SEROLOGICAL AND MOLECULAR CHARACTERIZATION OF FOOT-AND-MOUTH DISEASE SEROTYPE O VIRUSES ISOLATED FROM OUTBREAKS IN BRAZIL AND ARGENTINA BETWEEN 1958 AND 1983

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SUMMARY. **Genetic characterization of representative foot-and-mouth disease virus strains from major serotype O outbreaks which occurred between 1958 and 1983 in southeastern Brazil and centraleastern Argentina was obtained using T_1 maps. The results presented constitute the basis for a data bank to be applied in epidemiological studies.**

Control of foot-and-mouth disease (FMD) in endemic regions is compromised by the considerable variability of the virus (*16*), which is responsible for its complex serology. FMDV exists in seven immunologically different types: O, A, C, SAT-1, SAT-2, SAT-3 and Asia-1 (*22*), each of which comprises an ever-increasing number of subtypes (*21,24*).

In South America, continuous surveillance of the field situation for the emergence of new variants is basically limited to classical serological assays (*3*). Since these methods do not provide definitive data on the relationships among individual outbreak strains, the sources from which the infection is spreading may be difficult to trace. Such information becomes more relevant in view of the success of eradication programs in progress in South American countries.

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In contrast to serological assays, molecular approaches, such as RNA fingerprinting (*10,14*) and rapid sequencing (*23*), which analyze the primary genetic structure of the virus, allow a more precise measurement of the degree of relationship among viral strains, constituting a valuable epidemiological tool to follow the behavior of strains in the field.

Oligonucleotide fingerprinting has been especially useful to study the evolutionary relationships among closely related strains (*12,13,15,18, 25*). Furthermore, its diagnostic application has been clearly demonstrated for FMDV. In fact, it has been applied to determine close relationships between most European field and vaccine viruses (*11,17*).

For South American strains, RNase T_1 maps of the prototype viruses used presently for vaccine production were registered (*5,9*), and heterogeneity was established within one of these strains (*26*). Moreover, the T_1 maps of subtype C_3 viruses isolated in Argentina between 1981 and 1986 were

also recorded (*7*). The present report extends this analysis to several relevant outbreak field strains of serotype O which occurred between 1958 and 1983 in Argentina and Brazil.

Viral strains obtained from the Pan American Foot-and-Mouth Disease Center collection were passaged in baby hamster kidney cell monolayer cultures to the minimal extent needed to provide RNA for analysis. In most cases, this involved three to four passages from field material, without using virus plaque purification methods. Preparation of ^{32}P-labeled RNA from the cytoplasm of infected cells was as reported before (*5*). The method of separation of T_1 oligonucleotides used was a modification of earlier techniques, and was performed as described (*8*).

Six isolates representative of different episodes which occurred over a twenty five-year period in southeastern Brazil and centraleastern Argentina were studied. These were obtained from infected animals at the locations and dates indicated in table 1. Serological comparisons of these samples among themselves established a wide spectrum of relationships which were not proportional to the time elapsed between isolations (figure 1). This is shown by the relative similarity of virus O_1 C/58 with O_1 Arg/77 and O_1 Iri/83, isolated 19 and 25 years apart, respectively, and the marked difference between strains O_1 Cas/67 and O_1 Br/70, recovered three years apart.

Figure 2 shows the fingerprints from the RNAs of these six viruses. A schematic representation of the differences between them and the early strain O_1 C/58 is presented in figure 3. These comparisons revealed patterns which varied in many spots, except for virus O_1 Arg/77, which

Table 1. Representative FMDV strains isolated in Argentina and Brazil between 1958 and 1983

Strain designation	Origin County, State, Country	Date of isolation (Month/Year)
O_1 C/58	Campos, Rio de Janeiro, Brazil	01/1958
O_1 Cas/67	Caseros, Buenos Aires, Argentina	01/1967
O_1 Br/70	Bagé, Rio Grande do Sul, Brazil	09/1970
O_1 Arg/77	Unrecorded, Argentina	11/1977
O_1 Br/80	Dom Pedrito, Rio Grande do Sul, Brazil	06/1980
O_1 Iri/83	Hipólito Irigoyen, Buenos Aires, Argentina	09/1983

Abbreviations:
O_1 C/58, O_1 Campos Br 1/58; O_1 Cas/67, O_1 Caseros-Arg 2/67; O_1 Br/70, O_1 RS Brazil/70; O_1 Arg/77, O_1 Argentina/77; O_1 Br/80, O_1 RS Brazil/80; O_1 Iri/83, O_1 Irigoyen Argentina/83.

