UPDATING OF FOOT-AND-MOUTH DISEASE VIRUS STRAINS OF EPIDEMIOLOGICAL IMPORTANCE IN SOUTH AMERICA[1]

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SUMMARY

The high variability of foot-and-mouth disease, due to genetic recombination and the selective pressure of antibodies in areas of systematic vaccination, is the reason that one strain of virus causes only one epidemic wave in the field. The list of subtypes should therefore be revised periodically to eliminate those which are no longer found in the field. The diagnostic laboratories have to support the control campaigns of the disease by relating antigenically and immunologically the field strains with those used in the production of vaccines. The coverage of the vaccine strains, furthermore, is of great usefulness and interest for vaccine banks. One test which can be recommended for this type of study is the expected percentage of protection (EPP) determined through the serum protection test with sera from vaccinated and revaccinated cattle. An EPP of less than 75% with sera from revaccinated cattle is an indication of a low protection level in the field.

INTRODUCTION

Foot-and-mouth disease (FMD) is produced by a virus of the Picornaviridae family. Due to its physicochemical properties the virus is classified in the Aphtovirus genus.

Seven immunological serotypes (O, A, C, SAT_1, SAT_2, SAT_3 and Asia 1) have been identified within the Aphtovirus genus which do not give cross protection between each other.

Due to their high mutation capability, each serotype includes various subtypes that are characterized by immunological differences among themselves. The subtypes are represented by strains isolated from foci, outbreaks or epidemics, which have similar antigenic and immunogenic characteristics. To prevent errors in identification, the denomination of a strain must include the serotype, the virus subtype, the place and the year of isolation.

VARIABILITY OF THE VIRUS IN THE ENDEMIC AREAS

The great capability of variation of FMD virus is due to the high rate of mutation (*7*), the genetic recombination (*5*) and the selective action of antibody on virus replication in partially immune animals (*4*). These processes cause changes in the sequence of the nucleotides, which induce modifications in the amino acids of the capsid polypeptides.

The changes in the nucleotides can be detected by biochemical techniques like RNase T_1 resistent maps (*2*). But the modifications of the sequence of the amino acids, when located in the antigenic determinants, are detected by antigenic and immunogenic tests (*1, 6, 8*) or by the sequencing of the amino acids of the viral protein (*3*).

The changes in antigenicity and immunogenicity of the viruses in the field are of utmost importance for the FMD control programs, since the degree of protection of the vaccinated population depends on the quality of the vaccine applied and on the homology between the vaccine strain and the field strain. These changes also have a repercusion on the diagnosis and on the production and control of the vaccine.

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Because of high contagiousness of FMD and high capacity of variability of the virus, the success of the control programs depends initially on the efficiency of the epidemiological surveillance in detecting the foci, collecting samples and blocking the spread of the disease. This aspect is of vital importance in the countries having extensive livestock exploitation methods and much animal movement, which enormously facilitate propagation of the virus. The diagnosis laboratory, jointly with the quality control laboratory of vaccine, must determine the antigenic characteristics of the field strains, the immunological coverage of the vaccine strains, and the potency of the vaccines.

The antigenic characterization studies of the field virus and the analysis of the vaccine strain coverage against the epidemiologically important field viruses, must be available to other countries that vaccinate systematically and that have vaccine banks.

HISTORY OF THE VIRUS STRAINS IDENTIFIED IN SOUTH AMERICA FROM 1950 TO 1984

Retrospective studies of the FMD field viruses identified in South American countries have shown that in the Southern Cone the predominant viruses in the 1950's could be classified among the subtypes O_1, A_{24} and C_3. In the Andean countries, the viruses belonged to the O_1 and A_5 subtypes. Peru was an exception because it frequently imported bovine meat and by-products from Argentina and Colombia, which led to the identification of viruses from the two regions.

During the 1950's, FMD vaccine in the Southern Cone of South America was produced with the Waldmann method which led to the emergence of very different virus strains when partially immune cattle at slaughterhouses were inoculated to produce antigen. Thus subtypes O_8, A_{13}, A_{16} and A_{17} were identified in Brazil and A_{19} in Argentina. The isolation of those viruses was related to the sites of virus handling and vaccine production (slaughterhouses and laboratories).

