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Molecular Epizootiology and Evolution of Vesicular Stomatitis Virus New Jersey

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Vesicular stomatitis virus (VSV) has been shown previously to be capable of undergoing rapid mutational change during sequential experimental infections in various tissue culture cell systems (J. Holland, K. Spindler, F. Horodyski, E. Grabau, S. Nichol, and S. Vandepol, Science 215:1577–1585, 1982). The present study was undertaken to determine the degree of genetic diversity and evolution of the virus under natural infection conditions and to gain insight into the epizootiology of the disease. Between 1982 and 1985, numerous outbreaks of VSV of the New Jersey serotype were reported throughout regions of the United States and Mexico. A T1 RNase fingerprint analysis was performed on the RNA genomes of 43 virus isolates from areas of epizootic and enzootic virus activity. This indicates that virus populations were genetically relatively homogeneous within successive U.S. virus epizootics. The data included virus isolates from different epizootic stages, geographical locations, host animals, and host lesion sites. In contrast, only distant genome RNA T1 fingerprint similarities were observed among viruses of the different U.S. epizootics. However, Mexican viruses isolated before or concurrent with U.S. epizootics had very similar RNA genome fingerprints, suggesting that Mexico may have been the possible origin of virus initiating recent U.S. VSV New Jersey outbreaks. Comparison of T1 fingerprints of viruses within enzootic disease areas revealed a greater extent of virus genetic diversity in these areas relative to that observed in epizootic areas. The evolutionary significance of these findings and their relationship to experimental data on VSV evolution are discussed.

The vesicular stomatitis viruses (VSV) are members of the family *Rhabdoviridae* and are divided into two major serotypes, Indiana (IN) and New Jersey (NJ) (8). VSV IN has been extensively studied at the molecular level; however, VSV NJ is the more important cause of disease, being responsible for periodic and unpredictable major disease epizootics in cattle, horses, and swine throughout the Americas (9, 10, 15). Historically, VSV NJ epizootics in the United States have appeared at approximate 10-year intervals, virus activity first appearing in early summer and then disappearing with the first frost in early winter. Extensive enzootic virus activity also exists in regions of Mexico, Central America, and South America (15). In these areas, virus activity persists, with regular appearance of the disease in cattle populations. In addition, annual identification in the southeastern United States of VSV NJ-seropositive wildlife, particularly feral swine, has indicated that limited enzootic virus activity also exists in this region (6, 11, 24, 25).

The natural virus reservoir, the mechanism of maintenance of virus in enzootic regions, and the mode of virus transmission are still unidentified. Possible insect involvement in the transmission and maintenance of the disease has been suggested based on the typical seasonal nature of VSV NJ epizootics; numerous VSV isolations from insects (7, 14, 22, 27); demonstration of experimental transmission of VSV IN by horseflies, mosquitos, and sandflies (5, 28); and transovarial transmission of VSV IN in sandflies (28). However, viremias sufficiently high for virus transmission have not been demonstrated in VSV-infected animals. In addition, the spread of a recent major epizootic which occurred in the western United States from 1982 to 1983 continued throughout the winter months in the apparent absence of insect vectors (16). Recent VSV outbreaks have also been unusual in their frequency. Unlike the historic approximate 10-year cycles of VSV activity, after the large 1982 to 1983 western U.S. epizootic in cattle and horses, virus activity was confirmed in cattle in Texas in 1984 and another epizootic of cattle and horses occurred in Colorado, New Mexico, and Arizona in 1985. Epizootic and enzootic virus activity also occurred in various parts of Mexico during the same time period.

A number of possibilities exist as to the origin of the virus initiating these recent VSV NJ outbreaks in the United States. These include synchronous eruption of preexisting persistent or inapparent infections, initiation by virus preexisting in the U.S. wildlife populations of epizootic or enzootic areas, and introduction of the virus from outside the United States.

Considerable information is available concerning the molecular evolution of VSV IN under various conditions of tissue culture cell infections. It has been demonstrated that under conditions of serial undiluted virus passage and virus persistent infection, a rapid accumulation of virus RNA genome mutations can occur (12, 13, 18, 20, 23, 29–31). However, serial passage of the virus at low multiplicity of infection leads to the accumulation of very few, if any, genome mutations (23). The ability of the virus to evolve rapidly under certain tissue culture conditions may be owing to the inherent very high error rate of the virus RNA polymerase (26).

An extensive genetic study of VSV field isolates from various VSV NJ outbreaks and stages of epizootics from 1982 to 1985 was undertaken to clarify the epizootiology of VSV NJ and to compare the virus RNA genome evolution under conditions of natural infection with that observed experimentally.

