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EXPERIMENTAL INFECTION OF *DIDELPHIS MARSUPIALIS* WITH VESICULAR STOMATITIS NEW JERSEY VIRUS

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ABSTRACT: Although vesicular stomatitis has been present for many years in the Americas, many aspects of its natural history remain undefined. In this study, we challenged five adult Virginia opossums (*Didelphis marsupialis*) with vesicular stomatitis New Jersey serotype virus (VSNJV). Opossums had no detectable antibodies against VSNJV prior to being inoculated with $10^{6.5}$ median tissue culture infective doses ($TCID_{50}$) of VSNJV by two routes; intraepithelial/subepithelial (IE/SE) inoculation and scarification in the muzzle (SM). Clinical response was monitored daily and animals were tested for viral shedding. All infected animals developed vesicles and ulcers on the tongue and inflammation of the nasal alar folds. Virus was isolated from esophagus-pharynx, nasal, and from ocular swabs and lesions samples. The failure to detect viremia in these animals indicates that a source other than blood may be required for transmission to insect vectors. Our results suggest that *D. marsupialis* could play a role in the maintenance of VSNJV outside of domestic animal populations and could provide a model to study vesicular stomatitis virus pathogenesis.

Key words: *Didelphis marsupialis*, host, opossum, vesicular stomatitis virus.

INTRODUCTION

Vesicular stomatitis (VS) is caused by several viruses in the genus *Vesiculovirus*, family *Rhabdoviridae*. This disease primarily affects domestic cattle, horses, and swine, but also has been observed infrequently in camelids, sheep, goats, and some wildlife species; humans can also become infected, producing an acute, febrile, influenza-like illness (Thorne et al., 1983; Letchworth et al., 1999; McCluskey and Mumford, 2000).

Outbreaks of clinical disease are associated with infection by two vesicular stomatitis virus (VSV) serotypes, New Jersey (VSNJV) and Indiana (VSIV), with VSNJV being the most-common cause of clinical cases in Colombia, South America. Although VSV has been the object of exhaustive studies, limited advances have been achieved in understanding its ecology and epidemiology. Therefore, investigations of the wild and domestic maintenance and transmission cycles of the virus are needed to identify potential vectors and reservoirs and their role in transmission and persistence of VSV in enzootic areas. In addition, it is also important to identify the ecologic conditions (vegetation, temperature, rainfall, and relative humidity, among others), that allow the maintenance and re-emergence of VSV in endemic areas.

Some wild herbivores utilize areas occupied by domestic livestock, but their role as possible reservoir hosts for VSNJV remains speculative (Hanson and Brandly, 1957, cited by Yuill 1981; Stallknecht and Erickson, 1986; Webb et al., 1987). Currently, the natural reservoir for the VSVNJ has not been identified, but neutralizing antibodies have been detected in numerous wildlife species in enzootic areas of Central America including anteater (*Tamandua tetradactyla*), common opossum (*Didelphis marsupialis*), two-toed sloths (*Choleopus hoffmani*), spider monkeys (*Ateles* spp.), spiny rats (*Proechimys semispinosus*), and fruit bats (*Artibeus* spp.), among others (Tesh et al., 1969; Rodriguez et al., 1990). Experimental subcutaneous inoculation of VSNJV and VSIV into wildlife species from Panama demonstrated rapid development of antibodies against these viruses in 13

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species of rodents, two species of marsupials, two species of bats, two nonhuman primates, one species of rabbit, one species of edentate, and one carnivore species (Tesh et al., 1970). In the same study, age had a significant effect on susceptibility to both serotypes; adult mammals remained asymptomatic after a subcutaneous inoculation with VSV, while death resulted in sucking wild mice, hamsters, marmosets, opossums, and anteaters 2–4 days after a subcutaneous inoculation. These animals exhibited damages to the central nervous system, but vesicular lesions were not detected.

In Colombia, studies published by Zuluaga and Yuill (1979) and Arboleda et al. (2001) suggest that the common opossum is a good candidate to serve as a reservoir of VSV; it is abundant and usually has a high prevalence of VSV antibodies. This species also is highly adaptable to habitat changes related to human activity and could serve as a local source for VSV transmission to humans and domesticated animals.

Our objective was to determine experimentally if *D. marsupialis* could serve as a possible reservoir species, or amplifier host, of VSNJV.

