Genetic analysis of vesicular stomatitis virus–New Jersey from the 1995 outbreak in the western United States

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Objective—To compare molecular associations between the vesicular stomatitis virus (VSV)-New Jersey isolates of the 1995 outbreak with those from previous outbreaks between 1982 and 1985 in the western United States.

Sample Population—23 virus isolates considered representative of the 1995 outbreak of vesicular stomatitis.

Procedure—Viral gene coding for surface-envelope protein G was evaluated by use of nucleotide sequencing and phylogenetic analysis.

Results—Changes in up to 0.77% of the nucleotide bases and 1.35% of the amino acids were detected among the 1995 viral isolates, whereas changes in up to 3.2 and 2.9% of the nucleotides and amino acids, respectively, were found, compared with the 1982 to 1985 viruses. Insertions or deletions were not found in the entire gene, which spanned 1,554 nucleotide bases.

Conclusions and Clinical Relevance—Phylogenetic analysis indicated that the 1995 VSV-New Jersey virus belongs to a lineage distinct from that of the 1982 to 1985 viruses that caused previous outbreaks in the western United States. Furthermore, it also is distinct from strains from Central America and from the Georgian Hazelhurst strain. (Am J Vet Res 2000; 61:1358–1363)

Vesicular stomatitis virus (VSV) causes periodic outbreaks of disease in livestock and horses in the United States. In 1993, 367 premises located throughout Arizona, Colorado, New Mexico, Texas, Utah, and Wyoming were reported positive for vesicular stomatitis (VS). The first case of that outbreak was reported in Las Cruces, NM on May 9, 1995, and the disease migrated as far north as Thermopolis, Wyo. The outbreak lasted for 7 months, ending in November of 1995. For that outbreak, virus was isolated from bovids, equids, and camelids, and VSV-New Jersey (VSV-NJ) was the only viral serotype isolated from animals in which VS was diagnosed.

Vesicular stomatitis virus belongs to the family Rhabdoviridae, genus Vesiculovirus. The VSV has a broad host range, infecting livestock, horses, wildlife, rodents, insects, and humans. Vesicular stomatitis is a list A disease of the International Office of Epizootics, and laboratory confirmation of the disease is lawfully required, followed by notification of the international community that is responsible for surveillance of animal health. Therefore, trade is restricted as a result of quarantine measures and an exportation ban of animal products, causing great economic loss.

The 2 major VSV serotypes are VSV-Indiana (VSV-IN) and VSV-NJ. The 2 serotypes are distinct viruses, with only 50% similarity in the glycoprotein gene sequence. It appears that VSV-NJ is more predominant than VSV-IN in North America. Vesicular stomatitis virus-Indiana and VSV-NJ apparently are restricted to the New World, with only a few reports of VS outside the Americas.

Vesicular stomatitis virus is a negative-strand RNA virus with a genome size of approximately 11 kilobases. It contains 5 genes (in order from 3’ to 5’): nucleocapsid, phosphoprotein, matrix, glycoprotein (G), and large. The G gene of VSV-NJ encodes for 517 amino acids, including a signal peptide of 16 amino acids. The VSV G protein is anchored in the virus envelope and forms spikes on the surface of the virion. The G protein is the serotype-specific antigen that induces neutralizing antibodies.

Although researchers have established detailed molecular and biochemical aspects of VSV, its mode of transmission, animal reservoir, and contributing environmental factors remain enigmas. In the study reported here, virus isolates from the 1995 outbreak of VS in the western United States were selected for phylogenetic analysis to determine the molecular association with VSV previously sequenced for viruses isolated during the outbreaks reported between 1982 and 1985 in the western United States.

Materials and Methods

Viruses—Twenty-three VSV-NJ isolates from the 1995 outbreak were chosen for the study. Virus isolates from equids were obtained from the National Veterinary Services Laboratories, USDA Animal and Plant Health Inspection Service, Ames, Iowa. Viral isolates from bovids and camelids were provided by the Foreign Animal Disease and Diagnostic Laboratory, Plum Island Animal Disease Center, USDA, 

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Greenport, New York. Isolates were categorized on the basis of month of sample collection, state, and species of origin. Isolates for analysis were randomly selected from affected (case) cattle and horses in Colorado and New Mexico representing various months of viral activity. Additionally, an isolate from a bovid in Wyoming, 1 isolate each from an equid in Utah and another equid in Texas, and an isolate from a camelid in New Mexico also were used for genotypic analysis. Viruses from equids were propagated in baby hamster kidney cells, and isolates from boids and camels were grown in lamb kidney cells. Viruses were harvested from cell culture when cytopathic effect was observed, and culture fluid was clarified by use of centrifugation.