TABLE 1. VSV NJ isolates analyzed by T1 fingerprinting

Isolation date	Origin	Host or species	Host lesion site[a]	Nomenclature
USA				
1949	Ogden, Utah	Bovine		?/49-UT-B
1952	Hazelhurst, Ga.	Porcine		?/52-GA-P
08/06/82	Colorado	Bovine	Oral	8/82-CO-B
08/13/82	Wyoming	Bovine	Oral	8/82-WY-B
08/14/82	Colorado	*Culicoides*		8/82-CO-C
08/30/82	Idaho	Bovine	Tongue	8/82-ID-B
09/08/82	Idaho	Bovine	Oral	9/82-ID-B1
09/09/82	Colorado	Black fly		9/82-CO-BF
09/10/82	Montana	Equine		9/82-MT-E
09/16/82	Colorado	House fly		9/82-CO-HF
09/20/82	Idaho	Bovine		9/82-ID-B2
03/22/83	New Mexico	Bovine	Teat	3/83-NM-B
05/27/83	Nebraska	Bovine		5/83-NE-B
11/20/83	California	Bovine		11/83-CA-B
07/27/83	Ossabaw Island, Ga.	Porcine (feral)	Snout	7/83-GA-P
02/13/84	Texas	Bovine		2/84-TX-Bl
02/13/84	Texas	Bovine		2/84-TX-B2
06/28/85	New Mexico	Equine	Oral	6/85-NM-E
06/29/85	New Mexico	Bovine	Oral	6/85-NM-B
07/03/85	Arizona	Equine	Oral	7/85-AZ-E
07/19/85	New Mexico	Bovine		7/85-NM-B
07/26/85	Colorado	Bovine	Oral	7/85-CO-Bl
07/27/85	Colorado	Equine	Oral	7/85-CO-El
07/28/85	Colorado	Equine	Oral	7/85-CO-E2
07/30/85	Colorado	Bovine	Oral	7/85-CO-B2
08/04/85	Colorado	Equine	Oral	8/85-CO-E
08/07/85	Colorado	Bovine	Teat	8/85-CO-B
09/04/85	Colorado	Equine	Oral	9/85-CO-E
Mexico				
11/03/82	Veracruz	Bovine	Oral	11/82-VC-Bl
11/29/82	Veracruz	Bovine	Oral	11/82-VC-B2
02/17/83	Veracruz	Bovine	Oral	2/83-VC-B
11/22/83	Michoacan	Bovine	Teat	11/83-MH-B
01/18/84	Michoacan	Bovine	Oral	1/84-MH-B
01/26/84	Sonora	Porcine	Tongue	1/84-SN-P1
01/31/84	Sonora	Porcine	Snout	1/84-SN-P2
06/22/84	Chihuahua	Bovine	Oral	6/84-CH-B
07/29/84	Oaxaca	Bovine	Udder	7/84-OA-B
08/19/84	Chihuahua	Bovine	Tongue	8/84-CH-B1
08/27/84	Chihuahua	Bovine	Tongue	8/84-CH-B2
09/28/84	Chihuahua	Bovine	Udder	9/84-CH-B
11/15/84	Hidalgo	Bovine	Oral	11/84-HD-B
01/15/85	Nuevo Laredo	Bovine	Oral	1/85-NL-B
01/16/85	Morelos	Bovine	Oral	1/85-MR-B
05/18/85	Sonora	Bovine	Oral	5/85-SN-B
06/06/85	Chihuahua	Bovine	Oral	6/85-CH-B

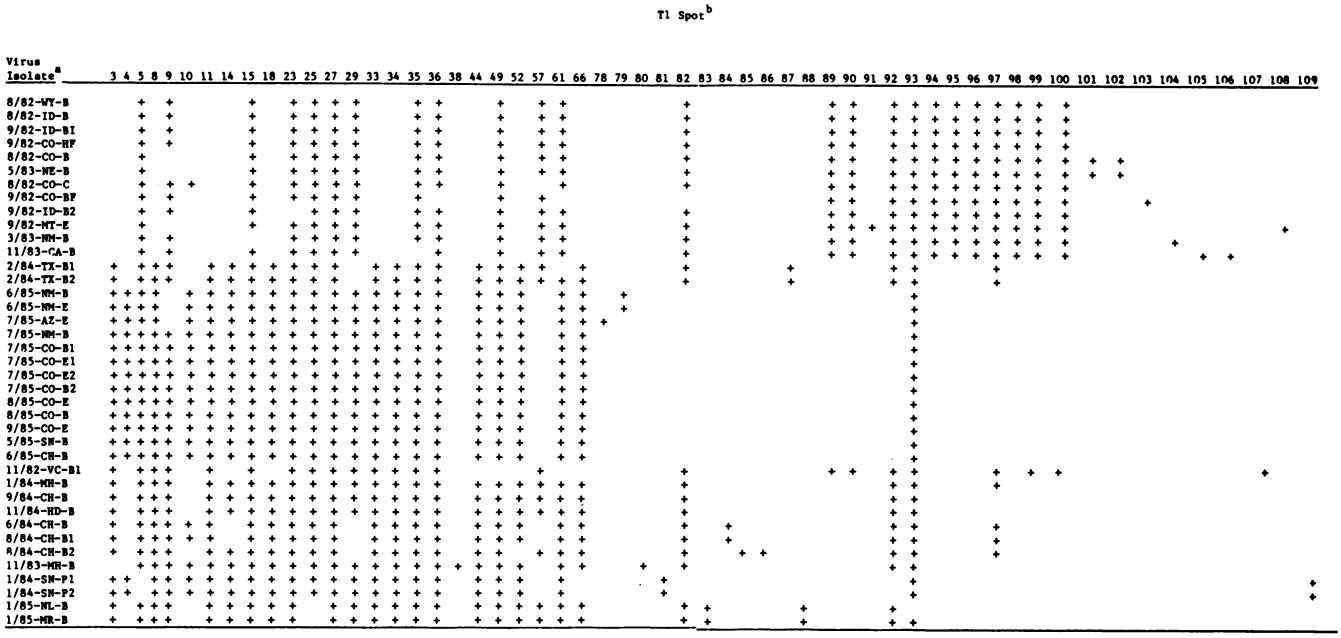
[a] Lesion site is indicated in cases for which it is known.

