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Genetic Diversity of Enzootic Isolates of Vesicular Stomatitis Virus New Jersey

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The RNA genomes of 43 vesicular stomatitis virus (VSV) isolates of the New Jersey (NJ) serotype were T_1-ribonuclease fingerprinted to compare the extent of genetic diversity of virus from regions of epizootic and enzootic disease activity. Forty of these viruses were obtained from Central America during 1982 to 1985. The other three were older isolates, including a 1970 isolate from *Culex nigripalpus* mosquitos in Guatemala, a 1960 bovine isolate from Panama, and a 1976 isolate from mosquitos (*Mansonia indubitans*) in Ecuador. The data indicate that extensive genetic diversity exists among virus isolates from this predominantly enzootic disease zone. Six distinct T_1 fingerprint groups were identified for the Central American VSV NJ isolates from 1982 to 1985. The 1960 VSV NJ isolate from Panama and the 1976 isolate from Ecuador formed two additional distinct fingerprint groups. This finding is in sharp contrast to the relatively close genetic relationship existing among VSV NJ isolates obtained from predominantly epizootic disease areas of the United States and Mexico during the same period (S. T. Nichol, J. Virol. 61:1029–1036, 1987). In this previous study, RNA genome T_1 fingerprint differences were observed among isolates from different epizootics; however, the isolates were all clearly members of one large T_1 fingerprint group. The eight T_1 fingerprint groups described here for Central American and Ecuadorian viruses are distinct from those characterized earlier for virus isolates from the United States and Mexico and for the common laboratory virus strains Ogden and Hazelhurst. Despite being isolated 14 years earlier, the 1970 insect isolate from Guatemala is clearly a member of one of the 1982 to 1985 Central American virus fingerprint groups. This indicates that although virus genetic diversity in the region is extensive, under certain natural conditions particular virus genotypes can be relatively stably maintained for an extended period. The implications of these findings for the evolution of VSV NJ and epizootiology of the disease are discussed.

Vesicular stomatitis viruses (VSV) of the New Jersey (NJ) serotype are responsible for extensive epizootic and enzootic disease activity in cattle, horses, and swine throughout the Americas (8–10). The United States and most regions of Mexico primarily experience periodic disease epizootics at varying intervals (14). Extensive enzootic disease activity exists in other regions of Mexico (particularly the states of Veracruz and Oaxaca) (13), throughout Central America (3), and in regions of South America (2). In addition, enzootic activity has also been reported in the southeastern United States on Ossabaw Island, Georgia (23).

A previous T_1 ribonuclease fingerprint analysis of 43 VSV NJ 1982 to 1985 isolates from the United States and Mexico revealed that genetically virus populations were relatively homogeneous within successive virus epizootics (14). Although considerable genetic change was evident on comparison of viruses from different epizootics, all virus isolates from epizootic regions were clearly members of one large T_1 fingerprint group. Three other T_1 fingerprint groups were evident in enzootic disease areas. These consisted of an isolate from Ossabaw Island, Georgia, two isolates from Veracruz, Mexico, and an isolate from Oaxaca, Mexico. Based on this small sample, it was suggested that virus genetic diversity may be greater in enzootic disease zones than in epizootic disease zones.

The present study reports the results of T_1 fingerprint analysis of the RNA genomes of 40 VSV NJ isolates during 1982 to 1985 from Central America, two VSV NJ strains isolated much earlier from this region, and one VSV NJ isolate from Ecuador. The aim of the study was to determine the degree of genetic diversity among VSV NJ isolates from this predominantly enzootic region.

Earlier analysis of U.S. and Mexican VSV NJ isolates had suggested that Mexico was the likely origin of the virus, initiating successive VSV NJ disease epizootics in the United States during the period 1982 to 1985 (14). However, it was unclear whether Mexico was the primary virus reservoir or whether it merely represented a stage in the transmission of virus from potential virus reservoirs further south in the enzootic regions of Central America. Therefore, information was sought concerning the epizootiology of the disease within this region and the genetic relationship among these viruses and those isolated in the United States and Mexico during the same period.

MATERIALS AND METHODS

Virus isolates. VSV NJ field isolates from Central America and Ecuador were generously provided by Robert B. Tesh, Yale Arbovirus Research Unit, Yale University School of Medicine, New Haven, Conn., and Luis C. Roquebert, Laboratory for the Diagnosis of Vesicular Diseases, Panama City, Panama. Viruses were analyzed after a minimum number of low-multiplicity virus passages in tissue culture. The two insect viruses were originally isolated by Centers for Disease Control research groups. The Ecuadorian virus was isolated at Fort Collins, Colo. (5), and the other virus was isolated at Atlanta, Ga.

