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MODIFIED LIVE VIRUS VACCINE AGAINST FOOT-AND-MOUTH DISEASE

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MODIFIED LIVE VIRUS VACCINE AGAINST FOOT-AND-MOUTH DISEASE*

Introduction

Foot-and-mouth disease (FMD) is an acute, highly communicable virus infection that affects all cloven-footed animals, but chiefly cattle, swine, sheep and goats. The disease occurs more or less enzootically in many countries of Europe, Asia, Africa and South America. The importance of the presence of FMD is due to the great economic losses which result from lowered livestock productivity and from the restrictions imposed upon the international trade. Annual losses in some countries of South America are estimated in 150 million dollars (11) and under unfavorable conditions may reach 25 per cent of the production of cattle, meat and milk. Being FMD one of the greatest socio-economic problems of the countries where the economy is basically agricultural, scientific research at basic and applied levels in this disease is being actively conducted at a number of institutes throughout the world. Among the important immunization studies are investigations to develop modified-live-virus vaccines. After the results of the investigations published by Rubino et al. in 1940 (38) (modification of the virus by serial passage in guinea-pigs and its laboratory and field trials as an immunogenic agent), after the discovery by Skinner (9) of the susceptibility of the unweaned mouse to FMD virus and the adaptation of this agent to embryonated eggs by Peragallo (1937), Traub and Schneider (1948), as referred by Brooksby (1) and tissue cultures, the technics used in FMD research for the development of modified-live-virus vaccines have been the adaptation of virus strains to various host or tissue culture systems with continued serial passage of the virus until pathogenicity for cattle has been reduced to a very low level.

In this report we will make a summary of the studies being done at the Pan American Foot-and-Mouth Disease Center (PAFMDC), at the Centro de Investigaciones Veterinarias (CIV), Venezuela, the present research program in South America, the field results obtained so far at local and country levels, and a brief comment on the application of live FMD vaccines in different parts of the world.

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*Prepared by Drs. Carlos Palacios, Mario V. Fernandes and Carlos Bernal, Pan American Foot-and-Mouth Disease Center for the Fifth Meeting of the PAHO/ACMR.

Application of Live FMD Vaccines in Different Parts of the World

Following the observations made by several investigators in England, Israel, Brazil, Holland, Venezuela and France (Skinner, Brooksby, Gillespie, Kemron, Cunha, Bernal, Palacios, Paraf, Mackowiak and others), that O, A, C, SAT_1 and SAT_2 FMD viruses, when modified by serial passage in various hosts, retained their antigenicity, as judged both by antibody response and challenge experiments, several field trials have been undertaken in different parts of the world. In a few cases, modified live FMD vaccines have been used in controlling outbreaks. A brief summary will be made of some of the results obtained in Israel, Kenya, South Africa, Transvaal, Southwest Africa and in Thailand. The experiments conducted in South America will be described later with more detail.

Israel

Using a live virus vaccine, modified by serial passages in embryonated eggs in an outbreak of FMD type $Asia_1$ in Israel, Kemron (5) reports that this vaccine was used successfully in arresting the spread of the disease from infected to uninfected herds about 8-10 days after vaccination, without causing any undesirable postvaccination reactions. A total of 86,000 head of cattle were vaccinated.

In more limited trials, Kemron and Goldsmit (6) found that following vaccination with this vaccine, there was a 100% immunogenic response as observed by the occurrence of antibodies in all cattle vaccinated, and that there was a considerable difference on the response to inoculations of different groups: the older the animal, the better the response. A significant rise in serum neutralization index (SNI) was observed following a booster dose given fourteen months after vaccination.

Kenya

Galloway (2) reported the use of a live vaccine prepared with a virus modified through 28 passages in unweaned mice and 89 passages in adult mice at the Animal Virus Research Institute, in Pirbright, in controlling an outbreak of FMD (type SAT_2) in Kenya, in the Nomyuki district. Although it has been shown that the field strain to which the vaccinated cattle were exposed was of a different subtype than the strain used in the vaccine (Rho 1) the results were very encouraging, with relatively mild reactions to the vaccine in only about 10 percent of the cattle, including high-grade Friesians, Guernsey, Ayrshire, Red Polls, with Borans, and crosses of these. About 80,000 cattle were vaccinated.