The exchange of strains among laboratories led to the accidental introduction of subtype A_{10} into Argentina. That subtype was used in the Netherlands for vaccine preparation. Strains C_2 and A_{30} caused epidemics in the 1940's and 50's in Rio Grande do Sul (Brazil) and in Uruguay.

Strains of the subtypes O_3, O_8, A_{10}, A_{13}, A_{16}, A_{17}, A_{19}, A_{25}, A_{30} and C_2 have totally disappeared and have not caused foci or any epidemic outbreaks since 1963.

In the 1960's, when the network of vesicular disease diagnosis laboratories was consolidated in South America (Fig. 1), several countries initiated FMD control programs based on systematic vaccination of the cattle population. That network of laboratories led to a striking improvement in epidemiological surveillance and in the characterization of viruses occurring in the field. At that time the control programs still suffered from several shortcomings due to their inexperience. In some countries, therefore, epidemics were caused by virus strains very similar to those used in vaccine production. For example, the A_{18} subtype was isolated in Venezuela in 1962. The disease was eliminated by massive field application of an attenuated live-virus vaccine of A_{24} Cruzeiro and, in 1969, of subtype A_{32} which still persists. Strains of the A_{26} and A_{29} subtypes, similar to A_{24}, were identified in Peru. Throughout Colombia the existence of viruses similar to those of subtype A_5 has been recorded. Those viruses were classified among the A_{27} subtype. In 1969 the plains in Bogotá were affected by virus A_{31}. It was eliminated by strict control of foci and strategic revaccination with homologous monovalent vaccine, thus preventing its spread into other areas of the country.

With respect to type C virus strain, a focus caused by C_4 Tierra del Fuego virus occurred in 1966 in Argentine Patagonia; it was different from those previously identified in the Southern Cone. Epidemiological data on that episode indicate that carrier cattle were possibly responsible for the focus. Argentina and Paraguay were affected in 1969 by an epidemic caused by a strain antigenically not much different from subtype C_3. The World Reference Laboratory (WRL) classified the Argentine strain as subtype C_5 and the strain found in Paraguay as C_3.

FIGURE 1. *Network of official laboratories for the diagnosis of vesicular diseases. South America, 1984.*

The 1970's were characterized by the consolidation of the vaccine-production and control laboratories, first in Uruguay and Chile, then in Argentina, Brazil, Colombia and Paraguay. The consistent production of vaccines and their official control led to improved immunization of the cattle population in the programs. Nevertheless, strains not very different from those used in vaccine preparation were isolated. For example, C_3 Indaial affected southern Brazil from 1971 to 1974, and Argentina and Uruguay in 1974-75. The A Venceslau and A Bagé strains were isolated in 1976 in Brazil. The latter strain affected cattle in Rio Grande do Sul before spreading into Argentina and Uruguay. During its process of evolution it originated A Argentina/79 and A Brazil/79. Because those two strains are very similar and also similar to A Venceslau and A Bagé, they are included in a single group.

The 1980's are characterized by better structured programs both in the field and in the laboratories. This factor is evident in the effort developed against the O RS-Brazil/80 strains, which is rather different from the O_1 Campos virus vaccine (Table 1). The O RS-Br/80 strain produced a severe epidemic in Rio Grande do Sul, Brazil, in 1980, but quick identification of the virus and the adoption of efficient epidemiological surveillance blocked its spreading into Uruguay and Argentina.

In 1981, virus strains A Argentina/81, A Brazil/81 and A Uruguay/81 emerged. Those strains are believed to be the end effect of the epidemic caused by the A Argentina–Brazil/79 strain. Those type A virus strains isolated in 1981 did not spread due to the epidemiological measures put into effect on that occasion.

The incidence of type C virus increased in Argentina in 1983 until it caused an epidemic in 1984. The epidemic was controlled by the use of a homologous monovalent vaccine jointly with the polyvalent vaccine. On that occasion the spread of the disease into the neighboring countries was likewise restrained, except for the occurrence of foci in the Bolivian Chaco area controlled by perifocal vaccination.