Virus growth and T_1 ribonuclease fingerprinting technique. Viruses were grown at 37°C in BHK-21 cells in minimum essential Eagle medium containing $^{32}P_i$ (80 μCi/ml). Virus

was harvested and purified, and RNA was extracted as described previously (15). The T_1 fingerprinting technique was used exactly as described previously (14). As before, reproducibility of each virus RNA fingerprint was checked at least once, and usually twice, for each virus isolate. An IBM PC-XT microcomputer was used to compare and catalog virus T_1 fingerprint spot differences.

RESULTS

T_1 ribonuclease fingerprinting analysis of virus RNA genomes was performed on 43 VSV NJ isolates from Central America and Ecuador (Table 1). The isolates represented 40 viruses isolated from cattle and swine in Central America during the period 1982 to 1985, one 1970 virus isolated from *Culex nigripalpus* mosquitoes in Guatemala, one 1960 bovine virus isolate from Panama, and one 1976 virus isolated from mosquitoes (*Mansonia indubitans*) in Ecuador (5). The majority of animal viruses were isolated from mouth lesions, although some were from teat lesions and the feet. Also, different host species, including cattle, horses, swine, and insects, were involved.

The spot patterns of RNA T_1 fingerprints were compared, and similarities and differences were cataloged (Fig. 1 and 2, Table 2). On the average, 80 to 90 of the larger oligonucleotide spots were analyzed on each T_1 fingerprint. Viruses were assigned to different groups if less than 50% of the spots were common between viruses. It has been previously estimated that the RNA genomes of viruses with less than 50% T_1 spots in common will exhibit less than approximately 95% nucleotide sequence similarity (1). By using this definition, eight T_1 fingerprint groups were clearly discernible, each sharing less than 50% of T_1 oligonucleotides in common with the other groups. Groups 1 through 6 contained all of the 1982 to 1985 Central American VSV NJ isolates in addition to the older ??/70-GM-C isolate. An estimate of the genetic relationship among virus members of groups 1 through 6 was obtained by calculating the number of T_1 spot differences within each group (Fig. 3, panels 1 through 6). Virus isolates ??/60-PN-B and ??/76-EC-M were the sole representatives of groups 7 and 8, respectively. The geographical distribution of virus T_1 fingerprint groups in Central America during 1982 to 1985 is shown (Fig. 4). Group 1 viruses occurred in Guatemala and El Salvador and included isolates from cattle, swine, and insects (Fig. 3). This group had persisted in the region for some time; the 1970 VSV NJ isolate from Guatemala (??/70-GM-C) was clearly identifiable as a member of this fingerprint group. Group 2 viruses were the most widely distributed, being present in Honduras, El Salvador, Nicaragua, and Guatemala (Fig. 3). Two isolates from Honduras (11/84-HD-B1 and 11/84-HD-B2) differed by only one T_1 spot. Interestingly, one virus, (10/82-HD-B) isolated more than 2 years earlier, differed by only five T_1 spots from the 11/84-HD-B1 isolate. Group 3 consisted of only three viruses isolated from cattle and horses in Honduras and Belize (Fig. 3). Group 4 was a rather diverse group of four viruses. Two isolates had identical T_1 fingerprints (10/85-HD-B1 and 10/85-HD-B2). Each of the other viruses differed from each other by at least 39 T_1 spots. This group had an interesting geographical distribution, being isolated from Honduras and Costa Rica but not the intervening country. Group 5 viruses were only identified in Nicaragua (Fig. 3). Some virus members were closely related to one another, with identical fingerprints or only small numbers of T_1 spot differences among them. Other fingerprint relationships were more distant, with some viruses having only approximately 75% of large T_1 oligonucleotides in common. Again, it is interesting to note that only four T_1 spot differences existed among a 1982 isolate (10/82-NC-B) and two viruses isolated 3 years later (07/85-NC-B1 and