MATERIALS AND METHODS

Six wild opossums, four males and two females (all adults), of approximately 1.5 kg, were captured using Tomahawk traps and a bait made of peanut butter, oatmeal flakes, and vanilla essence (Mills et al., 1995). The two females were nursing one, and three, suckling baby opossums; according to their size, young were about 1 mo old. All young were found inside the pouch and looked healthy. Animals were trapped on a farm 40 km to the north of the city of Medellín (Antioquia, Colombia) with approval from local authorities (Corporación Autónoma Regional de Antioquia). Before experimental infection, captured opossums were acclimated for 1 mo. Food consisting of a combination of fruits, eggs, and pet food concentrate and water were provided ad libitum. Opossums were kept in individual cages of 80×40×60 cm and handled according to the University's committee regulations for animal experimentation.

Prior to experimental infection, animals were clinically examined and did not show any evidence of illness, metabolic abnormalities, or physiologic stress. Furthermore, they were tested for VSNJV and VSIV antibodies using the serum neutralization assay.

The VSNJV used in this study was obtained from the immunovirology group of the University of Antioquia (Dr. María T. Rugeles). The virus had been originally isolated from a clinically ill cow at the Instituto Colombiano Agropecuario. This virus was intracerebrally inoculated in suckling mice and identified through serum neutralization assay in BHK-21 cells (American Type Culture Collection [ATCC], Rockville, Maryland, USA). Subsequently, the virus was amplified through one passage in BHK-21 cells and titered on Vero cells (ATCC) reaching $10^{7.5}$ median tissue culture infective doses ($TCID_{50}$).

Animals were housed in individual cages covered with a fine insect screen to avoid contact with vectors. Animals were sedated with an intramuscular injection of ketamine (100 mg/kg) and xylazine (10 mg/kg) and injected with $10^{6.5}$ $TCID_{50}$ of VSNJV in 100 µl of Eagle's minimum essential medium (MEM) with Earle's salts (Sigma Chemical Company, St. Louis, Missouri, USA) supplemented with 1% heat-inactivated fetal bovine serum (FBS; Difco Laboratories, Detroit, Michigan, USA). Three males were inoculated by intraepithelial/subepithelial (IE/SE) route and identified as 1IE/SE, 2IE/SE, and 3IE/SE; a male and a female (lactating with three offspring) were inoculated by scarification in the muzzle (SM) and identified as 4SM (male) and 5SM (female); and a remaining female was mock inoculated with viral isolation media by the IE/SE route.

For the inoculation, the virus was applied 2.5 cm proximal to the apex of the tongue. For the SM inoculation, each opossum was pricked 20 times on the snout and an inoculum of 100 µl of the virus suspension was added over the lesion and left there for 10 min; remaining liquid was dried off with a sterile gauze. All animals were examined daily for vesicular lesions and bled prior to inoculation and at 0, 12, 24, 48, 72, 96, 120, and 144 hr postinoculation (PI). Approximately 1.5 ml of blood per animal was obtained through cardiac puncture. A portion of the sample was transferred to tubes with anticoagulant (EDTA 100 UI/ml) for viral isolation in BHK-21 cell cultures; the other portion of the sample was placed in tubes without anticoagulant for

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VSNJV and VSIV antibody testing by serum neutralization.

Samples of vesicular lesions, esophagus-pharynx, nasal, ocular, and rectal mucosa were obtained using sterile swabs that were placed in 1.5 ml of transport media consisting of MEM supplemented with 2% FBS (Difco) and antibiotics (penicillin 1,000 U/ml, streptomycin 1 μg/ml, amphotericin B 2.5 μg/ml; Sigma). Swab samples were then stored at −70 C until they were processed. Urine and feces samples were collected from all the animals and were stored at −70 C in vials with 1.5 ml transport media. Samples of tonsils, submandibular ganglion, kidney, lung, spleen, liver, and brain were obtained at 21 days PI, after the animals were euthanized. Tissues were stored at −70 C in sterile plastic tubes with 5 ml of transport media.

