EPIDEMIOLOGIC, ANTIGENIC, IMMUNOGENIC AND BIOCHEMICAL CHARACTERIZATION OF APHTHOVIRUS A-81 ARGENTINA/87

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SHORT COMMUNICATION

Field Situation

From 1983 to 1986 the incidence of foot-and-mouth disease virus (FMDV) type A in Argentina, Uruguay and the state of Rio Grande do Sul (Brazil) was low (Table 1) and the majority of the virus samples isolated corresponded to subtype A_{24} and A-79.

Seroepidemiologic studies constantly carried out on field samples by the Diagnostic Reference Laboratory for the Americas at the Pan American Foot-and-Mouth Disease Center (PAFMDC) revealed, late in 1986, an antigenic variation of FMDV type A in relation to the usual A_{24} and A-A-79 field isolates in Argentina. The FMD situation started to present alarming characteristics in that country by the 11th week of 1987 (March 14-20). The number of affected grids (spacial dispersion) increased notably after the 16th week, going well beyond the most pessimistic forecast (Fig. 1). During that period, Argentina also suffered a series of floods which created an intense animal movement and concentration of susceptible animals in the more severely affected area, humid Pampa, thus contributing to the aggravation of the disease situation. The northern region and the Argentinian Mesopotamia also were affected by the epidemics.

The close relationship between the Argentinian livestock industry and its counterpart on the Uruguayan border, in conjuction with the presence of a variant of the FMDV-A may have contributed to the surge of FMDV in Uruguay, which started during week 18 of 1987, after a fairly calm period of four years. This hypothesis is also supported by the similar antigenic characteristics of the isolates from Uruguay and Argentina. These field samples were characterized as FMDV A-81 Argentina/87.

The Argentinian border area along the Uruguay river was the most affected area, although the disease spread all over the country.

The border between Uruguay and the state of Rio Grande do Sul, in Brazil, has no natural barriers, which is facilitating the diffusion of FMDV between these countries. The disease entered that state of Brazil during the second week in May (week 19).

The epidemiological situation observed in Rio Grande do Sul showed that the disease spread mainly throughout the Campanha region. The number of outbreaks was lower than that observed in Uruguay and Argentina and they occurred later than in these two countries (Fig. 1).

Antigenic Characterization

Bovine epithelial samples were received from the Agriculture Services of Argentina, Uruguay and Brazil which were collected from affected farms in the region. From the different samples received, we selected the sample designated A-81 Argentina/87, originally from the county of Castellanos, province of Santa Fé, as the most

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TABLE 1. *Herds affected by foot-and-mouth disease according to type of virus by year. 1980-1986*

Country	Type of virus	1980	1981	1982	1983	1984	1985	1986
Argentina	O	44	64	13	351	90	10	30
	A	339	429	39	23	6	5	11
	C	37	22	4	196	348	288	316
Rio Grande do Sul (Brazil)	O	379	60	6	5	12	2	1
	A	19	103	38	8	27	12	7
	C	1	13	11	7	1	5	4
Uruguay	O	127	4	1	–	10	15	2
	A	6	14	2	1	–	–	1
	C	–	–	–	4	6	3	28