TABLE 1. VSV NJ isolates analyzed by ribonuclease T_1 fingerprinting

Virus isolate[a]	Host or species	Host lesion site
Guatemala		
??/70-GM-C	*C. nigripalpus*	
12/82-GM-B	Bovine	Mouth
10/84-GM-P	Porcine	Foot
11/84-GM-B	Bovine	Mouth
12/84-GM-P	Porcine	Foot
04/85-GM-B	Bovine	Mouth
Belize		
10/84-BL-E	Equine	Mouth
Honduras		
09/82-HD-B	Bovine	Mouth
10/82-HD-B	Bovine	Mouth
12/82-HD-B	Bovine	Mouth
11/84-HD-B1	Bovine	Teat
11/84-HD-B2	Bovine	Teat
08/85-HD-B	Bovine	Mouth
10/85-HD-B1	Bovine	Mouth
10/85-HD-B2	Bovine	Mouth
El Salvador		
05/85-ES-B	Bovine	Mouth
07/85-ES-B	Bovine	Mouth
08/85-ES-B	Bovine	Mouth
10/85-ES-B	Bovine	Mouth
Nicaragua		
10/82-NC-B	Bovine	Mouth
07/83-NC-B	Bovine	Mouth
07/83-NC-P	Porcine	Mouth
09/83-NC-B	Bovine	Mouth
11/83-NC-B	Bovine	Mouth
01/85-NC-B	Bovine	Mouth
02/85-NC-B	Bovine	Mouth
05/85-NC-B	Bovine	Mouth
07/85-NC-B1	Bovine	Mouth
07/85-NC-B2	Bovine	Mouth
Costa Rica		
10/82-CR-B	Bovine	Mouth
12/82-CR-B1	Bovine	Mouth
12/82-CR-B2	Bovine	Mouth
01/83-CR-B	Bovine	Mouth
12/84-CR-B	Bovine	Teat
01/85-CR-B	Bovine	Teat
07/85-CR-B	Bovine	Teat
Panama		
??/60-PN-B	Bovine	Unknown
10/83-PN-B	Bovine	Teat
12/83-PN-B	Bovine	Mouth
01/85-PN-B1	Bovine	Teat
01/85-PN-B2	Bovine	Teat
07/85-PN-B	Bovine	Mouth
Equador		
??/76-EC-M	Mosquitos	

[a] Standard nomenclature adopted for all virus isolates included month/year of isolation, two-letter abbreviation of country of origin, and one-letter abbreviation of host of origin.