The A São Carlos virus was identified in 1984 in the state of São Paulo, Brazil (Tables 2 and 3). It was controlled through intensified epidemiological surveillance and strategic vaccination with classic A Cruzeiro and A Venceslau vaccine.

VIRUS STRAINS OF CURRENT INTEREST IDENTIFIED IN SOUTH AMERICA

Detailed analysis of the antigenic and immunogenic characteristics of the viruses identified in South America (Tables 4, 5, 6) leads to the conclusion that the currently predominant strains should be classed in the O_1, A_{24}, A_{32} and C_3 subtypes. Additionally the A Argentina–Brazil/79 and C Argentina/84 strains should be included (Table 7), pending their classification.

CLASSIFICATION OF FMD VIRUS

At the International Symposium on Variants and Immunity, held in Lyon, France, in 1967, the

TABLE 1. *Serological relationships (r), protection against foot generalization (PG), mouse protection indexes (MPI) and expected percentage of protection (EPP) in cattle revaccinated with strain O_1 Campos challenged against homologous virus and O RS-Br/80*

Challenge strains	Hyper. serum O_1 Campos r	O_1 Campos vaccine		
		PG	Mean MPI	EPP[a]
O_1 Campos-Br/58	1.00	16/16	3.62	87.7
O RS-Br/80	0.34	6/16	2.40	72.5

[a]Low limit of confidence 95%, expected percentage of protection. GOMES, I. & ASTUDILLO, V. *Bol. Centr. Panam. Fiebre Aftosa 17-18*:9-16, 1975.

TABLE 2. *Serological relationships (r) and expected percentage of protection (EPP) obtained from hyperimmune sera and from cattle vaccinated and revaccinated with A_{24} Cruzeiro-Br/55 and A Venceslau-Br/76 vaccines challenged against A São Carlos-Br/84*

Challenge strains	r – Hyper.serum		EPP – 30 DPV		EPP – 30 DPR	
	A_{24} Cruz.	A Venc.	A_{24} Cruz.	A Venc.	A_{24} Cruz.	A Venc.
A_{24} Cruz.-Br/55	1.00	–	69.7	–	99	–
A Venc.-Br/76	–	1.00	–	96.1	–	98.7
A S.Carlos-Br/84	0.31	0.05	⩽44.6	⩽61.0	73.2	⩽47.9

TABLE 3. *Protection against foot generalization (PG) in cattle vaccinated and revaccinated with A_{24} Cruzeiro-Br/55 and A Venceslau-Br/76 vaccine, challenged against A Venceslau-Br/76 and A São Carlos-Br/84*

Challenge strains	Cattle protected/used	
	30 DPV	21 DPR
A Venceslau-Br/76	14/16	18/18
A São Carlos-Br/84	3/16	11/18

TABLE 4. *Foot-and-mouth disease virus type O of historical or current interest, identified in South America. 1950-1984*

Subtype	Strain	Year		
		Classification	Identification	
		LMR	First	Last
O_1	O_1 Campos-Br/58	1967	1958	1984
O_3	O_3 Venezuela/50	1956	1950	1958
O_8	O_8 Bahia-Br/60	1962	1960	1962
O^a	O^a Rio Grande do Sul-Br/80	*a*	1980	1980

[a] It was not sent to the World Reference Laboratory (WRL).

WRL set down the standards for classifying FMD viruses. The standards were based on obtaining the serological relationships by means of the complement fixation test (*1, 8*). In view of the difficulty of applying those standards, due to the existence of many subtypes each with groups of various strains, the 1976 International Symposium on Variants and Immunity proposed to analyze only the relationships (r) against the different existing subtypes. When the value of both r was below 0.25, the strain under study was held to be a new subtype. Furthermore, it was proposed that only the epidemiologically important strains should be classified (*6*). The existing proposals have not resolved the problem, for no new subtypes have been identified since 1970 when virus A_{32} was identified.

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TABLE 5. *Foot-and-mouth disease virus type C of historical or current interest, identified in South America. 1950-1984*

Subtype	Strain	Year		
		Classification	Identification	
		LMR	First	Last
C_2	C_2 Pando-Uruguay/44	1969	1944	1974
C_3	C_3 Resende-Br/55	1969	1955	1984
C_3	C_3 Paraguay/69	1969	1969	1969
C_4	C_4 Tierra del Fuego-Arg/66	1969	1966	1966
C_5	C_5 Argentina/69	1969	1969	1974
C^a	C^a Argentina	*a*	1983	1984

[a]Pending classification by the World Reference Laboratory (WRL).

