1967. **71**: p. 371-85.
8. Jenney, E.W. and C.L. Brown, *Surveillance for Vesicular Stomatitis in the United States-January, 1968 through July, 1972.* Proc Annu Meet U S Anim Health Assoc, 1972. **76**: p. 183-193.
9. *OIE Disease timelines: Vesicular stomatitis*. [cited 2009 6/22/09]; Available from: http://www.oie.int/wahis/public.php?page=disease_timelines&disease_type=Terrestrial&disease_id=2&empty=999999.
10. Schmidtmann, E.T., et al., *1995 epizootic of vesicular stomatitis (New Jersey serotype) in the western United States: an entomologic perspective.* J Med Entomol, 1999. **36**(1): p. 1-7.
11. Jenney, E.W., G. A. Erickson, W. W. Buisch, W. C. Stewart and M. A. Mixson, *Surveillance for Vesicular Stomatitis in the United States 1972 through 1979.* Proc. Am. Assoc. Vet. Lab. Diagnost. , 1980. **23**: p. 83-89.
12. Hanson, R.P., et al., *Human infection with the virus of vesicular stomatitis.* J Lab Clin Med, 1950. **36**(5): p. 754-8.
13. Heiny, E., *Vesicular stomatitis in cattle and horses in Colorado.* North American Vet., 1945. **26**: p. 726-730.
14. Fields, B.N. and K. Hawkins, *Human Infection with the Virus of Vesicular Stomatitis During an Epizootic.* New England Journal of Medicine, 1967. **277**(19): p. 989-994.
15. Walton, T.E., et al., *Epizootic vesicular stomatitis in Colorado, 1982: epidemiologic and entomologic studies.* Am J Trop Med Hyg, 1987. **36**(1): p. 166-76.
16. Letchworth, G.J., L.L. Rodriguez, and J. Del cbarrera, *Vesicular stomatitis.* Vet J, 1999. **157**(3): p. 239-60.
17. Reif, J.S., et al., *Epizootic vesicular stomatitis in Colorado, 1982: infection in occupational risk groups.* Am J Trop Med Hyg, 1987. **36**(1): p. 177-82.
18. *Vesicular stomatitis*. Manual of diagnostic tests and vaccines for terrestrial animals. **Available at http://www.oie.int/eng/normes/mmanual/A_00025.htm**.
19. Howerth, E.W., et al., *Experimental vesicular stomatitis virus infection in horses: effect of route of inoculation and virus serotype.* Vet Pathol, 2006. **43**(6): p. 943-55.

20. Cotton, W.E., *Vesicular stomatitis in its relation to the diagnosis of foot-and-mouth disease.* J Am Vet Med Assoc, 1926. **69**: p. 313-332.
21. Cornish, T.E., et al., *Pathogenesis of experimental vesicular stomatitis virus (New Jersey serotype) infection in the deer mouse (Peromyscus maniculatus).* Vet Pathol, 2001. **38**(4): p. 396-406.
22. *Arboviruses and human disease.* WHO Chron, 1967. **21**(11): p. 460-462.
23. Clarke, G.R., D.E. Stallknecht, and E.W. Howerth, *Experimental infection of swine with a sandfly (Lutzomyia shannoni) isolate of vesicular stomatitis virus, New Jersey serotype.* J Vet Diagn Invest, 1996. **8**(1): p. 105-8.
24. Howerth, E.W., et al., *Experimental vesicular stomatitis in swine: effects of route of inoculation and steroid treatment.* J Vet Diagn Invest, 1997. **9**(2): p. 136-42.
25. Stallknecht, D.E., et al., *Potential for contact and mechanical vector transmission of vesicular stomatitis virus New Jersey in pigs.* Am J Vet Res, 1999. **60**(1): p. 43-8.
26. Alderink, F.J., *Vesicular stomatitis epidemic in Colorado: Clinical observations and financial losses reported by dairymen.* Prev Vet Med, 1984. **3**: p. 29-44.
27. *Foot and mouth disease*. [cited 2009 06/22/09]; Available from: http://www.oie.int/eng/info/en_fmd.htm.
28. Karstad, L.H., et al., *Evidence for the role of wildlife in epizootics of vesicular stomatitis.* J Am Vet Med Assoc, 1956. **129**(3): p. 95-6.
29. Brandly, C.A. and R.P. Hanson, *Epizootiology of vesicular stomatitis.* Am J Public Health Nations Health, 1957. **47**(2): p. 205-9.
30. Glazener, W.C., R.S. Cook and D.O. Trainer, *A serological study of diseases in the Rio Grande turkey.* J Wildl Mgm, 1967. **31**(1): p. 34-39.
31. Jenney, E.W., F.A. Hayes, and C.L. Brown, *Survey for vesicular stomatitis virus neutralizing antibodies in serums of white-tailed deer Odocoileus virginianus of the southeastern United States.* J Wildl Dis, 1970. **6**(4): p. 488-93.
32. Jenney, E.W., G. A. Erickson, W.W. Buisch, W.C. Stewart, and M.A. Mixon, *Surveillance for vesicular stomatitis in the United States in 1972 and 1979.* American Association of Veterinary Laboratory Diagnosticians, 1980(23 Annual Proceedings): p. 83-90.
33. Fletcher, W.O., D.E. Stallknecht, and E.W. Jenney, *Serologic surveillance for vesicular stomatitis virus on Ossabaw Island, Georgia.* J Wildl Dis, 1985. **21**(2): p. 100-4.
34. Rodriguez, L.L., et al., *Serological monitoring of vesicular stomatitis New Jersey virus in enzootic regions of Costa Rica.* Am J Trop Med Hyg, 1990. **42**(3): p. 272-81.
35. Aguirre, A.A., et al., *Serologic survey for selected arboviruses and other potential pathogens in wildlife from Mexico.* J Wildl Dis, 1992. **28**(3): p. 435-42.
36. Jimenez, A.E., et al., *Serological survey of small mammals in a vesicular stomatitis virus enzootic area.* J Wildl Dis, 1996. **32**(2): p. 274-9.
37. Miller, D.S., et al., *Serologic survey for selected infectious disease agents in swift and kit foxes from the western United States.* J Wildl Dis, 2000. **36**(4): p. 798-805.
38. Schnitzlein, W.M. and M.E. Reichmann, *Characterization of New Jersey vesicular stomatitis virus isolates from horses and black flies during the 1982 outbreak in Colorado.* Virology, 1985. **142**(2): p. 426-31.
39. Mitchell, C.J., et al., *Arbovirus isolations from mosquitoes collected during and after the 1982-1983 epizootic of western equine encephalitis in Argentina.* Am J Trop Med Hyg, 1987. **36**(1): p. 107-13.

