

# VSV-NJ on Ossabaw Island, Georgia

## The Truth Is Out There

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**ABSTRACT:** Ossabaw Island, Georgia, is the only recognized enzootic focus of vesicular stomatitis virus New Jersey (VSV-NJ) in the United States and has been the subject of VSV-NJ research since 1981. To date, VSV-NJ antibodies have been detected only from feral swine, cattle, equines, deer, and raccoons. VSV-NJ transmission occurs annually, is seasonal, and is associated with the maritime forest. Despite high transmission rates the clinical disease is rarely detected. A sand fly (*Lutzomyia shannoni*) occurs on the Island, and experimental and field data suggest that it is a biological vector of VSV-NJ at this site. Many questions relating to the epidemiology of VSV-NJ on Ossabaw remain. What is the maintenance cycle of VSV-NJ? Is a vertebrate amplifying host(s) needed? Are other insect vectors involved in mechanical or biological transmission? Why do vesicular lesions develop on some but not all infected animals? Do native and domestic animals play the same role in the maintenance cycle? These questions challenge researchers in all areas where VSV-NJ occurs. It is our hope that Ossabaw Island will provide a much needed model system for gaining insight into the epidemiology of this virus.

### INTRODUCTION

Ossabaw Island, the only recognized enzootic focus of vesicular stomatitis virus New Jersey (VSV-NJ) in the United States, is a 10,100 ha barrier island located off of the Georgia coast. The Island is divided almost evenly between salt marsh and upland habitats, the later consisting of pine, mixed-hardwood, and maritime forests. The fauna is diverse; however, some species common to mainland habitats are absent. In addition to wildlife species, introduced domestic and feral species have been or are present, including feral pigs, feral donkeys, horses, and cattle.

Serologic evidence of VSV-NJ on Ossabaw Island was first detected in 1965 during a serologic survey of white-tailed deer (*Odocoileus virginianus*) that included samples collected throughout the Southeastern United States.<sup>1</sup> Since vesicular stomatitis (VS) was common in the Southeast during the 1950s and 1960s,<sup>2</sup> the detection of seropositive deer was not regarded as a significant observation. In contrast, the detection of neutralizing antibodies to VSV-NJ from feral swine (*Sus scrofa*) on

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Ossabaw during 1979, when there were no recent reports of clinical VS in the Southeast, suggested that this virus was being maintained at this site.<sup>3</sup>

The long term goal of our VSV-NJ research on Ossabaw Island is to understand the epidemiology of this virus at this single enzootic focus. Because Ossabaw Island represents a relatively closed ecosystem, is in public ownership, and supports both wildlife and domestic animals, this small barrier island provides an ideal outdoor laboratory for these studies. The objectives of this review are to present what we have learned from these studies to date, and to discuss the limits of this knowledge in relation to understanding how VSV-NJ is maintained and transmitted in the natural environment.

## EPIDEMIOLOGY OF VSV-NJ ON OSSABAW

### *What We Have Learned*

Initial serologic testing of animals on Ossabaw Island for antibodies to VSV-NJ demonstrated the presence of seropositive white-tailed deer<sup>1</sup> and feral swine.<sup>2</sup> In order to determine the extent of this exposure, a serologic survey of representative mammals (17 species) and birds (7 species) was initiated.<sup>4</sup> Serum neutralizing antibodies (1:32 dilution or higher) were detected in six mammalian species including raccoons (*Procyon lotor*) (20%), white-tailed deer (33%), feral swine (53%), cattle (*Bos taurus*, 43%), horses (*Equus caballus*, 35%), and donkeys (*Equus asinus*, 40%). In all of these seropositive species, where age data were available, a strong relationship was observed between age and VSV-NJ antibody prevalence suggesting that this virus was transmitted annually. This pattern has persisted, and annual transmission of VSV-NJ has been confirmed through long-term serologic monitoring of hunter killed white tailed deer, from which antibodies have been detected every year from 1981 through 1997<sup>5,6</sup> (also Stallknecht, unpublished data). In addition to testing mammalian and avian species, several reptile and amphibian species also have been tested including rat snakes (*Elaphe obsoleta*), common garter snake (*Thamnophis sirtalis*), racer (*Coluber constrictor*), and Southern toad (*Bufo terrestris*). All of these have tested negative for antibodies to VSV-NJ (Stallknecht, unpublished data).

