PRODUCTION AND QUALITY CONTROL OF FOOT-AND-MOUTH DISEASE VACCINES IN SOUTH AMERICA[1]

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SUMMARY

In the production of foot-and-mouth disease (FMD) vaccines in South America, the 1950's, 60's and part of the 70's were dedicated to creating an infrastructure of production laboratories capable of supplying sufficient quantities of FMD vaccine to meet the needs of the programs.

That infrastructure has developed significantly in both the private and official sectors. Most of the South American countries now have qualified FMD vaccine laboratories and advanced production technology.

Concomitantly, the infrastructure of the official control laboratories was established. The standards for the production laboratories and the vaccines they produce were determined. Quality controls that should be applied to the vaccines and the minimum requirements of the protection tests for approval of each series were also defined.

At the present the most important step to control and eradicate FMD is to improve the epidemiological surveillance as well as the rational an strategic application of the vaccine, according to the ecosystems of the disease in the continent.

INTRODUCTION

In 1984 South America had a cattle population of 224 million, distributed in approximately 4,245,000 herds occupying an area of about 17 million square kms (*6*). This enormous population lives almost entirely on the open range. The animals are kept permanently outdoors and they are submitted to large movements in accordance with the types of management and commercialization.

Foot-and-mouth disease (FMD) is found in various countries of the region and its frequency depends on the ecosystems of the disease. Figure 1 shows the different ecosystems of the disease predominant in the South American countries in 1984.

The economic importance of FMD due to the losses it causes and the commerce inter-countries have led the governments of most of the South American countries to establish programs aimed to control and eradicate the disease. One of the major methods used in the programs to eliminate the disease has been massive immunization of the cattle population. This method has obliged the countries to create an important infrastructure to produce and control the FMD vaccines needed to meet the demands of the programs.

INACTIVATED FOOT-AND-MOUTH DISEASE VACCINE

Antigen

The first FMD vaccine available in South America was prepared in 1944 by the Institute of Veterinary Research Desidério Finamor, in Porto Alegre, Brazil. The Miguel C. Rubino Veterinary Research Center of the Ministry of Agriculture and Fisheries of Uruguay and the Santi Espiritu Laboratory of Argentina initiated the production

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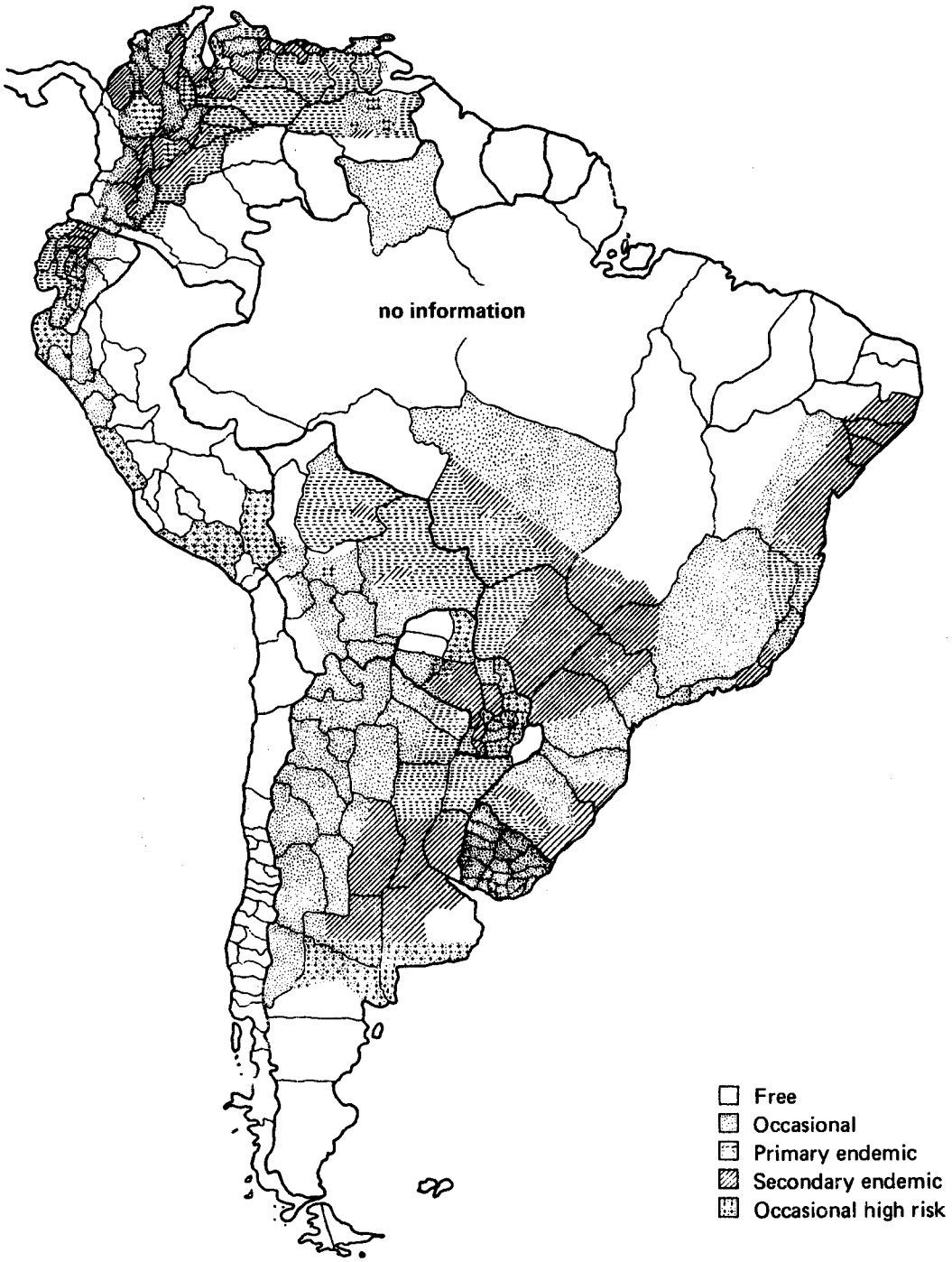

