FOOT-AND-MOUTH DISEASE VIRUSES USED IN VACCINE PRODUCTION AND CONTROL IN SOUTH AMERICA

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SUMMARY

The O, A and C viruses used in South America for foot-and-mouth disease vaccine production and control are examined. Strains that have recently shown some epidemiological significance in the field are also studied. With few exceptions, the control of vaccines is performed with the same samples utilized in the production.

All of the type O strains used for vaccine production in South America belong to the O_1 subtype. The type A viruses examined by the serum neutralization test can be divided into two groups, one group comprising A_{24} Cruzeiro, A_{24} Argentina/68 and A_{27} Cundinamarca, and the other group composed of A Venceslau, A Argentina/79 and A Brazil/79. All the C types studied are very similar with strains C_2 Pando and C_3 Resende having the higher antigenicity.

INTRODUCTION

The foot-and-mouth disease (FMD) virus' great capacity for mutation (*4, 6, 7*) leads to the constant appearance of new field strains. The World Reference Laboratory (WRL) was therefore organized in 1958 in Pirbright, England, with the major goal of classifying FMD viruses. And in 1951, when the Organization of American States (OAS) founded the Pan American Foot-and-Mouth Disease Center (PAFMDC), one of the Center's assigned tasks was to act as the diagnostic reference laboratory for the Americas. This activity was also acknowledged by the WRL, the United Nations Food and Agriculture Organization (FAO) and the International Bureau of Epizootics at its 28th General Meeting held in May, 1960.

Moreover because of the FMD virus variability, laboratories must produce vaccines from these varying strains. Thus, a vaccine that is suitable in one area may not be effective in another, due to the greater or lesser correlation between the strains active in the field and those used in vaccine production. It therefore becomes necessary to know the antigenic and immunogenic characteristics of the virus strains used in the formulation of the vaccines in the different countries, in order to determine which virus strain is most suitable for application in a given area.

This paper describes the antigenic and immunogenic characteristics of the virus strains utilized in the production and control of vaccines in South America. Strains that have been epidemiologically important in the region are also described.

MATERIALS AND METHODS

Virus strains studied

O_1 Campos–Brazil/58: Virus isolated in 1958 in the state of Rio de Janeiro, Brazil, in unvaccinated cattle. This virus is the reference strain for the Americas. The majority of the type O viruses identified in South America have antigenic characteristics similar to this virus.

O_1 Caseros–Argentina/67: Strain isolated from an FMD outbreak in cattle vaccinated with O_1 Campos, from the province of Santa Fe, in Argentina, in 1967. Its antigenic behavior is similar to the preceding strain.

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O_1 Urubamba–Peru/63: Isolated in unvaccinated cattle in Peru, in 1963, this strain is similar to the two strains mentioned above.

O_1 Cura–Venezuela/71: Collected in Venezuela in 1971 from FMD-diseased cattle that had been systematically inoculated with O_1 Campos modified live virus vaccine.

O_1 Magdalena–Colombia/78: This virus was collected from an outbreak of FMD in vaccinated cattle. Strains with antigenic characteristics similar to this one have been identified in Colombia since 1964.

O Rio Grande do Sul (RS)–Brazil/80: This strain caused an epidemic among cattle in Rio Grande do Sul, Brazil, which persisted from October 1979 to February 1981 and reached a 3.5% morbidity rate. It has not been identified again in subsequent years.

A_5 Westerwald/47: Subtype representative of Europe.

A_{24} Cruzeiro–Brazil/55: Isolated in 1955 in the state of São Paulo, Brazil, in unvaccinated cattle. The majority of the field strains in the countries of the Southern Cone had close relationship with A_{24} up to 1976. At the present time, only strains from the pantanal lowlands in Brazil's state of Mato Grosso, Ecuador, Peru, Colombia and Venezuela retain the antigenic characteristics of A_{24}.

A_{24} Argentina/68: This strain was isolated in 1968 from vaccinated cattle in Argentina, where it predominated until 1976.