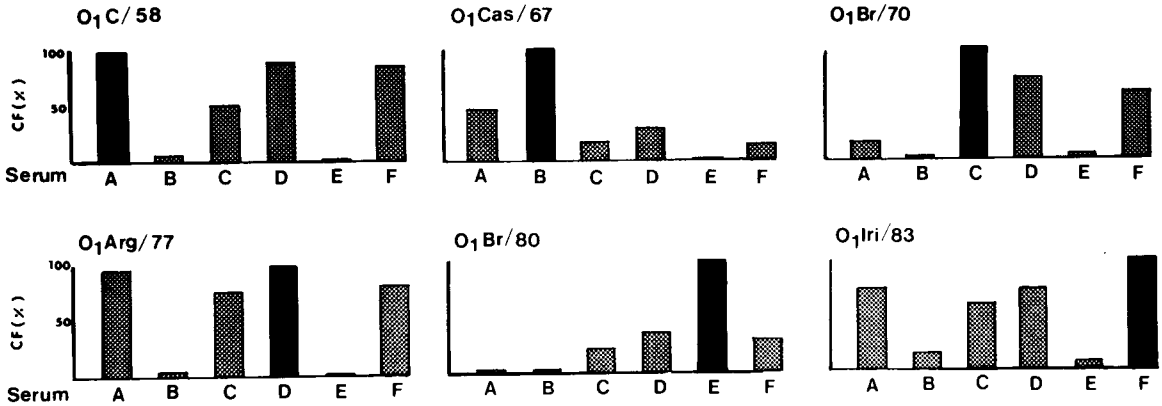

Figure 1. Serological analysis of the indicated viruses. Serological relationships were performed by complement fixation test at 50% hemolysis (CF) with the following sera: A, O_1 C/58; B, O_1 Cas/67; C, O_1 Br/70; D, O_1 Arg/77; E, O_1 Br/80; F, O_1 Iri/83

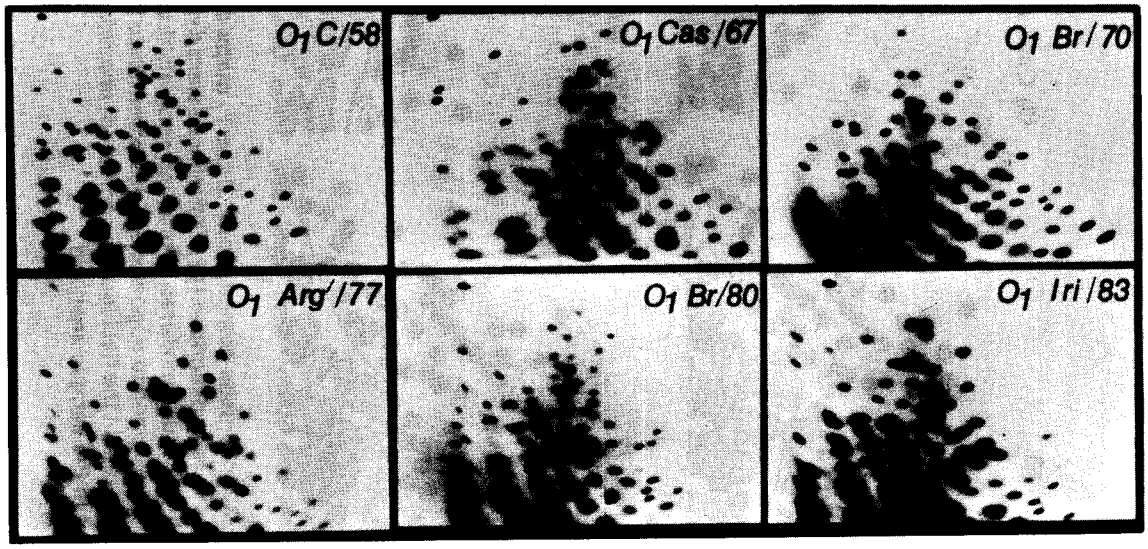

Figure 2. RNase T1 two-dimensional maps of ^{32}P-labeled RNA of the corresponding strains