MATERIALS AND METHODS

Virus isolates. VSV NJ field isolates from the United States were provided by the U.S. Department of Agriculture National Veterinary Services Laboratory, Ames, Iowa. Insect VSV NJ isolates were provided by the Centers for Disease Control, Fort Collins, Colo., and the U.S. Department of Agriculture Arthropod-Borne Animal Disease Research Laboratory, Laramie, Wyo. Mexican VSV NJ field isolates were provided by the Mexico-U.S. Commission for the Prevention of Foot and Mouth Disease, Mexico City, Mexico. The viruses were identified as VSV NJ by tissue complement fixation and the serum neutralization test before their dispatch from these laboratories. Viruses were analyzed after a minimum number of low-multiplicity virus passages in tissue culture. In most cases, this involved two or three virus passages from the field material, with no virus plaque purification methods used.

Virus growth and T1 RNase fingerprinting technique. Viruses were grown at 37°C in BHK-21 cells in minimal essential Eagle medium containing ^{32}P-labeled inorganic phosphate (80 μCi/ml). Virus was harvested and purified, and RNA was extracted as described previously (17). The T1 fingerprinting technique used was a modification of earlier techniques (4). Purified ^{32}P-labeled virus RNA (containing 10 μg of carrier tRNA) was thoroughly suspended in 8 μl of digest buffer (8.4 M urea, 12.5 mM sodium citrate [pH 5], 1.25 mM EDTA, 0.05% bromophenol blue, 0.05% xylene cyanol), boiled 1 min, and rapidly cooled on wet ice; 2 μl of T1 RNase was added, and the mixture was incubated for 15 min at 50°C. The T1-digested RNA was loaded directly onto a 0.8-mm standard first-dimension gel and electrophoresed at

TABLE 2. Catalog of T1 spot differences among VSV field isolates



[a] See Table 1 for nomenclature.
[b] See Fig. 1A. Only spots in which differences were seen are listed. +, Presence of spot.

approximately 250 V for 18 h. The gel track was then excised and transferred to a 0.4-mm standard second-dimension gel and electrophoresed at 475 V for 18 h. The reproducibility of each virus RNA fingerprint was checked at least once (usually twice) for each virus isolate. The comparison and cataloging of T1 fingerprint spot differences was aided by the use of an IBM PC-XT microcomputer.

RESULTS

T1 RNase fingerprinting analysis was carried out on 45 VSV NJ isolates from the United States and Mexico (Table 1). The isolates included two commonly studied standard virus lab strains, Ogden and Hazelhurst, representing the two previously defined subtypes of VSV NJ (19). A total of 43 were actual field isolates from the period 1982 to 1985. U.S. isolates consisted of 12 virus isolates from the 1982 to 1983 VSV epizootic in the western United States, including 3 insect isolates, one from *Culicoides veriipennis* midges, another from *Simulium bivatatum* black flies, and the other from *Musca domestica* house flies; a 1983 isolate from a feral swine on Ossabaw Island, Ga., a region of apparent enzootic virus activity (6); 2 isolates from a 1984 VSV outbreak in Texas; and 11 isolates from the 1985 VSV epizootic in Arizona, New Mexico, and Colorado. Mexican isolates included 17 viruses from various epizootic and enzootic disease regions from 1982 through 1985. These virus RNA fingerprints were compared, and spot differences and similarities were indexed and correlated (Table 2 and Fig. 1A). Table 3 shows the number of T1 spot differences between virus isolates. The exact number of T1 spot differences is not given for viruses with genome RNA fingerprints demonstrating little similarity (more than 30 spot differences) to the other viruses analyzed.