South Africa

Using the same vaccine, Galloway (2) reported its application in a severe outbreak in South Africa, in an area where there were 80,580 cattle and the disease had been spreading for three weeks before vaccination was started. Approximately 44,000 cattle were vaccinated, starting from the periphery towards the infected area. Close to this latter zone the vaccine was used on farms on which there was already some infected animals. Only 0.7 percent of the 44,000 vaccinated animals contracted FMD, although the field strain (SAT_2) was a different subtype than the strain used in the vaccine (Rho 1). There were a few cattle (1.5 percent) of the 44,000 vaccinated that developed lesions of FMD. A second dose of vaccine was applied to 18,000 of these animals and no further cases of the disease were recorded. The rapid spread of the outbreak was halted and when the previous established cordons were removed and the cattle released from this area, there was no evidence of spread of infection from the vaccinated cattle to susceptible animals with which they were in contact.

Transvaal

Using a mouse-adapted FMD live virus vaccine (Rho 1), Martin and Edwards (8) reported that in a field trial in Eastern Transvaal, mild lesions were observed in only 0.35% of over 3,400 vaccinated animals. These investigators did not note abortion or other undesirable reactions attributable to the vaccine; although all ages and sexes were vaccinated, the virus did not appear to spread to unvaccinated control cattle. This trial indicates that this modified virus might be used and be expected to provide protection for a high percentage of adult cattle in outbreaks in which the strain involved does not differ widely in antigenic structure from that of the Rho 1.

Southwest Africa

The use of a live virus vaccine (RV II) in a severe outbreak in South Africa has been described by Galloway (2). Around half a million cattle were vaccinated in the central infected zone with this mouse modified virus. Inactivated vaccines were also used in order to create immune belts of cattle round the zone vaccinated with the live vaccine. 431,219 cattle vaccinated with the live vaccine did not develop FMD, what gave a protection of 90 percent. These results are of great significance considered the fact that the vaccine strain was antigenically different (subtype) from the field strain to which the vaccinated cattle were exposed and that great difficulties were encountered in the campaign.

Thailand

Girard et al. (3-4) found in preliminary trials in Thailand that the mouse-passed $Asia_1$ virus vaccine prepared by the Animal Virus Research Institute, in Pirbright, was innocuous for Devon and Thai cattle and buffalo, and that the mouse-passed type A virus vaccine, also prepared at Pirbright, was innocuous for the Devon breed but caused erosive lesions on the tongue of Thai cattle and was still partially virulent for buffalo. The immunity obtained was around 80 percent.

Research on Foot-and-Mouth Disease Modified-Live-Virus Vaccines in South America

The first information on research and application of FMD modified-live-virus vaccines in South America was reported by Rubino and collaborators in 1940, in the Monthly Bulletin of the Ministry of Livestock and Agriculture of Uruguay (38). This paper reports the work done during the period 1933-1939, using a virus serially passed in guinea-pigs which showed low pathogenicity and good immunity in laboratory tests. This virus was then passed in sheep in order to obtain the necessary amount of virus for field experiments. 7,300 cattle were vaccinated in several farms of Uruguay. Postvaccinal reactions were observed only in 30 of the vaccinated animals (0.4%). In 20 out of the 27 experiments performed, an adequate field protection was observed. Rubino finished his appreciations by pointing out that there was no contagiousness and that the modified virus appeared also to have a marked reduction of pathogenicity for sheep.

Following the research work iniciated in Europe some papers were brought out to report results on adaptation to several laboratory animals, such as suckling mice, rats and embryonated eggs (García Mata et al. 22, 23, 24); adult mice and rabbits (Cunha et al. 12, 13, 14); 1-day old chicks (Palacios et al. 28, 29), and hamsters (Schmidt Funes, 39).

The progress of the above-mentioned research work led to the modification of several virus strains of the types A and O Vallée and C Waldmann, which showed a low pathogenicity for cattle while maintaining their immunizing antigenic properties. These achievements gave rise to new hopes as to the utilization of these modified viruses as vaccines. This work was reported in papers published by García Mata et al. (23); Palacios et al. (29-35); Cunha et al. (14-20); Henderson and Cunha (25, 26); Epstein (21); Zahran (41, 42); Nóbrega (27); Villegas (40) and Pustiglione (36).

A summary of the results of the research work on the development of a FMD live vaccine against the three types present in South America (A, O and C) is shown in Tables I to V. These studies were performed at the PAFMDC and the CIV in Venezuela, through serial passage of the viruses in rabbits, embryonated eggs and one-day old chickens.