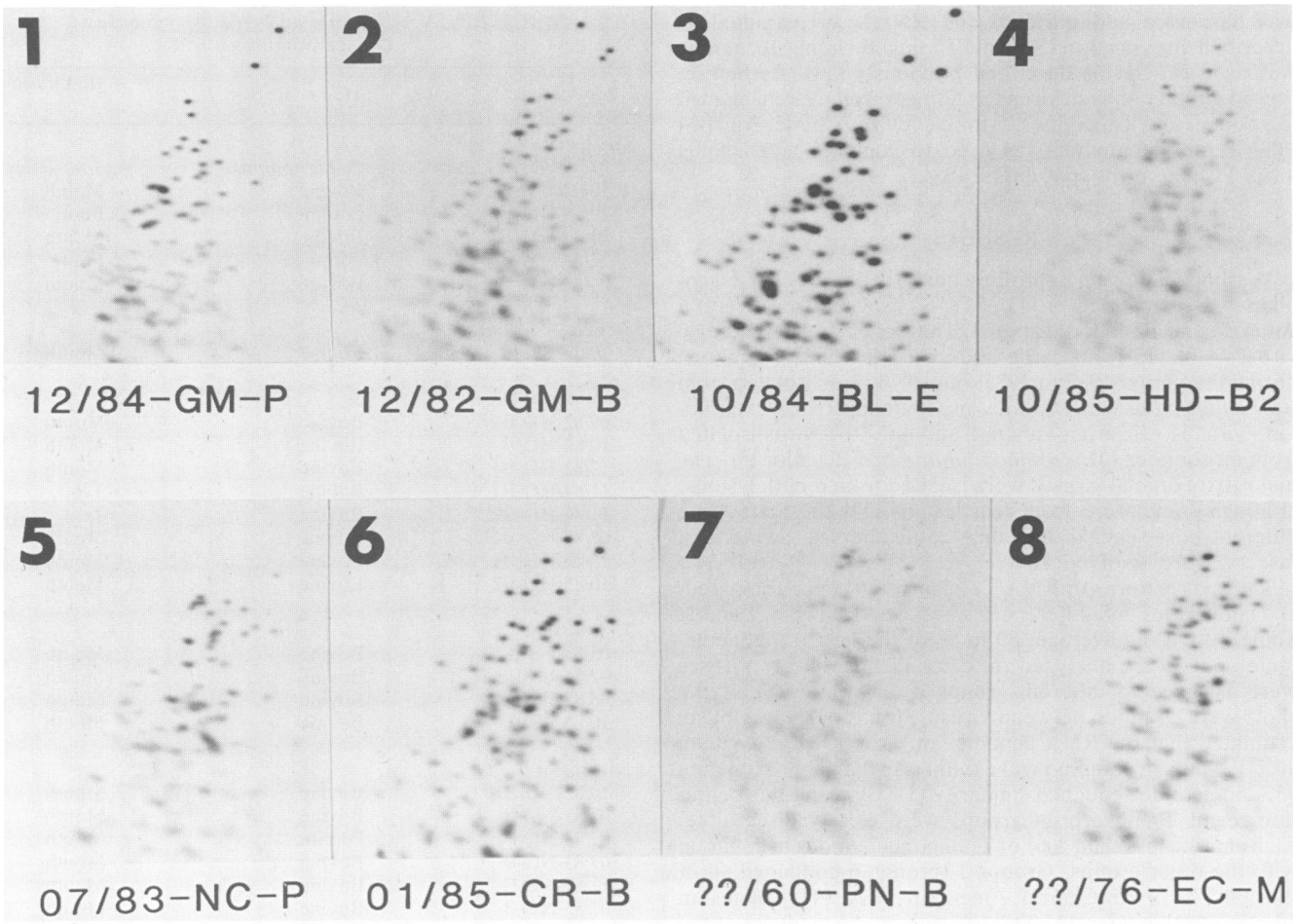

FIG. 1. T_1 fingerprints of the RNA genomes of representative VSV NJ isolates. T_1 fingerprint groups 1 through 8 are represented.

07/85-NC-B2). Group 6 viruses were isolated in Costa Rica and Panama (Fig. 3). Although the fingerprints of these viruses were obviously related, they appeared to fall into two subgroups. One subgroup was present in Costa Rica, and the other subgroup was present in Panama. Within group 6, further evidence was seen of instances in which few T_1 spot differences existed among viruses isolated several years apart.

The eight T_1 fingerprint group patterns defined here were distinct from those of the common laboratory strains Ogden and Hazelhurst, which represent the two previously defined subtypes of VSV NJ (17). In addition, they were also dissimilar to the four T_1 fingerprint groups recently described for VSV NJ isolates from 1982 to 1985 from the United States and Mexico (14).

DISCUSSION

The data presented show that a high degree of genetic diversity exists among VSV NJ serotype isolates from the Central American region. This result is rather surprising considering the relatively small geographical area represented and is in sharp contrast to the situation in the United States and most of Mexico, in which mainly epizootic disease activity is seen. A previous T_1 fingerprinting study had demonstrated that of 43 VSV NJ isolates during 1982 to 1985 from throughout the United States and Mexico, 39 belonged to a single T_1 fingerprint group (14). The other four viruses were isolated from regions of primarily enzootic disease activity. Not only were a greater number of fingerprint groups identified in Central America, but larger numbers of T_1 spot differences were seen among members of several of these virus groups.

The reason for the much higher degree of virus genetic diversity seen in enzootic disease areas compared with that in epizootic regions is unclear, as are the mechanisms for maintenance and transmission of the virus in these regions. It is possible that virus could be maintained in enzootic disease regions in insect populations or in the form of persistent or inapparent infections of animals (10, 13, 21, 23, 28). The evidence for possible involvement of insects in this process include the typical cessation of VSV outbreaks on the arrival of an insect-killing frost, the isolation of VSV NJ and Indiana (IND) serotypes from numerous insect species, and the experimental infection and transovarial transmission of VSV IND in insects (7, 20, 25, 26, 28). However, insects are not the sole means of virus transmission, because U.S. virus outbreaks whose spread continued through the winter months in the apparent absence of insects have been reported (10).