A_{27} Cundinamarca–Colombia/76: Isolated from vaccinated cattle, this virus strain has been representative for the strains acting in Colombia since 1967. It is closely related to the A_5 subtype. This characteristic was greater in the A_{27} Colombia/67 strain.

A_{32} Venezuela/70: The predominant strain in Venezuela from 1969 to 1980.

A Venceslau–Brazil/76: Collected from cattle vaccinated with A_{24} Cruzeiro, predominated in Brazil, except in Rio Grande do Sul, from 1976 to 1980.

A Bage–Brazil/76: Isolated from cattle vaccinated with A_{24} Cruzeiro in Rio Grande do Sul in 1976. The majority of the viruses identified from 1967 to 1981 in the field in Rio Grande do Sul, Uruguay and Argentina exhibit antigenic characteristics similar to this strain.

A Argentina/79: This strain was isolated in 1979 from cattle vaccinated with A_{24} Argentina/68 in the province of Santa Fe. Its antigenic behavior is similar to that of A Brazil/79 and it is also very similar to A Bage.

A Brazil/79: Identified in vaccinated cattle in Rio Grande do Sul in 1979, is closely related to A Bage and A Argentina/79. It has been the predominant strain in Brazil since 1980.

A RS–Brazil/81: This strain was isolated from an outbreak in vaccinated cattle in Rio Grande do Sul in 1981.

C_1 GC: Strain isolated in Holland during the 1962 epidemic.

C_2 Pando–Uruguay/44: Isolated in the department of Colonia in 1944, caused an epidemic outbreak in Rio Grande do Sul (Brazil), Uruguay, and Argentina from 1943 to 1945.

C_2 997: Was responsible for the outbreaks in Great Britain in 1953 and 1965.

C_3 Resende–Brazil/55: Identified in unvaccinated cattle in the state of Rio de Janeiro in 1955, and is the representative subtype of the countries affected by type C virus in South America.

C_3 Indaial–Brazil/71: This virus was isolated from vaccinated cattle in 1971 in the state of Santa Catarina and is closely related to C_3 Resende.

C_4 Tierra del Fuego–Argentina/66: This virus caused an outbreak in 1966 in cattle in Tierra del Fuego.

C_5 Argentina/69: Predominated in Argentina from 1969 to 1974, the last year it was identified.

C Chaco–Paraguay/74: Isolated in the Chaco region of Paraguay in vaccinated cattle in 1974. This strain is closely related to all the type C strains studied in this experiment.

Serological relationships

The serological relationships were obtained by the methods described in (*2*) using the WRL-proposed standards (*8*); i.e., the hyperimmune sera were titered against 2.5 50% complement fixing units (CFU_{50}) of each of the antigens, using four hemolytic CFU_{50} and incubation at 4°C hemolysis times of 30 minutes. The serological

relationships were calculated by dividing the reciprocal of the titer of each serum against the heterologous antigen by the homologous antigen.

$$\text{That is: Relationship r} = \frac{\text{reciprocal titer of hyperimmune sera against heterologous antigen}}{\text{reciprocal titer of hyperimmune sera against homologous antigen}}$$

The antigens to determine the serological relationships were the same suspensions of virulent virus used to prepare the vaccine and conduct the serum neutralization and mouse protection tests. The suspensions were obtained by passaging virus obtained from cattle two or three times in BHK_{21} cells, clone 13, grown in roller bottles. For the C_1 GC and C_2 997 strains, however, guinea pig virus was used because of the unavailability of virus passed in cattle.

The hyperimmune sera were produced in guinea pigs utilizing suspensions of virulent virus adapted to the species. Six weeks after infection, the guinea pigs were hyperimmunized by two footpad inoculations of 0.2 ml of a suspension of virulent virus adapted to guinea pigs, a week apart. The suspension contained 0.1% of saponin. Seven days after the last hyperimmunization the guinea pigs were bled by cardiac puncture and the serum inactivated at 56° C for 30 minutes and stored at -20° C.