Comparison of RNA fingerprints of virus isolates from the major VSV NJ epizootic of the western United States in 1982 to 1983 (Table 3) indicates a high degree of genetic similarity among the isolates (e.g., Fig. 1B). The results are represented diagrammatically (Fig. 2). Four isolates had identical fingerprints, and a maximum of four spot differences was observed between closest pairings of the other viruses of this epizootic. The viruses from this epizootic included isolates from cattle, horses, and insects, demonstrating that within this epizootic the viruses were relatively genetically homogeneous, despite the fact that viruses were isolated from areas of considerable geographical separation over a 15-month period. This group of isolates had completely different fingerprints from those of historic VSV NJ isolates, Ogden and Hazelhurst, which represent the two existing subtypes of the VSV NJ serotype (Fig. 1E and J). Previous work has demonstrated that serial dilute passage of VSV and the passage of VSV strains for many years in different laboratories lead to very few T1 spot changes in the virus fingerprint (3, 23). Thus, it is unlikely that the differences in T1 fingerprints observed between the historic and recent VSV isolates are due to the long period of maintenance of the historic isolates in tissue culture. In addition, the VSV NJ isolate from the region of enzootic virus activity in feral swine on Ossabaw Island, Ga., also had a completely different fingerprint (Fig. 1F). However, a 1982 isolate from Veracruz, Mexico, was only 12 spots different from the main group of U.S. 1982 to 1983 VSV NJ isolates (Fig. 1G and Fig. 2).

The relationships among the two 1984 VSV NJ Texas isolates, a representative isolate of the preceding U.S. outbreak, and the 1984 Mexican isolates are illustrated in Fig. 3. Clearly, the Texas isolates are closely related to each other and to the 1984 Mexican isolates. The bovine isolate from Michoacan (1/84-MH-B) had the closest relationship to the Texas isolates, being only two T1 spots different, and was isolated 1 month before the appearance of the disease in the United States. A considerably more distant relationship (at least 20 spots different) is evident between the 1984 Texas virus isolates and isolates from the preceding VSV NJ U.S. outbreak between 1982 and 1983 (Fig. 1B and C).

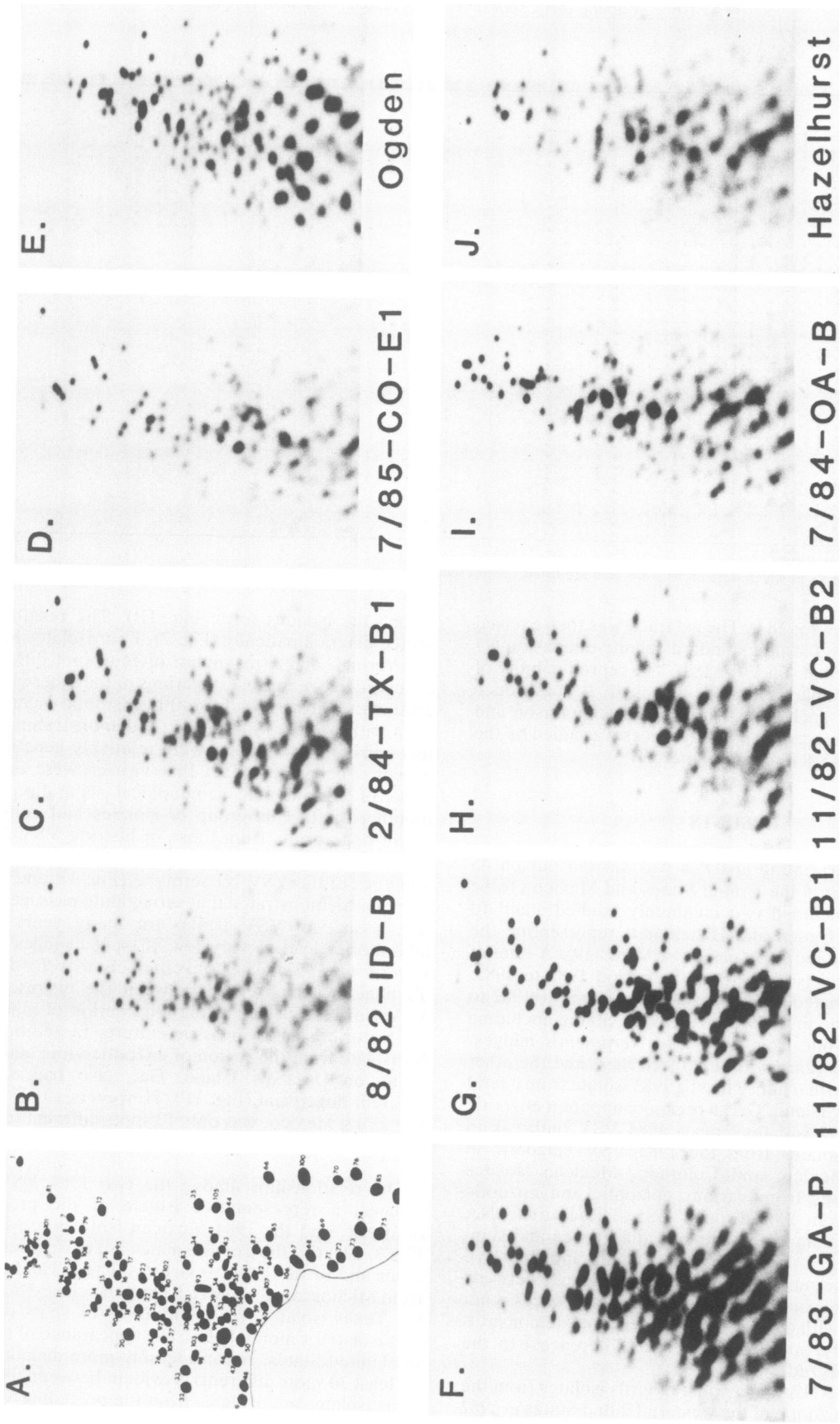


FIG. 1. T1 fingerprints of the RNA genomes of representative VSV NJ isolates. (A) Diagrammatic representation of the T1 spot number index. (B to J) T1 fingerprints of the virus RNA genomes indicated.

