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Response to the experimental inoculation of *Sigmodon hispidus* (Rodentia: Muridae) with vesicular stomatitis virus, New Jersey serotype

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ABSTRACT

Sigmodon hispidus (cotton rat) was previously found to have antibodies against Vesicular Stomatitis Virus, New Jersey serotype (VSV-NJ) under field conditions in Costa Rica. In order to evaluate their response to the virus and potential role as reservoirs, a total of 30 adult laboratory-raised cotton rats were experimentally inoculated with 10^7 $TCID_{50}$/ml VSV-NJ, Greentree strain, as follows: 10 by subcutaneous route (group A), 10 by oral abrasion (group B) and 10 by intranasal route (group C). Two negative control animals were included in each group. Animals developed neutralizing antibody titers ranging between 1:10-1:2560 six days after the inoculation. This species showed an immune response to Vesicular Stomatitis Virus by increasing antibody titers after inoculation, and most individuals maintained their antibody titers over time. However, live virus and replication of the virus in the host could not be demonstrated by virus isolation attempts in tissue culture methods. Hyperactivity was the only clinical sign observed in the subcutaneous and oral abrasion groups. The intranasal group presented dyspnea, depression, and weight loss. In this group, 7 of the inoculated animals died 6 days post inoculation and the last one died on day 37 post inoculation. The necropsy of the animals in this group revealed pneumonia, hyperemia in the intranasal bone, and cerebral and hepatic congestion. Control animals did not show antibody development or clinical signs. After 122 days post-inoculation, case fatality rate was 12.5% in group A, 43% in group B, and 100% in group C.

KEYWORDS: *Sigmodon hispidus*, vesicular stomatitis, Costa Rica, cotton rat, transmission.

Respuesta de *Sigmodon hispidus* (Rodentia: Muridae) a la inoculación experimental con el virus de la estomatitis vesicular serotipo New Jersey

RESUMEN

Se han reportado anticuerpos contra el Virus de Estomatitis Vesicular, serotipo New Jersey (VEV-NJ), en *Sigmodon hispidus* (rata algodonera) libres en Costa Rica. Con el fin de evaluar su respuesta ante el virus y su potencial rol como reservorio, treinta ratas algodoneras adultas, criadas en laboratorio, fueron inoculadas experimentalmente con 10^7 $TCID_{50}$/ml VEV-NJ de la siguiente manera: 10 vía subcutánea (grupo A), 10 por abrasión oral (grupo B) y 10 por vía intranasal (grupo C). Se incluyeron dos animales como control negativo para cada grupo. Todas las ratas inoculadas presentaron títulos de anticuerpos neutralizantes en un rango entre 1:10-1:2560 a partir del día 6 post-inoculación. Esta especie mostró un incremento en el título de anticuerpos contra el VEV-NJ, que se mantuvo durante todo el tiempo de observación. Sin embargo, la presencia del virus y su replicación no se pudieron determinar en las muestras recolectadas por medio de la técnica de aislamiento viral. La hiperactividad fue el único signo clínico observado en los animales inoculados por vía subcutánea y abrasión oral. En el grupo inoculado por vía intranasal se observó disnea, depresión y pérdida de peso. En este grupo, 7 de los animales inoculados murieron en los primeros 6 días y uno al día 37 post-inoculación. A la necropsia estos presentaron neumonía, hiperemia en el hueso intranasal, así como congestión cerebral y hepática. Los animales control no presentaron títulos de anticuerpos neutralizantes, ni signos

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clínicos durante todo el estudio. Luego de 122 días post- inoculación, la tasa de mortalidad fue de 12.5% en el grupo A, 43% en el grupo B y 100% en grupo C.

PALABRAS CLAVES: *Sigmodon hispidus*, estomatitis vesicular, Costa Rica, rata algodonera, transmisión.

ABBREVIATION KEY

D-MEM= Dulbecco's Minimal Essential Medium
EDTA= Ethylenediaminetetraacetic acid
IN=Intranasal
ND=Not determined
OB=Oral abrasion
RNA= Ribonucleic acid
SC= Subcutaneous
$TCID_{50}$= Median tissue culture infective dose
VSV= Vesicular Stomatitis Virus
VSV-NJ= Vesicular Stomatitis Virus, New Jersey serotype

INTRODUCTION

Vesicular Stomatitis Virus (VSV) is a negative sense, single stranded RNA virus belonging to the Rhabdoviridae family. VSV causes symptoms that resemble foot and mouth disease (Chamizo, 1995). It targets primarily cattle, pigs and horses causing lesions on their mouth, tongue and coronary bands of the hooves (McCluskey and Mumford, 2000). The loss to cattle herds can be considerable, as animals must be quarantined until cleared from foot and mouth disease (Rodríguez, 2002). Humans can develop flu-like symptoms, and in severe cases, encephalitis (Letchworth et al*.,* 1999). In endemic areas like the Western Hemisphere, rodents have been identified as possible reservoirs (McCluskey and Mumford, 2000).