40. Hayek, A.M., et al., *Financial impact of the 1995 outbreak of vesicular stomatitis on 16 beef ranches in Colorado.* Journal of the American Veterinary Medical Association, 1998. **212**(6): p. 820-3.
41. Rainwater-Lovett, K., et al., *Molecular epidemiology of vesicular stomatitis New Jersey virus from the 2004-2005 US outbreak indicates a common origin with Mexican strains.* J Gen Virol, 2007. **88**(Pt 7): p. 2042-51.
42. McCluskey, B.J., H.S. Hurd, and E.L. Mumford, *Review of the 1997 outbreak of vesicular stomatitis in the western United States.* Journal of the American Veterinary Medical Association, 1999. **215**(9): p. 1259-62.
43. Rodriguez, L.L., *Emergence and re-emergence of vesicular stomatitis in the United States.* Virus Res, 2002. **85**(2): p. 211-9.
44. Acree, J.A., D.R. Hodgson, and R.W. Page, *Epizootic Indiana vesicular stomatitis in southwestern U.S.* Proc Annu Meet U S Anim Health Assoc, 1964. **68**: p. 375-9.
45. Hanson, R.P., *Discussion of the natural history of vesicular stomatitis.* Am J Epidemiol, 1968. **87**(2): p. 264-6.
46. Webb, P.A., et al., *Epizootic vesicular stomatitis in Colorado, 1982: epidemiologic studies along the northern Colorado front range.* Am J Trop Med Hyg, 1987. **36**(1): p. 183-8.
47. Hurd, H.S., B.J. McCluskey, and E.L. Mumford, *Management factors affecting the risk for vesicular stomatitis in livestock operations in the western United States.* Journal of the American Veterinary Medical Association, 1999. **215**(9): p. 1263-8.
48. Hanson, R.P., and L. Karstad, *Further Studies on Enzootic Vesicular Stomatitis.* Proc U.S. Livestock Sanit. Assoc., 1957. **71**: p. 371-385.
49. Bridges, V.E., et al., *Review of the 1995 vesicular stomatitis outbreak in the western United States.* J Am Vet Med Assoc, 1997. **211**(5): p. 556-60.
50. McCluskey, B.J., B.J. Beaty, and M.D. Salman, *Climatic factors and the occurrence of vesicular stomatitis in New Mexico, United States of America.* Revue scientifique et technique, 2003. **22**(3): p. 849-56.
51. Stallknecht, D.E., et al., *Contact transmission of vesicular stomatitis virus New Jersey in pigs.* Am J Vet Res, 2001. **62**(4): p. 516-20.
52. Stallknecht, D.E., et al., *Effect of strain and serotype of vesicular stomatitis virus on viral shedding, vesicular lesion development, and contact transmission in pigs.* Am J Vet Res, 2004. **65**(9): p. 1233-9.
53. Francy, D.B., et al., *Epizootic vesicular stomatitis in Colorado, 1982: isolation of virus from insects collected along the northern Colorado Rocky Mountain Front Range.* J Med Entomol, 1988. **25**(5): p. 343-7.
54. Kramer, W.L., et al., *Isolation of arboviruses from Culicoides midges (Diptera: Ceratopogonidae) in Colorado during an epizootic of vesicular stomatitis New Jersey.* J Med Entomol, 1990. **27**(4): p. 487-93.
55. Nunamaker, R.A., et al., *Grasshoppers (Orthoptera: Acrididae) could serve as reservoirs and vectors of vesicular stomatitis virus.* J Med Entomol, 2003. **40**(6): p. 957-63.
56. Mead, D.G., et al., *Transmission of vesicular stomatitis virus from infected to noninfected black flies co-feeding on nonviremic deer mice.* Science, 2000. **287**(5452): p. 485-7.
57. Mead, D.G., et al., *Black fly involvement in the epidemic transmission of vesicular stomatitis New Jersey virus (Rhabdoviridae: Vesiculovirus).* Vector Borne Zoonotic Dis, 2004. **4**(4): p. 351-9.

58. Redelman, D., et al., *Experimental vesicular stomatitis virus infection of swine: extent of infection and immunological response.* Vet Immunol Immunopathol, 1989. **20**(4): p. 345-61.
59. Mason, J., A. Herrera Saldana, and W.J. Turner, *Vesicular stomatitis in Mexico.* Proc Annu Meet U S Anim Health Assoc, 1976(80): p. 234-53.
60. Atwill, E.R., L. L. Rodriguez, D. W. Hird and O. Rojas, *Environmental and host factors associated with seropositivity to New Jersey and Indiana vesicular stomatitis virus in Costa Rican cattle.* Prev Vet Med, 1993. **15**: p. 303-314.
61. Blickwede, M., et al., *Neutralizing antibodies against vesicular stomatitis viruses (serotypes New Jersey and Indiana) in horses in Costa Rica.* J Vet Diagn Invest, 2002. **14**(5): p. 438-41.
62. Herrero, M.V., et al., *Phlebotomines (Diptera: Psychodidae) collected at a Costa Rican dairy farm in a vesicular stomatitis endemic area.* J Med Entomol, 1994. **31**(6): p. 912-4.
63. Jimenez, A.E., et al., *Survey of small rodents and hematophagous flies in three sentinel farms in a Costa Rican vesicular stomatitis endemic region.* Ann N Y Acad Sci, 2000. **916**: p. 453-63.
64. Remmers, L., et al., *Longitudinal studies in the epidemiology of vesicular stomatitis on Costa Rican dairy farms.* Ann N Y Acad Sci, 2000. **916**: p. 417-30.
65. Galindo, P., et al., *An ecological survey for arboviruses in Almirante, Panama, 1959-1962.* The American journal of tropical medicine and hygiene, 1966. **15**(3): p. 385-400.
66. Peralta, P.H. and A. Shelokov, *Isolation and characterization of arboviruses from Almirante, Republic of Panama.* The American journal of tropical medicine and hygiene, 1966. **15**(3): p. 369-78.
67. Stallknecht, D.E., et al., *Enzootic vesicular stomatitis New Jersey type in an insular feral swine population.* Am J Epidemiol, 1985. **122**(5): p. 876-83.
68. Comer, J.A., et al., *Vesicular stomatitis virus, New Jersey serotype: replication in and transmission by Lutzomyia shannoni (Diptera: Psychodidae).* Am J Trop Med Hyg, 1990. **42**(5): p. 483-90.
69. Brody, J.A., G.F. Fischer, and P.H. Peralta, *Vesicular stomatitis virus in Panama. Human serologic patterns in a cattle raising area.* Am J Epidemiol, 1967. **86**(1): p. 158-61.
70. Shelokov, A. and P.H. Peralta, *Vesicular stomatitis virus, Indiana type: an arbovirus infection of tropical sandflies and humans?* American journal of epidemiology, 1967. **86**(1): p. 149-57.
71. Tesh, R.B., P.H. Peralta, and K.M. Johnson, *Ecologic studies of vesicular stomatitis virus. I. Prevalence of infection among animals and humans living in an area of endemic VSV activity.* Am J Epidemiol, 1969. **90**(3): p. 255-61.
72. Vanleeuwen, J.A., L.L. Rodriguez, and D. Waltner-Toews, *Cow, farm, and ecologic risk factors of clinical vesicular stomatitis on Costa Rican dairy farms.* Am J Trop Med Hyg, 1995. **53**(4): p. 342-50.
73. Comer, J.A., et al., *Effect of forest type on the distribution of Lutzomyia shannoni (Diptera: Psychodidae) and vesicular stomatitis virus on Ossabaw Island, Georgia.* J Med Entomol, 1993. **30**(3): p. 555-60.
74. Stallknecht, D.E., et al., *Antibodies to vesicular stomatitis virus in populations of feral swine in the United States.* J Wildl Dis, 1986. **22**(3): p. 320-5.

75. Fletcher, W.O., et al., *Antibodies to vesicular stomatitis New Jersey type virus in white-tailed deer on Ossabaw Island, Georgia, 1985 to 1989.* J Wildl Dis, 1991. **27**(4): p. 675-80.
76. Stallknecht, D.E. and G.A. Erickson, *Antibodies to vesicular stomatitis New Jersey type virus in a population of white-tailed deer.* J Wildl Dis, 1986. **22**(2): p. 250-4.
77. Stallknecht, D.E., et al., *Antibodies to vesicular stomatitis New Jersey type virus in wild and domestic sentinel swine.* Am J Epidemiol, 1987. **125**(6): p. 1058-65.
78. Stallknecht, D.E., et al., *Feral swine as a potential amplifying host for vesicular stomatitis virus New Jersey serotype on Ossabaw Island, Georgia.* J Wildl Dis, 1993. **29**(3): p. 377-83.
79. Comer, J.A., et al., *Lutzomyia shannoni (Diptera: Psychodidae): a biological vector of the New Jersey serotype of vesicular stomatitis virus on Ossabaw Island, Georgia.* Parassitologia, 1991. **33 Suppl**: p. 151-8.
80. Comer, J.A., et al., *Population dynamics of Lutzomyia shannoni (Diptera: Psychodidae) in relation to the epizootiology of vesicular stomatitis virus on Ossabaw Island, Georgia.* J Med Entomol, 1994. **31**(6): p. 850-4.
81. Comer, J.A., et al., *Titers of vesicular stomatitis virus, New Jersey serotype, in naturally infected male and female Lutzomyia shannoni (Diptera: Psychodidae) in Georgia.* J Med Entomol, 1992. **29**(2): p. 368-70.
82. Comer, J.A., W.S. Irby, and D.M. Kavanaugh, *Hosts of Lutzomyia shannoni (Diptera: Psychodidae) in relation to vesicular stomatitis virus on Ossabaw Island, Georgia, U.S.A.* Med Vet Entomol, 1994. **8**(4): p. 325-30.
83. Comer, J.A., D.E. Stallknecht, and V.F. Nettles, *Incompetence of domestic pigs as amplifying hosts of vesicular stomatitis virus for Lutzomyia shannoni (Diptera: Psychodidae).* J Med Entomol, 1995. **32**(5): p. 741-4.
84. Comer, J.A., D.E. Stallknecht, and V.F. Nettles, *Incompetence of white-tailed deer as amplifying hosts of vesicular stomatitis virus for Lutzomyia shannoni (Diptera: Psychodidae).* J Med Entomol, 1995. **32**(5): p. 738-40.