TABLE 3. Number of T1 spot differences among RNA genome fingerprints of VSV isolates

	?/49-UT-B	?/52-GA-P	8/82-WY-B	8/82-ID-B	9/82-ID-B1	9/82-CO-HF	8/82-CO-B	5/83-NE-B	8/82-CO-C	9/82-CO-BF	9/82-ID-B2	9/82-MT-E	3/83-NM-B	11/83-CA-B	7/83-GA-P	2/84-TX-B1	2/84-TX-B2	6/85-NM-B	6/85-NM-E	7/85-AZ-E	7/85-NM-B	7/85-CO-B1	7/85-CO-E1	7/85-CO-E2	7/85-CO-B2	8/85-CO-E	8/85-CO-B	9/85-CO-E	5/85-SN-B	6/85-CH-B	11/82-VC-B1	1/84-MH-B	9/84-CH-B	11/84-HD-B	6/84-CH-B	8/84-CH-B1	8/84-CH-B2	11/83-MH-B	1/84-SN-P1	1/84-SN-P2	1/85-NL-B	1/85-MR-B	11/82-VC-B2	2/83-VC-B	7/84-OA-B
?/49-UT-B	0																																											
?/52-GA-P	>[a]	0																																										
8/82-WY-B	>	>	0																																									
8/82-ID-B	>	>	0	0																																								
9/82-ID-B1	>	>	0	0	0																																							
9/82-CO-HF	>	>	0	0	0	0																																						
8/82-CO-B	>	>	3	3	3	3	0																																					
5/83-NE-B	>	>	3	3	3	3	0	0																																			
8/82-CO-C	>	>	2	2	2	2	5	5	0																																		
9/82-CO-BF	>	>	4	4	4	4	7	7	6	0																																	
9/82-ID-B2	>	>	1	1	1	1	4	4	3	5	0																																
9/82-MT-E	>	>	2	2	2	2	3	3	4	6	3	0																															
3/83-NM-B	>	>	3	3	3	3	6	6	5	7	4	5	0																														
11/83-CA-B	>	>	3	3	3	3	6	6	5	7	4	5	6	0																													
7/83-GA-P	>	>	>	>	>	>	>	>	>	>	>	>	>	>	0																												
2/84-TX-B1	>	>	21	21	21	21	24	24	23	23	22	23	24	24	>	0																											
2/84-TX-B2	>	>	20	20	20	20	23	23	22	24	21	22	23	23	>	1	0																										
6/85-NM-B	>	>	26	26	26	26	27	27	24	28	27	26	29	29	>	11	10	0																									
6/85-NM-E	>	>	26	26	26	26	27	27	24	28	27	26	29	29	>	11	10	0	0																								
7/85-AZ-E	>	>	26	26	26	26	27	27	24	28	27	26	29	29	>	11	10	2	2	0																							
7/85-NM-B	>	>	24	24	24	24	27	27	22	26	25	26	27	27	>	9	8	2	2	2	0																						
7/85-CO-B1	>	>	24	24	24	24	27	27	22	26	25	26	27	27	>	9	8	2	2	2	0	0																					
7/85-CO-E1	>	>	24	24	24	24	27	27	22	26	25	26	27	27	>	9	8	2	2	2	0	0	0																			
7/85-CO-E2	>	>	24	24	24	24	27	27	22	26	25	26	27	27	>	9	8	2	2	2	0	0	0	0																		
7/85-CO-B2	>	>	24	24	24	24	27	27	22	26	25	26	27	27	>	9	8	2	2	2	0	0	0	0	0																	
8/85-CO-E	>	>	24	24	24	24	27	27	22	26	25	26	27	27	>	9	8	2	2	2	0	0	0	0	0	0																
8/85-CO-B	>	>	24	24	24	24	27	27	22	26	25	26	27	27	>	9	8	2	2	2	0	0	0	0	0	0	0															
9/85-CO-E	>	>	24	24	24	24	27	27	22	26	25	26	27	27	>	9	8	2	2	2	0	0	0	0	0	0	0	0														
5/85-SN-B	>	>	24	24	24	24	27	27	22	26	25	26	27	27	>	9	8	2	2	2	0	0	0	0	0	0	0	0	0													
6/85-CH-B	>	>	24	24	24	24	27	27	22	26	25	26	27	27	>	9	8	2	2	2	0	0	0	0	0	0	0	0	0	0												
11/82-VC-B1	>	>	12	12	12	12	15	15	14	16	13	12	15	15	>	15	14	20	20	20	18	18	18	18	18	18	18	18	18	0												
1/84-MH-B	>	>	18	18	18	18	21	21	20	22	19	20	21	21	>	3	2	8	8	8	6	6	6	6	6	6	6	6	6	12	0										
9/84-CH-B	>	>	19	19	19	19	22	22	21	23	20	21	22	22	>	4	3	7	7	7	5	5	5	5	5	5	5	5	5	13	1	0									
11/84-HD-B	>	>	19	19	19	19	22	22	21	23	20	21	22	22	>	4	3	7	7	7	5	5	5	5	5	5	5	5	5	13	1	0	0								
6/84-CH-B	>	>	21	21	21	21	24	24	19	25	22	23	24	24	>	6	5	9	9	9	7	7	7	7	7	7	7	7	7	15	5	6	6	0							
8/84-CH-B1	>	>	21	21	21	21	24	24	19	25	22	23	24	24	>	6	5	9	9	9	7	7	7	7	7	7	7	7	7	15	5	6	6	0	0						
8/84-CH-B2	>	>	20	20	20	20	23	23	22	24	21	22	23	23	>	5	4	12	12	12	10	10	10	10	10	10	10	10	10	14	4	5	5	7	7	0					
11/83-MH-B	>	>	22	22	22	22	25	25	20	26	23	24	25	25	>	9	8	8	8	8	6	6	6	6	6	6	6	6	6	18	6	5	5	7	7	10	0				
1/84-SN-P1	>	>	26	26	26	26	29	29	24	28	27	28	29	29	>	13	12	6	6	6	4	4	4	4	4	4	4	4	4	20	10	9	9	11	11	14	10	0			
1/84-SN-P2	>	>	26	26	26	26	29	29	24	28	27	28	29	29	>	13	12	6	6	6	4	4	4	4	4	4	4	4	4	20	10	9	9	11	11	14	10	0	0		
1/85-NL-B	>	>	23	23	23	23	26	26	25	27	24	25	26	26	>	8	7	11	11	11	9	9	9	9	9	9	9	9	9	17	5	4	4	10	10	9	9	13	13	0	
1/85-MR-B	>	>	24	24	24	24	27	27	26	28	25	26	27	27	>	9	8	12	12	12	10	10	10	10	10	10	10	10	10	18	6	5	5	11	11	10	10	14	14	1	0
11/82-VC-B2	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>	0
2/83-VC-B	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>	6	0
7/84-OA-B	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>	0