TABLE 6. *Foot-and-mouth disease virus type A of historical or current interest, identified in South America. 1950-1984*

Subtype	Strain	Year		
		Classification	Identification	
		LMR	First	Last
A_{10}	A_{10} Argentina/61	1961	1961	1961
A_{13}	A_{13} Santos-Br/58	1962	1958	1958
A_{16}	A_{16} Belém-Br/59	1964	1959	1960
A_{17}	A_{17} Guarulhos-Br/59	1964	1959	1962
A18	A_{18} Zulia-Ven/62	1964	1962	1963
A_{19}	A_{19} Suipacha-Arg/62	1964	1963	1963
A_{24}	A_{24} Cruzeiro-Br/55	1967	1955	1984
A_{25}	A_{25} Argentina/59	1967	1959	1964
A_{26}	A_{26} Argentina/66	1967	1963	1967
A_{27}	A_{27} Colombia/67	1967	1967	1983
A_{29}	A_{29} Peru/69	1970	1969	1970
A_{30}	A_{30} Uruguay/45	1970	1945	1960
A_{31}	A_{31} Colombia/69	1970	1969	1970
A_{32}	A_{32} Venezuela/70	1970	1969	1984
A^a	A^a Argentina/76	*a*	1976	1976
A^b	A^b Ecuador/75	*b*	1975	1978
A^b	A^b Venceslau-Br/76	*b*	1976	1984
A^b	A^b Bagé-Br/76	*b*	1976	1984
A^b	A^b Argentina/79	*b*	1979	1984
A^b	A^b Brasil/79	*b*	1979	1984
A^b	A^b Argentina/81	*b*	1981	1982
A^b	A^b Brasil/81	*b*	1981	1981
A^a	A^a São Carlos-Br/84	*a*	1984	1984

[a]It was not sent to the World Reference Laboratory (WRL).
[b]Pending classification by the WRL.

TABLE 7. *Foot-and-mouth disease virus strains and subtypes epidemiologically important at present, identified in South America. 1984*

Country	Subtypes and strains					
	O_1	A_{24}	A_{32}	A Arg/79 Br/79	C_3	C Arg/84
Argentina	x	–	–	x	x	x
Bolivia	x	x	–	–	x	–
Brazil	x	x	–	x	x	–
Colombia	x	x	–	–	–	–
Ecuador	x	x	–	–	–	–
Paraguay	x	–	–	–	x	–
Peru	–	x	–	–	–	–
Uruguay	x	–	–	–	x	–
Venezuela	x	x	x	–	–	–

laboratories have jointly sought to resolve the situation for the countries of the region by applying the following procedure:

1. *Preparation of the virus strain collection*

– Identify the existing strains in the field and those handled by the official and private laboratories in the region.

– Designate the representative strain of each recognized subtype.

– Prepare antigens and hyperimmune sera from the virus strains listed in the two previous points, for CF_{50} and ELISA tests.

– Prepare virulent virus suspensions for potency tests.

– Compile RNase T_1 resistants maps (one and two dimensional "fingerprinting").

– Keep "Serum Bank" of sera from cattle vaccinated and revaccinated with vaccines prepared with the various vaccine strains used in South America up-to-date.

– Establish the serological relationships.

– Determine the immunological coverage of the vaccine strains by means of the mouse protection and/or serum neutralization tests, with sera from vaccinated and revaccinated cattle (Serum Bank), and by direct testing.

2. *Identification of new strains*

– Collect samples of all the field foci.

– Characterize the strains antigenically by subtyping.

– Obtain the r_1 values against the standard strains used in vaccine preparation.

– Establish the immunological coverage of the "Serum Bank" sera from cattle vaccinated and revaccinated with the vaccine strains used in the region.

– Epidemiological information.

– When the strain persists in the field, it is included in the strain collection and all the work stated in point 1 is performed. If the strain used in the vaccine provides protection at revaccination equal to or greater than 75%, it will be included in the subtype to which the vaccine strain belongs.