CHAPTER 3

THE DISAPPEARANCE OF AN ENDEMIC FOCUS OF VESICULAR STOMATITIS VIRUS ON OSSABAW ISLAND, GEORGIA[1]

[1] Killmaster, L. F., D.M. Mead, E. W. Howerth and D. E. Stallknecht. To be submitted to the American Journal of Tropical Medicine and Hygiene

Introduction

Vesicular Stomatitis (VS) is a reportable disease affecting livestock, notably cattle, horses and swine [1]. The disease is characterized by the occurrence of blister-like vesicles on the nose, mouth, teats and coronary band of hooves, and is caused by four viruses in the family *Rhabdoviridae.* Two of these viruses occur in the United States: *Vesicular Stomatitis Indiana Virus* (VSIV), and *Vesicular Stomatitis New Jersey Virus* (VSNJV). Antibodies to VSNJV and VSIV have been detected in a variety of wildlife species [2-8], but infection is generally subclinical and the role of wildlife species in the epidemiology of these viruses is not clearly defined.

Ossabaw Island, Georgia is the only known endemic focus of VSNJV in the United States, and antibodies to VSNJV have been repeatedly reported from wild and domestic animals sampled at this site [4, 7, 9-14]. In addition, a sand fly (*Lutzomyia shannoni*) has been identified as the endemic vector on the island [15-18]. Early surveillance of wildlife on Ossabaw Island demonstrated that of the 16 species sampled, white-tailed deer (*Odocoileus virginianus*) (83%) and feral swine (*Sus scrofa*) (60%) had the highest antibody prevalence of VSNJV neutralizing antibodies [7]. However, vesicular lesions have only been detected in a small proportion of sampled pigs [10, 19]. Although viremia has not been detected in any naturally infected wild or domestic animals to date [20-31], feral swine are thought to play a major role in the persistence of the virus in this endemic focus [4, 7, 10, 12, 13, 19, 20, 25, 29, 31-35].

Based on previously published serologic data [4, 9, 11, 14] from white-tailed deer sampled on Ossabaw Island, the prevalence of VSNJV neutralizing antibodies decreased from 60% and 62% in 1956 [7] and 1983 [11], respectively, to 38% and 23% in 1988 and 1989,

respectively [14] (Table 3.1, Figure 3.1). A decrease in antibody prevalence may reflect a sampling bias or an actual measure of decreasing transmission at this site. With regard to sampling bias, both age and location have been shown to influence VSNJV antibody prevalence in white-tailed deer and feral swine on Ossabaw Island [10, 11, 13, 14]. A change in antibody prevalence related to changing transmission patterns may be related to population changes in either the biological vector, *Lutzomyia shannoni*, or suspected amplifying hosts. With regard to the latter, there has been a notable decrease in the feral swine population since 1997 with more than 14,000 feral swine removed as part of island management practices [Georgia Department of Natural Resources (GADNR), personal communication].

The objectives of this study were to determine the current status of VSV antibody prevalence in the white-tailed deer population and to compare these results with antibody prevalence estimates from feral swine. In order to understand any observed changes in antibody prevalence in both species, the age structure of these populations and the location of sampled animals were considered as potential sources of bias. In addition, a complete survey and qualitative analysis of the vector habitat (quantification and characterization of tree holes within the three forest types), an attempt to isolate VSNJV from the sand flies, and an analysis of bloodmeal sources was done to possible explain observed changes in VSNJV antibody prevalence estimates.

Materials and Methods

Serum Collection

Blood samples were obtained from hunter-killed white-tailed deer and feral swine shot annually during quota hunts operated by the GADNR, as well as from feral swine routinely removed during population control efforts. Age, sex, and the location of the animal when it was killed were obtained for each blood sample taken. Age was determined by tooth wear and replacement. Swine were categorized into four age classes (<8 months, 8-14 months, 14-24 months, and 24+ months), pigs less than 14 months old were considered juveniles and those over 14 months old were considered adults. White-tailed deer were categorized into seven age classes (0.5 years, 1.5 years, 2.5 years, 3.5 years, 4.5 years, 5.5 years, and 6.5+ years). The mean age of deer was determined for each sample year and compared over time using a SAS linear regression model. Blood was stored at 4° C; serum was separated by centrifugation and was stored at -20° C prior to testing.

Serology

Serum samples from hunter-killed white-tailed deer and feral swine were tested for VSNJV neutralizing antibodies using the virus neutralization assay with Becton Dickinson Microtest® 96 well plates. Heat inactivated serum (56˚C for 30 minutes) from each animal was added in 2- fold serial dilutions to one column of the 96 well plate. Individual wells were inoculated with 10^3 $TCID_{50}$/mL of an Ossabaw Island isolate of VSNJV. Plates were allowed to incubate at 37˚ C for 90 minutes. Confluent Vero MARU cells from a 75 cm^2 cell culture flask were resuspended in 50 mLs of Minimal Essential Media (MEM) supplemented with 3% fetal

bovine serum (FBS) and antibiotic/antimycotic. One hundred and fifty microliters of the cell suspension was transferred to each well of the 96 well plate. Plates were observed for cytopathic effect over a 72 hour period, samples were considered negative if no cytopathic effect was noted. To remain consistent with previously reported results, white-tailed deer samples were considered positive at titers of 8 or greater and feral swine samples were considered positive at titers of 32 or greater [10-14]

Sand Fly Collection

Phlebotomine sand flies, *Lutzomyia shannoni*, were captured with CDC light traps augmented with dry ice [16, 36] and funnel traps [16, 36]. A modified funnel trap based on the original design by Comer et al. (1991) was used (Ed Rowton, personal communication). The modified funnel trap utilizes a cylindrical plastic container with a lid in lieu of a 2-liter bottle. A piece of polyester organza was placed over the opening of the container and secured with a plastic, circular lid, the center of which was cut out to allow for air flow. A small hole, approximately 1 cm in radius, was cut in the side of the container where the funnel spout was inserted. The hole was covered with 2, 1" x 1" latex sheets, each with a diagonal cut creating an "x" to allow for a tight seal between the container and the spout of a funnel (Figure 3.2). A funnel, chosen based on the size of the tree hole, was placed in the opening of the tree hole and any cracks or openings along the edges were sealed with Spanish moss (*Tillandsia usneoides*). Traps were set in late afternoon, usually between 1600 and 1800 and retrieved by 0800 the next morning.

Light traps were hung from trees on the southern end of the island in the live oak (*Quercus spp.*) forest. Light traps were placed so that the collection nets hung approximately

one meter off the ground. A cylindrical water cooler with a flip up spout was filled with dry ice and suspended next to the light traps as an attractant.

Upon collection, funnel trap containers and nets from light traps were placed at 4° C for 15 minutes and flies were removed for sorting. Males and females were separated and placed in Nalgene sample tubes in pools of no more than 22 flies. Females that had obtained a bloodmeal were placed singly in sample tubes. Tubes were stored and transported in liquid nitrogen. Trapping commenced on May 15, 2006 and continued until September 8, 2006.