[a] >, More than 30 spot differences.

The relationships between VSV NJ isolates from the 1985 epizootic in Colorado, New Mexico, and Arizona are represented in Fig. 4. All these isolates were found to have identical or extremely similar T1 fingerprints (e.g., Fig. 1D), despite the fact that the viruses were isolated from both cattle and horses. Comparison of the T1 fingerprints of these viruses with those isolated from this region during the 1982 to 1983 U.S. epizootic indicates a distant relationship, the closest member being 22 spots different. In contrast, 1985 Mexican isolates had very similar T1 fingerprints (Fig. 4). Two isolates had fingerprints identical to the 1985 U.S. isolates, including one, 5/85-SN-B, which was isolated 1

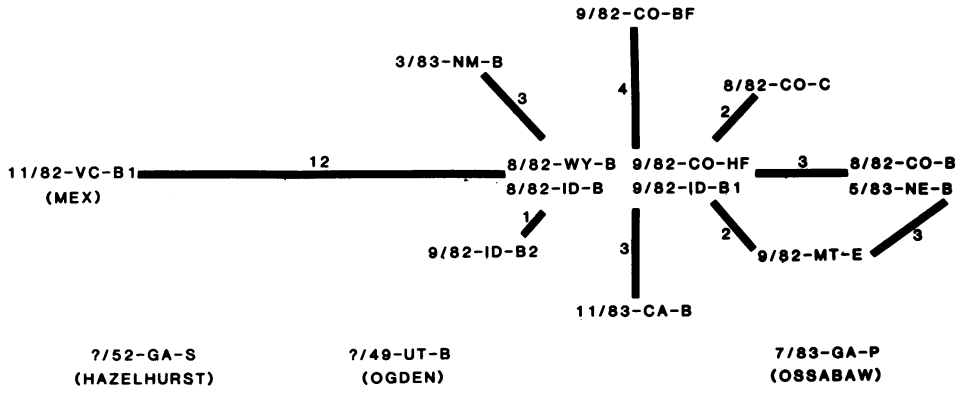


FIG. 2. Diagrammatic representation of the relationships of the T1 fingerprints of viruses of the 1982 to 1983 western U.S. VSV epizootic. Solid lines indicate close similarity. Numbers indicate the number of T1 spot differences among isolates.