For sample preparation for virus isolation, blood samples were thawed, mixed by vortex, and diluted 1:5 in MEM supplemented with 2% FBS. For swab samples, fluid from swabs was drained against the walls of the vials and swabs were discarded. Samples were then mixed by vortex and centrifuged at 1500 × G for 10 min at 4 C. Tissues were thawed, sliced in 3×3×3 mm cubes, placed in vials with 0.5 ml MEM supplemented with 2% of FBS, and macerated with sterile homogenizers. An additional 0.5 ml of medium was added and mixed by vortex, and tubes were centrifuged at 1500 × G for 20 min at 4 C. Urine and feces were thawed and mixed in vortex, diluted 1:10 in MEM supplemented with 2% FBS and 2% antibiotics, and then centrifuged at 12,000 × G for 20 min.

For virus isolation, 100 μl of the diluted sample (blood), supernatant (swab, feces/urine/tissue) was inoculated on 1-day-old monolayers of BHK-21 cells (2×10^5 cells/well) cultured in 24-well plates. Plates were incubated for 1 hr at 37 C with rocking every 10 min. After this time, inoculum was removed and replaced by 1 ml of fresh medium (MEM supplemented with 2% of FBS); inoculated cells were incubated at 37 C in 5% CO_2. Plates were subsequently observed at 24, 48, and 72 hr PI for cytopathic effect (CPE). Two passages in BHK-21 cells were done, and samples were considered negative if CPE was not observed. The $TCID_{50}$ for positive samples was determined in a 96-well plate following the protocol described by Rovozzo and Burke (1973) and Ballew (1992); viral titers were calculated by the method of Reed and Muench (1938).

Sera from opossums were tested by VSVNJ serum neutralization assay in 96-well plates as previously described by Arboleda et al. (2001). Briefly, sera were inactivated at 56 C for 30 min, and twofold dilutions were performed beginning with 1:4 up to 1:1,024. A challenge dose of 100 $TCID_{50}$ of each viral serotype (VSNJV and VSIV) in a volume of 50 μl were combined with equal volumes of each serum dilution. The mixture was allowed to incubate for 60 min at 37 C, and 100 μl was added to each of four wells in 96-well plates with BHK-21 cells (seeded 24 hr prior to testing). Plates were incubated at 37 C with 5% CO_2 and observed daily for 72 hr for CPE.

Cell controls (MEM and cell monolayers), test sera controls (diluted serum plus maintenance medium), as well as virus controls (MEM and viruses infecting cell monolayer) were included in each plate. A positive bovine serum, with a neutralizing titer of 4.05 $TCID_{50}$ (expressed as the reciprocal logarithm of the dilution) to both VSNJV and VSIV (kindly provided by Dr. Guillermo Suarez Restrepo, VECOL SA, Bogotá), and a negative opossum serum that had previously been shown to be negative for the presence of antibodies against VSNJV, were also included in each test as positive and negative controls, respectively, for the serum neutralization tests. Serum samples were considered positive if the antibody titer was ≥32 (Cuartas and Muñoz, 2003; Nunamaker et al., 2003)

In order to determine the susceptibility of the possum's offspring to VSNJV, two out of the three offspring from one of the females (5SM) were subcutaneously inoculated with $10^{6.5}$ $TCID_{50}$ of VSNJV in a 40-μl volume; a third one was used as a negative control. Inoculated animals were observed for 7 days.

RESULTS

Lesions or clinical signs (weakness, anorexia, diarrhea, and salivation) were not observed at 24 hr PI (Table 1). Two of the animals inoculated in the tongue had lesions at 48 hr PI, with loss of continuity in epithelium, and irregular borders, but without vesicles or blood. Other lesions were observed; on cheeks, with hemorrhage (ecchymoses and petechiae), but without any type of secretion. By 72 hr PI, all animals inoculated via IE/SE had lesions at the inoculation site with ulcers that extended farther than the region of inoculation. Further, the lateral tongue epithelium sloughed off, leaving a reddened superficial ulcer with ragged mar-

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TABLE 1. Clinical monitoring of opossums infected with VSNJV.

Animal ID (via inoculation)[a]	Days postinfection (DPI)[b]								
	0.5	1	2	3	4	5	6	14	21
1 IE/SE ♂	−	−	++	+++++	+++++	+H	+H	CH	−
2 IE/SE ♂	−	−	+++++	+++++	+++++	+H	+H	CH	−
3 IE/SE ♂	−	−	+++++	+++++	+++++	+H			
4 SM ♂	−	−	−	+++	+++	+H	+H	CH	−
5 SM ♀	−	−	+	++++	+++	+H	+H	CH	−
Mock ♀	−	−	−	−	−	−	−	−	−

[a] IE/SE = intraepithelial/subepithelial; SM = inoculation by scarification in the muzzle; ♂ = male; ♀ = female.