Virus Isolation

Sand flies were transferred directly from Nalgene tubes in liquid nitrogen into Sarstedt tubes containing 0.1mm glass beads and 500 microliters of Minimal Essential Media supplemented with 3% FBS and antibiotic/antimycotic. Samples were macerated using a Biospec Mini Bead Beater-8 at high speed. Samples were then centrifuged at 9200 x g (10,000 rpm) for 10 minutes. One hundred microliters of the supernatant was transferred to confluent Vero cells on 12-well plates. Cells were incubated at 37° C and observed for 72 hours for cytopathic effect.

Sand Fly Bloodmeal Analysis

Blood fed flies were placed in 300 microliters Dulbecco's Phosphate Buffer Solution (DPBS) to avoid serum interaction during bloodmeal analysis; samples were macerated as described above. Extractions were performed with QiaAmp viral RNA mini kit (Qiagen Sciences, Maryland). PCR amplification of mammalian and avian DNA was performed from 50µl solution composed of 5 µl 10X buffer, 3µL of $MgCl_2$ (25mM), 2.5µL dNTPs (5mM), 0.5

µL each of the forward and reverse primers, 0.15 µL *taq* polymerase, 35.85 µL H2O, and 2.5 µL of template. Primers were designed to target the 16s ribosomal subunit. The sequences of primers are as follows: mammalian f---5' CCTGTTTACCAAAAACATCAC 3', r---5' AYTGTCGATAKGRACTCTWRARTAG 3'; avian f--- 5' MMCAAGTATTGAAGGTGA 3', r---5' CTGATCCAACATCGAGGTCGT 3'; amphibian f---5'CTGTTTACCAAAAACATCG3'; reptile f---5' CCRACTGTTTACCAAAAACATA3'; ectotherm universal (used with reptile and amphibian forward primers) r---5' ATCCAACATCGAGGTCGT3'. Mammalian, avian and reptilian reactions were incubated at 94ºC for 2 minutes 30 seconds, followed by 39 cycles as follows: 94ºC for 45 s, 55 ºC for 1min, 72 ºC for 1 min 15 s, and concluded with an indefinite cooling step at 4 ºC. Amphibian reactions followed a modified protocol with an annealing temperature of 52 ºC instead of 55 ºC. PCR products were separated by electrophoresis and visualized with ethidium bromide staining under ultraviolet light. Samples were compared to known avian, mammalian, amphibian, and reptilian controls. Bands from amplified gene products were extracted and purified with the QIAquick gel extraction kit (QIAGEN, Inc.). DNA was sequenced in forward and reverse directions at the Molecular Genetics Instrumentation Facility, The University of Georgia, with an ABI 3100 automated sequencer (Applied Biosystems, Perkins Elmer Corp., Foster City, CA). The sequences were assembled and edited using the Sequencher software package, version 4.1.4 (Gene Codes Corp,. Ann Arbor, MI). A nucleotide-nucleotide BLAST (blastn) search was performed to determine the most similar sequences of the target genes published in GenBank (http://www.ncbi.nlm.nih.gov/).

Tree Hole Survey

In temperate regions, such as on Ossabaw Island, GA, sand flies utilize tree holes as habitat for overwintering and ovipositing, and as a diurnal resting shelter [37]. Tree holes provide a humid environment and protection from fluctuating temperatures, and in 1993, a tree hole survey was conducted on Ossabaw Island to determine which of the island's three major forest types (pine forest, mixed hardwood forest and live oak/maritime forest) were suitable for supporting sand fly populations [38]. In 2007, a new tree hole survey was conducted, as previously described, for each of the forest types. Fixed radius circular plots, each measuring 1000 m², were assigned randomly to each forest type and the number of tree holes, as defined by Comer et al. 1993, were counted. Differences in prevalence of tree holes within forest types were tested by analysis of variance (ANOVA) using SAS statistical software. Differences among the same forest type over time were also analyzed using SAS ANOVA.

Results

VSNJV Serology

From November 1990 to December 2006 serum samples were obtained from 1291 white-tailed deer (Table 3.1). All age classes of white-tailed deer were sampled each year except for the 6.5+ age class in 2001. The mean age of white-tailed deer sampled decreased slightly from 1981-2006, but the decrease was not statistically significant (p=0.08, R^2=0.13). From November 2005 to February 2007, serum samples were obtained from 373 feral swine; an additional 220 serum samples were tested from hunter-killed feral swine harvested in 1993, 1996 and 1997 (Table

3.2). Of the feral swine sampled from 2005 to 2007, 49% were juveniles and 51% were adults. The distribution of white-tailed deer across age classes from 1981 to the present is presented in Figure 3.3. Ages of pigs sampled in 1992, 1993 and 1996 are unknown. Antibodies to VSNJV were detected in deer from 1981-2002 with seroprevalence ranging from 12% (2002) to 62% (1983) (Table 3.1). One 6.5+ year old deer was positive with a low titer (16) in 2004. Antibodies were found in 42% (30/72), 23% (16/69), and 46% (31/67) of feral swine sampled from 1993, 1996, and 1997, respectively (Table 3.2). Antibodies were not detected in white-tailed deer or feral swine from 2005 to the present.

Virus Isolation

Sand flies were trapped from May 18, 2006 to September 7, 2006; 880 female and 4259 male sand flies were captured over 611 trap nights. Use of funnel traps accounted for the majority of the trap nights (558 of 611). Light traps were introduced into the study on July 8, 2006, and account for 3167 sand flies in 53 trap nights. Virus isolation was attempted from 313 pools of sand flies, 11 individual sand flies, and 56 blood fed females; VSNJV was not isolated.

Sand Fly Bloodmeal Analysis

Of the 5,139 sand flies, bloodmeals were detected in 56 females. Fifty-one blood fed females were captured in the mixed hardwood areas with funnel traps, and five females were captured in the maritime forest with CO_2 light traps. Of those captured in the mixed hardwood forest, 28 (55%) were obtained from a single tree over the course of the study. Using mammalian, avian, reptilian and amphibian primers, DNA was amplified from 35 of the 56 sand flies and all products were mammalian in origin. Sources of the bloodmeal were identified for all

PCR products. Only two species, white-tailed deer, and feral swine were identified as a bloodmeal source. White-tailed deer accounted for 97% (34 of 35) of the species-identified bloodmeals; the remaining bloodmeal was of swine origin (1 of 35, 3%) (Table 3.3). The percentage of identified bloodmeals from deer (97%) was higher than reported by Comer et al. (1994) (81%) [32]. In contrast, the percentage of bloodmeals from swine decreased from 16% in 1993 [32] to 3% in 2006 (Table 3.4). The majority (34/35) of the bloodmeals that were identified came from sand flies captured in the mixed hardwood forest type.

Tree Hole Survey

In March 2007, each of the three forest types on the island was sampled to determine the mean number of tree holes per 1,000 m2 plot. The number of plots sampled from each forest type are as follows: 35 plots in the live oak forest, 50 plots in the mixed hardwood forest, and 12 plots in the pine forest. The mean number of tree holes was calculated for each forest type and compared to data from 1993. Results are shown in tables 3.5 and 3.6. In the live oak forest, there was a mean of 6.14 tree holes/plot. This forest type showed no significant change from the data obtained by Comer et al in 1993 [38] which determined the mean number of tree holes to be 7.93 (p=0.1164). The mean number of tree holes in the mixed hardwood forest increased from 5.2 in 1993 to 7.58 in 2007 (p=0.002). This forest type had the greatest number of tree holes in the 2007 survey in contrast to Comer's findings in 1993 which found the greatest number of tree holes in the maritime forest. The pine forest also experienced an increase in mean number of tree holes from 1.6 in 1993 to 3.08 in 2007, however the change was not significant (p=0.2370). As was the case in 1993, the tree holes occurring in the pine forest area were found in the sparsely

occurring hardwood trees. The mean number of tree holes sampled was significantly different between each forest type (p=0.0022).

