

## Biological Transmission of Vesicular Stomatitis Virus (New Jersey Serotype) by *Simulium vittatum* (Diptera: Simuliidae) to Domestic Swine (*Sus scrofa*)

DANIEL G. MEAD,<sup>1</sup> ELMER W. GRAY,<sup>2</sup> RAYMOND NOBLET,<sup>2</sup> MOLLY D. MURPHY,<sup>3</sup>  
ELIZABETH W. HOWERTH,<sup>3</sup> AND DAVID E. STALLKNECHT<sup>4</sup>

Southeastern Cooperative Wildlife Disease Study, College of Veterinary Medicine, The University of Georgia,  
Athens, GA 30602

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**ABSTRACT** The role of hematophagous arthropods in vesicular stomatitis virus (New Jersey serotype; VSV-NJ) transmission during epizootics has remained unclear for decades in part because it has never been shown that clinical or subclinical disease in a livestock host results from the bite of an infected insect. In this study, we investigated the ability of VSV-NJ-infected black flies (*Simulium vittatum* Zetterstedt) to transmit the virus to domestic swine, *Sus scrofa* L. Experimental evidence presented here clearly demonstrates that VSV-NJ was transmitted from black flies to the swine. Transmission was confirmed by seroconversion or by the presence of clinical vesicular stomatitis followed by seroconversion. Our results represent the first report of clinical vesicular stomatitis in a livestock host after virus transmission by an insect.

**KEY WORDS** vesicular stomatitis, insect transmission, black fly, Simuliidae, clinical disease, vector competence

THE NEW JERSEY SEROTYPE of vesicular stomatitis virus (VSV-NJ) is one of the causative agents of vesicular stomatitis (VS), an economically important disease of livestock. This virus is enzootic from northern South America to southern Mexico (Rodriguez 2002). In the United States, VSV-NJ is enzootic on Ossabaw Island, GA (Comer et al. 1990, 1992) and is the serotype most often associated with the recurring and unpredictable VS epizootics in the western United States.

Virus isolations from field collected insects followed by vector competency studies of suspected vectors have led to the identification of sand flies and black flies as enzootic and epizootic vectors, respectively. While sand flies (*Lutzomyia* spp.) are recognized as important enzootic vectors of VSV-NJ, they are not believed to be important epizootic vectors because of their limited flight range (Ward 1989). Black flies have been on the list of suspected VSV-NJ epizootic vectors since the 1950s (Hanson 1952); however, the vectorial competence of black flies for VSV-NJ using an animal model has only recently been documented (Mead et al. 1999).

On Ossabaw Island, GA, *Lutzomyia shannoni* Dyar has been identified as a biological vector of VSV-NJ (Comer et al. 1990). Experimental infection of *L. shannoni* by oral and intrathoracic inoculation resulted in virus replication by both routes. Both orally and parenterally infected *L. shannoni* transmitted VSV-NJ by bite to susceptible rodents (Comer et al. 1990). In addition, field evidence (Comer et al. 1992) coupled with laboratory confirmation demonstrated that transovarial transmission of VSV-NJ occurs in *L. shannoni* (Comer et al. 1990).

Theiler and Downs (1973) first reported the isolation of VSV-NJ from black flies, *Simulium exiguum* Roubaud, collected near Medellin, Colombia. Since then, VSV-NJ has been isolated from two mixed pools of *S. vittatum* Zetterstedt and *S. bivittatum* Malloch collected in Colorado during the 1982–1983 VSV-NJ epizootic (Francy et al. 1988) and from three pools of *Simulium* (*Psilopelmia*) spp. collected during the 1995 epizootic in New Mexico (Schmidtman et al. 1999). Laboratory findings have demonstrated that VSV-NJ isolates from enzootic and epizootic sites replicate in colonized (Cupp et al. 1992) and wild black flies (Mead et al. 1997) and that virus is present in the saliva of experimentally infected black flies. Recently, a mouse model was used to confirm the vectorial competence of *S. vittatum* for VSV-NJ (Mead et al. 1999).

All studies with suspected VSV-NJ vectors have used a capillary tube, rodent model, or both to determine vector competence. Similarly, investigations of

<sup>1</sup> E-mail: dmead@vet.uga.edu.