[b] − = without typical signs of the illness; + = lesion beginning in inoculation site; ++ = beginning of vesicular laceration outside of the inoculation site; +++ = inflammation and erosion in nasal nostril; ++++ = inflammation and erosion in nasal nostril with nasal discharge; +++++ = injury widespread with loss of the epithelium of the tongue; +H = beginning of healing process; HC = complete healing.

gins (Table 1). Later, erosions with irregular borders became deeper and bloody, but no vesicles appeared (Fig. 1). Animals infected via SM had inflammation of the nasal alar folds with erosions on the nasal septum, and one of the opossum had abundant nasal serous discharge in both nostrils. By day 5 PI, the healing process of the lesions began, and no vesicles were detected. At days 14 and 21 PI, the lesions had healed completely, and the animals remained in good health.

The suckling young of the female infected by scarification (5SM) had a normal growth without any type of disease or lesions. The mock-inoculated female did not show any clinical, physiologic, or behavioral change during the 21 days of observation.

The identity of isolated viruses was confirmed by a virus neutralization test using a specific serum against VSNJV. All blood samples tested negative by virus isolation, but VSNJV was isolated from swabs obtained from the esophagus-pharyngeal tract of all five of five infected opossums. In IE/SE inoculated animals, virus was detected from days 1–5 PI, whereas in animals infected via SM, virus was not detected in the oral cavity until day 3 PI. Nasal swabs from four of the five infected opossums were positive by virus isolation. In one male (3IE/SE), virus was isolated from 12 hr until day 5 PI (Table 2); VSNJV was also isolated from 48 hr to day 5 PI from tongue lesions of animals infected via IE/SE (Table 2). Virus was isolated from ocular swabs from two opossums (5SM and 3IE/SE) on days 2 and 3 PI, respectively (Table 2). We did not detect the virus at the site of inoculation of the snout of opossums infected via SM, and we did not detect any other virus from day 6 through day 21 PI. All samples of urine and tissues from infected opossums were negative for virus isolation; VSNJV was isolated only from a single stool sample, taken from animal 2IE/SE on day 3 PI (Table 2).

We isolated VSNJV from samples collected from 12 hr until day 5 PI, with viral titers ranging from 1.0 to 5.75 $TCID_{50}$/ml. Highest viral titers were found in samples taken of esophageal-pharyngeal (5.25 $TCID_{50}$/ml) and nasal swabs (5.75 $TCID_{50}$/ml) from opossum 2IE/SE, samples which were obtained 24 hr PI before showing any clinical signs compatible with VSV infection (Table 3).

Samples obtained from animals inoculated via IE/SE had higher viral titers than those of animals that were infected via SM. Similarly, higher titers were found in samples that were taken between days 1 and 2 PI prior to the detection of lesions (Table 3).

Before the experimental infection, all opossums were negative for the presence

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FIGURE 1. Lesion with loss of the epithelium extending towards the apex of the tongue.

TABLE 2. Viral isolation from samples of experimentally infected opossum with VSNJV.

Route[a]	ID/Isolation source[b]		Days postinfection (DPI)[c]								
			0.5	1	2	3	4	5	6	14	21
IE/SE	1 ♂	EP	−	+	+	+	+	−	−	−	−
		L	−	−	+	+	−	−	−	−	−
	2 ♂	EP	−	+	+	+	+	+	−	−	−
		N		+	+	+	+	−	−	−	−
		L	−	−	+	+	−	−	−	−	−
		F	−	−	−	+	−	−	−	−	−
	3 ♂	EP	−	+	+	+	+	+	−	−	−
		N	+	+	+	+	+	+	−	−	−
		L	−	−	+	+	+	+	−	−	−
		O	−	−	−	+	−	−	−	−	−
SM	4 ♂	EP	−	−	−	−	+	−	−	−	−
		N	−	−	+	+	−	−	−	−	−
	5 ♀	EP	−	−	−	+	+	+	−	−	−
		N	−		+	−	−	−	−	−	−
		L	−	−	+	−	−	−	−	−	−
		O	−	−	+	−	−	−	−	−	−
Mock ♀			−	−	−	−	−	−	−	−	−

[a] IE/SE = intraepithelial/subepithelial; SM = inoculation by scarification in the muzzle.