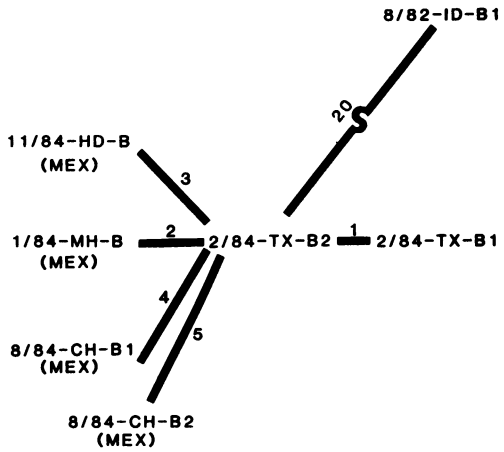


FIG. 3. Diagrammatic representation of the relationships of the T1 fingerprints of viruses of the 1984 Texas VSV outbreak.

month before the appearance of the disease in the United States (Fig. 4).

Analysis of T1 fingerprints of VSV NJ isolates from epizootic and enzootic regions of Mexico between 1982 and 1985 reveals a different pattern (Fig. 5). Two viruses (11/82-VC-B1 and 11/82-VC-B2) isolated the same month from the state of Veracruz (an enzootic region) had very different T1 fingerprints (Fig. 1G and H). An isolate (7/84-OA-B) from Oaxaca, another enzootic region, also had a T1 fingerprint with many spot differences relative to other VSV NJ isolates (Fig. 1I). In contrast, other isolates had clearly closely related T1 fingerprints. For instance, 11/82-VC-B2 and 2/83-VC-B had very similar fingerprints, and the majority of the other Mexican isolates were closely similar to one another (Fig. 5). Again, this was evident even for isolates from different host species, in this case, cattle and swine.

DISCUSSION

In analyzing the 1982 to 1983, 1984, and 1985 U.S. VSV NJ outbreaks, a common pattern emerges in which within a given outbreak the viruses were all closely related, having identical or very similar T1 fingerprints. This pattern was observed even for viruses isolated from different epizootic stages; separate geographical locations; different host species, including cattle, horses, and insects; and from different host lesion sites. This suggests that within a given epizootic the virus population is genetically relatively homogeneous. No evidence was found to suggest that very genetically distinct viruses were involved in the infection of animals and insects or in different host animals or lesion sites.

In contrast to the very close T1 fingerprint relationships observed among isolates within U.S. epizootics, only distant relationships were observed among viruses from the three different outbreaks analyzed. These data provide an indication of the possible origin of virus initiating these VSV NJ outbreaks. It is highly unlikely that these U.S. epizootics arose owing to the synchronous eruption of preexisting persistent infections in these areas. It has been demonstrated earlier that VSV undergoes rapid and random evolution during experimental persistent infections (12, 13). Synchronous eruption of persistent infections preexisting in each affected area would not lead to such homogeneous virus populations as observed here for each virus outbreak. In addition, the completely dissimilar T1 fingerprint of the virus isolate from feral swine on Ossabaw Island, Ga. (the only well-documented region of enzootic virus activity in the United States), provided no evidence to support its being the source of virus initiating the 1982 to 1983 VSV NJ epizootic in the western United States. In contrast, for each U.S. VSV NJ outbreak, a close relationship to earlier or concurrent Mexican virus isolates can be demonstrated. These data suggest that Mexico may be the source of virus initiating recent VSV NJ outbreaks in the United States. This is supported by epidemiological investigations indicating a northwards spread of virus activity during the early stages of recent VSV NJ epizootics (2, 16).

However, it is still unclear how the virus is transmitted during an epizootic. The isolation of virus from insects (during the 1982 to 1983 U.S. epizootic) which had few, if any, T1 fingerprint differences from cattle and horse virus isolates demonstrates that insects were involved in the epizootic but does not indicate their exact role in virus transmission. Clearly, other factors must also play a role. Direct-contact transmission has been suggested as the means of transmission, at least in the absence of insects during winter months (16). Movement of infected cattle may also play a role. The proposed involvement of a cattle dispersal sale in Idaho in the continued spread of VSV during the 1982 to 1983 U.S. epizootic (16) correlates well with T1 fingerprint relationships of viruses from this epizootic (Fig. 2).

Comparison of T1 fingerprints of the RNA genomes of VSV NJ isolates from enzootic and epizootic regions indi-

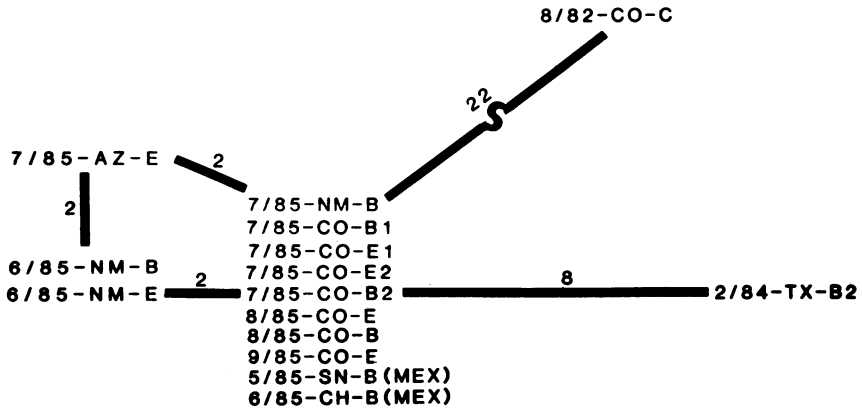


FIG. 4. Diagrammatic representation of the relationships of the T1 fingerprints of viruses of the 1985 U.S. VSV epizootic.