The high genetic diversity among enzootic virus isolates may provide an indication as to how virus is maintained in these regions. In common with most RNA viruses, VSV has been shown to be capable of high rates of accumulation of

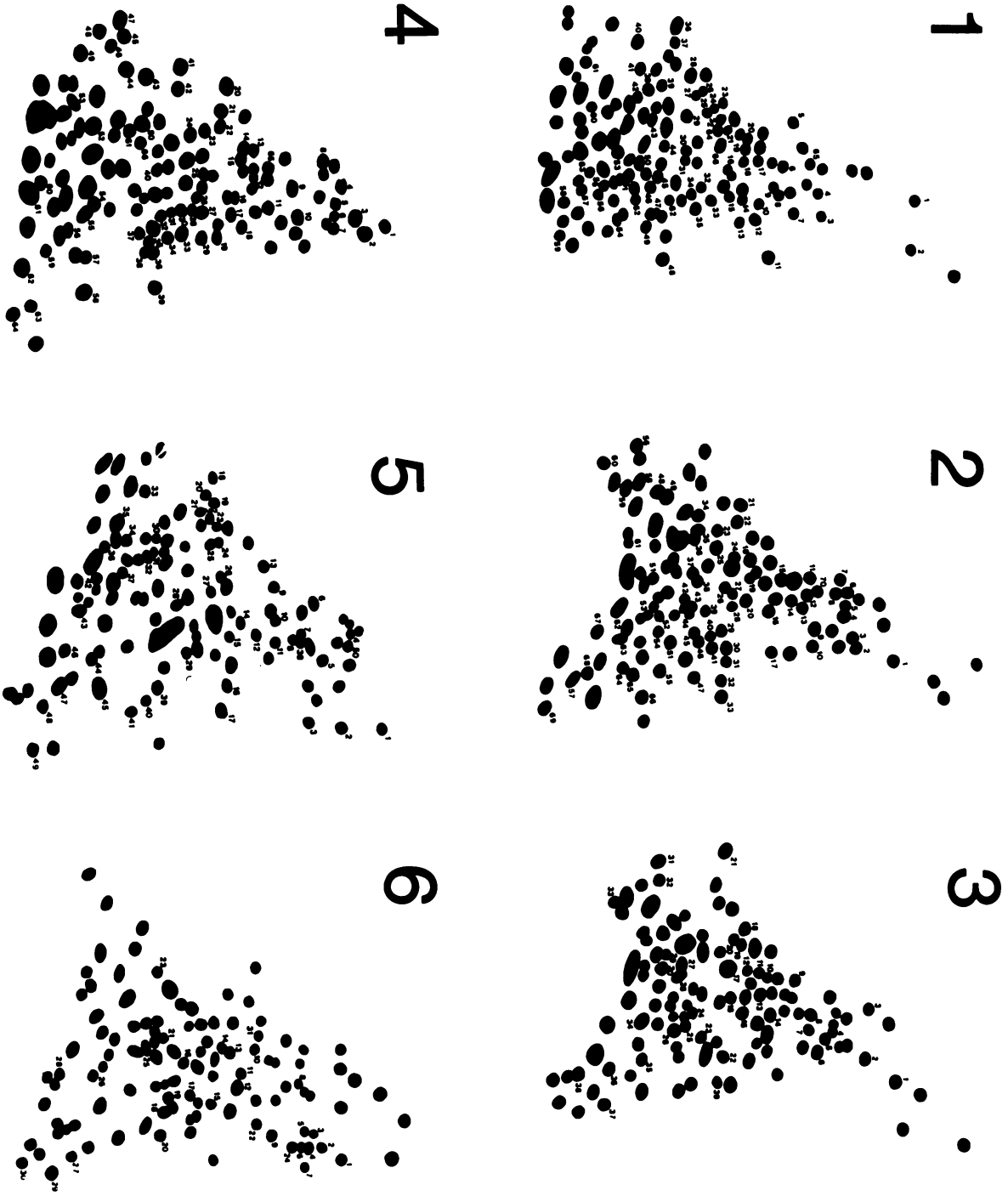

FIG. 2. Diagrammatic representation of the T_1 spot numbering system for virus fingerprint groups 1 through 6.