FIGURE 1. *Ecosystems of foot-and-mouth disease. South America, 1984*

of FMD vaccine in 1946 and 1948 respectively. The three laboratories used the Waldmann method. In 1953 the Foot-and-Mouth Disease Institute of the Venezuelan Ministry of Agriculture and Livestock initiated production by the Frenkel method. And in 1954, the Cooper McDougall and Robertson Laboratory in Uruguay produced the first private vaccine by the Frenkel method.

In the 1950's and 60's the countries produced FMD vaccines by the Waldmann and Frenkel methods. Brazil was the exception; for most of the 1960's and 70's it prepared a high percentage of vaccines with antigen obtained in newborn rabbits. Also in the 60's some private laboratories in Argentina produced limited quantities of vaccine using antigen obtained from newborn calves. The 1970's brought large-scale production of FMD antigen in cell cultures, mainly in BHK cell suspensions.

About 350 million doses of vaccine are now annually prepared from antigen obtained in cell cultures, plus 75 million doses by the Frenkel method, totalling 425 million doses of which 391 million are produced with O, A and C antigens and 34 million are bivalent O and A. Table 1 illustrates the production of vaccines in the South American countries in the 1980-84 period. Figure 2 shows the number and location of vaccine-producing laboratories in South America.

Inactivants

Most of the laboratories, mainly those that produce antigens in cell cultures, utilize first-order inactivants, of which binary ethylenimine (BEI) is generally preferred (*3*). However, several laboratories that use the Frenkel method for antigen production still use formalin as the inactivant. Brazil and Colombia allow only first-order inactivants, while both inactivants are used in Argentina, Paraguay and Uruguay.

Adjuvants

The adjuvants most used in the production of the FMD vaccines are aluminum hydroxide with saponin. Only Peru does not use saponin.

Approximately 20 million doses of trivalent vaccine are prepared with Freund's incomplete adjuvant as a primary emulsion (*2*) with regular inactivated antigen or with inactivated antigen concentrated on aluminum hydroxide (*1*).