Discussion

The decline of VSNJV serum-neutralizing antibodies in white-tailed deer was confirmed by the thorough sampling of all age classes of white-tailed deer and validated by the same trend in seroprevalence among feral swine. Antibodies to VSNJV were not detected in any white-tailed deer or feral swine from 2005 to the present. Previous studies indicated seroprevalence rates as high as 62% in deer (Table 3.2) and 60% in feral swine (Table 3.3) [10, 11]. The lack of antibodies in the oldest age class of feral swine indicates that sampled pigs had not been exposed to VSNJV for a period of at least two years prior to sampling, or since 2003. Likewise, the most recent evidence of seropositive white-tailed deer was a low titer (16) in a 6.5+ year old deer in 2004. An absence of antibodies in the youngest age classes of deer from 2003-2006 indicates that VSNJV was not circulating during this time period and this single seropositive deer was likely exposed in years prior. Additionally, attempts to isolate virus from 5139 *L. shannoni*, captured in 2006, were unsuccessful. Previous isolation rates are highly varied and range from 0.01% to 0.15% for non-blooded female sand flies [16, 18], and 0.008% to 0.017% for male sand flies [18]. Given these rates we could expect at most one isolate of VSNJV, so it is possible that the sample size was not adequate, however the complete lack of antibodies in both white-tailed deer and feral swine indicates a lack of virus transmission and possibly an absence of virus in the vector.

Failure to detect antibodies in both white-tailed deer and feral swine was not associated with a sampling bias. Regarding age bias, the mean age of white-tailed deer decreased slightly in deer sampled from 1981 to 2006, but this decrease was not statistically significant. Despite this small drop in mean age, all age classes were sampled, and antibodies were not detected in any age class from the 2004-2006 seasons, with the exception of the single deer from the oldest age class in 2004. The feral swine sampled for our study were distributed evenly between adults (51%), who had historically high seroprevalence rates, and juveniles (49%). Seroconversion in susceptible feral swine (juveniles) is known to occur in the summer months [13], and the feral swine in this study were sampled in the Fall, after exposure to the virus would have taken place. In regards to location bias, a significant relationship between forest type and antibody prevalence exists for both white-tailed deer [14] and feral swine [10, 13, 19] on Ossabaw with the highest antibody prevalence associated with the maritime/live oak forest. Of the 186 negative white-tailed deer and 347 negative feral swine with known harvest locations, 74 (40%) white-tailed deer and 164 (47%) feral swine were sampled from this forest type. Previous studies in these areas indicate seroprevalence rates as high as 83% (1988) in white-tailed deer [11] and 71% in feral swine(1990) [19].

The decline in antibody prevalence in white-tailed deer was also not associated with an obvious change in the vector population. Sand flies are still present in abundance on the island, and results from this study show that the island habitat remains viable for the support of *L. shannoni*. In fact, when compared to the last study 17 years ago, the only statistically significant change is an increase in the number of tree holes available as sand fly shelters [38].

There has been a clear change in host feeding behavior as was evidenced by bloodmeal analysis of blood fed female sand flies. White-tailed deer are still the primary host for sand flies

(97%), but there has been an obvious reduction in utilization of feral swine for bloodmeals (16% in 1993 to 3% in 2006) [32], likely an artifact related to the feral swine removal efforts established by DNR in the late 1990's.

Although their role is still not entirely defined, evidence thus far implicates feral swine as major players in the persistence of VSNJV on Ossabaw Island. Feral swine were initially regarded as amplifying hosts of VSNJV. High antibody titers have been detected in feral swine on Ossabaw and throughout southeast [12, 19], and pigs are present in strong numbers in other areas where VSNJV antibodies have been found in white-tailed deer [9, 12, 19]. They are the only wildlife species in which naturally occurring VSNJV infection has been confirmed by virus isolation [13, 19] and laboratory studies have confirmed the capability of feral swine to transmit VSNJV by contact with naïve animals [31, 34]. Biological transmission by insect vectors feeding on swine has been demonstrated [33], as well as cofeeding transmission between infected and uninfected insects feeding on a non-viremic pig [39]. Finally, the high reproductive rates of feral swine [40] ensured large numbers of susceptible animals were being introduced simultaneously with the emergence of the vector [10, 13, 41]. As the only known source of virus (in the form of clinical lesions) in this endemic region, it is presumed that pigs are required for the periodic reintroduction of VSNJV to the sand fly population, and hence long-term maintenance of the virus. Although there are no population estimates available for Ossabaw, there has been a dramatic documented decrease in numbers of feral pigs on the island. Since the institution of population control measures, the number of feral swine removed from the island has exceeded 14,000 pigs [GADNR personal communication].

To our knowledge, this is the only record of the disappearance of an endemic focus of VSNJV, and it is interesting that this system seems dependant upon the presence of feral swine.

If so, this implies that the now extinct focus of VSNJV occurred as a result of anthropogenic activity, specifically the introduction and establishment of an invasive species, feral swine, in this ecological niche. Vesicular stomatitis New Jersey virus was first isolated in the southeastern United States in 1943 when an outbreak occurred at a hog cholera vaccine plant [42, 43], incidentally, this is also the first report of VSNJV in swine in the United States. It has been loosely conjectured that the spread of VS in the southeast occurred due to the dissemination of contaminated vaccine from this facility, in fact, the Ossabaw Island strain of VSNJV is most closely related to the original isolates associated with this outbreak [44-46]. Although outbreaks of VS occurred in livestock in the 1950's and 1960's [47, 48], clinical VS has not been reported in livestock in the southeastern United States since 1977 [2]. It has been speculated that this disappearance of VS may have been related to the end of free-range farming. This practice was heavily utilized by swine producers until incidences of psuedorabies and brucellosis pushed farmers to eliminate systems that allowed for contact between domestic pigs and feral swine. At the same time, harvesting of old growth forests for timber may have eliminated potential vector habitat. It is possible that due the conservation of old growth forest and the high numbers of feral swine on the island, VSNJV on Ossabaw represented a long term carryover, and last cases, of a VSNJV epidemic that began in 1943, and that the conclusion of this epidemic is a consequence of the reduction of the feral swine population.

It is apparent that VSNJV has seen its better days on Ossabaw, and that the island is no longer a viable candidate for long-term study of the ecology of this virus. We cannot say with absolute certainty that VSNJV has completely vanished from this area nor can we say definitively that the decline in the feral swine population was the direct cause of the

disappearance of VSNJV from this endemic focus; however, the evidence strongly suggests that vesicular stomatitis is gone with the pigs.

Table 3.1

Prevalence of Vesicular Stomatitis serum neutralizing antibodies in white-tailed deer by year and age class, Ossabaw Island, Georgia