Serum neutralization

The test was conducted by the described microneutralization technique (*5*) using sera from cattle collected at 30 days post vaccination with a trivalent inactivated vaccine adjuvanted with aluminum hydroxide and saponin as described in (*1*).

Mouse protection

The test was performed in suckling mice with the cattle sera previously described using the technique indicated in (*3*).

RESULTS

Table 1 shows the different FMD virus strains presently utilized for vaccine production in South America. The viruses used most are O_1 Campos, A_{24} Cruzeiro, and C_3 Resende.

TABLE 1. *Strains used in South America for FMD vaccine production*

Countries	Strains		
	O	A	C
Argentina	O_1 Caseros[a]	A_{24} Argentina/68	C_3 Resende[a]
	O_1 Campos	A Argentina/79[a]	
Brazil	O_1 Campos[a]	A Venceslau[a]	C_3 Indaial[a]
		A_{24} Cruzeiro	
Colombia	O_1 Magdalena[a]	A_{27} Cundinamarca[a]	–
Ecuador	O_1 Urubamba[a]	A_{24} Cruzeiro[a]	–
Paraguay	O_1 Campos[a]	A_{24} Cruzeiro[a]	C_3 Resende[a]
Peru	O_1 Urubamba[a]	A_{24} Cruzeiro[a]	C_3 Resende[a]
Uruguay	O_1 Campos[a]	A_{24} Cruzeiro[a]	C_3 Resende
			C_2 Pando[a]
Venezuela	O_1 Campos[b]	A_{32} Venezuela/70[b]	
	O_1 Campos	A_{24} Cruzeiro	

[a]Strains used for control. In Venezuela O_1 Cura and A_{32} Venezuela strains are used for vaccine control.

[b]Strains used to produce attenuated live virus vaccine.

Tables 2, 4 and 6 show the serological relationships obtained with type O, A and C of the FMD virus strains, respectively. In addition to the viruses used in vaccine production, South American field strains and the European strains A_5 Westerwald, C_1 GC and C_2 997 were used.

Columns (r_1) indicate the complement fixing capacity of each one of the hyperimmune sera with the various antigens. Rows r_2 show the capacity with which each antigen is fixed by the different hyperimmune sera.

Accordingly, the amplitude of the hyperimmune serum is determined by r_1. Hence, the virus strains that induced hyperimmune sera with higher r_1 values are those that provide broader antigenic coverage.

Tables 3, 5 and 7 show the mean titers of the micro-neutralization of 5 sera from cattle taken at 30 days post vaccination (DPV), challenged with type O, A and C virus strains, respectively.

TABLE 2. *Serological relationships of FMD virus type O strains*

	Hyperimmune sera (r_1)					
Virus strains (r_2)	O_1 Camp.	O_1 Cas.	O_1 Urub.	O_1 Cura	O Mag.	O RS
O_1 Campos-Brazil/58	1.00	0.96	0.67	0.81	0.44	0.37
O_1 Caseros-Argentina/67	0.87	1.00	0.73	0.92	0.67	0.35
O_1 Urubamba-Peru/63	0.77	0.88	1.00	0.78	0.48	0.26
O_1 Cura-Venezuela/71	0.80	0.88	0.65	1.00	0.56	0.29
O_1 Magdalena-Colombia/78	0.51	0.45	0.54	0.37	1.00	0.28
O RS-Brazil/80	0.34	0.27	0.24	0.31	0.27	1.00

TABLE 3. *Mean cross neutralization titers of FMD virus type O of sera from cattle bled 30 days after vaccination*