Table I summarizes the data obtained with the strain O Campos (type O Vallée) attenuated by serial passages in rabbits (Cunha et al., 13-18). Cattle inoculated with virus of the 110, 111 and 138 passages showed a variable degree of pathogenicity (0-20%) and a poor immunizing effect. A very high pathogenicity was detected in swine, followed by a good immunity. The studies carried out in sheep revealed no pathogenicity (0/14) and a high degree of immunity (14/14). The virus was contagious from vaccinated to nonvaccinated animals of the same species (cattle and swine). One experiment performed with sheep was negative.

Table II presents the results obtained with the 66th and 67th serial passages in rabbits of a virus type C Waldmann (Cunha et al., 20). A low degree of pathogenicity (3/96) and a good immunity (91/100) was observed in cattle. In contrast, all swine inoculated had generalized FMD (10/10) and seven of them died. No contagiousness was detected among cattle, but it was quite high among swine.

Table III summarizes the results of the studies performed by Bernal et al. (44) with the strain O Campos (type O Vallée) modified through serial passage in 14 days embryonated eggs. Inoculations in cattle using virus from several passages showed that its pathogenicity decreases progressively, maintaining a good immunity capacity even at the 99th passage level. These results are much more encouraging than the ones obtained with the same strain attenuated through serial passage in rabbits (Table I).

Data obtained with the 24th, 37th and 49th passages of the strain A Cruzeiro (type A Vallée) in 14 days embryonated eggs (Bernal, 44; Cunha, 19; Palacios, 35) are shown in Table IV.

"Live foot-and-mouth disease vaccines, with special reference to South America", by Carlos Palacios, Mario V. Fernandes and Carlos Bernal.

E r r a t a

Table I (between pages 6 and 7): Change the title to "Lapinized O Vallée virus (Deodoro)".

Page 6, line 6: Change "with the strain O Campos" to "with the strain O Deodoro".

TABLE I

Lapinized O Vallée virus (Campos)

Preparation of vaccines			Testing											
			Cattle				Swine				Sheep			
Passage No.	Material used	Virus titer** $LD_{50\%}$/nbm	LD_{50} p/dose	Pathogenicity	Immunity*	Contagiousness	LD_{50} p/dose	Pathogenicity	Immunity	Contagiousness	LD_{50} p/dose	Pathogenicity	Immunity	Contagiousness
110+1nbm	nbm	9.4	7.7-9.1	0/24	4/24	-	-	-	-	-			-	-
111	rabbit	7.6-8.6	6.9-7.6	19/98	51/98	5/32					7.1-7.8	0/14	14/14	0/4
138	rabbit	7.6	6.9	0/16	3/14	0/5	6.2	3/6	6/6	4/4	-	-	-	-

* Tested by innoculation of $4 \times 10,000$ $LD_{50\%}$ newborn mice (nbm)

** log 6.10 per g of tissue

TABLE II

Lapinized C Waldmann virus

Preparation of vaccine			Testing							
			Cattle				Swine			
Passage No.	Material used	Virus titer (LD_{50}nbm)	LD_{50} p/dose	Pathogenicity	Immunity	Contagiousness	LD_{50} p/dose	Pathogenicity	Immunity	Contagiousness
66-1nbm	nbm	8 8 9 6	8 1 8 9	3/55	51/54	0/1	6 4-6 7	10/10	3/3	3/4
66-1TC*	BHK 21	8.0	8.7 6.7	0/18	15/18					
67	rabbit muscle	7 1 8 5	6 8-8 2	0/23	25/34	0/3				
Total 66-67	nbm BHK rabbit	7 1-9 6	6 8-8 9	3/96	91/106	0/4				

* TC - Tissue culture.

TABLE III

Avianized O Vallée virus (Campos)

Preparation of vaccines			Testing with cattle			
Passage No.	Material used	Virus titer (LD_{50}/nbm)	LD_{50} p/dose	Pathogenicity	Immunity	Contagiousness
17	heart	7.1	6.1	2/4	4/4	
50	heart + liver	8.4	8 1	1/10	9/10	0/2
56	heart + liver	8 6	8 3	1/12	10/12	0/2
68	heart + liver + gizzard	8.0	7 4	0/8	7/8	0/2
71	heart + liver	8.4	7 8	1/12	11/12	
91+1	nbm	8 8	8 1	0/6	6/6	
99+1	nbm	7 5	6 1-7 5	0/35	24/35	

TABLE IV

Avianized A Vallée virus (Cruzeiro)