Year	0.5		1.5		2.5		3.5		4.5		5.5		6.5+		Total		Mean Age
1981[11]	1/17	6%	2/13	15%	8/17	47%	10/19	53%	9/14	64%	5/9	55%	6/7	86%	**41/96**	**43%**	**3.21**
1982[11]	3/18	17%	5/11	45%	5/12	42%	10/18	55%	8/16	50%	9/11	82%	10/10	100%	**50/96**	**52%**	**3.29**
1983[11]	9/21	43%	11/28	39%	7/9	78%	8/10	80%	10/10	100%	9/10	90%	6/7	86%	**60/96***	**62%**	**2.66**
1984[11]	4/15	27%	9/27	33%	15/28	54%	11/17	65%	5/8	62%	2/5	40%	9/12	75%	**55/112**	**49%**	**2.81**
1985[14]	2/12	17%	18/27	67%	12/23	52%	15/23	65%	6/7	86%	3/3	100%	7/13	54%	**63/108**	**58%**	**2.93**
1986[14]	3/7	43%	5/15	33%	13/24	54%	19/29	66%	4/5	80%	4/6	67%	8/10	80%	**56/96**	**58%**	**3.2**
1987[14]	1/12	8%	3/10	30%	7/15	47%	6/15	40%	8/15	5%	1/3	33%	4/6	67%	**30/76**	**39%**	**3.07**
1988[14]	3/21	36%	1/18	54%	11/19	54%	8/18	25%	¼	44%	7/13	58%	7/13	6%	**38/106**	**36%**	**3.03**
1989[[14]	1/17	6%	6/25	24%	5/23	22%	2/20	10%	5/9	56%	1/4	25%	4/7	57%	**24/105**	**23%**	**2.68**
1990	0/2	0%	6/12	50%	6/16	38%	11/21	52%	3/12	25%	3/5	60%	7/10	70%	**36/78**	**46%**	**3.57**
1991	0/14	0%	3/23	13%	8/34	24%	5/32	16%	2/11	18%	4/10	40%	10/15	67%	**32/139**	**23%**	**3.12**
1992															**16/101**	**16%**	**2.8**
1993															**37/118**	**31%**	**2.7**
1994															**22/100**	**22%**	**3.09**
1995															**15/94**	**16%**	**2.23**
1996	2/10	20%	8/33	24%	8/26	31%	4/11	36%	3/8	38%	3/9	33%	6/8	75%	**34/105**	**32%**	**2.7**
1997	1/4	25%	1/9	11%	8/16	50%	0/2	0%	½	50%	0/1	0%	1/1	100%	**12/35**	**34%**	**2.38**
1998	1/12	8%	0/11	0%	3/8	37%	7/16	44%	2/5	40%	1/3	33%	0/2	0%	**14/57**	**25%**	**2.55**
1999	0/6	0%	5/9	56%	2/17	50%	6/12	50%	4/12	33%	1/3	33%	1/2	50%	**19/63***	**30%**	**2.92**
2000	0/4	0%	1/6	17%	8/16	50%	2/6	33%	2/4	50%	1/2	50%	0/1	0%	**14/39**	**36%**	**2.35**
2001	0/3	0%	1/3	33%	0/6	0%	3/7	43%	4/8	50%	1/2	50%	0/0	0%	**9/30***	**30%**	**3.08**
2002	0/7	0%	1/26	4%	1/7	14%	1/9	11%	3/5	60%	1/2	50%	0/2	0%	**7/59***	**12%**	**2.33**
2003															**0/31**	**0%**	**2.89**
2004	0/9	0%	0/10	0%	0/19	0%	0/6	0%	0/2	0%	0/1	0%	1/4	25%	**1/51**	**2%**	**2.52**
2005	0/14	0%	0/19	0%	0/25	0%	0/12	0%	0/8	0%	0/6	0%	0/4	0%	**0/83**	**0%**	**2.83**
2006	0/29	0%	0/29	0%	0/22	0%	0/12	0%	0/8	0%	0/3	0%	0/2	0%	**0/105**	**0%**	**2.18**

*Total includes samples of unknown age

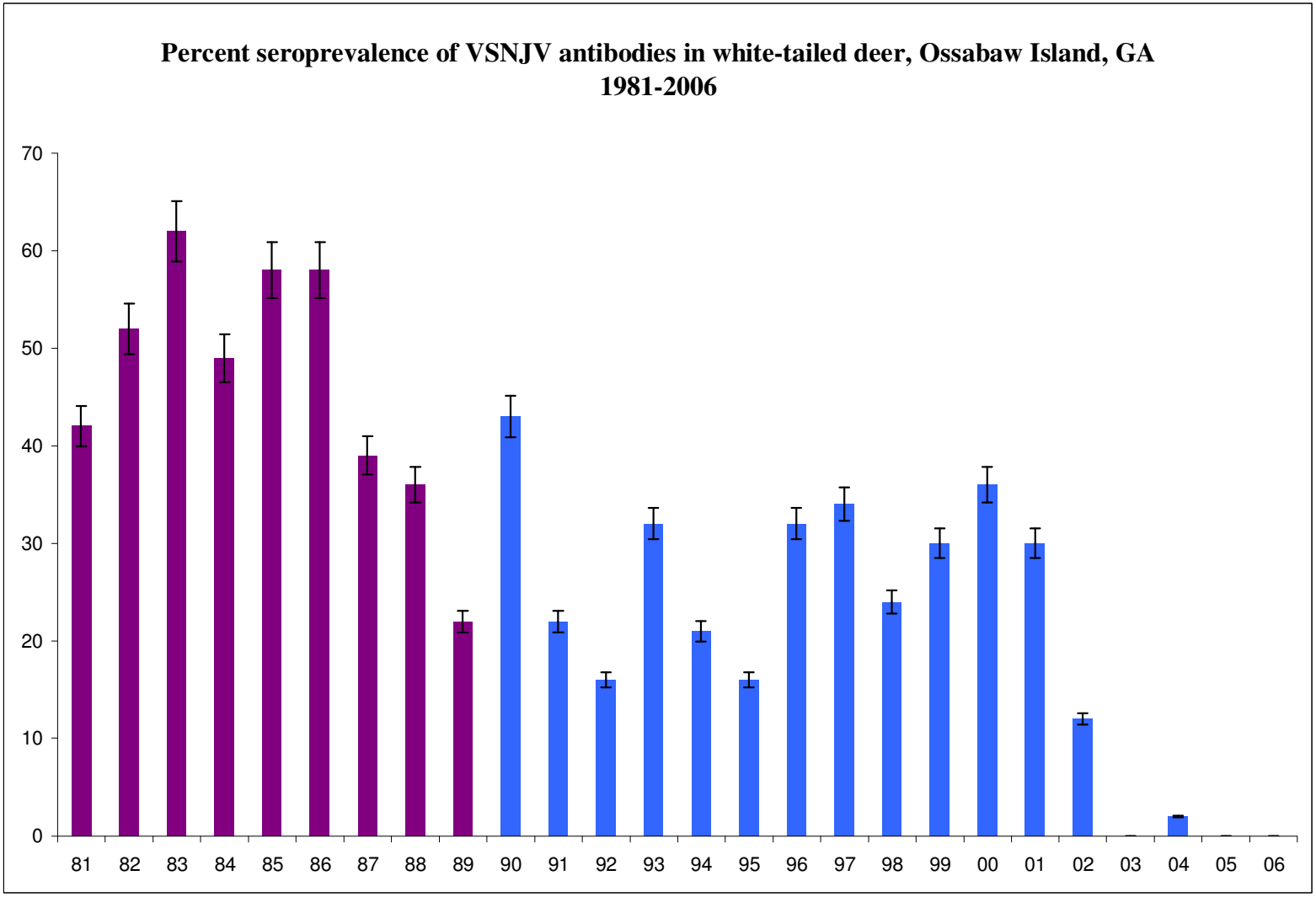
Percent seroprevalence of VSNJV antibodies in white-tailed deer, Ossabaw Island, GA
1981-2006
70
60
50
40
30
20
10
0
81
82
83
84
85
86
87
88
89
90
91
92
93
94
95
96
97
98
99
00
01
02
03
04
05
06

Figure 3.1

Figure 3.2 Photo of Funnel Trap courtesy of Danny Mead

Table 3.2

Prevalence of VSNJV serum neutralizing antibodies in feral swine, Ossabaw Island, GA 1956-2007

Year	#Pos	% Pos
1956[7]	29/34	83%
1979[9]	5/10	50%
1980[4]	48/92	52%
1981[4]	31/56	55%
1982[10]		12%
1983[10]		60%
1984*[13]		32%
1985*[13]		26%
1990[19]	58/243	24%
1991*[38]	57/176	32%
1993	30/72	42%
1996	16/69	23%
1997	31/67	46%
2005	0/37	0%
2006	0/181	0%
2007	0/129	0%

* data based on seroconversion rates of sentinel swine

Representation of Age Classes Among Deer Sampled for Antibodies to VSNJV on Ossabaw Island, GA 1981-2006