	Sera from vaccinated cattle					
Virus strains	O_1 Camp.	O_1 Cas.	O_1 Urub.	O_1 Cura	O Mag.	O RS
O_1 Campos-Brazil/58	2.50	2.60	2.46	2.25	1.65	$\leqslant$ 1.35
O_1 Caseros-Argentina/67	3.12	3.15	3.00	2.76	1.89	1.86
O_1 Urubamba-Peru/63	2.85	3.18	3.34	2.67	2.01	1.92
O_1 Cura-Venezuela/71	2.63	2.73	2.64	2.42	1.80	$\leqslant$ 1.35
O_1 Magdalena-Colombia/78	1.98	1.97	$\leqslant$ 1.50	$\leqslant$ 1.50	2.43	$\leqslant$ 1.35
O RS-Brazil/80	$\leqslant$ 1.44	$\leqslant$ 1.47	$\leqslant$ 1.35	1.63	$\leqslant$ 1.41	3.12

TABLE 4. *Serological relationships of FMD virus type A strains*

	Hyperimmune sera (r_1)									
Virus strains (r_2)	A_5 West.	A_{24} Cruz.	A_{24} Arg.	A_{27} Cund.	A_{32} Ven.	A Venc.	A Bage	A Arg/79	A Br/79	A RS/81
A_5 Westerwald/47	1.00	0.51	0.53	0.54	0.23	0.24	0.26	0.60	0.55	0.07
A_{24} Cruzeiro-Br/55	0.29	1.00	0.48	0.27	0.15	0.08	0.14	0.49	0.32	0.05
A_{24} Argentina/68	0.46	0.61	1.00	0.45	0.27	0.24	0.32	0.76	0.61	0.14
A_{27} Cundinamarca-Col/76	0.50	0.37	0.48	1.00	0.14	0.20	0.18	0.52	0.37	0.05
A_{32} Venezuela/70	0.30	0.19	0.39	0.16	1.00	0.14	0.27	0.52	0.68	0.28
A Venceslau-Br/76	0.13	0.22	0.30	0.15	0.15	1.00	0.44	0.60	0.37	0.05
A Bage-Br/76	0.18	0.28	0.40	0.25	0.23	0.37	1.00	0.92	0.61	0.06
A Argentina/79	0.12	0.21	0.28	0.16	0.15	0.30	0.51	1.00	0.95	0.06
A Brazil/79	0.16	0.24	0.30	0.18	0.23	0.44	0.58	0.93	1.00	0.05
A RS-Brazil/81	0.09	0.05	0.12	0.05	0.16	0.06	0.10	0.15	0.21	1.00

TABLE 5. *Mean cross neutralization titers of FMD virus type A or sera from cattle bled 30 days after vaccination*

	Sera from vaccinated cattle					
Virus strains	A_{24} Cruz.	A_{24} Arg.	A Cund.	A Venc.	A Arg/79	A Br/79
A_{24} Cruzeiro-Brazil/55	2.68	2.28	1.71	$\leqslant$ 1.35	$\leqslant$ 1.53	$\leqslant$ 1.35
A_{24} Argentina/68	2.42	2.97	2.07	$\leqslant$ 1.43	1.83	$\leqslant$ 1.50
A_{27} Cundinamarca-Col/76	2.05	2.52	2.85	$\leqslant$ 1.50	$\leqslant$ 1.43	$\leqslant$ 1.35
A Venceslau-Brazil/76	$\leqslant$ 1.59	2.16	$\leqslant$ 1.43	2.94	2.40	2.34
A Argentina/79	$\leqslant$ 1.53	1.98	$\leqslant$ 1.35	2.13	2.31	2.22
A Brazil/79	$\leqslant$ 1.50	1.96	$\leqslant$ 1.43	2.25	2.40	2.22

TABLE 6. *Serological relationships of FMD virus type C*

	Hyperimmune sera (r_1)							
Virus strains (r_2)	C_1 GC	C_2 997	C_3 Res.	C_4 TF	C_5 Arg.	C_3 Ind.	C_2 Pando	C Chaco
C_1 GC Holland/62	1.00	0.42	0.28	0.20	0.44	0.32	0.47	0.79
C_2 997 Great Britain/53	0.80	1.00	0.38	0.37	0.30	0.47	0.97	0.76
C_3 Resende-Brazil/55	0.54	0.46	1.00	0.17	0.30	0.54	0.27	0.65
C_4 Tierra del Fuego-Arg/66	0.37	0.49	0.14	1.00	0.29	0.27	0.28	0.59
C_5 Argentina/69	0.42	0.34	0.30	0.34	1.00	0.40	0.17	0.63
C_3 Indaial-Brazil/71	0.67	0.49	0.44	0.25	0.29	1.00	0.38	0.85
C_2 Pando-Uruguay/44	0.64	0.74	0.16	0.29	0.21	0.42	1.00	0.72
C Chaco-Paraguay/74	0.63	0.45	0.31	0.41	0.49	0.44	0.46	1.00