TABLE 2. Catalog of T_1 spot differences among VSV NJ isolate groups 1 though 6

Virus	T1 spots[b]																																																																					
Isolate[a]	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70
Group 1:																																																																						
11/84-GM-B		+	+	+			+	+	+		+	+		+		+			+	+						+	+	+	+					+	+	+		+		+	+				+					+				+			+							+	+					
12/84-GM-P		+		+			+	+	+		+	+		+		+			+				+	+				+	+					+	+	+		+	+	+	+		+		+					+				+			+							+	+					
10/85-ES-B				+		+	+	+			+	+		+	+	+	+	+	+			+						+	+	+			+		+	+		+				+					+		+		+			+	+	+				+	+			+	+					
10/84-GM-P				+			+	+			+	+	+		+	+		+		+	+			+				+	+	+		+		+		+		+				+	+	+	+		+				+		+	+									+		+	+				
08/85-ES-B			+								+	+		+	+	+	+	+		+	+							+	+	+	+	+		+		+		+	+			+	+		+		+				+			+								+			+	+				
??/70-GM-C	+			+			+	+			+	+	+		+	+		+		+	+			+					+	+	+	+		+		+						+	+		+	+		+			+	+						+	+							+				
04/85-GM-B				+	+		+	+		+				+	+			+		+					+			+		+	+	+		+			+	+				+	+		+		+				+						+		+					+	+					
Group 2:																																																																						
11/84-HD-B1		+	+	+		+	+	+	+			+			+	+		+		+		+		+	+	+			+	+							+	+			+		+				+		+			+	+	+	+		+	+	+			+	+			+	+			
11/84-HD-B2		+	+	+		+	+	+	+			+			+	+		+		+		+		+	+	+			+	+			+				+	+			+		+				+		+			+	+	+	+		+	+	+			+	+			+	+			
10/82-HD-B		+	+	+		+	+	+	+			+			+	+		+		+		+		+		+			+	+						+		+	+		+		+				+		+				+	+	+		+	+	+			+	+			+	+			
12/82-GM-B		+			+			+	+		+	+				+			+	+		+			+	+	+	+		+		+			+		+				+		+	+		+	+	+		+		+	+	+	+		+	+	+		+		+		+		+		+	
05/85-ES-B		+		+			+	+	+			+				+			+	+		+			+	+	+	+		+		+			+						+	+		+	+		+	+			+	+	+	+			+	+	+		+		+		+			+	+	
07/85-ES-B			+	+			+			+	+		+	+				+		+		+	+	+	+	+		+	+		+				+		+	+	+	+			+		+						+	+			+			+	+				+		+		+	+		
09/83-NC-B	+	+		+			+	+	+			+			+	+	+		+		+				+				+	+		+		+	+			+			+	+			+		+		+	+		+	+	+	+	+	+			+				+	+	+	+			+
Group 3:																																																																						
09/82-HD-B		+	+			+		+	+		+		+						+	+	+		+	+			+	+	+			+			+	+			+																																		
10/84-BL-E		+			+		+			+		+		+			+	+				+			+						+			+		+		+																																		
08/85-HD-B	+		+	+		+			+		+	+	+		+	+		+	+		+				+	+	+	+		+	+	+	+		+		+	+	+																																	
Group 4:																																																																						
10/85-HD-B1 / 10/85-HD-B2	+		+		+	+							+	+		+		+				+	+		+		+			+		+	+	+	+				+	+	+		+	+		+		+	+		+				+	+	+		+		+	+			+	+				
10/82-CR-B		+		+		+			+	+	+	+	+		+		+				+		+	+		+	+	+	+					+		+	+	+	+	+		+	+	+	+	+			+	+	+	+	+	+		+		+	+		+	+	+	+		+				
12/82-HD-B			+	+	+		+	+	+	+	+	+			+		+		+	+		+			+				+	+	+	+			+			+			+						+				+	+	+		+		+			+		+			+					
Group 5:																																																																						
07/83-NC-B / 07/83-NC-P	+		+	+	+			+			+		+	+			+	+				+			+		+					+		+	+			+	+			+	+	+	+		+		+																					
05/85-NC-B		+	+			+	+					+	+			+			+	+				+	+			+							+	+	+	+	+			+	+	+	+	+		+		+																				
02/85-NC-B		+	+			+	+					+	+			+			+	+				+	+			+					+		+	+	+	+	+			+	+	+	+	+		+		+																				
01/85-NC-B		+	+			+	+					+	+			+			+	+				+	+			+		+	+				+	+	+	+	+			+	+	+	+	+		+		+																				
07/85-NC-B1 / 07/85-NC-B2		+						+	+			+	+			+		+	+	+					+	+						+			+			+			+	+					+	+																						
10/32-NC-B		+						+	+	+		+	+			+		+	+	+					+				+			+			+						+	+					+	+																						
11/83-NC-B		+	+					+			+				+				+		+		+									+					+	+	+	+			+		+		+	+																						
Group 6:																																																																						
12/82-CR-B2				+	+			+	+		+			+					+	+			+	+	+	+		+		+	+																																								
01/83-CR-B			+	+	+			+	+		+			+			+		+	+		+	+	+	+	+		+		+	+																																								
12/82/CR-B1		+		+	+			+	+		+								+	+			+	+	+	+		+		+	+																																								
12/84-CR-B / 01/85-CR-B					+		+	+			+			+	+		+		+	+		+	+	+	+	+		+		+	+																																								
07/85-CR-B					+		+	+			+			+	+		+		+	+		+		+	+	+		+		+	+																																								
01/85-PN-B1 / 01/85-PN-B2				+	+				+	+		+	+	+		+		+			+	+	+	+			+		+		+																																								
07/85-PN-B				+		+			+	+		+	+	+		+		+			+	+	+	+			+		+		+																																								
10/83-PN-B				+		+			+	+		+	+	+		+		+	+		+	+	+	+			+		+		+																																								
12/83-PN-B	+			+	+					+		+	+	+		+		+	+	+	+		+				+		+																																									