<sup>2</sup> Department of Entomology, The University of Georgia, Athens, GA 30602

<sup>3</sup> Department of Pathology, The University of Georgia, Athens, GA 30602

<sup>4</sup> Department of Medical Microbiology and Parasitology, The University of Georgia, Athens, GA 30602.

**Table 1.** Summary of clinical and serological response of domestic swine after exposure to VSV-NJ-infected and noninfected black flies

Pig ID	Route (no. of black flies feeding)	Serum antibody level			Clinical VS
		0	7	12	
60	Snout (5)	<1:8	(Euthanized on PID 3)		Yes
61	Abdomen (11) <sup>a</sup>	<1:8	<1:8	<1:8	No
62	Abdomen (2)	<1:8	1:256	1:512	No
63	Abdomen (4)	<1:8	1:128	1:256	No
64	Abdomen (1)	<1:8	1:64	1:64	No
65	Snout (14)	<1:8	1:256	1:1,024	Yes
66	Abdomen (11)	<1:8	1:256	1:2,048	No

<sup>a</sup> Noninfected black flies.

the pathologic and immunologic effects of experimental VSV-NJ infection in rodents or livestock hosts have relied on parenteral inoculation of VSV-NJ to produce infection. Clinical VS in a livestock host has yet to be produced experimentally by the bite of an infected insect. Here, our objectives were to determine if VSV-NJ infected black flies transmit the virus to a livestock host when blood-feeding and to document the clinical response to VSV-NJ infection associated with this route of exposure in the host by measuring the duration, source, and extent of virus shedding associated with infection. The use of animals in this study was approved by the University of Georgia's Institutional Animal Care and Use Committee (approval A2001-10076-m1).

### Materials and Methods

One- to 3-d-old *S. vittatum* females (IS-7 cytotype) from a continuous laboratory colony (Bernardo et al. 1986) were infected through intrathoracic inoculation with 1  $\mu$ l of a suspension containing 10<sup>6.9</sup> plaque forming units (pfu)/ml of a 1997 Colorado equine VSV-NJ isolate. Baseline infection rates and viral counts were determined in three black flies immediately after injection as previously described (Mead et al. 1997). Remaining infected black flies were maintained on 15% dextrose at 26 C for 3–4 d extrinsic incubation (EI).

Seven 30-lb pigs (*Sus scrofa* L.) were anesthetized (Telazol 2 mg/kg and xylazine 2 mg/kg, intramuscularly), and feeding cages containing between 30 and 50 black flies/cage were manually held on the abdomen or planum rostrale (snout) of each animal for at least 20 min. An estimate of the number of black flies that fed on each pig, as determined by dissection and observation of blood in the fly, was recorded (Table 1). Noninfected black flies were allowed to feed on the negative control animal.

Animals were examined daily for lesions, and blood was collected on postinfection days (PID) 1–5 through cranial vena cava puncture for virus isolation using Vero cells. Additional samples for virus isolation included swab samples from buccal cavity/tonsil of the soft palate (oral), and if vesicular lesions were present, epithelial surfaces of lesion sites. Swabs were placed in individual cryovials containing 1 ml transport medium (minimum essential medium supple-

mented with 1,000 U penicillin G, 1 mg streptomycin, 0.25 mg gentamicin sulfate, 0.5 mg kanamycin monosulfate, and 2.5  $\mu$ g/ml amphotericin B) and centrifuged at 3,000 rpm for 15 min. Then, 0.1 ml of the resulting supernatant fluids was used for virus isolation.

One pig (60) was killed on PID 3 by intravenous inoculation of barbiturate and necropsied. Pigs 62–66 were killed and necropsied on PID 12. Samples representing black fly bite sites, any lesions, pharynx and tonsil of the soft palate, and cervical, mandibular, and retropharyngeal lymph nodes were collected at necropsy for virus isolation.

Specific VSV-NJ neutralizing antibody response was determined using microtiter serum neutralization. A four-fold rise in VSV-NJ neutralizing antibodies (NA) in the presence or absence of clinical signs was considered evidence of virus transmission.

### Results

The baseline black fly infection rate was 100%. Infection virus titers ranged from 10<sup>3.4</sup> to 10<sup>3.9</sup> pfu/black fly at baseline and from 10<sup>4.5</sup> to 10<sup>5.7</sup> pfu/black fly after EI. As many as 14 infected black flies fed on an individual pig (mean, six black flies/pig; range, 1–14 black flies/pig; Table 1).