VSV is transmitted through contact with infected lesions, saliva and fomites. VSV maintenance and transmission under natural conditions is still poorly studied. A natural reservoir that may serve as maintenance or amplifying host or as a source of the virus for haematophagous insects has been proposed (Davis et al*.,* 1972) but not demonstrated.

Observational studies have shown that white tailed deer (*Odocoileus virginianus)* (Jenney et al*.,* 1970), swines (Stallknecht et al., 1987), rodents (Jiménez et al*.,* 1996) and other mammal species (Yuill and Zuluaga, 1979; Aguirre et al., 1992) present antibodies against VSV-NJ in their natural environment.

Experimental studies to evaluate the role of wildlife animals as potential reservoirs have been conducted. White tailed deer, feral swine (Comer et al*.,* 1994), Syrian hamsters (Fultz and Holland, 1985), *Peromyscus manipulatus* (deer mouse) (Cornish et al*.,* 2001) and cotton rats (Sun et al*.,* 1984) have been tested; however, only viraemia was found in *P. manipulatus*, using immunohistochemistry and *in situ* hybridization.

In Costa Rica *Sigmodon hispidus* were found to have antibodies against VSV (Jiménez et al*.,* 1996). The present study was conducted in order to evaluate the susceptibility of this species to the experimental inoculation with VSV-NJ.

MATERIAL AND METHODS

Sigmodon hispidus rodents were obtained from a newly established laboratory colony reared at the School of Veterinary Medicine at Universidad Nacional, Costa Rica, making sure that the introduced field rodents were negative to VSV- serotype New Jersey and VSV- serotype Indiana antibodies by serum neutralization assay (titers less than 1:10), following the method described by Rodríguez et al. (1990) and modified by Jiménez et al. (1996), and that close related rats were not mixed. The rats used in the experiment were all born in the colony; no new rats were introduced to the colony 6 months prior to the beginning of the study. Thirty *S. hispidus* of individual body weight of 110g or more and healthy to the physical examination were selected. Prior to inoculation, all rodents were bled and tested for VSV-NJ by serum neutralization. All rodents selected for this experiment were seronegative to VSV-NJ. In order to ensure that animals were negative, they were bled and tested twice within two weeks preceding experimental inoculation. During the experiment, rodents with titers equal to or higher than 1:20 were considered seropositive to VSV-NJ.

Experimental Design

Each rodent was maintained in an individual cage. Animals were anesthetized with halothane before inoculation. Three groups were formed with 10 individuals each, according to the route of inoculation. Group A was inoculated subcutaneously (SC), group B by oral abrasion (OB) and group C was inoculated intranasally (IN). In each group, 8 individuals were treated and two were negative controls. Each treated individual was inoculated with 100*ul* VSV-NJ, Greentree strain (10^7 $TCID_{50}$/ml) plus 100*ul* D-MEM (Dulbecco's Minimal Essential Medium). Controls were inoculated with 200*ul* D-MEM. The antibody response, evidence of viraemia, clinical signs and behavioral changes were monitored for 122 days to determine the response of adult *S. hispidus* to the experimental inoculation with VSV-NJ.

Rodents were bled from the retroorbital sinus of the eye using a micro capillary tube and blood was collected in tubes with EDTA, which is used routinely for viral isolations (Rovid and Roth, 2008).Bleeding was performed 6 and 12 hours after inoculation, and on days 1, 6, 12, 17, 23, 33, 37, 51, 65, 80, 93, 107 and 122 post inoculation. Plasma was separated by centrifugation at 5000 x g for 10 minutes at room temperature. Plasma and the clotted portion of the blood were stored frozen at -20ºC and -70ºC, respectively. Serum neutralization and virus isolation on Vero cells were conducted with these samples following the method described by Rodríguez et al. (1990), and as modified by Jiménez et al. (1996). Antibody titers were expressed as logarithms. The mean and standard deviation were calculated for each bleeding day and for each inoculation route.

Additional data were obtained during bleeding by collecting a saliva sample from each rodent and determining body weight. All individuals were observed daily for clinical signs and behavioral changes. All rodents had either succumbed or were euthanized at 122 days post inoculation. Upon death, each animal was dissected and internal organs (liver, lung, kidney, spleen, brain) were removed for necropsy. Each organ and the saliva samples were stored for virus isolation in Vero cells at -70°C.