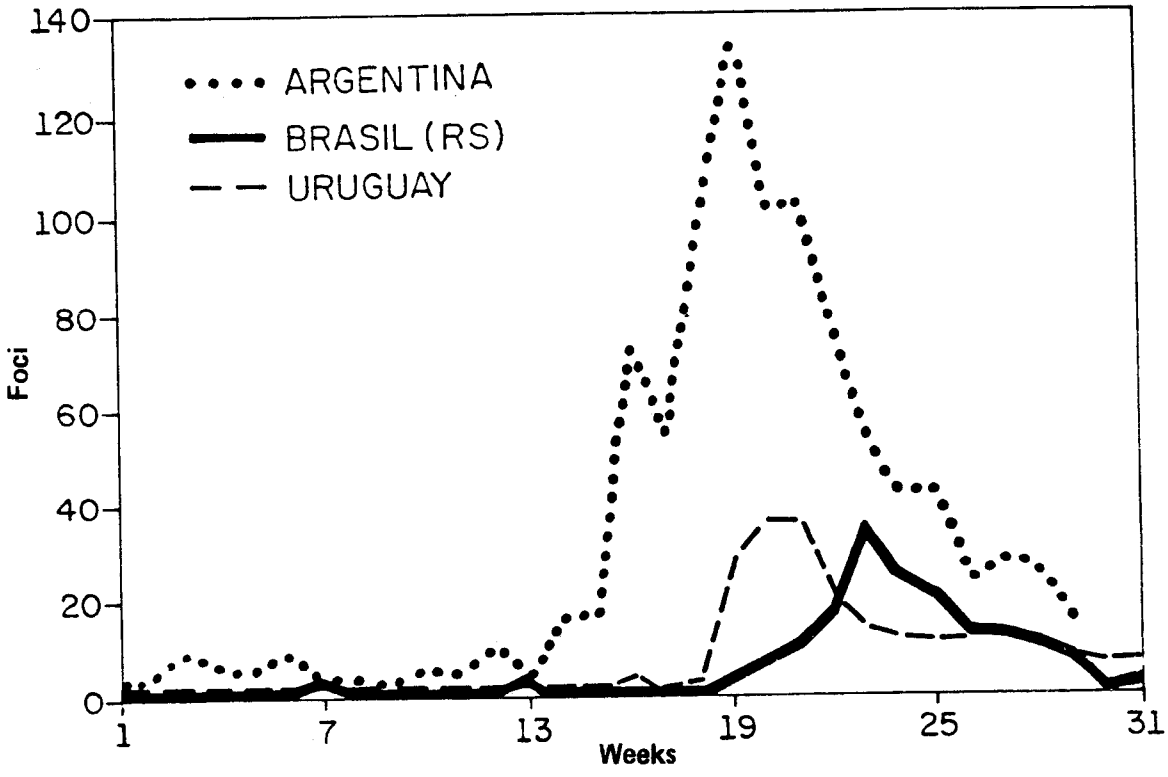

FIGURE 1. *Time distribution of outbreaks. Argentina, Uruguay and Rio Grande do Sul (Brazil), 1987*

representative, due to its antigenicity, immunogenicity, replication and stability. All the samples received showed a similar serological scope.

The serological relationships (r and R) carried out by 50% complement fixing (CF_{50}) (*1, 6*), of the strain A-81 Argentina/87 shown in Table 2 are very low excluding the r and R against A-81 Brasil/81 and A-81 Argentina/81. The results of antigens used to study FMDV are shown in Table 3.

The serological studies with ELISA using polyclonal antibodies in comparison to our CF_{50} showed a higher sensitivity but lower specificity which does not allow a correct interpretation of results. To avoid this problem we are attempting to use monoclonal antibodies in the ELISA test.

TABLE 2. *Serological relatioships (r) and parentage(R) of foot-and-mouth disease virus strain A-81 Argentina/87*

Strains	r_1	r_2	R
A_5 Kelderman-Belgium	<0.02	0.03	<2
A_5 Allier-Fr/60	<0.02	0.07	<4
A_{10} Argentina/61	<0.02	0.02	<2
A_{12} UK/32 (A119)	<0.02	0.05	<3
A_{24} Cruzeiro-Br/55	<0.02	0.06	<3
A-79 Venceslau-Br/76	<0.02	0.03	<2
A-79 Argentina/79	<0.02	0.04	<3
A-85 Sabana-Col/85	<0.02	0.08	<4
A-81 Uruguay/79	0.25	0.25	25
A-81 Brazil/81	0.50	0.42	46
A-81 Argentina/81	0.65	0.42	52
A-81 Argentina/87	1.00	1.00	100

r_1 = CF capacity of guinea pig A-81 Argentina/87 hyperimmune serum.

r_2 = CF capacity of A-81 Argentina/87 antigen by heterologous hyperimmune sera.