ATTENUATED LIVE VIRUS VACCINE

In the 1960's various countries, including South Africa, Brazil, England, Israel, Thailand and Venezueia, dedicated time and efforts to obtain attenuated live virus FMD vaccines which were used experimentally. However, various problems arose to advise against the use of such vaccines:

TABLE 1. *Available production (in thousand of doses) of FMD vaccine by country and year. South America, 1980-1984*

Country	1980	1981	1982	1983	1984
Argentina	182,400	169,600	116,200	131,000	158,300
Bolivia	700	200	1,000	400	400
Brazil	224,100	219,100	233,200	177,400	203,300
Colombia	32,700	27,000	22,800	22,400	24,600
Chile	400	50[a]	100[a]	50[a]	50[a]
Ecuador	1,400	1,700	1,900	1,800	1,200
Paraguay	11,900	10,400	13,900	9,700	12,800
Peru	1,600	...	1,300	1,500	1,500
Uruguay	51,500	44,700	30,400	38,000	29,800
Venezuela	12,400	12,700	12,000	12,200	8,300
Total	519,100	485,450	432,800	394,450	440,250

[a] For emergency situations, produced at the PAFMDC.

... No data available.

FIGURE 2. *Laboratories of production of foot-and-mouth disease vaccine. South America, 1984*

the difficulty of attenuation without loss of immunogenicity; varying degrees of sensitivity among the susceptible species; pathogenicity in pigs; difficulty in preserving the vaccines; virus neutralization induced by antibodies in previously vaccinated animals; the possibility of reversion to virulence, etc. Moreover, the fact that meat-importing countries objected to receiving meat from regions using this type of vaccine strongly influenced the gradual termination of the experiments of FMD virus attenuation. Only Venezuela still uses this method of vaccine production as a substitute for inactivated vaccine (*9*).

NEEDS AND SITUATION OF FOOT-AND-MOUTH DISEASE VACCINE IN SOUTH AMERICA

Table 2 shows that the FMD control programs in South America covered 184,663,200 cattle in 1984. Therefore, just to maintain this species suitably immunized throughout the year, using aluminum hydroxide-saponin vaccines, would require approximately 550 million doses.

The ecological conditions in South America require the use of vaccines that confer the longest possible immunity. In this regard the Pan American Foot-and-Mouth Disease Center/Pan American Health Organization (PAFMDC/PAHO) has given considerable effort and technical support into developing an oil-adjuvanted vaccine for use in cattle.

The use of vaccines providing greater and longer lasting immunity (*4*) would mean a substantial economy in transportation, storage and cooling of vaccines, handling of herds, etc. The expenses of administering the vaccines would likewise be reduced and would facilitate the official application of the vaccine. It would also lead to a more efficient surveillance, supervision and inspection,

TABLE 2. *Vaccination coverage of cattle against foot-and-mouth disease. South America, 1984*

Country	Herds			Population		
	program	vaccinated	coverage (%)	program (x 1000)	vaccinated (x 1000)	coverage (%)
Argentina	323,495	300,019	92.74	52,670.0	46,558.0	88.40
Bolivia[a]	23,380	2,804	11.99	383.0	157.2	41.04
Brazil[b]	1,522,690	800,453	52.57	79,239.7	54,134.3	68.32
Colombia	441,818[c]	268,507[d]	60.77	16,043.8	9,750.4[d]	60.77
Ecuador	246,958	66,404	26.89	3,200.4	1,284.2	40.13
Paraguay	197,164[e]	106,199	53.86	7,186.2	3,870.7[f]	53.86
Peru	464,182[g]	44,197	9.52	3,391.5	432.3[g]	12.75
Uruguay	64,929	45,405[h]	69.93	11,236.6	8,129.0[h]	72.34
Venezuela	115,404	21,557	18.68	11,312.0	3,497.2	30.92
Total	3,400,020	1,655,545	46.13	184,663.2	127,813.3	69.21

[a] Two annual vaccinations in Cochabamba and Santa Cruz Departments. Does not include 186,116 vaccinated cattle from Beni Pilot Plan area.
[b] Does not include figures for: Amazonas, Pará, Maranhão, Piauí, Amapá and Rondonia.
[c] Does not include figures for: Caquetá, Chocó, Arauca, Putumayo, Amazonas, Guainía, Vichada and San Andrés Islands.
[d] Estimated figures as per number of doses injected.
[e] Data estimated by the PAFMDC.
[f] Mean of vaccination stages.
[g] No information from several departments.
[h] Mean of two vaccination stages.
SOURCE: Country reports to COSALFA XII.

resulting in a more effective protection of the animal population in the program.

The aluminum hydroxide-saponin vaccines presently used in most of the countries are administered every four months to maintain adequate and sustained immunization of the animal population. The costs of handling the vaccine are high and inspection is expensive and difficult due to the frequent vaccination cycles.