Rodents were kept isolated from each other in individual cages during this experiment. To monitor the absence of insects in the room during the experiment a

CDC-miniature light trap was set in the room and its content observed daily.

Statistical Analysis

Only means and standard deviations of antibody titers and weight of *S.hispidus* were determined.

RESULTS

Most animals in each group developed neutralizing antibodies against VSV-NJ in a range between 1:20-1:2560 six days after the inoculation (Figure 1). In the IN group all animals seroconverted until day 6; however only one rat survived longer than 33 days. All animals in the OB group seroconverted between days 6 and 12, whereas in the SC group most animals showed antibodies on day 6; however, 2 animals never seroconverted during the experiment. The means and standard deviation of the antibody titers for each bleeding day and for each inoculation route are shown in Table 1. Control animals in all three groups remained seronegative during the study. No virus was isolated from blood, organs and oral swabs collected during the experiment.

Seropositive animals did not show vesicular lesions, but other clinical signs were observed (Table 2). Rodents inoculated by the intranasal route were greatly affected, as shown by the mortality rate of 100% and the significant weight loss prior to their death (Table 3). Only one animal from this group survived longer than 6 days, succumbing at 37 days (1:2560), and presenting a dry ulcerous lesion on its nose. It was also observed that these rodents had respiratory difficulties (Table 2). The other two groups had lower fatalities: 12.5% and 43.2% in group SC and group OB, respectively, and their clinical

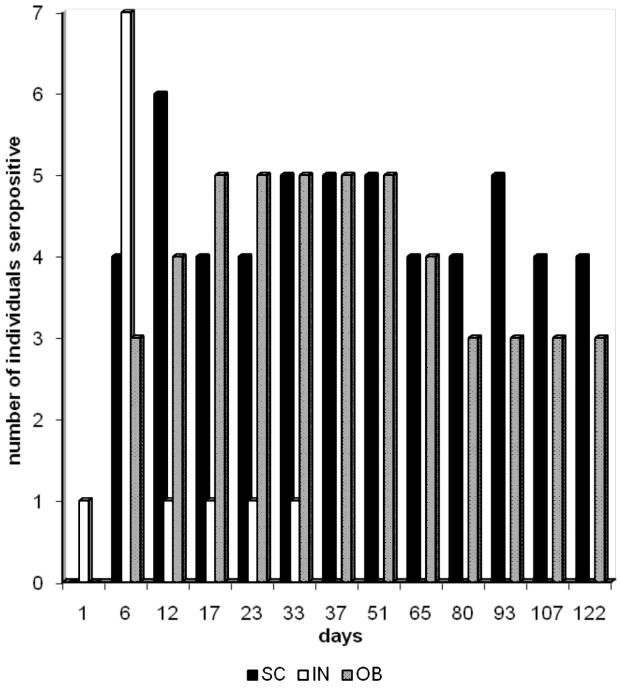

Figure 1. Number of *Sigmodon hispidus* seropositive to VSV-NJ by days over the study period (SC: subcutaneous, IN: intranasal, OB: oral abrasion).

Table 1: Antibody titers of *Sigmodon hispidus* inoculated with VSV-NJ by different inoculation routes during the study period.

Day	Subcutaneous		Oral Abrasion		Intranasal	
	Mean	SD	Mean	SD	Mean	SD
1	0	0	0	0	0.142	0.377
6	1.134	0.759	0.851	0.976	2.806	0.301
12	2.153	0.482	1.903	0.998	2.806	ND
17	1.903	0.877	2.143	0.862	2.806	ND
23	1.722	0.724	1.963	0.779	3.408	ND
33	1.903	0.737	2.444	0.494	3.408	ND
37	1.782	0.867	2.444	0.616	D	D
51	1.963	0.652	2.083	0.892	D	D
65	1.180	0.403	1.703	1.072	D	D
80	1.782	0.892	1.301	0.650	D	D
93	1.963	0.686	1.903	1.312	D	D
107	1.722	0.784	2.404	1.057	D	D
122	1.642	1.108	2.404	1.057	D	D

*Antibody titers were expressed as logarithms (≥ 1.3 = positive result). Mean values and standard deviation (SD) were calculated for each treatment day and for each inoculation route. Day: days post inoculation. ND: not determined because the number of animals was low, D: no data available, since all animals died.

signs were aggressiveness, nervousness, oral bleeding, weight loss but respiratory difficulties were not observed (Tables 2 and 3). Control animals in all three groups did not show any clinical signs during the study.

