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SEROLOGICAL SURVEY OF SMALL MAMMALS IN A VESICULAR STOMATITIS VIRUS ENZOOTIC AREA

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ABSTRACT: Small mammals were captured in a Costa Rican dairy farm located in a vesicular stomatitis virus (VSV) enzootic focus, in order to determine which species were naturally infected by this virus. Monthly captures were performed from March 1989 to February 1990. Eighty-four individuals belonging to the orders Rodentia (n = 52), Insectivora (n = 31) and Marsupialia (n = 1) were captured. Only *Sigmodon hispidus* had neutralizing antibodies to VSV; among 21 animals, six had antibodies to Indiana, one to New Jersey, and two to both serotypes. In addition, groups of 40 sentinel mice (*Mus musculus*, strain C_3H) were placed in cages distributed throughout the farm. Each group was exposed for 1 mo over a period of 1 yr. None of 312 sentinel mice developed antibodies against either VSV serotype. Based on these results, we believe that *S. hispidus* might be part of the natural cycle of VSV in this enzootic focus. Caged *Mus musculus* do not seem appropriate for monitoring VSV activity in this area.

Key words: Vesicular stomatitis virus, natural cycle, small mammals, *Sigmodon hispidus*, serologic survey.

INTRODUCTION

Vesicular stomatitis (VS) is caused by vesicular stomatitis virus (VSV), a member of the Rhabdoviridae family. The disease, which affects cattle, horses, and pigs, produces vesicular lesions that often lead to loss of epithelium of the mouth, tongue, coronary band, and udder (Nichol, 1994). Two VSV serotypes, New Jersey (VSV-NJ) and Indiana (VSV-IN), cause most clinical cases of VS. Despite extensive knowledge of the molecular characteristics of this virus, many questions about virus transmission and maintenance in nature remain unanswered. Vesicular stomatitis virus is an arthropod-transmitted virus. Both serotypes have been isolated from sand flies (*Lutzomyia* spp.) and other hematophagous insects in enzootic areas and during epizootics of disease in North America (Tesh et al., 1974; Francy et al., 1988). Two insect groups, sand flies (*Lutzomyia* spp.) and black flies (*Simulium* spp.), are capable of transovarial transmission and infection of susceptible hosts (Tesh et al., 1971, Comer et al., 1990; Cupp et al., 1992). However, the low frequency of transovarial transmission in these insects, has led many to believe that there are other natural reservoirs from which insect vectors obtain the virus (Tesh et al., 1971; Webb et al., 1987).

Antibodies to VSV have been detected in wild primates, edentates, marsupials, bats, carnivores, artiodactyls and rodents throughout the Americas (Tesh et al., 1970; Jenney et al., 1970; Fletcher et al., 1985; Stallnecht et al., 1987). However, few studies exist in agricultural areas of enzootic VSV activity. This study was performed in a well characterized enzootic focus of VSV activity (Rodriguez et al., 1990). Our objective was to determine if wild small mammals were naturally infected by VSV and therefore could be involved in the natural cycle of this virus.

MATERIALS AND METHODS

The study site was a dairy farm near the town of Grecia, province of Alajuela, Costa Rica (10°07′N, 84°17′W). This farm was located 1,300 m above sea level in a premontane wet forest ecological zone, with an average annual rainfall of 200 to 300 cm and a mean annual temperature of 24 C (range 15 to 29 C). The approximately 20-ha farm had four distinct areas; buildings (storage shed, milking barn, pigpen and a small house), pastures which consisted mostly of African star grass (*Cynodon dactylon*), coffee (*Coffea arabica*) fields, and a

small secondary forest along a river with medium size trees (5 m tall) and shrubs. The pasture land represented 70%, coffee fields 25%, and buildings and forest 5%, of the total area of the farm.

Between 25 and 30 adult Jersey cows were present on the farm during the time of the study. This farm is a VSV enzootic focus, where clinical cases of VSV are observed every year. The herd was closed, except for two cows that entered the herd during the study period. However, their introduction to the herd was not associated to the occurrence of clinical VS (Rodriguez et al., 1990). Cattle grazed during the day and night in the pasture areas and were in the barn only at the times of milking early in the morning and at mid-afternoon. Cattle on the farm were observed for the presence of clinical vesicular stomatitis from March 1989 to February 1990. When clinical signs were observed tissue samples from the lesions were submitted to the Regional Laboratory for Vesicular Disease Diagnosis in Panama where they were tested by complement fixation and virus isolation (Rodriguez et al., 1993). In addition, serum samples were taken from all adult cattle bimonthly during the same period and analyzed by virus neutralization test for both VSV-NJ and VSV-IN at our laboratory (Rodriguez et al., 1990).