To understand the temporal and spatial characteristics of VSV-NJ transmission on Ossabaw, we elected to use the indigenous feral swine population in a series of mark-recapture studies. Feral swine are ideally suited for this study design owing to their abundance, ease of capture and recapture, and their reproductive patterns. Peak breeding on Ossabaw peaks in the Fall with farrowing occurring in late Winter. This results in an influx of susceptible animals every year. Maternally derived antibodies to VSV-NJ in these animals persist for approximately 2–3 months, so these animals are fully susceptible to VSV-NJ in early Spring of each year. During 1982 and 1983, respectively, 307 and 340 juvenile feral swine were repeatedly tested from March through September.<sup>7</sup> The results indicated a seroconversion incidence of 12% and 60% during 1982 and 1983, respectively, with seroconversion initially detected during the first 10-day period of June during both years. Also during both years, the earliest detected seroconversions and the highest incidences of seroconversion occurred on the Southern portion of the Island. These same patterns were confirmed in a follow up study during 1984 and 1985, but in these years the initial seroconversions

were detected during mid May.<sup>8</sup> These studies confirmed that annual transmission of VSV-NJ occurs on this Island. The isolation of VSV-NJ from vesicular lesions on two sentinel swine during 1983 validated these serologic results.<sup>7</sup>

Despite the high incidence of seroconversion observed in sentinel feral swine<sup>7,8</sup> and the relatively high prevalence of antibodies to VSV-NJ observed in other species such as raccoons, white-tailed deer, horses, and cattle,<sup>3</sup> clinical disease (the presence of vesicular lesions) has either not been reported (deer, raccoons, cattle, horses, and donkeys) or occurs infrequently (feral swine). In the case of our sentinel feral swine studies, vesicular lesions were recorded only during 1983 and then only in two of 340 animals, of which 60% seroconverted. It is interesting that both of these animals were detected in July, well after the onset of transmission as detected by seroconversion. These field results strongly suggest that clinical VS is the exception rather than the rule in natural infections. Since VS detection in livestock is based on detection of clinical disease, this observation has important implications for VS control strategies.

The seasonal pattern of transmission of VSV-NJ on Ossabaw suggested that this virus was being transmitted by arthropod vectors. In order to test this hypothesis, we constructed four isolation pens on the Southern portion of the Island.<sup>8</sup> These pens were designed to eliminate native food, ground water, and contact with other animals. During both 1984 and 1985, seroconversions were detected in domestic swine in these pens and the timing and locations of these seroconversions matched observations from feral swine. Insect trapping at these pens and adjacent areas revealed the presence of the sand fly species *Lutzomyia shannoni*, and through additional trapping and virus isolation attempts, VSV-NJ was isolated from this species.<sup>9</sup> Subsequent experimental infections of *L. shannoni* by Comer *et al.*<sup>10</sup> demonstrated that laboratory-reared sand flies could be infected by oral and intrathoracic inoculation of VSV-NJ. Viral replication was documented in these flies with viral titers exceeding  $10^4$  plaque forming units (PFU) in the heads of individual flies. Transovarial transmission to a small proportion of F<sub>1</sub> progeny and bite transmission to suckling mice were also demonstrated. These findings indicated that *L. shannoni* was a biological vector of VSV-NJ. Additional evidence supporting *L. shannoni* as a biological vector came from field studies and included: the isolation of VSV-NJ from field-caught male sand flies,<sup>11</sup> demonstration that flight activity for this species began in April and peaked in May corresponding to the seasonal pattern of VSV-NJ on Ossabaw;<sup>12</sup> demonstration that VSV-NJ titers in field caught sand flies were similar to titers observed in experimental trials;<sup>11</sup> and identification of deer and feral swine as the primary sources for blood meals,<sup>13</sup> which corresponds to the high prevalence of antibodies detected in these species. Although VSV-NJ can be routinely isolated from *L. shannoni* at this site, the infection rate is relatively low. Based on three years of data, Comer *et al.*<sup>14</sup> reported VSV-NJ isolation rates of 1 per 4,407 total sand flies and 1 per 915 females.