[b] ♂ = male; ♀ = female; EF = esophagus-pharyngeal swab; N = nasal swab; O = ocular swab; F = feces; L = lesions.

[c] − = virus not detected; + = virus isolated.

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TABLE 3. Viral titers from samples of opossum infected with VSNJV.

Animal identification (inoculation via)[a]	Days postinfection (DPI)[b,c]								
	0.5	1	2	3	4	5	6	14	21
1 IE/SE ♂	ND	EP=2.0	EP=2.0 L=2.25	EP=2.0 L=2.0	EP=2.0	ND	ND	ND	ND
2 IE/SE ♂	ND	EP=5.25 N=5.75	EP=2.25 N=4.25 L=4.0	EP=3.25 N=4.0 L=2.75 F=3.0	EP=1.0 N=2.0	EP=1.0	ND	ND	ND
3 IE/SE ♂	N=2.25	EP=2.0N=2.25	EP=1.0 N=2.25 L=3.0	EP=2.25 N=2.25 O =2.25 L=2.0	EP=1.0- N=1.75 L=1.0	EP=2.0 N=1.0 L=1.0	ND	ND	ND
4 SM ♂	ND	ND	N=2.25	N=2.0	EP=2.0	ND	ND	ND	ND
5 SM ♀	ND	N=3.25	N=2.25 O =3.75 L=4.25	EP=1.0	EP=1.0	EP=1.0	ND	ND	ND
Mock ♀	ND	ND	ND	ND	ND	ND	ND	ND	ND

[a] IE/SE = intraepithelial/subepithelial; SM = inoculation by scarification in the muzzle; ♂ = male; ♀ = female.

[b] Viral titers expressed in $TICD_{50}$/0.1 ml.

[c] ND = virus not detected; EF = esophagus-pharyngeal swab; N = nasal swab; O = ocular swab; F = feces; L = lesions.

of antibodies to VSNJV and VSIV (titer <1.5 log correspondent to 1:32 dilution). All sera remained negative up to 48 hr PI. On day 3 PI, neutralizing antibodies were detected in three of the five infected opossums, with titers of 32. In general, at 5 days PI, all experimentally infected animals seroconverted, showing increasing titers throughout the experiment that reached >512 by 14 days PI (Table 4). Seroconversion was delayed in animals that were experimentally inoculated via SM. None of the three suckling opposums with VSVNJ had lesions, but they did seroconvert with antibody titers >128 (data not shown).

DISCUSSION

Results of this experimental infection demonstrate the susceptibility of the opossum, *Didelphis marsupialis*, to VSNJV through two different inoculation routes, IE/SE and SM. Our results were partially consistent with the ones reported by Tesh et al. (1970). We did not observe mortality or any signs of disease in suckling opposums, but we did observe infection with seroconversion between day 4 and 6 PI in adults. Because we detected antibodies in both challenged and negative control suckling opossums, we believe that passively transferred antibodies from the mother (5SM female) may have protected these progeny from viral effects.

Antibody titers were determined based on 100 $TCID_{50}$ of VSNJV. While this allowed for accurate detection of an antibody response, reported antibody titers cannot be directly related to regulatory positive thresholds. The Office International des Epizooties (OIE, currently World Organisation for Animal Health), protocol recommends using 1,000 $TCID_{50}$ for the serum neutralization assay for domestic animals (OIE, 2008).

Although virus was isolated from many samples, titers were normally lower than the inoculated dose. While this does not demonstrate significant amplification, virus

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TABLE 4. Neutralizing antibodies titers from opossum infected with VSNJV by IE/SE and SM.