Small mammals were captured during four consecutive days, once a month, between March 1989 and February 1990. One hundred Sherman traps (Sherman Traps, Inc, Tallahassee, Florida, USA) were placed in randomly selected transects on the different areas of the farm. Sixteen traps were placed in buildings, 40 in pastures, 30 in secondary forest, and 14 in coffee fields. Baited traps were placed on the ground at a distance between traps of 5 m in buildings area or 10 m in other areas (Teska, 1980). Bait consisted of rat food pellets covered with peanut butter and vanilla. Traps were baited and checked daily. Captured specimens were anesthetized by ether inhalation, eartagged (Finger Ling Tag, Salt Lake Stamp Company, Salt Lake Utah, USA) and released after two to eight heparinized capillary tubes of blood were collected from the orbital sinus. A few individuals from each species were killed by ether overdose and identified according to species keys (Goodwing, 1946; Scott, 1967; Nowak and Paradiso, 1983). Ages of *Sigmodon hispidus* were determined by weight: Juvenile (<40 g), subadult (41 to 80 g), adult (>80 g) (Goertz, 1965).

Sentinel mice (*Mus musculus*, strain C_3H) were used to attempt detecting seroconvertion to VSV from June 1989 to May 1990. Adult C_3H mice produced neutralizing antibodies to both VSV serotypes within 1 wk after intradermal inoculation with 500 tissue culture infectious doses ($TCID_{50}$) of VSV-NJ or VSV-IN (L. Rodriguez, unpubl.). Before placing them in the field, all mice were bled and confirmed to be seronegative to both VSV serotypes by virus neutralization test, as described by Rodriguez et al. (1990). Animals were kept five to a cage, with food and water available at all times. Cages were placed 1 m above the ground and distributed as follows: three in buildings, three in pasture, and one each in coffee plantation and secondary forest. The cages measured 18 cm by 20 cm by 27 cm and were made with galvanized mesh of 13 by 25 mm which allowed free access of blood sucking insects. Each group of mice was exposed in the field for 30 days, brought to the laboratory and bled within 2 days. During the first 3 mo, the same sentinel mice were maintained in the field. For the remaining 9 mo, groups of 40 mice were exposed for 1 mo. Exposures that might have occurred during the last week in the field could not be detected, since sentinels were not held to allow for seroconvertions. Of 400 mice exposed, 88 were lost due to predators, stolen cages, and cannibalism.

Wild rodents and sentinels were lightly anesthetized and bled from the orbital sinus using heparinized capillary tubes. Approximately 350 µl of blood were obtained from each animal. Plasma was separated after centrifugation at $800 \times G$ for 5 min, diluted 1:10 with Minimum Essential Medium (MEM), (Eagle, Sigma Chemical Company, St. Louis, Missouri, USA) and stored at −70 C until tested.

Plasmas were tested by virus neutralization against 100 $TCID_{50}$ of VSV-NJ (Greentree strain) or VSV-IN (Laboratory strain) in twofold dilutions from 1:20 to 1:160 using Madin Darby bovine kidney (MDBK) cells (American Type Culture Collection, Rockville, Maryland, USA) as indicators. Plasmas were heat-inactivated at 56 C for 30 min and treated with kaolin (Sigma Chemical Co.) to remove non-specific reactions at low serum dilutions (Clarke and Casals, 1958). No plasmas were cytotoxic to MDBK cells at any dilutions tested. Known VSV positive and negative control sera (kindly provided by Dr. J. Pearson, National Veterinary Services Laboratory, Ames, Iowa, USA) were included on each plate.

RESULTS

Eighty-four individuals of eight species were captured. Only three individuals, two *S. hispidus* and one *Peromyscus nudipes*, were recaptured during the study. Most

TABLE 1. Species of small mammals captured in San Roque de Grecia, Alajuela, Costa Rica, from March 1989 to February 1990.