Observed spatial patterns in VSV-NJ on Ossabaw, suggested that the virus was maintained and transmitted on a very small portion of this Island. Given the size of Ossabaw, this was not expected. Since forest types varied significantly across the Island, this potential variable was addressed in two independent studies one involving deer<sup>6</sup> and the other using feral swine.<sup>15</sup> In both cases, prevalence was significantly associated with forest type, specifically with the old growth maritime forest. The

reason for this association probably relates to the behavior and ecological needs of *L. shannoni*. The maritime forest, due to a preponderance of older trees, has an abundance of tree holes, which were identified as an important resting site for *L. shannoni*.<sup>16</sup> Subsequent work indicated that the presence of *L. shannoni* not only correlated with number of tree holes, but also to the prevalence of VSV-NJ neutralizing antibodies in sentinel swine. It is interesting that the distribution of this forest type can be associated with barrier island agriculture, which terminated at the end of the nineteenth century.<sup>15</sup> Because the Holocene soils were geologically new and infertile, they were not cleared for agriculture. The result, 100 years later, is that these areas support old growth forests, tree holes, sand flies, and VSV-NJ.

As stated by Jonkers,<sup>17</sup> the primary argument against VSV-NJ being maintained in a traditional vertebrate–vector viral maintenance cycle is the failure to demonstrate a sustained viremia in any wildlife or domestic animal host. We have not resolved this argument. In an effort to understand the potential role of feral swine as an amplifying host for VSV-NJ on Ossabaw, we incorporated virus isolation attempts into our sentinel swine mark–recapture studies.<sup>18</sup> Although 21 of 54 pigs seroconverted, we did not isolate VSV-NJ from the blood of any of these animals. However, VSV-NJ was recovered from tonsil and nasal swabs from five animals, all of which were frequenting a single trap. It was interesting to observe that VSV-NJ was isolated from this single group of animals every week for a period exceeding one month. In addition, only two of these animals showed any indication of vesicular lesions.

It is well known that VSV-NJ can be transmitted by contact, and from our previous studies<sup>7,8,18</sup> it became readily apparent that the transmission of this virus among swine on Ossabaw Island probably involved multiple transmission routes. To better understand the potential for contact and mechanical transmission among swine, we have conducted a series of experimental infections of domestic swine with the Ossabaw strain of VSV-NJ.<sup>19,20,21</sup> Results from these studies indicate that: swine can be infected by a variety of routes that are consistent with contact and mechanical vector transmission; swine can be infected with as little as  $10^{0.7}$  TCID<sub>50</sub> of virus administered by intradermal inoculation; clinical response is related to dose and route of inoculation; pigs do not develop a viremia during infection; both clinically and subclinically affected animals shed virus for up to seven days via the tonsil, nasal secretions, and saliva; limited shedding can occur in the feces; and viral shedding ceases upon detection of a serum neutralizing antibody response. Contact transmission can occur within 24 hours of contact with an infected animal, but appears to be lesion dependent. However, these lesions may be so superficial that lesion detection in the field would be unlikely (Stallknecht, unpublished data). Pigs represent a very dependable laboratory animal for the *in vivo* study of VSV-NJ and we are currently evaluating their response to challenge with recent VSV-NJ, and VSV Indiana strains from the Western United States.

#### *What We Need to Learn*

The critical questions concerning the epidemiology of VSV-NJ relate to the maintenance cycle. Is a vertebrate amplifying host needed for maintenance? To date, with the possible exception of rodents, we have not identified any species that support a viremia capable of infecting a biting arthropod. We have conducted feeding trials