[a] See Table 1, footnote a, for virus nomenclature.
[b] See Fig. 2 for spot difference numbering for each group. +, Presence of spot.

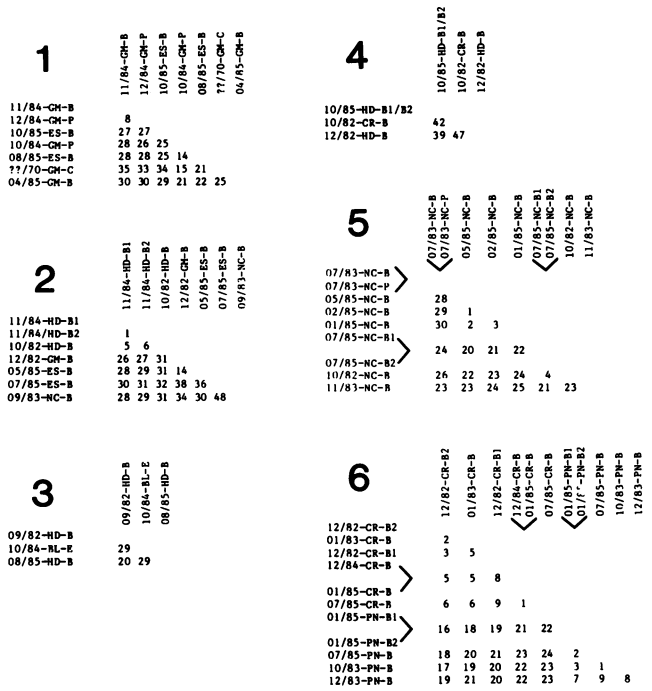

FIG. 3. Number of T_1 spot differences among RNA genome fingerprints of VSV NJ isolates for groups 1 through 6.

RNA genome mutations (11). The inherent rate of error of the VSV RNA polymerase appears to be as high as 1×10^{-4} to 4×10^{-4} substitutions per base incorporated (24). In laboratory studies with animal tissue culture cells, VSV IND has been shown to exhibit rapid genetic diversity or genome evolution under conditions of serial undilute virus passage, persistent infection, and immune pressure (11, 12, 16, 18, 22, 27, 29–31). However, virus genetic stability was observed on repeated serial passage of the virus at low virus multiplicities of infection of cells (4, 22). The yearly cycling of VSV NJ in enzootic regions in cattle possessing some degree of immunity to VSV due to past virus exposure could increase virus genetic diversity in these regions. It is likely that the situation would be different in virus epizootic regions, because the virus would less frequently encounter animals with some level of preexisting virus immunity. It is also possible that if virus is being frequently maintained in enzootic regions in the form of persistent virus infections of animals, this would also lead to a higher degree of virus genetic diversity.

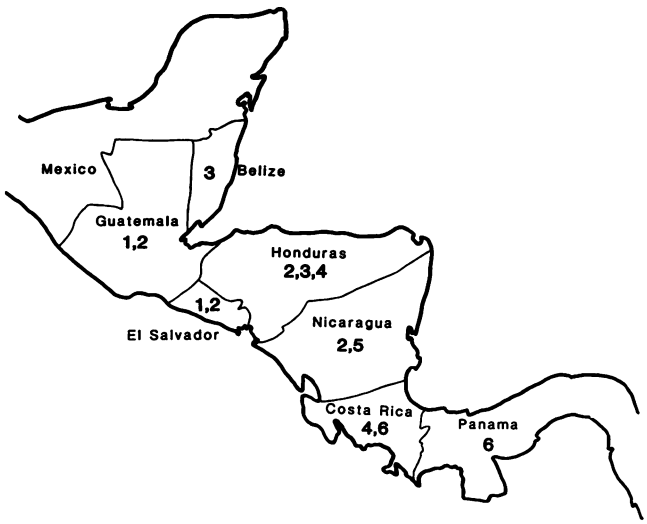

FIG. 4. Geographical distribution of Central American VSV NJ fingerprint groups 1 through 6 during 1982 to 1985.