Species	Capture location				
	Buildings	Pasture	Coffee plantation	Forest	Total (%)
Rodentia					
Mus musculus	15	4	0	4	23 (27)
Sigmodon hispidus	0	19	1	1	21 (25)
Peromyscus nudipes	0	0	0	4	4 (5)
Heteromys desmarestianus	0	0	0	2	2 (2)
Rattus rattus	1	0	0	0	1 (1)
Reithrodontomys mexicanus	0	0	0	1	1 (1)
Insectivora					
Cryptotis parva	0	31	0	0	31 (37)
Marsupialia					
Didelphis marsupialis	0	0	0	1	1 (1)
Total (%)	16 (19)	54 (64)	1 (1)	13 (16)	84 (100)

(64%) individuals were captured in pastures, followed by 19% in farm buildings and 17% in secondary forest and coffee plots. *Cryptotis parva* (37%), *M. musculus* (27%) and *S. hispidus* (25%) were the most abundant species captured (Table 1).

TABLE 2. Neutralizing antibody prevalence and clinical case occurrence of vesicular stomatitis in cattle at San Roque de Grecia, Alajuela, Costa Rica, from March 1989 to February 1990.

Month	Clinical cases[a]		Antibody prevalence (%)		Population[c]
	VSV-NJ[b]	VSV-IN	VSV-NJ	VSV-IN	
March	0	0	ND[d]	ND	ND
April	0	0	ND	ND	ND
May	0	0	ND	ND	ND
June	0	0	71	50	30
July	0	0	ND	ND	ND
August	0	0	74	19	27
September	1	1	ND	ND	ND
October	0	0	89	75	26
November	1	0	ND	ND	ND
December	2	0	100	65	25
January	5	0	ND	ND	ND
February	2	0	ND	ND	ND
Total	11	1	NA[e]	NA	NA

[a] Laboratory confirmed cases of vesicular stomatitis virus (VSV).

[b] VSV-NJ is the New Jersey serotype of VSV; VSV-IN is the Indiana serotype of VSV.

[c] Animals present on the farm at the time of sampling.

[d] ND, not determined.

[e] NA, not apply.

Sigmodon hispidus was the only species in which neutralizing antibodies to VSV were found. Nine (43%) of 21 individuals captured had antibodies to one or both serotypes of VSV. Six individuals (66%) had antibodies only to VSV-IN, two (22%) had antibodies to both serotypes and one (11%) had antibodies only to VSV-NJ. Neutralizing antibody titers ranged from 1:20 to 1:80. According to their body weight, five of the seropositive rats were adults (weight >80 g) and four were sub-adults (weight 41 to 80 g). Seven of the positive individuals were males and two were females. None of the seropositive individuals were re-captured.

During the study period there were 12 laboratory-confirmed clinical cases of VSV in the premises; 11 were caused by VSV-NJ and one by VSV-IN (Table 2). Further confirmation of VSV activity was obtained from the prevalence of neutralizing antibodies to both serotypes in the cattle population throughout the study period (Table 2). Most clinical cases occurred during the dry season (December through May), and were not associated with the introduction of animals to the farm nor with large epi-

zootics of VSV in the region. None of the 312 sentinel mice exposed in the field seroconverted to either VSV serotype.

DISCUSSION

Our objective was to determine what species of small mammals were naturally infected by VSV in a well documented enzootic focus and therefore could be involved in the natural cycle of this virus. The species of rodents captured during this study were expected (McPherson, 1985). Although this study was not designed to analyze the population dynamics of the captured species in the different management areas of the farm, it is interesting that the species most frequently captured in buildings was *M. musculus*. In contrast, areas of pasture were preferred by *S. hispidus* which feeds mainly on grazing land and frequently uses areas of high and dense cover (Goertz, 1964).