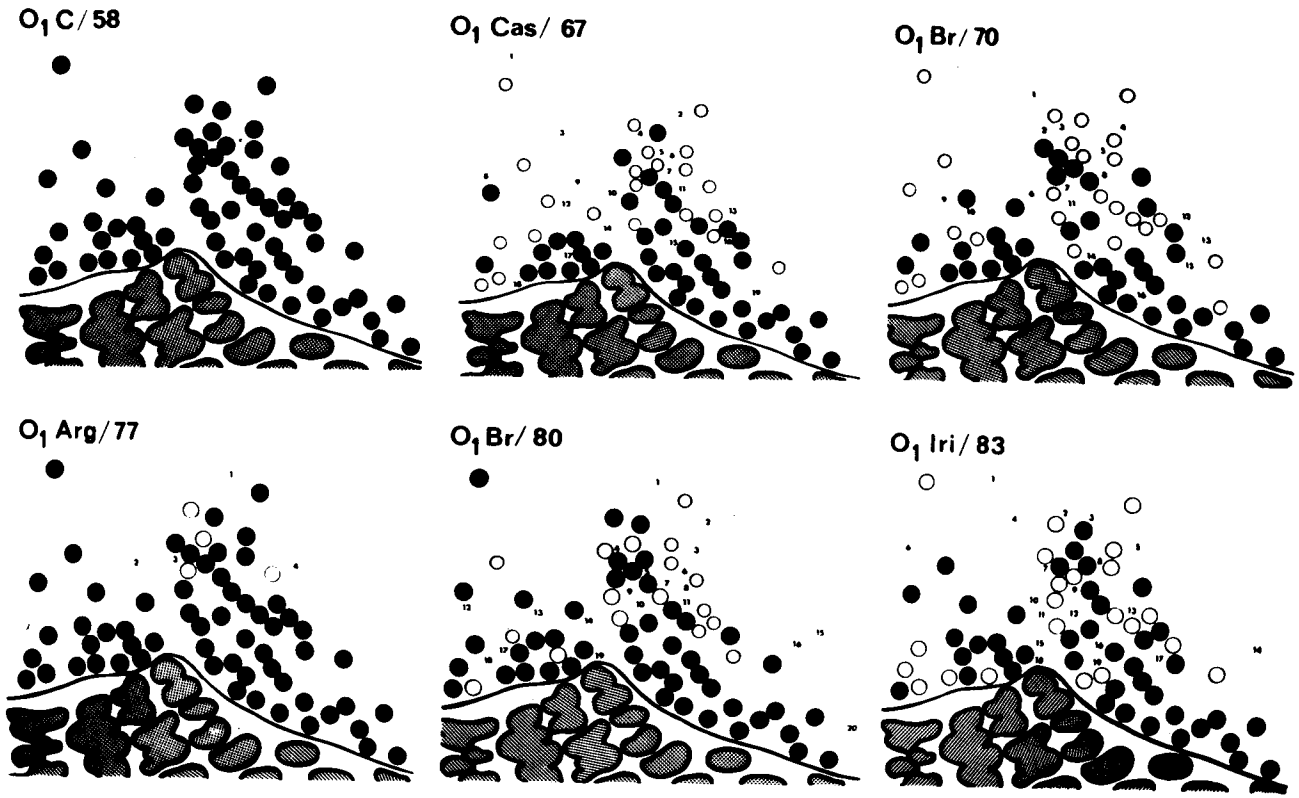

Figure 3. Schematic comparisons of the T_1 maps of the indicated strains with the early strain O_1 C/58. Criteria for analysis were as described (*18*). Differences were determined by comparing the migration of the viral oligonucleotides individually and in mixtures containing equivalent amounts of both T_1 digests. Keys for comparative analyses: solid circles correspond to oligonucleotides common to the strains compared; open circles represent spots of virus O_1 C/58 not present in the field isolate compared; uncircled numbers represent spots absent in the early strain O_1 C/58, but present in the sample analyzed

showed a T_1 map quite similar to that of the early strain, with only four additional and four missing spots. Values deduced from figure 3, calculated as reported previously (*20*), indicated that the genetic homology was always over 96% (O_1 Cas/67, 96.2%; O_1 Br/70, 96%; O_1 Arg/77, 99.3%; O_1 Br/80, 96.5% and O_1 Iri/83, 96.2%), falling within the expected range in which T_1 map differences of various RNA(s) may be compared (*1*).

In agreement with the serological data, the degree of variation did not correlate with the time elapsed between isolations. Moreover, there seemed to be no accumulation of variant oligonucleotides among the consecutive isolates. Similar conclusions could be drawn from nucleotide sequencing results (*18*).

Such patterns of variation suggest that the outbreak strains are likely to represent fluctuations of heterogeneous populations, with close to distant relationships to the consensus sequence. These populations may evolve independently from each other and may be selected under certain epidemiological situations. In the case of virus O_1 Arg/77 an alternative explanation could also be the accidental reintroduction of vaccine viruses in the field, as was suggested for many European outbreak strains (*6,11*).

From the data presented, it is clear that, in contrast to the situation in Europe, FMDV isolates in South America showed considerable genetic diversity. Similar results have been demonstrated among field isolates of type C representative of outbreaks in Argentina (*7*), of type O in Peru Colombia and Venezuela (Malirat et al., manuscript in preparation), and in other endemic regions (*4,19*).

Overall, T_1 map differences were associated with antigenic variations, as revealed by complement fixation (figure 1), and by their reactivity in an ELISA test with a selected panel of monoclonal antibodies (*2*). However, considering the high precision of molecular characterization by fingerprinting, the information is useful for inclusion in a data bank for FMDV strains acting in South America. This will favor future epidemiological studies on the maintenance and spread of FMDV in this region.

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