TABLE 3. *Subtyping of antigens used to study foot-and-mouth disease strain A-81 Argentina/87. PAFMDC, 1987*

	Antisera													
Antigen	A_5 Keld.	A_5 Allier	A_{10} Arg/61	A_{12} (119)	A_{24} Cruz.	A-79 Venc.	A-79 Arg/79	A Sab. Col/85	A-81 Uru/79	A-81 Bra/81	A-81 Arg/81	A-81 Arg/87	A Poliv.	Control antigen
A_5 Kelderman-Belgium	00	02	29	46	22	65	66	65	67	66	67	67	03	64
A_5 Allier-Fr/60	01	00	54	54	64	64	66	66	67	66	66	67	02	65
A_{10} Argentina/61	64	59	00	62	64	64	65	65	66	67	66	68	05	66
A_{12} UK/32 (A119)	69	27	03	00	58	67	67	67	66	65	66	67	04	67
A_{24} Cruzeiro-Br/55	09	06	36	14	00	65	45	61	60	60	61	64	02	67
A-79 Venceslau-Br/76	48	48	44	40	59	00	09	62	61	65	67	64	04	65
A-79 Argentina/79	64	64	65	64	65	18	00	65	64	65	66	67	03	66
A-85 Sabana-Col/85	64	65	64	65	66	62	60	00	64	65	66	67	02	68
A-81 Uruguay/79	65	64	61	62	65	64	45	66	00	04	05	06	01	66
A-81 Brazil/81	64	65	65	62	64	64	63	63	09	00	01	01	03	66
A-81 Argentina/81	65	64	60	66	64	61	68	66	07	08	00	03	05	67
A-81 Argentina/87	67	66	67	67	66	66	66	64	35	07	04	00	05	68
Antisera control	65	66	65	67	65	65	64	65	65	65	67	67	65	

Immunological Coverage

The immunological coverage of this virus was studied by the mouse protection test (*3*) and seroneutralization test (*4*), using sera from our serum bank, taken from vaccinated and revaccinated cattle. Results are expressed as mean expected percentage of protection (*5, 7*) in Table 4. Also Argentina and Brazil have carried out tests in vaccinated and revaccinated cattle. Results are shown in Tables 5 and 6.

Biochemical Studies

The ribonucleic acid (RNA) of the strains A-81 Argentina/81 and A-81 Argentina/87 (A-81 Castellanos-Arg/87, A-81 Rivera-Uru/87 and A-81 Palmar-Br/87) were studied by fingerprinting (*2*) (Fig. 2). Other studies in progress are: RNA sequencing using synthetic probes from Centro de Biología Molecular (Madrid, Spain), electrofocusing and polyacrylamide gels (PAGE) analysis.

TABLE 4. *Foot-and-mouth disease: coverage of several vaccine virus strains from Europe and South America*

Tests	Challenge virus				
	A-79 Venceslau	A_5 Kelderman	A_{24} Cruzeiro	A-79 Arg/79	A-81 Arg/81
EPP 30 DPV bovine serum vaccinated with A-79 Argentina/79	–	–	–	61.1	⩽38.4
EPP 30 DPR bovine serum revaccinated with A-79 Argentina/79	–	–	–	90.1	52.3
EPP 30 DPV bovine serum vaccinated with A_{24} Cruzeiro-Br/55	–	–	67.5	–	⩽37.5
EPP 30 DPR bovine serum revaccinated with A_{24} Cruzeiro-Br/55	–	–	91.9	--	79.3
EPP 30 DPV bovine serum vaccinated with A_5 Kelderman-Belg.	–	61.2	–	–	⩽39.0
EPP 30 DPR bovine serum revaccinated with A_5 Kelderman-Belg.	–	83.3	–	–	56.0
EPP 30 DPV bovine serum vaccinated with A-79 Venceslau-Br/76	68.8	–	–	–	⩽39.0
EPP 30 DPR bovine serum revaccinated with A-79 Venceslau-Br/76	91.7	–	–	–	51.3
EPP 30 DPV bovine serum vaccinated with A_{24} Cruzeiro + A-79 Venceslau	59.6	–	62.0	–	⩽38.1
EPP 30 DPR bovine serum revaccinated with A_{24} Cruzeiro + A-79 Venceslau	94.9	–	94.9	–	76.5