Table 3 shows the characteristics of the FMD vaccines prepared during 1983, which was similar to the 1984 production.

EVOLUTION OF THE FOOT-AND-MOUTH DISEASE VACCINE QUALITY CONTROL IN SOUTH AMERICA

The use of vaccines with bacterial contamination was allowed in the 1950's and part of the 60's provided that such contamination was not caused by pathogenic or proteolytic bacteria. The innocuity tests were not strict. There existed, therefore, the possibility of the presence of active virus that would be inactivated by formalin, the only inactivant used at that time, during storage and maturation of the vaccine at 4°C.

The potency control in several countries were not very frequent and, in many cases, the immunogenicity of the vaccine was only determined by the infectivity and complement fixation titers obtained in the antigen suspensions prior to their inactivation.

In the 1970's emphasis was placed on the sterility and innocuity controls, which were complemented in several countries by direct potency tests in cattle and indirect tests on guinea pigs or by determination of antibody levels in the sera of vaccinated cattle. In this field important work was done by Chile and Uruguay in the early 1970's.

In the beginning, Argentina, Brazil, Paraguay, and Uruguay adopted the quality-control methodology used in France (*8*). But later, with the technical cooperation provided through the PAFMDC/PAHO, their own observations, and the requirements of the European standards, several countries gradually relinquished the Lucam test in favor of the 50% bovine protective dose (BPD_{50}), the

TABLE 3. *Formulation and potency control of FMD vaccine. South America, 1983*

Country	Formulation system			No. of series prod.	Vaccine (in thousand of doses)			
	Antig.	Inact.	Adj.		Prod.	Control.	Approv.	Availab.
Argentina	63% FK	60% Formol	HS	86	126,500	126,500	126,000	131,000
	37% CC	40% 1st.ord.	OL	4	5,000	5,000	5,000	
Bolivia	–	–	–	–	–	–	–	400
Brazil	100% CC	100% 1st.ord.	HS	133	222,600	222,600	173,100	177,400
			OL	7	600	600	600	
Colombia	100% CC	100% 1st.ord.	HS	23	24,000	24,400	24,400	22,400
			OL	1	107	107	107	
Chile	–	–	–	–	–	–	–	50
Ecuador	100% FK	100% 1st.ord.	HS	8	1,600	1,600	1,400	1,800
Paraguay	51% FK	51% Formol						
	49% CC	49% 1st.ord.	HS	9	9,400	9,400	9,400	9,700
Peru	100% FK	100% 1st.ord.	HS	6	1,900	1,900	1,500	1,500
Uruguay	34% FK	34% Formol						
	66% CC	66% 1st.ord.	HS	70	39,000	39,000	39,000	38,000
Venezuela	100% VVA	–	–	–	11,100	11,100	11,100	12,200

FK = Frenkel. CC = Cell culture. ALV = Attenuated live virus. 1st.ord. = First order.
HS = Aluminum hydroxide-saponin. OL = Oil.

protection against generalized foot lesion (PG), the 50% guinea pig protective dose ($GPPD_{50}$), and the serum protection test conducted in suckling mice (mouse protection test - MPT).

The direct tests in cattle (BPD_{50} and PG) and the MPT were gradually expanded as the countries, lacking FMD-free areas from which to get susceptible cattle, created better systems for the selection of cattle coming from herds protected from and free of FMD. Calves that had never been vaccinated were identified and selected in these herds. It had been previously established that the immunological response of such animals was comparable to that of cattle from disease-free areas. This fact was again demonstrated with BPD_{50} tests conducted in Brazil and Colombia, using such selected animals. Argentina used cattle from Patagonia, an area free of FMD (Table 4).

Some countries have used indirect testing in guinea pigs (C Index or $GPPD_{50}$) or the mouse protection and serum neutralization tests as alternative for the direct control tests using challenge in cattle.

A correlation between the direct test in cattle and the test in guinea pigs has never been determined. Generally, the vaccines rejected in guinea pigs are also rejected in cattle. However, a certain percentage of the vaccines approved in guinea pigs are rejected when tested in cattle. The guinea pig test could therefore perhaps be useful when used for screening.

A good correlation has been found between the levels of circulating antibodies of vaccinated cattle and the results of virus challenge tests in cattle when the mouse protection or serum neutralization tests are used.