The demonstration of only a small number of spot differences among viruses isolated several years apart would suggest that despite the apparent extensive virus genetic diversity in this region, under certain natural circumstances virus genomes can be relatively stably maintained. This is particularly evident in group 1, with virus ??/70-GM-C, which was isolated 14 years earlier than 10/84-GM-P, only differing by 15 T_1 spots. An average of one T_1 spot change per year in this specific case would correlate with virus genome variation of only approximately 0.1% per year (1).

The mechanism by which VSV with such a high inherent polymerase error level can in some instances remain genetically stable for extended periods in nature is not clearly understood. Several explanations are possible. Under certain natural conditions virus evolutionary constraints must be high or selective pressures must be low. For instance, if insects do play a role in the natural history of VSV as is currently supposed (9, 10, 14, 23, 28), then the necessity of VSV to replicate with high efficiency in both insect and mammalian hosts may place constraints on the rate at which virus genetic variability will occur. Also, it is possible that maintenance of virus in insect hosts for extended periods may lead to virus genetic stability due to the avoidance of mammalian host immune selection. Indeed, recent evidence obtained by using another negative-strand RNA virus, Toscana virus, suggests that virus genome stability can be maintained for extended periods of virus maintenance solely in the insect host (P. A. Bilsel, R. B. Tesh, and S. T. Nichol, submitted for publication).

Epizootiological questions concerning the relationship of Central American VSV NJ isolates to each other and to viruses isolated during the same period in the United States and Mexico were also addressed by this study. In Central America, a predominantly enzootic disease area, the apparent situation exists whereby several distinct groups of VSV NJ can coexist in one country (e.g., three groups of viruses existed in Honduras during 1982 to 1985) and one virus group can be present in several countries (e.g., group 2 viruses are present in four different countries). It would appear that virus groups do not exclude one another and can be transmitted within these enzootic regions.

The T_1 fingerprinting analysis of U.S. and Mexican VSV NJ isolates from 1982 to 1985 previously demonstrated that all isolates from epizootic regions belonged to a single T_1 fingerprint group and suggested that Mexico was the origin of viruses initiating epizootics in the United States. It was, however, unclear whether Mexico was the primary reservoir of these viruses or merely represented a stage in the transmission of virus from potential virus reservoirs further south in the enzootic regions of Central America. The dissimilarity of all the Central American VSV NJ isolate T_1 fingerprint spot patterns to those of all the U.S. and Mexican isolates would indicate that the disease activity in this region is unrelated to that occurring further north. This would support the earlier suggestion of Mexico being the primary reservoir of viruses that initiated VSV NJ epizootics in the United States during 1982 to 1985 (14).

This study, together with the earlier study (14), reports the

genetic analysis of a total of 86 VSV NJ isolates from cattle, horses, swine, and insects. No evidence was found to support a conclusion that genetically distinct viruses caused disease in different hosts or at different host lesion sites. Instances of viruses from different hosts and lesion sites possessing few or no T_1 spot differences were numerous. The data indicate that at least 14 distinct VSV NJ fingerprint groups exist throughout the Americas. The degree of relatedness of the different fingerprint groups to one another cannot be accurately ascertained by T_1 fingerprinting technique alone. Virus G gene nucleotide sequencing studies are currently in progress in this laboratory that support the genetic grouping of VSV NJ isolates presented, on the basis of these T_1 fingerprinting studies.

Previous studies have suggested that the main U.S. and Mexican epizootic virus T_1 fingerprint group is related to the defined Concan subgroup of VSV NJ (14, 19). Results obtained in this and previous studies on the 1970 Guatemalan VSV NJ isolate would suggest that Central American VSV NJ fingerprint group 1 viruses are related to the Ogden subgroup of VSV NJ (6, 17). The relative relationship of the other virus fingerprint groups to the two defined subgroups of VSV NJ should become evident on completion of ongoing nucleotide sequencing studies of the virus isolate G genes. In addition, monoclonal antibody analysis of these virus isolates has been initiated to determine whether diversity present at the virus genetic level results in virus antigenic diversity.

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