TABLE 7. *Mean cross neutralization titers of FMD virus type C of sera from cattle bled 30 days after vaccination*

Virus strains	Sera from vaccinated cattle			
	C_3 Res.	C_3 Ind.	C_2 Pando	C Chaco
C_3 Resende-Brazil/55	2.50	1.70	2.30	1.80
C_3 Indaial-Brazil/71	1.60	2.20	1.80	⩽ 1.40
C_2 Pando-Uruguay/44	2.10	2.10	2.80	2.10
C Chaco-Paraguay/74	2.10	1.70	2.30	2.40

Table 8 shows the protection reached in cattle revaccinated 21 DPV with O_1 Campos and challenged 35 days after the revaccination (DPR). The serum protection values for the O_1 Campos and the O RS–Brazil/80 viruses are the mean of the sera from the 32 cattle used in the study.

TABLE 8. *Protection against foot lesions and mouse protection indexes (MPI) in cattle revaccinated with O_1 Campos and challenged with same virus and O RS-Brazil/81*

Challenge virus	No. of cattle protected/used	Mean MPI
O_1 Campos-Brazil/58	16/16	3.84
O RS-Brazil/80	6/16	2.99

Table 9 indicates the protection against development of foot lesions in cattle revaccinated 30 DPV with A Venceslau vaccine and challenged 30 DPR. The mouse protection indexes (MPI) for the A Venceslau and A RS–Brazil/81 viruses represent the mean of the value of the sera from the 18 cattle utilized.

Table 10 summarizes the results of a PBD_{50} protection test in cattle at 21 DPV. The challenge was done with A Argentina/68 and A Argentina/79 strains.

TABLE 9. *Protection against foot lesions and mouse protection indexes (MPI) in cattle revaccinated with A Venceslau and challenged with same virus and A RS-Brazil/81*

Challenge virus	No. of cattle protected/used	Mean MPI
A Venceslau	9/9	4.75
A RS-Brazil/81	5/9	1.44

DISCUSSION

With few exceptions, the FMD vaccines in South America are customarily prepared with the O_1 Campos, A_{24} Cruzeiro and C_3 Resende strains. The potency control is almost always done with viruses homologous to those used in the production (Table 1). Venezuela is the only country that uses modified live-virus vaccines.

Among the O strains, it has been observed that the O_1 Campos, O_1 Caseros, O_1 Urubamba and O_1 Cura are antigenically (Table 2) and immunogenically (Table 3) closely related and provide good cross protection. The data shown in these Tables also indicate that the O Magdalena and O RS strains differ from the foregoing O strains. Immunologically, however, it is observed that revaccination with the O_1 Campos strain affords acceptable protection against the O RS sample (Table 8). The complement fixation

TABLE 10. *Cross protection test of 50% protective bovine dose (PBD_{50}) between A Argentina/68 and A Argentina/79 strains*

	Vac. A_{24} Argentina/68		Vac. A Argentina/79	
	Virus		Virus	
Vaccine dilution	A Arg/68	A Arg/79	A Arg/79	A Arg/68
1/1	5/5	4/5	5/5	4/5
1/3	5/5	1/5	5/5	2/5
1/9	4/5	1/5	1/5	0/5
PBD_{50}	$\geqslant 12.5$	2.2	6.5	2.2
Immunological relationship =	$\frac{2.2}{\geqslant 12.5}$ =	$\leqslant 0.18$	$\frac{2.2}{6.5}$ =	0.34

Challenge was done 21 days after vaccination.

data (Table 2) and the serum neutralization data (Table 3) jointly enable the O Magdalena strain to be fitted into the pattern of subtype O_1.