Post mortem examination showed that from the group inoculated by the intranasal route, four had pneumonia with mucous secretion and four had intranasal hyperemic cornets. From the group inoculated by oral abrasion one had pneumonia, one showed hepatic congestion, whereas one animal died 2 days after developing an abdominal mass on day 107 after inoculation. No post mortem findings were determined in the group inoculated by the subcutaneous route. During the experiment, no insects were found in the light trap.

DISCUSSION

In this study, a great variability of antibody titers was determined in all animals, regardless of the inoculation route, probably due to the low inbreeding of the animals of the colony used. No differences were observed between titers at each observational time when both routes were compared. Differences were determined in antibody titers over time, which could be due to repeated exposure to the virus. As the individual rats were maintained isolated from each other and protected from insects during the study, the persistence of the virus in some individuals is suggested. This is in accordance with Fultz and Holland (1985) who reported that injections of less than 10^7

Table 2. Clinical signs of *Sigmodon hispidus* inoculated by different routes with VSV-NJ.

Clinical signs	Inoculation route		
	Subcutaneous	Oral abrasion	Intranasal
Aggressiveness	+ (3)*	+ (2)	-
Nervousness	+ (3)	+ (2)	-
Oral bleeding	+ (5)	+ (2)	-
Dyspnea	-	-	+ (3)
Depression	-	-	+ (3)
Lack of appetite	-	-	+ (3)
Weight loss	+ (2)	+ (1)	+ (5)
Mucous nasal secretions	-	-	+ (4)
Mucous ocular secretions	-	-	+ (1)
Nasal ulceration	-	-	+ (1)

* + positive, - negative, (n) number of animals that showed the signs.

Table 3: Weight changes of *Sigmodon hispidus* experimentally infected by different routes with VSV-NJ during the observation period.

Inoculation route	ID	Initial weight (g)		Final weight (g)		Weight difference (g)	
		Mean	SD	Mean	SD	Mean	SD
Subcutaneous	Control	266.5	16.3	249	11.3	-17.5	4.9
	Treated	175.1	30.4	184.9	25.6	9.7	27.7
Oral abrasion	Control	141.5	26.2	186.5	4.9	45	31.1
	Treated	159.1	31.2	195.8	20.9	29.4	26.6
Intranasal	Control	160.5	17.7	192	25.5	31.5	7.8
	Treated	186.6	37.8	181	30.8	-7.3	21

$TCID_{50}$/ml of VSV-NJ induced persistent infections in LSH hamsters.

The isolation of VSV-NJ from saliva, tissues or blood was not possible, which agrees with the findings of Barrera and Letchworth (1996), who were unable to recover infectious virus from tissue of convalescent hamster by conventional serial passages in Vero cells. Although Trujillo et al. (2010) were able to isolate VSV-NJ from swabs obtained from the esophagus-pharyngeal tract and nasal swabs from *O. marsupialis*, these samples were not analyzed in rats for the present study. In accordance with our results, all blood samples tested negative by virus isolation, which could indicate a localized rather than a systemic infection (Trujillo et al., 2010). However, an interfering effect of EDTA could not be ruled out. Further studies should be conducted to detect virus by molecular techniques.

Intranasal inoculation proved to be 100% lethal, affecting greatly the respiratory system, suggesting that this route might not be the natural route of infection of the virus for *S. hispidus*, as proven for *Peromyscus maniculatus* (Cornish et al*.,* 2001). Subcutaneous and oral abrasion routes of viral inoculation were not lethal for rats and induced the development of antibodies, which suggests that both routes may serve to infect rats in their natural environment, as reported for *Didelphis marsupialis* by Trujillo et al. (2010).

The negative control rats were timid throughout the experiment; in contrast, experimentally infected animals showed an aggressive and self-destructive behavior. These changes suggest that the virus may have migrated to the brain. Many researchers have found viral particles or viral RNA in the brain of rats experimentally inoculated with VSV-NJ (Cave et al., 1985, Barrera and Letchworth, 1996; Cornish et al., 2001). Further studies are needed to test the presence of VSV in rats during experimental studies.

CONCLUSIONS

Behavioral changes and antibody titers proved the susceptibility of experimentally infected rats with VSV-NJ. Although viral isolation was not possible, further studies are needed to rule out viraemia or presence of the virus in tissues like brain with molecular techniques. Since in the present study the animals were euthanized before the antibodies diminished to pre-inoculation levels, more data may be obtainable in future studies continuing the experiment until antibodies disappear.

Susceptibility of *S. hispidus* to VSV-NJ through different inoculation routes was demonstrated. This is the first report which shows the role *S. hispidus* could play in VSV-NJ epidemiology, leaving the door open for future studies.

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