using laboratory-reared *L. shannoni* on both pigs<sup>23</sup> and deer,<sup>24</sup> and transmission from experimentally infected host to sand fly was not detected. This was surprising considering that 98% of all blood meals identified from sand flies on Ossabaw were from these two species.<sup>13</sup> Perhaps a viremia is not needed and flies are infected via cofeeding with other infected flies or become infected through ingestion of VSV-NJ infected fluids such as saliva or vesicular fluid. The later seems unlikely due the relatively few times we have detected such lesions. From the vector side, are other insect species involved in transmission at this site, such as, black flies and *Culicoides*, which have been suggested as potential vectors in the Western states. Although we have tested other insect species from Ossabaw, are the numbers adequate to detect a 0.1% infection rate? What mechanical vectors may be involved? To date, we have not looked at this. Do native and domestic animals play the same role in VSV-NJ epizootics or in the maintenance of this virus at enzootic sites? Our work on Ossabaw suggests that VSV-NJ epidemiology may involve two distinct cycles, one involving maintenance in a wildlife–biological vector system and another involving clinical disease in a dead end domestic animal cycle primarily sustained by contact and mechanical vector transmission. Why do vesicular lesions develop on some but not all animals? Our contact transmission work suggests that the development of lesions is extremely important in transmission and the detection of such lesions forms the basis of all of our current control efforts. Finally, is Ossabaw Island the only VSV-NJ focus in the Southeastern United States? The answer to this simple question would greatly help us to understand if the Ossabaw focus represents something relatively new or simply represents the remnants of what was once a much larger enzootic area dependent on now gone old growth forest habitats? In conclusion, although progress has been made, the epidemiology of VSV-NJ on Ossabaw Island is not fully understood. It is our hope that the information gained to date from this tiny Island in Georgia will assist other researchers in developing and implementing VS-related field research in other enzootic areas, and will provide a relevant basis for comparison when such research is completed.

#### REFERENCES

1. JENNEY, E.W., F.A. HAYES & C.L. BROWN. 1970. Survey for vesicular stomatitis virus neutralizing antibodies in serums of white-tailed deer *Odocoileus virginianus* of the southeastern United States. *J. Wildl. Dis.* **6**: 488–493.
2. JENNEY, E.W. 1967. Vesicular stomatitis in the United States during the last five years (1963–1967). *Proc. U.S. Animal Hlth. Assoc.* **71**: 371–385.
3. STALLKNECHT, D.E., V.F. NETTLES, G.A. ERICKSON & D.A. JESSUP. 1986. Antibodies to vesicular stomatitis virus in populations of feral swine in the United States. *J. Wildl. Dis.* **22**: 320–325.
4. FLETCHER, W.O., D.E. STALLKNECHT & E.W. JENNEY. 1985. Serologic surveillance for vesicular stomatitis virus on Ossabaw Island, Georgia. *J. Wildl. Dis.* **21**: 100–104.
5. STALLKNECHT, D.E. & G.A. ERICKSON. 1986. Antibodies to vesicular stomatitis New Jersey type virus in a population of white-tailed deer. *J. Wildl. Dis.* **22**: 250–254.
6. FLETCHER, W.O., D.E. STALLKNECHT, M.T. KEARNEY & K.A. EERNISSE. 1991. Antibodies to vesicular stomatitis New Jersey type virus in white-tailed deer on Ossabaw Island, Georgia, 1985–1989. *J. Wildl. Dis.* **27**: 675–680.