Reverse transcription and polymerase chain reaction (RT-PCR)—Viral genomic RNA was extracted from 140 µl of viral suspension, using a viral RNA isolation kit. To obtain G-gene DNA for nucleotide sequencing, viral RNA was used as a template to obtain first-strand cDNA, which was subsequently subjected to PCR amplification. Briefly, for first-strand synthesis, the reaction mix contained 2 µg of RNA, 0.5 µg of primer G1, 10 µl of 5X first-strand buffer, 25 nM of dNTP, 20 to 40 units of Rnasin, 10.5 µl of diethyl pyrocatecholate water, and 500 units of Moloney murine leukemia virus reverse transcriptase. The reaction mix was incubated at 37 C for 1.5 hours (Appendix).

The PCR mix contained 5 µl of cDNA, 20 nM of dNTP, 10 µl of 10X PCR buffer, 250 nM MgCl2, 50 µM each of G1 and G1a primer, and 72.2 µl of water (Appendix). The reaction mixture was heated at 95 C for 5 minutes, followed by addition of 1.25 units of Taq polymerase. The PCR reaction was 30 cycles, each of which was as follows: denaturation at 94 C for 1 minute, annealing at 55 C for 1 minute, and extension at 72 C for 2 minutes. A final extension step was performed at 72 C for 5 minutes. Amplified products were analyzed on 1.0% agarose gels, as described elsewhere.

For PCR amplification of isolates from bovids was performed at Plum Island Animal Disease Center, and the DNA was shipped to Colorado State University after being tested to ensure that it was safe for transport. This resulted in inadequate DNA concentrations for sequence reactions, and, therefore, each sample was subjected to another round of PCR amplification to obtain sufficient DNA for sequence analysis.

DNA sequencing and analysis of sequence data—The PCR products were gel purified, using a gel extraction kit, and approximately 80 ng of purified DNA template was used for direct cycle sequencing. Nucleotide sequencing was performed, using an automated sequencer. Eight of the sequencing primers used have been described previously by Nichol et al., and the remaining primers were designed to sequence the entire gene. The DNA sequence data were analyzed by use of a sequencing program. For a comparative analysis, previously published VSV G-gene sequences were retrieved from Genbank, using the following accession numbers: M21430, M21416, M21431, M21435, M21434, M21429, M21425, M21424, M21432, M23437, M21436, M21433, M21428, M21427, M21426, M21417, M21419, M21559, M21558, M21421, M21420, M21423, M21422, M21560, M21566, M21568, M21567, M21565, M21561, M21564, M21563, and M21562.

Phylogenetic analysis—A multiple sequence alignment program was used for the multiple DNA sequence alignment. Another program was used to produce the maximum likelihood and maximum parsimony trees, using a heuristic search and a branch-swapping method. An unrooted neighbor-joining tree was produced by use of another computer program. The reliability of the tree was estimated by bootstrap, using maximum parsimony. One of the computer programs also was used for calculations of nucleotide, amino acid, nonsynonymous, and synonymous proportional distances.

Results

PCR and sequence analysis—The full-length G gene for various 1995 VSV isolates was amplified by PCR, and the entire G gene for all isolates was sequenced from both directions. The gene length was 1,554 nucleotide bases, and insertions, deletions, or rearrangements were not detected. These sequences have been submitted to Genbank (accession No. AF170602 to AF170624). Sequence comparisons indicated a difference of up to 0.77% in nucleotides and up to 1.35% in amino acids among the viruses from the 1995 outbreak (Table 1). Mean differences in nucleotides and amino acids were 0.46 and 0.49%, respectively. All differences remained within the range of mean + SD, except for viruses 6/95-NM-E2 and 6/95-NM-B1, which contained the highest divergence in nucleotides and amino acids. This observation also was supported by the proportional differences of synonymous and nonsynonymous changes. Isolates 5/95-NM-E1, 7/95-CO-E3, 7/95-TX-E1, and 9/95-CO-E1, all of which were obtained from equids, had identical sequences. Isolates 7/95-NM-B3 and 10/95-CO-B1, obtained from bovids, also had an identical sequence. Substantial distinctions could not be made between isolates for the 1995 outbreak on the basis of date of collection or geographic location. Changes in amino acids were distributed throughout the G gene, including the signal peptide region, glycosylation site, transmembrane domain, and hydrophilic cytoplasmic domain (Fig 1). All isolates from boids and camels, except for 10/95-WY-B1, contained an amino acid conversion (from that of isolates from equids in 1995) of aspartate to alanine at amino acid 510 in the cytoplasmic domain. A representative sample of the differences in amino acids among isolates collected during the first month of the 1995 outbreak from boids and equids was compared. Previously, the identity of the specific regions of the G proteins that contain the functional