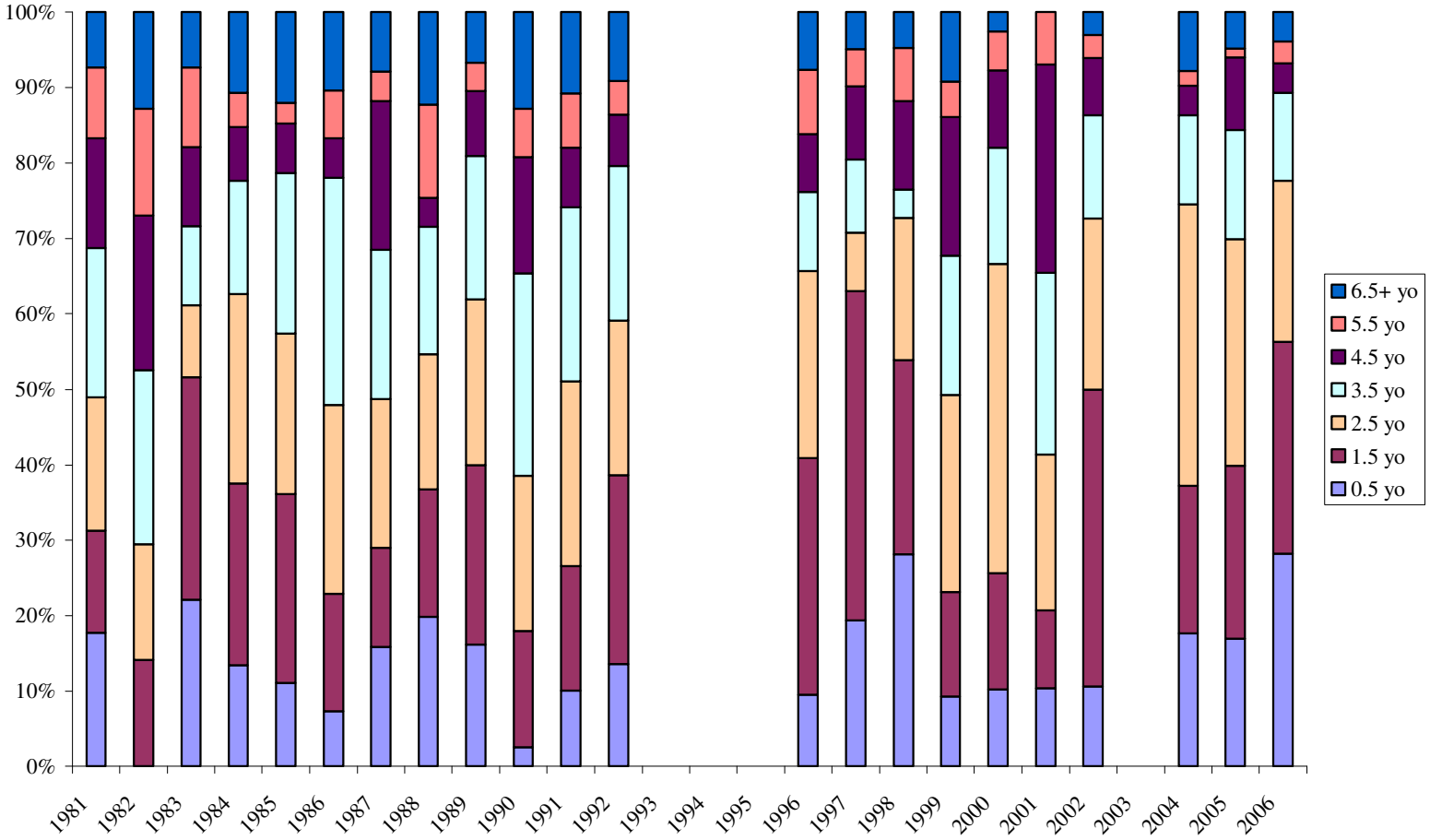

Figure 3.3

Table 3.3

Hosts of *Lutzomyia shannoni* on Ossabaw Island, Georgia, 2006 as determined by PCR analysis of bloodmeals	
Host	**No. of meals**
White-tailed deer	34 (97%)
Swine	1(3%)

Table 3.4

Comparison of bloodmeal hosts of *Lutzomyia shannoni* on Ossabaw Island, Georgia, 1990 and 2006		
Host	**No. of meals**	**No. of meals**
	1990	2006
White-tailed deer	265 (81%)	34 (97%)
Swine	53 (16%)	1 (3%)
Horse	5	0
Raccoon	2	0
Mixed	2	0
Unidentified	6 (2%)	

Table 3.5

Number of tree holes per 1,000 m^2 plots among forest types on Ossabaw Island, GA 2007			
Forest type	**No. of plots**	**No. of tree holes**	
		Mean ± SE	**Range**
Maritime live oak	35	6.14 ± .64	1-21
Mixed hardwood	50	7.58 ± .62	0-18
Pine	12	3.08 ± .78	0-9

Table 3.6

Comparison of mean number of tree holes per forest type on Ossabaw Island, GA, 1993 and 2007			
Forest Type	No. of tree holes		*p*-value
	1993	2007	
Maritime live oak	7.9	6.14	0.1164
Mixed hardwood	5.2	7.58	0.002
Pine	1.6	3.08	0.237

References

1. *OIE Listed diseases*. 2009; Available from: http://www.oie.int/eng/maladies/en_classification2009.htm?e1d7.
2. Jenney, E.W., G. A. Erickson, W.W. Buisch, W.C. Stewart and M.A. Mixson, *Surveillance for Vesicular Stomatitis in the United States 1972 through 1979.* Proc. Am. Assoc. Vet. Lab. Diagnost. , 1980. **23**: p. 83-89.
3. Brandly, C.A. and R.P. Hanson, *Epizootiology of vesicular stomatitis.* Am J Public Health Nations Health, 1957. **47**(2): p. 205-9.
4. Fletcher, W.O., D.E. Stallknecht, and E.W. Jenney, *Serologic surveillance for vesicular stomatitis virus on Ossabaw Island, Georgia.* J Wildl Dis, 1985. **21**(2): p. 100-4.
5. Glazener, W.C., R.S. Cook and D.O. Trainer, *A serological study of diseases in the Rio Grande turkey.* J Wildl Mgm, 1967. **31**(1): p. 34-39.
6. Jimenez, A.E., et al., *Serological survey of small mammals in a vesicular stomatitis virus enzootic area.* J Wildl Dis, 1996. **32**(2): p. 274-9.
7. Karstad, L.H., et al., *Evidence for the role of wildlife in epizootics of vesicular stomatitis.* J Am Vet Med Assoc, 1956. **129**(3): p. 95-6.
8. Miller, D.S., et al., *Serologic survey for selected infectious disease agents in swift and kit foxes from the western United States.* J Wildl Dis, 2000. **36**(4): p. 798-805.
9. Jenney, E.W., F.A. Hayes, and C.L. Brown, *Survey for vesicular stomatitis virus neutralizing antibodies in serums of white-tailed deer Odocoileus virginianus of the southeastern United States.* J Wildl Dis, 1970. **6**(4): p. 488-93.
10. Stallknecht, D.E., et al., *Enzootic vesicular stomatitis New Jersey type in an insular feral swine population.* Am J Epidemiol, 1985. **122**(5): p. 876-83.
11. Stallknecht, D.E. and G.A. Erickson, *Antibodies to vesicular stomatitis New Jersey type virus in a population of white-tailed deer.* J Wildl Dis, 1986. **22**(2): p. 250-4.
12. Stallknecht, D.E., et al., *Antibodies to vesicular stomatitis virus in populations of feral swine in the United States.* J Wildl Dis, 1986. **22**(3): p. 320-5.
13. Stallknecht, D.E., et al., *Antibodies to vesicular stomatitis New Jersey type virus in wild and domestic sentinel swine.* Am J Epidemiol, 1987. **125**(6): p. 1058-65.
14. Fletcher, W.O., et al., *Antibodies to vesicular stomatitis New Jersey type virus in white-tailed deer on Ossabaw Island, Georgia, 1985 to 1989.* J Wildl Dis, 1991. **27**(4): p. 675-80.
15. Comer, J.A., et al., *Vesicular stomatitis virus, New Jersey serotype: replication in and transmission by Lutzomyia shannoni (Diptera: Psychodidae).* Am J Trop Med Hyg, 1990. **42**(5): p. 483-90.
16. Corn, J.L., et al., *Isolation of vesicular stomatitis virus New Jersey serotype from phlebotomine sand flies in Georgia.* Am J Trop Med Hyg, 1990. **42**(5): p. 476-82.
17. Comer, J.A., et al., *Lutzomyia shannoni (Diptera: Psychodidae): a biological vector of the New Jersey serotype of vesicular stomatitis virus on Ossabaw Island, Georgia.* Parassitologia, 1991. **33 Suppl**: p. 151-8.
18. Comer, J.A., et al., *Titers of vesicular stomatitis virus, New Jersey serotype, in naturally infected male and female Lutzomyia shannoni (Diptera: Psychodidae) in Georgia.* J Med Entomol, 1992. **29**(2): p. 368-70.