All pigs were seronegative on PID 0. Specific VSV-NJ neutralizing antibody titers rose with time in all pigs fed on by VSV-NJ infected black flies. At necropsy, seroconversion (as defined by a four-fold increase in NA titer) was detected in all pigs except for the control. Antibody titers were variable and ranged from 1:64 to 1:2,048 (Table 1).

Virus shedding and clinical disease, characterized by vesicular lesions, was present only in pigs (60 and 65) on which infected black flies fed on the planum rostrale. In both pigs, lesions began to develop on the planum rostrale of the snout on PID 1. In pig 65, almost the entire surface of the snout ventral to the nostrils was reddened and swollen, with pinpoint pale raised areas on PID 1 (Fig. 1). This proceeded to vesiculation of the area on PID 2 and subsequent rupture, erosion, and crusting by PID 3. Erosion persisted for several days, but by PID 7 the vesiculated area was almost healed. This pig developed secondary vesiculation of the upper lips and the tip of the tongue on PID 3. In pig 60, the skin immediately surrounding fly bites

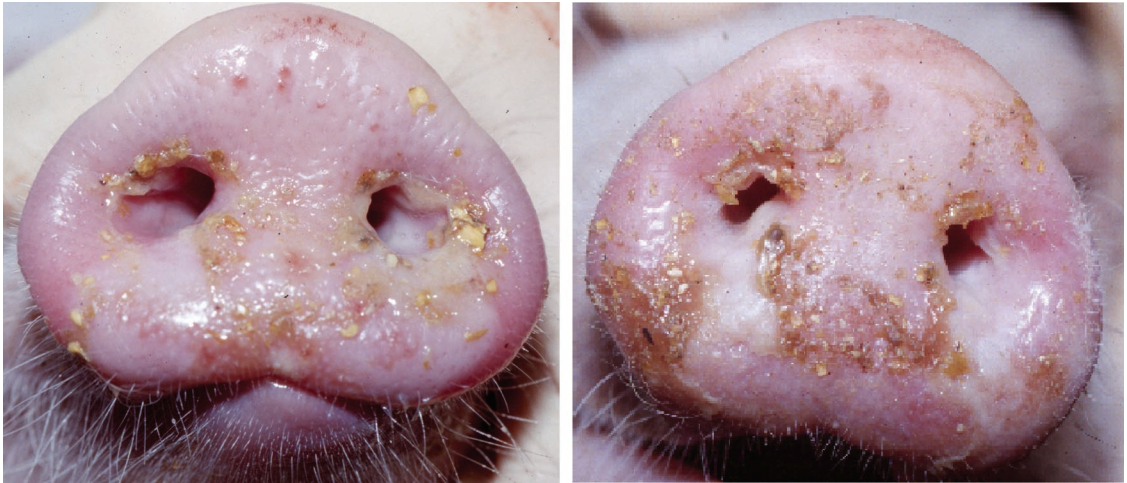


Fig. 1. Lesion on snout of pig 65 in area of black fly bites on PID 2 (left) and PID 5 (right). On PID 2, there is a large, raised area of vesiculation ventral to the nostrils. By PID 5, the vesicle had ruptured and the area was eroded.

ventral to the nostrils was elevated on PID 1. This proceeded to expanding vesiculation of the skin around these bites on PID 2 and 3. This pig also developed secondary vesicles on the rostral lower lip and tip of tongue on PID 3.

VSV-NJ was isolated from oral swabs from pigs 60 and 65 (snout exposure) on PIDs 2–3 and 3–5, respectively. VSV-NJ was recovered from snout lesion swabs from pig 60 on PIDs 1–3, and on PIDs 4–5 from pig 65. Maximum VSV-NJ titers from oral and snout lesion swabs were 5.26 and 3.60 TCID<sub>50</sub>, respectively.

Virus isolation attempts on whole blood and plasma from all pigs on PIDs 0–5 were negative. In addition, attempts to recover VSV-NJ from tissue samples taken at necropsy on PID 12 from pigs 62–66 were also negative. However, VSV-NJ was recovered from tonsil of the soft palate and snout tissue from pig 60 taken at necropsy on PID 3. Virus titers were 4.93 and 3.26 TCID<sub>50</sub> for these tonsil and snout tissues, respectively.