EPP = Expected percentage of protection. Lower confidence limit (0.95).
DPV = Days post-vaccination. DPR = Days post-revaccination.

TABLE 5. *Cross immunity three weeks vaccinated cattle performed in SELAB, Argentina*

Vaccines	Challenge virus				
	A-79 Arg/79	A-81 Arg/81	A-81 Arg/87 (Utracán)	A-81 Arg/87 (25 de Mayo)	A-81 Arg/87 (Castellanos)
A-79 Argentina/79	14/16[a]	–	–	–	7/16
A-79 Argentina/79 + A-81 Argentina/81	10/10	10/10	9/10	8/10	10/10

[a]Protected cattle/vaccinated cattle.

TABLE 6. *Cross immunity in vaccinated and revaccinated cattle performed in LARA-Porto Alegre, Brazil*

Vaccines	Challenge virus	
	A-79 Venceslau/76	A-81 Argentina/87 (Castellanos)
Vaccinated cattle with A-81 Castellanos/87	–	16/16[a]
Revaccinated cattle with A_{24}Cruzeiro-Br/55 + A-79 Venceslau-Br/76	16/16	16/16

[a]Protected cattle/vaccinated cattle.

Measures Taken

After subtying of field samples at the PAFMDC the countries were notified in 24 hours, about antigenic variations in strains A-81 Argentina/87. In addition to the above, the immunologic coverage of vaccine strains normally used in the area against A-81 Argentina/87 could be determined within a week by seroneutralization and mouse protection tests using our serum bank (Table 4).

Animal fairs and expositions were prohibited in all affected areas. Movement of herds for the purpose of slaughter were only permitted after confirmation of absence of disease from the farm of origin. Monovalent vaccines with the strains A-81 Argentina/81 or A-81 Argentina/87 were prepared and in some cases this strain is being added to trivalent vaccine.

Measures taken were as follows:

a) Massive vaccination with monovalent A-81 Argentina/87 vaccine in Rio Grande do Sul and Uruguay.
b) Selective vaccination monovalent A-81 Argentina/81 vaccine in Argentina.
c) Advance regular vaccination program using the normal trivalent vaccine.

At present, a clear decrease in incidence of disease is observed and strain A-81 Argentina/87 has not been found in any areas of Brazil or any other countries.

REFERENCES

1. ALONSO FERNANDEZ, A., VIANNA FILHO, Y.L., DURINI, L.A.E., SUTMÖLLER, P. Los virus de fiebre aftosa usados en la producción y control de vacunas en América del Sur. Foot-and-mouth disease viruses used in vaccine production and control in South America. *Bol.Centr.Panam.Fiebre Aftosa 43-44*: 21-28, 29-36, 1981.
2. AUGÉ DE MELLO, P., CASAS OLASCOAGA, R., COSTA GIOMI, M.P., ALONSO FERNANDEZ, A., SCODELLER, E.A., LA TORRE, J.L., BERGMANN, I.E. RNA fingerprinting of South American prototype aphthovirus strains. *Vaccine 4*: 105-110, 1986.