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<tr>
<th>Comparison</th>
<th>Nucleotide</th>
<th>Amino acid</th>
<th>Synonymous</th>
<th>Nonsynonymous</th>
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<tr>
<td>Equine vs equine</td>
<td>0.22 ± 0.14 (0.00–0.52)</td>
<td>0.43 ± 0.31 (0.00–0.16)</td>
<td>0.32 ± 0.24 (0.00–0.84)</td>
<td>0.19 ± 0.13 (0.00–0.50)</td>
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<td>Bovine vs bovine</td>
<td>0.24 ± 0.11 (0.00–0.45)</td>
<td>0.26 ± 0.15 (0.00–0.58)</td>
<td>0.65 ± 0.36 (0.00–1.40)</td>
<td>0.11 ± 0.07 (0.00–0.25)</td>
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<td>Equine vs bovine</td>
<td>0.46 ± 0.12 (0.26–0.77)</td>
<td>0.49 ± 0.27 (0.15–0.35)</td>
<td>1.26 ± 0.26 (0.64–1.92)</td>
<td>0.21 ± 0.12 (0.00–0.59)</td>
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<td>1995 vs 1996</td>
<td>2.97 ± 0.15 (2.71–3.22)</td>
<td>2.26 ± 0.22 (1.93–2.56)</td>
<td>6.65 ± 0.98 (1.01–14.04)</td>
<td>0.98 ± 0.10 (0.84–1.26)</td>
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1Values reported are mean ± SD (range) percentage difference.
domains, glycosylation sites, and major neutralizing epitopes had been established.\textsuperscript{12-27} Substantial changes in the overall hydrophobicity or hydrophilicity of these regions could not be detected for the 1995 isolates. Furthermore, changes in amino acids were not found in any of the major neutralization epitopes.

The VSV-NJ G-gene sequences that were previously published\textsuperscript{14} were compared with those for 1995 VSV-NJ. Analysis of the results indicated there is a difference of 2.71 to 3.22% in nucleotides and a sequence divergence of 1.93 to 2.90% in amino acids between the 1995 isolates and the isolates obtained between 1982 and 1985 from the western United States. The samples obtained in 1995 from the western United States had a difference of approximately 4% in nucleotides and a divergence of 3 to 4% in amino acids from the Hazelhurst strain (Ga52 and Ga83; data not shown). Previous analysis of VSV-NJ isolates in samples obtained from the western United States and samples obtained from Mexico, Central America, and South America revealed a heterogeneous population of VSV-NJ isolates.\textsuperscript{14} The 1995 isolates had a divergence of 12.4 to 20% in nucleotides and a divergence of 3.0 to 8.5% in amino acids from that of the Central and South American isolates (data not shown).

**Phylogenetic analysis**—Information included in a report\textsuperscript{14} on the studies conducted on the phylogeny of the G gene of VSV-NJ during the past 10 years allowed us to compare the 1995 samples with other isolates to construct a full phylogeny on current viruses. The maximum parsimony analysis with bootstrap values of the entire G-gene sequence for VSV-NJ was rooted with all Central and South American isolates (Fig 2). A similar maximum-likelihood tree and an unrooted neighbor-joining tree were produced on the basis of the entire G-gene nucleotide sequence (data not shown). Maximum-parsimony analysis produced 2 main clades of viruses from the western United States in samples that were collected 10 years apart. One clade contained exclusively viruses isolated in 1995, whereas another clade contained viruses collected from 1982 to 1985. The Hazelhurst strain from Georgia forms a branch that is distinct from the viruses obtained from the western United States, revealing the genetic divergence of VSV. Isolates from Mexico collected between 1982 and 1987 were closely related to isolates from the United States.