### Discussion

Information relating to the transmission of VSV-NJ is based largely on the observational, serological, and entomological studies conducted on Ossabaw Island, GA, and during the sporadic epizootics in the western United States. Despite repeated isolations of VSV-NJ from insects, their role in virus transmission during epizootics remains enigmatic. Previous efforts to elucidate the role of biting insects in VSV-NJ transmission have relied on experimental models that did not include natural livestock hosts. The current study clearly demonstrates that VSV-NJ infected black flies readily transmit the virus to swine when blood-feeding. Here, we describe the first report of experimental insect transmission of VSV-NJ to a livestock host. Swine were chosen as a livestock model because they are a natural host of VSV-NJ (Hanson 1952) and because clinical outcomes and patterns of virus shedding associated with different VSV-NJ exposure

routes are known for this animal (Howerth et al. 1997, Stallknecht et al. 1999).

The number of VSV-NJ infected black flies that fed on each pig was variable, and the number of potentially infectious bites received by each pig while the black flies were probing is unknown. On average, six VSV-NJ infected black flies blood fed on each pig. It is noteworthy that when a single infected black fly blood fed, sufficient virus was transferred to cause subsequent infection. The amount of VSV-NJ shed in saliva during probing and blood feeding is not known; however, in previous studies that used an in vitro method to collect saliva, VSV-NJ titers between 30 and 10<sup>4</sup> pfu/ml were detected in the saliva of experimentally infected *S. vittatum* (Cupp et al. 1992).

Our failure to detect viremia is consistent with previous experiments in swine (Van der Maaten 1986, Redelman et al. 1989, Clarke et al. 1996, Howerth et al. 1997, Stallknecht et al. 1999, 2001). To date, viremia has not been detected in any domestic animal species naturally or experimentally infected with VSV-NJ. Our findings suggest that clinical course of infection is related to infected black fly feeding site, which corroborates previous studies that demonstrated that route of VSV-NJ inoculation greatly influenced clinical outcome in swine (Clarke et al. 1996, Howerth et al. 1997, Stallknecht et al. 1999, 2001). Introduction of VSV-NJ into the snout, either through needle inoculation or insect bite, consistently resulted in lesion formation at the inoculation or bite site, respectively. Conversely, inoculation of the virus by needle or insect bite on haired areas, such as the abdomen, consistently resulted in seroconversion in the absence of lesion formation. These findings may offer an explanation for serological surveys conducted after VSV-NJ outbreaks in the western United States, which revealed that the majority of livestock exposed to VSV-NJ (as determined by seroconversion) never developed clinical VS (Walton et al. 1987, Mumford et al. 1998). The formation of vesicular lesions after VSV-NJ

transmission by black flies has added significance because VSV-NJ transmission through animal-to-animal contact also is possible if vesicular lesions are present (Shahan et al. 1946, Patterson et al. 1955, Howerth et al. 1997, Stallknecht et al. 2001). Additionally, vesicular lesions may provide a source of virus for insects feeding on or close to them as proposed by Francy et al. (1988).

The potential for vectors to amplify and transmit VSV-NJ during epizootics was suggested in 1942 (Heiny 1945); however, little has been done since then to identify the specific role of arthropods in the epizootology of this virus. The recent outbreaks of VS in livestock in the western United States have emphasized the need for a better understanding of the ecology of the disease in nature. Collectively, when one considers the previous field isolations of VSV-NJ from black flies (Theiler and Downs 1973, Francy et al. 1988, Schmidtman et al. 1999), the findings that black flies are competent laboratory vectors of VSV-NJ enzootic and epizootic isolates (Cupp et al. 1992, Mead et al. 1997), the demonstration of black fly transmission of VSV-NJ to rodents (Mead et al. 1999, 2000) and now swine, and the finding that a viremic host is not necessary for black fly infection (Mead et al. 2000), a major role for black flies, and possibly other hematophagous insects, in the epizootic aspects of the ecology of VS in the western United States is established.

Identification of the specific mechanisms of VSV-NJ transmission has important implications in regard to USDA-APHIS control measures. Based on these results, limiting VSV-NJ animal to animal contact transmission through the imposition of livestock quarantines on VS positive premises would provide only a partial solution. Restrictions on animal movement would be less effective where it has been shown that insects, such as black flies, play a role in biological transmission of VSV-NJ. Therefore, the presence of blood-feeding insects in VS epizootic regions must be considered in the development of VS control and eradication programs. In addition, these data support the need for protecting animals against insect feeding, as well as the need for basic insect control measures.

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