19. Stallknecht, D.E., et al., *Feral swine as a potential amplifying host for vesicular stomatitis virus New Jersey serotype on Ossabaw Island, Georgia.* J Wildl Dis, 1993. **29**(3): p. 377-83.
20. Clarke, G.R., D.E. Stallknecht, and E.W. Howerth, *Experimental infection of swine with a sandfly (Lutzomyia shannoni) isolate of vesicular stomatitis virus, New Jersey serotype.* J Vet Diagn Invest, 1996. **8**(1): p. 105-8.
21. Comer, J.A., D.E. Stallknecht, and V.F. Nettles, *Incompetence of domestic pigs as amplifying hosts of vesicular stomatitis virus for Lutzomyia shannoni (Diptera: Psychodidae).* J Med Entomol, 1995. **32**(5): p. 741-4.
22. Comer, J.A., D.E. Stallknecht, and V.F. Nettles, *Incompetence of white-tailed deer as amplifying hosts of vesicular stomatitis virus for Lutzomyia shannoni (Diptera: Psychodidae).* J Med Entomol, 1995. **32**(5): p. 738-40.
23. Cornish, T.E., et al., *Pathogenesis of experimental vesicular stomatitis virus (New Jersey serotype) infection in the deer mouse (Peromyscus maniculatus).* Vet Pathol, 2001. **38**(4): p. 396-406.
24. Howerth, E.W., et al., *Experimental vesicular stomatitis virus infection in horses: effect of route of inoculation and virus serotype.* Vet Pathol, 2006. **43**(6): p. 943-55.
25. Howerth, E.W., et al., *Experimental vesicular stomatitis in swine: effects of route of inoculation and steroid treatment.* J Vet Diagn Invest, 1997. **9**(2): p. 136-42.
26. Mead, D.G., C.J. Mare, and F.B. Ramberg, *Bite transmission of vesicular stomatitis virus (New Jersey serotype) to laboratory mice by Simulium vittatum (Diptera: Simuliidae).* J Med Entomol, 1999. **36**(4): p. 410-3.
27. Perez De Leon, A.A., D. O'Toole, and W.J. Tabachnick, *Infection of guinea pigs with vesicular stomatitis New Jersey virus Transmitted by Culicoides sonorensis (Diptera: Ceratopogonidae).* J Med Entomol, 2006. **43**(3): p. 568-73.
28. Perez de Leon, A.A. and W.J. Tabachnick, *Transmission of vesicular stomatitis New Jersey virus to cattle by the biting midge Culicoides sonorensis (Diptera: Ceratopogonidae).* J Med Entomol, 2006. **43**(2): p. 323-9.
29. Redelman, D., et al., *Experimental vesicular stomatitis virus infection of swine: extent of infection and immunological response.* Vet Immunol Immunopathol, 1989. **20**(4): p. 345-61.
30. Schnitzlein, W.M. and M.E. Reichmann, *Characterization of New Jersey vesicular stomatitis virus isolates from horses and black flies during the 1982 outbreak in Colorado.* Virology, 1985. **142**(2): p. 426-31.
31. Stallknecht, D.E., et al., *Effect of strain and serotype of vesicular stomatitis virus on viral shedding, vesicular lesion development, and contact transmission in pigs.* Am J Vet Res, 2004. **65**(9): p. 1233-9.
32. Comer, J.A., W.S. Irby, and D.M. Kavanaugh, *Hosts of Lutzomyia shannoni (Diptera: Psychodidae) in relation to vesicular stomatitis virus on Ossabaw Island, Georgia, U.S.A.* Med Vet Entomol, 1994. **8**(4): p. 325-30.
33. Mead, D.G., et al., *Biological transmission of vesicular stomatitis virus (New Jersey serotype) by Simulium vittatum (Diptera: Simuliidae) to domestic swine (Sus scrofa).* J Med Entomol, 2004. **41**(1): p. 78-82.
34. Stallknecht, D.E., et al., *Potential for contact and mechanical vector transmission of vesicular stomatitis virus New Jersey in pigs.* Am J Vet Res, 1999. **60**(1): p. 43-8.

35. Stallknecht, D.E., et al., *Contact transmission of vesicular stomatitis virus New Jersey in pigs.* Am J Vet Res, 2001. **62**(4): p. 516-20.
36. Comer, J.A. and J. L. Corn, *Funnel Trap for the Capture of Phlebotomine Sand Flies (Diptera: Psychodidae) from Tree Holes.* J Med Entomol, 1991. **28**(2): p. 289-292.
37. Comer, J.A., and J. Brown, *Use of Hollow Trees as Diurnal Resting Shelter by Lutzomyia shannoni (Diptera: Psychodidae) on Ossabaw Island, Georgia.* Population Ecology, 1993. **22**(3): p. 613-617.
38. Comer, J.A., et al., *Effect of forest type on the distribution of Lutzomyia shannoni (Diptera: Psychodidae) and vesicular stomatitis virus on Ossabaw Island, Georgia.* J Med Entomol, 1993. **30**(3): p. 555-60.
39. Mead, D.G., et al., *Black fly involvement in the epidemic transmission of vesicular stomatitis New Jersey virus (Rhabdoviridae: Vesiculovirus).* Vector Borne Zoonotic Dis, 2004. **4**(4): p. 351-9.
40. Taylor, R.B., et al., *Reproduction of feral pigs in southern Texas.* Journal of Mammalogy, 1998. **79**(4): p. 1325-1331.
41. Comer, J.A., et al., *Population dynamics of Lutzomyia shannoni (Diptera: Psychodidae) in relation to the epizootiology of vesicular stomatitis virus on Ossabaw Island, Georgia.* J Med Entomol, 1994. **31**(6): p. 850-4.
42. Shahan M. S., A.H. Frank, and L.O. Mott, *Studies of Vesicular Stomatitis with Special Reference to a Virus of Swine Origin.* J Am Vet Med Assoc, 1946. **108**: p. 5-19.
43. Hanson, R.P. and L. Karstad, *Vesicular stomatitis in deer.* Am J Vet Res, 1957. **18**(66): p. 162-6.
44. Bilsel, P.A., et al., *Phosphoprotein and nucleocapsid protein evolution of vesicular stomatitis virus New Jersey.* J Virol, 1990. **64**(6): p. 2498-504.
45. Vernon, S.D., L.L. Rodriguez, and G.J. Letchworth, *Vesicular stomatitis New Jersey virus glycoprotein gene sequence and neutralizing epitope stability in an enzootic focus.* Virology, 1990. **177**(1): p. 209-15.
46. Rainwater-Lovett, K., et al., *Molecular epidemiology of vesicular stomatitis New Jersey virus from the 2004-2005 US outbreak indicates a common origin with Mexican strains.* J Gen Virol, 2007. **88**(Pt 7): p. 2042-51.
47. Hanson, R.P., *The natural history of vesicular stomatitis.* Bacteriol Rev, 1952. **16**(3): p. 179-204.
48. Jenney, E.W., *Vesicular stomatitis in the United States during the last five years (1963-1967).* Proc Annu Meet U S Anim Health Assoc, 1967. **71**: p. 371-85.