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<tr>
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<td>VQDSG0K3V</td>
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<tr>
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<td>G6K5IFG2R</td>
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<td>TD</td>
<td>G6K5IFG2R</td>
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Figure 1—Amino acid sequences of the glycoprotein gene of vesicular stomatitis virus-New Jersey (VSV-NJ) isolates collected from the western United States during the 1995 outbreak of vesicular stomatitis. These sequences are representative of changes between isolates from equids and bovids during the 1995 outbreak and the 1985 outbreak. Changes in amino acids are seen throughout the glycoprotein gene in the signal peptide, transmembrane domain, and cytoplasmic domain. SP = Signal peptide, GS = Glycosylation site, ES = Neutralizing epitopes, TD = Transmembrane domain, CD = Cytoplasmic domain.
collected between 1982 and 1995 and were included within the clade for each US outbreak. Clear genetic divergence existed between the 1995 US isolates and the VSV-NJ isolates from Central and South America.

**Discussion**

Analysis of the nucleotide sequence of the G gene of the 1995 VSV-NJ isolates and comparison with that of viruses causing similar outbreaks revealed important
findings. First, the sequence alignment of the various 1995 isolates revealed a fairly homogeneous population with a mean divergence in nucleotides of 0.46% and a mean difference of 0.49% in amino acids, with differences among isolates of up to 0.77% in nucleotides and 1.35% in amino acids (Table 1). The phylogenetic analysis suggests that the same virus is responsible for all the outbreaks at various premises, among species, and at various periods during each outbreak. Therefore, it is evident that a primary outbreak resulted in subsequent spread to many states and to several species. Interestingly, viruses 69/5-NM-E2 and 6/95-NM-B1 had a higher divergence between each other and from other isolates. These isolates were obtained from the same state during the same month, but they were obtained from animals on different premises.

The VSV genome has a high mutation rate attributable to the lack of proofreading function of the RNA polymerase. It is apparent from the sequence analysis reported here that the VSV-NJ G gene undergoes mutations that only mutants that do not have major biological change in the functional domains can exist. Although there are amino acid substitutions in the hydrophobic signal peptide region, transmembrane domain, and cytoplasmic domain, these substitutions have not resulted in substantial change in the overall hydrophobicity or hydrophilicity of these regions and, therefore, do not disrupt the functional properties of this protein. Changes in amino acids were not found in the major neutralization epitopes defined by sequence analysis of monoclonal antibody resistant mutants. This suggests that immune selection may not play a major role in the evolution of VSV.

To determine the evolutionary relationship of the 1995 isolates with that of other viruses, detailed phylogenetic analysis was performed. Maximum-parsimony, maximum-likelihood, and neighbor-joining analyses placed the 1995 isolates on a branch separate from that of viruses for the 1982 to 1985 outbreak, suggesting that viruses causing these 2 outbreaks were distinct isolates. Therefore, the 1995 outbreak was not caused by the previously reported viruses, which persisted in an unknown host between the outbreaks. It has not been proven that VS is an endemic disease in the western United States. However, endemic activity exists in Ossabaw Island, Ga in the southeastern United States. Phylogenetic analysis clearly separated the 1995 isolates from that of isolates related to the southeastern Georgian strains. Analysis of the results rule out the possibility of the endemic strains in the southeastern United States spreading to the western United States and causing the 1995 outbreak. Analysis also indicated that the 1995 isolates are quite different from the viruses from Central and South America, ruling out those areas as the origin for the virus that caused the 1995 outbreak in the western United States. However, a close genetic relationship can be seen between the viruses from Mexico and the viruses in 1982 and 1995 from North America. The current database for VSV-NJ G gene is limited for comparison of field isolates obtained from the western United States prior to 1982. Therefore, it is not possible to place the VSV-NJ G gene into a timeline to monitor whether the current changes in amino acids and nucleotide bases are similar to that of the viruses isolated in the United States prior to 1982.

Additional work is being conducted on the G gene of VSV-NJ to determine the evolutionary pattern of VSV-NJ in the United States and Mexico. Sequence analysis of isolates obtained in 1997 could help answer questions about VSV-NJ persistence in the United States.

References