7. STALLKNECHT, D.E., V.F. NETTLES, W.O. FLETCHER & G.A. ERICKSON. 1985. Enzootic vesicular stomatitis New Jersey type in an insular feral swine population. *Am. J. Epidemiol.* **122**: 876–883.
8. STALLKNECHT, D.E., W.O. FLETCHER, G.A. ERICKSON & V.F. NETTLES. 1987. Antibodies to vesicular stomatitis New Jersey type virus in feral and domestic sentinel swine. *Am. J. Epidemiol.* **125**: 1058–1065.
9. CORN, J.L., J.A. COMER, G.A. ERICKSON & V.F. NETTLES. 1990. Isolation of vesicular stomatitis virus New Jersey serotype from phlebotomine sand flies in Georgia. *Am. J. Trop. Med. Hyg.* **42**: 476–482.
10. COMER, J.A., R.B. TESH, G.B. MODI, J.L. CORN & V.F. NETTLES. 1990. Vesicular stomatitis virus, New Jersey serotype: replication in and transmission by *Lutzomyia shannoni* (Diptera: Psychodidae). *Am. J. Trop. Med. Hyg.* **42**: 483–490.
11. COMER, J.A., J.L. CORN, D.E. STALLKNECHT, J.G. LANDGRAF & V.F. NETTLES. 1992. Titers of vesicular stomatitis virus, New Jersey serotype, in *Lutzomyia shannoni* (Diptera: Psychodidae) in Georgia. *J. Med. Entomol.* **29**: 369–370.
12. BRINSON, F.J., D.V. HAGAN, J.A. COMER & D.A. STROHLEIN. 1992. Seasonal abundance of *Lutzomyia shannoni* (Diptera: Psychodidae) on Ossabaw Island, Georgia. *J. Med. Ent.* **29**: 178–182.
13. COMER, J.A., W.S. IRBY & D.M. KAVANAUGH. 1994. Hosts of *Lutzomyia shannoni* (Diptera: Psychodidae) in relation to vesicular stomatitis virus on Ossabaw Island, Georgia, USA. *Med. Vet. Ent.* **8**: 325–330.
14. COMER, J.A., D.E. STALLKNECHT, J.L. CORN & V.F. NETTLES. 1992. *Lutzomyia shannoni* (Diptera: Psychodidae): A biological vector of the New Jersey serotype of vesicular stomatitis virus on Ossabaw Island, Georgia. *Parassitologia* **55**: 151–158.
15. COMER, J.A., D.M. KAVANAUGH, D.E. STALLKNECHT, G.O. WARE, J.L. CORN & V.F. NETTLES. 1993. Effect of forest type on the distribution of *Lutzomyia shannoni* (Diptera: Psychodidae) and vesicular stomatitis virus on Ossabaw Island, Georgia. *J. Med. Ent.* **30**: 555–560.
16. COMER, J.A. & J. BROWN. 1993. Use of hollow trees as diurnal resting shelter by *Lutzomyia shannoni* (Diptera: Psychodidae) on Ossabaw Island, Georgia. *Environmental Entomol.* **22**: 613–617.
17. JONKERS, A.H. 1967. The epidemiology of vesicular stomatitis viruses: a reappraisal. *Am. J. Epidemiol.* **86**: 286–291.
18. STALLKNECHT, D.E., D.M. KAVANAUGH, J.L. CORN, K.A. EERNISSE, J.A. COMER & V.F. NETTLES. 1993. Feral swine as a potential amplifying host for vesicular stomatitis virus New Jersey serotype on Ossabaw Island, Georgia. *J. Wildl. Dis.* **29**: 377–383.
19. CLARKE, G.R., D.E. STALLKNECHT & E.W. HOWERTH. 1996. Experimental infection of swine with a sandfly (*Lutzomyia shannoni*) isolate of vesicular stomatitis virus, New Jersey serotype. *J. Vet. Diag. Invest.* **8**: 105–108.
20. HOWERTH, E.W., D.E. STALLKNECHT, M. DORMINY, T. PISELL & G.R. CLARKE. 1997. Experimental vesicular stomatitis in swine: Effects of route of inoculation and steroid treatment. *J. Vet. Diag. Inv.* **9**: 136–142.
21. STALLKNECHT, D.E., E.W. HOWERTH, C.L. REEVES & B.S. SEAL. 1999. Potential for contact and mechanical vector transmission of vesicular stomatitis virus New Jersey in pigs. *Am. J. Vet. Res.* **60**: 43–48.
22. COMER, J.A., D.E. STALLKNECHT & V.F. NETTLES. 1995. Are vesicular stomatitis viruses maintained by sand flies by vertical transmission. *Boletin de la Direccion de Malariologia.* **35**: 115–120.
23. COMER, J.A., D.E. STALLKNECHT & V.F. NETTLES. 1995. Incompetence of domestic pigs as amplifying host of vesicular stomatitis virus for *Lutzomyia shannoni* (Diptera: Psychodidae). *J. Med. Ent.* **32**: 741–744.
24. COMER, J.A., D.E. STALLKNECHT & V.F. NETTLES. 1995. Incompetence of white-tailed deer as amplifying host of vesicular stomatitis virus for *Lutzomyia shannoni* (Diptera: Psychodidae). *J. Med. Ent.* **32**: 738–740.