The expected percentage of protection (EPP) values were determined from tests conducted by the PAFMDC in 1975, comparing circulating antibody levels and virus challenge results in more than 700 cattle. The EPP represent the percentage of probability that a vaccinated animal will be protected against infection with a given level of antibodies (*7*). In 1983 the EPPs and the results of virus challenge in groups of vaccinated cattle were compared. Considering the standard error of the mean of the EPP, the limits of confidence were defined and the test made more accurate (*10*).

PRESENT SITUATION OF THE QUALITY CONTROL OF FOOT-AND-MOUTH DISEASE VACCINE

The quality control of FMD vaccines in Argentina, Brazil, Colombia, Paraguay and Uruguay is conducted by official control laboratories, which are independent of the vaccine-producing laboratories.

Quality control includes determination of sterility, innocuity and potency of all the series of vaccines produced. These controls are performed on each series of vaccine after it is completely bottled. Random samples are selected for testing by the official control services.

The vaccine is released after tests confirm that it is both sterile and innocuous and gives the protection required by the control standards.

TABLE 4. *Results of BPD_{50} tests against O_1 Campos-Br/85, carried out by several countries with saponin-aluminum hydroxide vaccine produced by the PAFMDC*

Vaccine dilution	Argentina	Brazil							Colombia
Inert dil.	1	1st	2nd	3rd	4th	5th	6th	Total	1
1/1	5/5	4/4	3/4	3/4	4/4	3/4	3/4	20/24	5/5
1/3	5/5	4/4	4/4	4/4	3/4	3/4	4/4	22/24	3/5
1/9	2/5	3/4	3/4	1/3	2/4	2/4	2/4	12/23	4/5
BPD_{50}	7.8	11.4	8.7	5.5	5.1	5.1	6.6	6.8	7.8

Efficacy evaluation is measured by direct or indirect challenge against the strains homologous to those used in producing the vaccine. The FMD virus strains used in challenge tests in 1984 are indicated in Table 5.

The control methods, as well as the requirements, were systematically analyzed and updated at the international seminars on FMD vaccine quality control sponsored by the PAFMDC/PAHO in 1970, 1978, 1980 and 1983, in Uruguay, Argentina, Colombia and Paraguay, respectively (*5*).

The systematic application of the quality control of FMD vaccines substantially improved the production and commercialization of a product providing greater potency and biological safety. The stricter requirements gradually applied in quality control generated a constant increase in the quality of the vaccines in Argentina, Brazil, Colombia, Paraguay and Uruguay resulting in a better protection of the herds.

At the same time production laboratories were remodelled or built with the capability to adjust to production systems using cell cultures and to the stricter potency requirements imposed by the official services.

The methods used for potency control, the requirements by each country and the mean potency values of the vaccines released for the market in 1983 in South America are shown in Table 6.

1. *Protection against generalized foot lesions (PG).* Sixteen cattle are vaccinated. At 21-30 days post-vaccination (DPV) they are challenged by intradermolingual inoculation of $10^{4.0}$ ID_{50}. The vaccine is released for use if at least 12 animals are protected against PG.

2. *50% bovine protection dose (BPD_{50}).* A pure vaccine and vaccine diluted 1:3 and 1:9 in an inert diluent is used. Five cattle each are vaccinated with the pure vaccine and with the dilutions used. At 21-30 DPV each of the animals is challenged by intradermolingual inoculation of $10^{4.0}$ ID_{50}. A reading is done at 7 days post-inoculation, the BPD_{50} is calculated, and the vaccines providing a BPD_{50} of 3 or higher are passed for release.

The current requirements were gradually established. In the case of Argentina, a level of 1.2 BPD_{50} was first required in 1975. The level was subsequently raised to 1.6 in 1977, then to 2.3 in January 1978, and, in March 1978, to the 3 BPD_{50} now required. Table 7 shows the relation

TABLE 5. *Strains used for the control of FMD vaccine. South America, 1984*

Country	Strains		
	O	A	C
Argentina[a]	O_1 Caseros-Arg/67	A Argentina/79 A Argentina/81	C_3 Resende-Br/55
Brazil	O_1 Campos-Br/58	A_{24} Cruzeiro-Br/55 A Venceslau-Br/76	C_3 Indaial-Br/71
Colombia	O_1 Campos-Br/58	A_{24} Cruzeiro-Br/55	–
Ecuador	O_1 Urubamba-Per/63	A_{24} Cruzeiro-Br/55	–
Paraguay	O_1 Campos-Br/58	A_{24} Cruzeiro-Br/55	C_3 Resende-Br/55
Peru	O_1 Urubamba-Per/63	A_{24} Cruzeiro-Br/55	C_3 Resende-Br/55
Uruguay	O_1 Campos-Br/58	A_{24} Cruzeiro-Br/55	C_3 Resende-Br/55
Venezuela[b]	O_1 Campos-Br/58	A_{32} Venezuela/70	–

[a] Strain O_1 Campos-Br/58 is also used for production.