– When the field strain is different from the strain used to produce the vaccines a strain will be selected to be used for vaccine production considering its antigenicity, replicability, stability and immunogenicity.

CONCLUSIONS

The great capacity of FMD virus for frequent mutation, plus the selective action affecting virus replication in field conditions, as well as its passage through different hosts, make it practically impossible for a strain to cause more than one epidemic wave in areas of systematic vaccination. Therefore,

the list of subtypes and strains should be reviewed periodically to eliminate those that have not been identified in the field in recent years.

The initial concern regarding emerging strains should be to restrain their spread in the field. The viruses causing foci in the interepidemic periods should be carefully studied: their antigenic structure should be compared with that of the vaccine strains used in the region, and the immunological coverage of the sera from animals vaccinated and revaccinated with the vaccine strain should be determined against the field strain. This test can be complemented by tests of protection against development of foot lesions in cattle vaccinated and revaccinated with the vaccine strain.

When the degree of protection in cattle at three weeks post-revaccination is equal to or exceeds 75%, and the vaccination coverage in the region is broad enough, then revaccination with the available vaccine and stringent control of animal movement will suffice to stop the spread of the new strain. The new strain should then be classified within the same subtype as the vaccine strain. When the immunological coverage is below 75%, the spread becomes epidemic and its control is difficult. In this case it is necessary to prepare and use an specific monovalent vaccine or to incorporate the strain to complement the antigenic profile of the vaccine commonly used in the region.

Countries where FMD is controlled and where the susceptible population is still protected by systematic vaccination —such as Western Europe— or disease-free countries that possess vaccine banks, should be aware of the coverage of their vaccines against the epidemiologically important strains in the endemic countries. The mouse protection test with sera from cattle vaccinated and revaccinated with their vaccines are very suitable for this type of work. The Pan American Foot-and-Mouth Disease Center through the network of vesicular-disease diagnosis laboratories and the countries of the region have organized those studies.

REFERENCES

1. ALONSO FERNANDEZ, A., SÖNDAHL, M.S., ROSENBERG, F.J., ASTUDILLO, V.M. Variabilidad antigénica e inmunogénica del virus de la fiebre aftosa. (Antigenic and immunogenic variability of the foot-and-mouth disease virus). *Bol. Centr. Panam. Fiebre Aftosa 47-48*: 11-22, 1983.
2. AUGÉ DE MELLO, P., CASAS OLASCOAGA, R., COSTA GIOMMI, M., ALONSO FERNANDEZ, A., SCODELLER, E.A., LA TORRE, J.L., BERGMAN, I.E. RNA fingerprinting of South American prototype aphtovirus strains. *Vaccine 4*: 105-110, 1986.
3. BECK, E., FEIL, G., STROHMAIER, K. The molecular basis of the antigenic variation of foot-and-mouth disease. *The EMBO Journal 2* (4): 555-559, 1983.
4. HYSLOP, N.St.G. & FAGG, R.N. Isolation of variant during passage of strains of foot-and-mouth disease virus in partly immunized cattle. *J. Hyg.* (Camb.) *63*: 357-368, 1965.
5. McCAHON, D., KING, A.M.Q., SLADE, W.R., SKINNER, H.H., HARESHAPE, J. The potential of recombination for the production of foot-and-mouth disease virus vaccine strains. 3rd. General Meeting of ESECT, Oxford 1979. *Develop. biol. Standard. 46*: 223-230 (S. Karger, Basel, 1980).
6. PEREIRA, H.G. Foot-and-mouth disease virus subtypes. Int. Symp. on FMD Variants and Immunity, Lyon, 1976. *Develop. biol. Standard. 35*: 165-174 (S. Karger, Basel, 1977).
7. PRINGLE, C.R. Genetic aspects of the thermal inactivation properties of foot-and-mouth disease virus strains. *Bull. Off. Int. Epiz. 61* (7-8): 619-628, 1964.
8. RESOLUTIONS. Int. Symp. on FMD Variants and Immunity, Lyon, 1967, Symp. Series Imm. Stand. *8*: 169-170 (Karger.B/N.Y., 1968).