The only species with neutralizing antibodies to VSV was *S. hispidus*. Based on the serological evidence, *S. hispidus* was exposed to both VSV serotypes. In previous studies in Panama, Tesh et al. (1970) found low prevalences of antibodies to VSV-IN (6%) and VSV-NJ (0%) in this same species. *Sigmodon hispidus* captured in a tropical dry forest area of Costa Rica had similar prevalences of antibodies to VSV as those captured in this study (L. Rodriguez, unpubl.). The most prevalent serotype of VSV in cattle at the study farm was VSV-NJ (Table 2). In addition, 11 of 12 clinical VSV cases in cattle at this farm during the study period were caused by VSV-NJ (Table 2). A similar pattern of VSV occurrence was documented in this farm from 1986 to 1990 (Rodriguez et al., 1990). In contrast, the seroprevalence in *S. hispidus* was higher for VSV-IN than for VSV-NJ. Thus, transmission cycles of VSV to cattle and to *S. hispidus* may be different. It could be argued, that the rat and cattle populations cannot be compared, since we do not know the time when the rats were exposed to VSV. However, the serological pattern in cattle had been maintained for at least 3 yr prior to the study and half of the positive rats were sub-adults (<1 yr of age). Thus, they were in contact with the virus shortly before or during the study period.

Neutralizing antibody titers against VSV-IN and VSV-NJ found in *S. hispidus* were relatively low (1:20 to 1:80) compared to titers obtained after experimental intradermal inoculation of C_3H mice which ranged from 1:400 to 1:1,600 (L. Rodriguez, unpubl.). It could be argued that these antibody responses were nonspecific reactions. However, the kaolin treatment of the samples and the fact that some were positive only to one VSV serotype, make this possibility unlikely. Fletcher et al. (1985) reported that titers >1:32 are considered positive; we considered positive titers ≥1:20. Previous studies in which *S. hispidus* was captured and the presence of antibodies against VSV was determined lack information about neutralizing antibody titers and therefore precluded comparison with our results (Tesh et al., 1969; Zuluaga and Yuill, 1979).

Evidence of infection by VSV was limited to *S. hispidus* which were captured mostly on pastures. This rodent could have contracted the infection either through ingestion of grass contaminated with the saliva of sick animals, by ingestion of virus-infected arthropods, or through bites from virus-infected insects. There is strong evidence supporting VSV transmission through insect vectors (Tesh et al., 1971; Comer et al., 1990). The recent findings that *Lutzomyia shannoni* become infected naturally, and can replicate and transmit VSV-NJ in natural areas reinforces the hypothesis of transmission by arthropods (Corn et al., 1990). The insect vector for VSV in Costa Rica is not known. However, at least eight species of sand flies have been described for the ecological zone where the study site is located (Herrero et al., 1994). In Colombia, higher prevalences of VSV antibodies were found in several species of terrestrial rodents (Zuluaga and Yuill, 1979). However, *S. hispi-*

dus was not tested in that study. In Panama, the prevalence to VSV-IN was high in arboreal and semi-arboreal mammals and VSV-NJ was limited to carnivores, bats, and rodents (Tesh et al., 1969). In a sampling of wild animals carried out in an enzootic VSV-NJ site in the southeastern USA, only raccoons (*Procyon lotor*), feral swine (*Sus scrofa*), and white-tailed deer (*Odocoileus virginianus*) had neutralizing antibodies to this virus. None of three species of rodents captured were positive (Fletcher et al., 1985). These differences are evidence of more than one VSV transmission cycle, which could depend on the characteristics of each particular ecological zone.

All *Mus musculus* captured were seronegative despite their susceptibility to VSV under laboratory conditions (L. Rodriguez, unpubl.). However, most *M. musculus* were captured in buildings, where they might not be exposed to VSV. None of 312 sentinel mice exposed in groups of 40 over a period of 1 yr, seroconverted. This could be due to a number of reasons: the sites sampled might have not been appropriate for VSV transmission, the number of mice exposed was not sufficient, or the VSV insect vector(s) do not feed on mice. Another possible explanation is that VSV did not circulate in vectors during the study period. However, the latter is unlikely since during the study period there were 12 confirmed clinical cases of VSV in cattle and this farm had documented VSV enzootic activity every year for at least three years before this study took place. None of these cases were due to introduction of livestock to the farm and there is no evidence supporting infectious virus persistence in cattle (Vernon et al., 1990; Rodriguez et al., 1990).

In conclusion, *S. hispidus* was the only rodent species tested with antibodies to VSV in this enzootic focus. Its role in the natural cycle of VSV remains unknown; however, the fact that this species shares the pasture habitat with cattle makes it a candidate for a reservoir host to VSV. These preliminary results warrant further studies in order to determine the capacity of *S. hispidus* as VSV reservoir, such as its ability to produce sustained viremias and serve as feeding host to vector sand flies.

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