[b] The only country which produces attenuated-live-virus vaccine.

TABLE 6. *Potency control of FMD vaccine. South America, 1983*

	Countries									
	Argentina		Brazil		Colombia		Paraguay		Uruguay	
Control methods	A	B	A	B	A	B	A	B	A	B
50% bovine protection dose	3.0	6.9	3.0	6.2	3.0	4.2	–	–	–	–
Protection against foot generalization	87.5	94.5	81.3	87.5	75.0	88.6	75.0	93.7	–	–
K Index	–	–	–	–	–	–	–	–	1.8	2.6
Mouse protection	–	–	–	–	2.5	3.6	2.5	3.2	2.5	3.3
Serum neutralization	–	–	–	–	1.5	1.6	1.5	2.0	1.5	1.7
C Index	–	–	2.0	3.8	–	–	–	–	2.5	3.0

A = Minimum value for vaccine approval.
B = Mean value of vaccines approved.

between the BPD_{50} and the PG obtained by the official control laboratory (SELAB) of the Argentine Secretary of Agriculture.

This example is valid for all the methods and countries. There is currently no intention of raising the vaccine-approval levels. However, work is being developed to select the most reliable methods, to reduce their variability, and to improve vaccine-handling techniques.

3. *Circulating antibodies.* In general, the tests most used with sera from vaccinated cattle are the mouse protection test and the serum neutralization test. Some countries that use these vaccine control methods have established a serum protection index of 2.5 and a serum neutralization index of 1.5 and require a mimimum of 75% protection of vaccinated animals as criterion for approval of the vaccine.

As criterion to evaluate vaccine potency, the PAFMDC currently utilizes the EPP based on the mouse protection index in susceptible cattle 9-12 months old (*7*). It has been established that the lower confidence limit of 95% should be equal to or greater than a protection of 75%. This method requires the use of at least 16 cattle between 18-24 months old per vaccine. The accuracy of the test increases as the number of test animals is increased.

The EPP's main advantage over the direct tests is that all the valencies of a vaccine can be tested in a single group of cattle, whereas the use of the PG and the BPD_{50} make it economically unfeasible to obtain these data in all the series of vaccines produced. Argentina and Brazil, which use the PG and BPD_{50} tests, can test the vaccines only against a single valency. Recolection of sera previously to virus inoculation allows to determine antigenic rates for three valencies obtaining an information of great value to evaluate their efficacy. Also, when using the EPP, it is not necessary to challenge the vaccinated cattle with live virus. This eliminates the high risks of virus escape from diseased animals. After the test is concluded they can be returned to the field, thus eliminating the inconvenience of sacrificing or destroying the animals.

TABLE 7. *Relationship between protection against foot generalization and 50% protective dose in cattle according BPD_{50} tests carried out in SELAB-SENASA, Argentina. 1977-1981.*

BPD_{50} range	O_1 Caseros			A_{24} & A_{79}			C_3 Resende		
	P/U[a]	% PG[b]	BPD_{50}[c]	P/U	% PG	BPD_{50}	P/U	% PG	BPD_{50}
< 2	73/140	52	1.37	67/145	46	1.30	16/25	64	1.88
2.1-4	208/250	83	2.99	167/225	74	2.94	69/85	81	3.23
4.1-6	220/240	92	5.03	325/355	92	4.94	121/135	90	4.93

[a]Cattle protected/cattle used, pure vaccine.
[b]Percentage of protection against foot generalization.
[c]Mean 50% bovine protective dose of the group.

Cummulative results of PG and BPD_{50} of range 2.1-4 for the three valencies

BPD_{50} range	O_1 Caseros, A_{24}, A_{79}, C_3 Resende		
	P/U	% PG	BPD_{50}
2.1-4	444/560	79.28	3.01

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