Animal identification[a]	Days postinfection (DPI)								
	0	1	2	3	4	5	6	14	21
1 IE/SE ♂	0.60[b]	0.60	0.91	1.20	1.50	2.11	2.61	2.71	2.71
2 IE/SE ♂	0.60	0.92	0.90	1.50	2.33	2.41	2.41	≥2.71	>3.01
3 IE/SE ♂	0.60	0.60	0.90	1.52	2.11	2.11			
4 SM ♂	0.60	0.60	1.22	1.20	1.20	1.50	1.82	2.11	2.71
5 SM ♀	0.60	1.20	1.20	1.50	1.52	1.80	2.11	2.63	>2.71
Mock ♀	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60

[a] IE/SE = intraepithelial/subepithelial; SM = inoculation by scarification in the muzzle; ♂ = male; ♀ = female.
[b] Reciprocal logarithm of the serum dilution.

was detected at relatively high viral titers between days 2 and 6. This demonstrates both replication and the possibility that this species could serve as a source of infection for other susceptible host or vector species. In our work, viremia was never detected, which could indicate a localized rather than a systemic infection. This is similar to what has been previously shown in cattle and swine by Rodriguez et al. (1990) and Stallknecht et al. (2004), respectively, but is in contrast with previous studies by Arbeláez and Rocha (1984) and Arbeláez and Valbuena (1987), who demonstrated VSNJV in the blood of a bovine animal experimentally infected by the IE/SE route and by nasal instillation. In these experiments, however, the duration of this viremia was only 24 hr and, to date, these results have not been replicated in any subsequent studies. On the other hand, viremia has also been previously reported in young rodents (Cornish et al., 2001).

The inability to isolate VSV from the blood of experimentally infected wild animals has been previously shown by other investigators. Karstad and Hanson (1957) described the presence of fever and mouth lesions in three out of four deer infected via IE/SE and no viremia in the first 72 hr after PI. The experimental infection of two pronghorn antelope (*Antilocapra americana*) with VSNJV via IE/SE (Thorne et al., 1983, cited by Webb et al., 1987) produced similar results to the ones described in deer. We were also able to detect virus excretion through different routes, but never in blood samples; therefore, we propose that these sites could be a possible source of infection for mechanical vectors, a possibility that has been proposed by others in earlier investigations (Ferris et al., 1955; Mason, 1978; Mead et al., 2000).

The apparent incubation time of VSV in opossums experimentally infected through the routes used in this study was 48 hr, which is similar to the time described for cattle experimentally infected with VSNJV and VSIV (Mason, 1978; Yuill, 1981; Arbeláez and Rocha, 1984; Letchworth et al., 1999; Schmitt, 2002). The clinical signs, and the extension and kinetics of lesions observed in experimentally infected opossums, were similar to the ones described for bovine, equine, and porcine subjects when these were experimentally inoculated (Howerth et al., 2006). However, our marsupials did not show profuse salivation, vesicles, or lesions in the feet, as occurs in some of the other above-mentioned domestic mammals.

The two routes used for inoculation, IE/SE and SM, caused different lesions, with the IE/SE route being responsible for the most-severe clinical manifestations. This observation was confirmed in one of the infected animals, which had severe lesions not only at the inoculation site, but also developed lesions on the right cheek, located in a place distant from the inoculation site. In general, after 72 hr

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PI, secondary vesicles were not observed in the five experimentally infected opossums.

From a clinical point of view, it was possible to establish the susceptibility of this species to experimental infection as well as its capacity of shedding the virus through several routes. To the best of our knowledge, this is the first report which shows that *D. marsupialis* could play a role in the epidemiology of the virus, and it leaves the door open for future studies about its use as an animal model for the infection and the illness. Even though *Sus scrofa* (domestic pig), a natural host for VSV infection, has also been used and proposed as a good model to study mechanisms of viral pathogenesis for this agent (Redelman et al., 1989), the opossum *D. marsupialis* may also be considered a good and convenient model, based on low sustainability expenses, high prolific capability, its abundance in tropic areas of the world where VSV is endemic, and its adaptability to be kept in farms under captivity or laboratory conditions (De Souza et al., 1992; Jaramillo et al., 1992; Cuartas and Muñoz, 2003). *Didelphis marsupialis* could become an excellent alternative to the domestic species in the investigation of routes of infection and dissemination of VSV under natural conditions and could help to experimentally test the real capacity of vectors in the transmission of this virus. These findings support the susceptibility of *D. marsupialis* to VSNJV and, because it is one of the species most abundantly found not only in the wilderness but also in peridomestic environments of tropical regions of Colombia, we propose that opossums could participate in the VS viral cycle. The opposum could not only serve as a host for different vectors in the forest, but also as an intermediary host for transmission of VSNJV to other susceptible animals such as pigs, cows, and horses in rural areas, through direct contact or by contamination of inanimate objects (fomites and vehicles).

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