The A strains identified from 1976 to 1979 in Argentina, Brazil and Uruguay are seen to be closely related antigenically (Table 4). This observation is confirmed by microneutralization tests performed with sera from cattle vaccinated for the first time (Table 5). This test also shows that the strains studied can be divided into two groups, one comprising A_{24} Cruzeiro, A_{24} Argentina and A_{27} Cundinamarca, and the second composed of A Venceslau, A Argentina/79 and A Brazil/79. These data suggest that the vaccines should be formulated with a strain from the first group and another from the second group, thus providing wider coverage than what is obtained with one or two strains from the same group.

It is important to note that A_{27} Cundinamarca is closely related to A_5 Westerwald, and has an even stronger relationship with A_{27} Colombia/67.

The A RS–Brazil/81 strain identified during an outbreak among cattle in Rio Grande do Sul (Brazil) in 1981 is serologically quite different from all the strains discussed in this study. It was eliminated by means of strategic vaccination and control of animal movement. The immunity tests conducted with cattle revaccinated with A Venceslau again indicate this strain's poor protection against field virus (Table 9).

Studies of the immunogenic coverage of the A_{24} Argentina/68 and A Argentina/79 strains with PBD_{50} show that the latter strain is dominant, since it provides an immunological relationship of 0.34 in comparison to $\leqslant 0.18$ for A_{24} Argentina/68 (Table 10).

The type C strains exhibit the least antigenic differences when compared with the other types (Table 6). The microneutralization studies in Table 7 confirm the foregoing and likewise indicate that the C_2 Pando and C_3 Resende strains provide the widest antigenic coverage.

A subsequent study will assess the behavior of these virus strains in revaccinated cattle.

REFERENCES

1. ABARACON, D., GOMES, I. Respuesta inmunitaria en bovinos vacunados con vacunas antiaftosa inactivadas y producidas con 5 cepas del subtipo O_1. (Immune response of cattle after vaccination with inactivated foot-and-mouth disease vaccine of 5 strains of subtype O_1). *Bol. Centr. Panam. Fiebre Aftosa 23-24:* 3-10, 1976.
2. ALONSO FERNANDEZ, A., FEDERER, K.E., GOMES, I., VIEIRA, A., Comparación inmunológica y serológica de dos subtipos del virus aftoso tipo

C Waldmann. *Bol. Centr. Panam. Fiebre Aftosa 4:* 9-20, 1971.

3. CUNHA, R.G., BAPTISTA Jr., J.A., SERRÃO, U.M., TORTURELLA, I. El uso de los ratones lactantes en la evaluación de los anticuerpos contra el virus de la fiebre aftosa y su significación inmunológica. *Gac. vet.* B.Aires, *19* (110): 243-267, 1957.
4. FEDERER, K.E., ALONSO FERNANDEZ, A. Développement d'un nouveau soustype du virus de la fièvre aphteuse par passages en séries sur bovins partiellement immuns. Int. Symp. on FMD Variants and Immunity, Lyon, 1967, Symp. Series Imm. Stand., *8:* 65-72 (Karger, B./N.Y., 1968).
5. FERREIRA, M.E.V. Prueba de microneutralización para estudio de anticuerpos de la fiebre aftosa. (Microtiter neutralization test for the study of foot-and-mouth disease antibodies). *Bol. Centr. Panam. Fiebre Aftosa 21-22:* 17-20, 21-24, 1976.
6. HYSLOP, N.St.G., FAGG, R.H. Isolation of variants during passage of strain of foot-and-mouth disease virus in partly immunized cattle. *J.Hyg.*, Camb. *63:* 357-368, 1965.
7. PRINGLE, C.R. Genetic aspects of the thermal inactivation properties of foot-and-mouth disease virus strains. *Bull. Off. Int. Épizoot. 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).