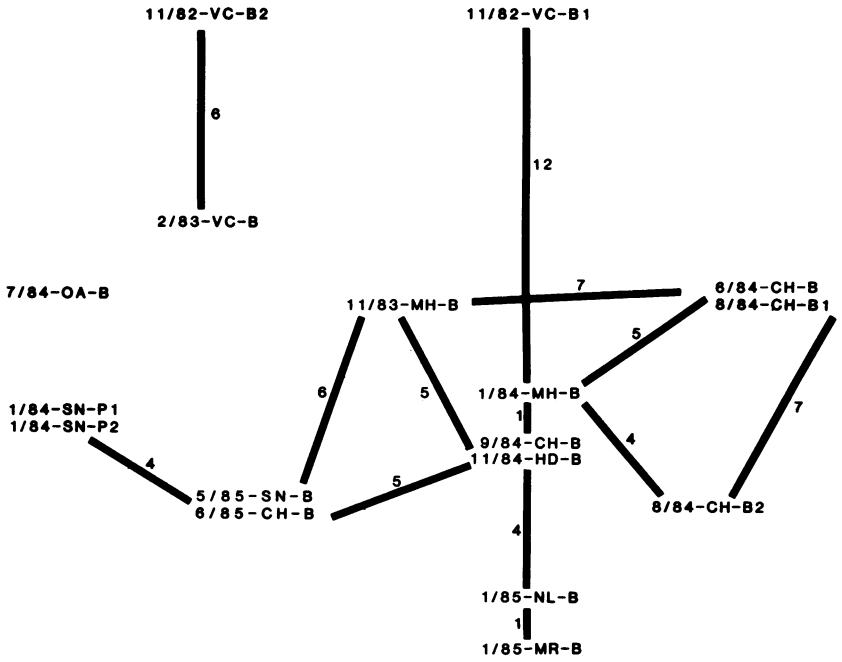


FIG. 5. Diagrammatic representation of the relationships of the T1 fingerprints of VSV isolates from Mexico from 1982 to 1985.

cates that a much greater genetic diversity exists among virus isolates from enzootic areas. It appears that, unlike epizootic regions, enzootic regions can exhibit more than one distinct type of VSV NJ. Preliminary fingerprint analysis of VSV NJ isolates from enzootic regions of Central America confirms this observation; these isolates form several different groups on the basis of their fingerprints and show little similarity to the U.S. and Mexican isolates (unpublished observation). It is currently unclear how the virus is maintained in enzootic regions. The possible involvement of persistent infections in the maintenance of the virus in enzootic regions would be consistent with the data. Since VSV RNA genomes quickly accumulate mutations during experimental persistent infections, this type of infection process could explain the extensive virus genetic diversity observed in areas of enzootic virus activity.

The data presented here identify at least six distinct VSV NJ subgroups based on virus RNA genome T1 fingerprints. Two are the currently defined groups represented here by the Ogden and Hazelhurst VSV NJ isolates. The other four include the main group of U.S. and Mexican isolates which have fingerprints varying from identical to more distantly related: the Ossabaw Island, Ga., isolate, 7/83-GA-P; the Veracruz isolates 11/82-VC-B2 and 2/83-VC-B; and the Oaxaca isolate, 7/84-OA-B. Virus RNA genomes which share less than 25% of their T1 oligonucleotides by comparison of their T1 fingerprints are approximately less than 90% homologous (1). Consequently, it is not possible to determine the exact relationship of these more distantly related VSV NJ subgroups based on their T1 fingerprints. Previous hybridization studies have demonstrated that the western U.S. isolate 9/82-CO-BF is more closely related to the Hazelhurst subgroup than to the Ogden subgroup (21). This result would place the majority of the U.S. and Mexican VSV NJ isolates studied here in the Hazelhurst subgroup and would extend the host range of that group to include cattle, house flies, and culicoides midges in addition to swine, horses, and black flies. Whether the other three fingerprint groups defined here represent new subgroups or more distantly related members of the existing two subgroups remains to be determined.

Although the degree of virus genetic diversity in enzootic regions appears to be greater than that in epizootic regions, isolates within an epizootic are not all identical. The ability of VSV to undergo rapid evolution, quickly accumulating genome mutations under various experimental conditions (12, 13, 18, 20, 23, 29–31), and the inherent high error rate of VSV RNA polymerase (26) suggest that virus genome variation within VSV NJ epizootic isolates represents the relatively rapid natural evolution of the virus during the spread of the disease. RNA sequencing and monoclonal antibody reactivity studies are under way to determine more precisely the genomic and antigenic variation of these naturally occurring virus variants.

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