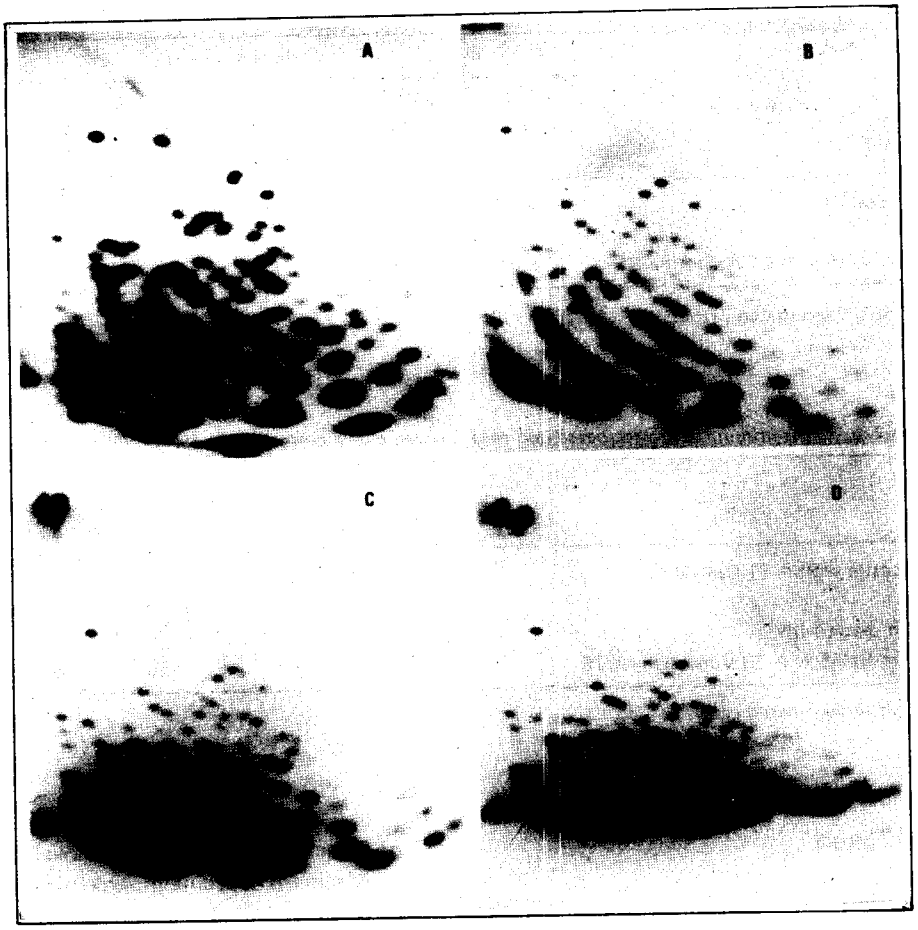

FIGURE 2. *RNase T_1 Two-D maps (Fingerprints) of FMDV type A strains. (A) A-81 (Alen-Arg) Argentina/81, (B) A-81 (Castellanos-Arg) Argentina/87, (C) A-81 (Rivera-Uru) Argentina/87, and (D) A-81 (Palmar-Br) Argentina/87.*

3. CUNHA, R.G., BAPTISTA Jr., J.A., SERRAO, 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. 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.
5. GOMES, I. & ASTUDILLO V. Foot-and-mouth disease evaluation of mouse protection test results in relation to cattle immunity. *Bol.Centr.Panam.Fiebre Aftosa 17-18*: 9-16, 1975.
6. RESOLUTIONS. Int. Symp. on FMD Variants and Immunity, Lyon, 1967. Symp. Series Imm. Stand. *8*: 169-170 (Karger, B/NY, 1968).
7. SUTMÖLLER, P., COSTA, K. de F., GOMES, I. Prueba de microneutralización por microtécnica para fiebre aftosa: cálculo de la expectativa porcentual de protección. The serum microneutralization test for foot-and-mouth disease: establishment of an expected percentage of protection. *Bol.Centr.Panam.Fiebre Aftosa 39-40*: 31-36, 37-42, 1980.