Preparation of vaccines			Testing									
			Cattle							Swine			
				Pathogenicity		Immunity						
Passage No	Material used	Virus titer (LD_{50}/nbm)	LD_{50} p/dose	A	B	A	B	Contagiousness	LD_{50} p/dose	Pathogenicity	Immunity	Contagiousness
24	heart or total embryo	5 9-7 7	5 6 6 7	16/32	6/30	32/32(1)	30/30(2)	0/14	2 6 6 0	18/18	18/18	-
37	heart or total embryo	7 0	5.9 6.7	13/32	-	30/32(3)	-	-	-	-	-	-
49	heart or total embryo	6 0-9 2	6 4-8 2	15/87	0/66	65/86(3)	55/66(2)	0/2	6.7	8/16	16/16	1/14

A In highly susceptible animals from areas free of the disease (Venezuela, Ecuador, Colombia, Chile)

B In susceptible animals from enzootic areas

(1) Against a serologically homologous and heterologous virus (A_{18})

(2) Against an homologous and heterologous virus (A_{19})

(3) Against an heterologous virus (A_{18})

TABLE V

Avianized O_3 Vallée virus (Lara)

Preparation of vaccines			Testing							
				Cattle			Swine			
Passage No.	Material used	Virus titre LD_{50} nbm	LD_{50} p/dose	Pathogenicity	Immunity (1)	Contagiousness	LD_{50} p/dose	Pathogenicity	Immunity	Contagiousness
101-103	total embryo + heart + 1 day old chick gizzard	5.4 6 8* 6 9 7.8**	5.2 7.3	43/164	124/143	0/14	5.9	6/6	9/10***	4/6
162 163	chicken heart	7 0-8.7	5.4-8 3	0/56	45/53					
182	chicken heart	7 9-8 5	6 0-7 5	0/12	12/12					
202	chicken heart	7.8-8 9	6.2 8 3	2/24	16/24			6/6		
301	chicken heart	7.4-8 6	5 8-7.0	0/6	5/6			6/6		

(1) - Tested by innoculation of 2 × 10.000 LD_{50}/nbm of pathogenic virus

* - In total embryo

** In chicken heart and gizzard innoculated the first day

*** By seroneutralization tests

TABLE VI

Results of the Calabozo campaign

Year	No of cattle vaccinated	LD_{50}	Pathogenicity	Contagiousness	Remarks
1961	3,729	6^{0}	3/3.729	0	Noncontagiousness noted
1962	6,417	$6^{\cdot 0}$	7/6,417	0	" " "
1963	10,928	6^{7}	0/10,928	0	1 cattle showed symptoms of anaphylactic shocks.
Total	21,074	$6^{\cdot 0}$ to $6^{\cdot 7}$	10/21,074	0	

The pathogenicity of this virus, even at the 49th passage level for highly susceptible cattle (from FMD free zones in Venezuela, Colombia, Ecuador and Chile) was quite high: 16/32 for virus at the 24th passage and 15/87 at the 49th. In susceptible cattle from an enzootic zone (Brazil), the pathogenicity was milder: 6/30 and 0/66 respectively for the 24th and the 49th passage levels. A good immunity was attained in all cattle vaccinated, even in the cases where the challenge had been made using an antigenically different virus (subtypes A_{18} and A_{19}). There was no evidence of spread of infection from the vaccinated cattle to susceptible contact animals. This virus still retains a high degree of pathogenicity for pigs even at the 49th passage level.

Table V refers to the results obtained by Palacios et al. (28-35, 47) with the strain O Lara (type O Vallée) modified through serial passages in embryonated eggs and one-day old chicks. Starting at the passages 162-163 a marked decrease in the pathogenicity for cattle was observed. A good immunity was obtained in cattle inoculated with virus from all passages studied, with the exception of the 202nd. Susceptible cattle maintained in contact with vaccinated animals did not contract the disease. The pathogenicity of this virus for swine, even at the 301st passage level, is very high.

Data obtained by Palacios et al. (35) with the application of the virus from passages 101-103, under field conditions in an enzootic zone in Venezuela (Map 1) are summarized in Table VI. In 21,074 cattle vaccinated only 10 developed lesions of FMD, which gives a percentage of 0.05%. This extremely low percentage of pathogenicity, in contrast to the one referred in Table V, seems to be related to the fact that these animals have been previously vaccinated with inactivated vaccine.

Venezuela

Foot-and-mouth disease (type O Vallée) appeared for the first time in Venezuela, in 1950, spreading practically throughout the centralwest part of the country where it has remained enzootic up to the present time. The type A Vallée was found in two areas, at the north of the country (Puerto Cabello, 1951) and along the border with Colombia (Táchira, 1954), from where it spread out to the centralwest area due to an epizootic outbreak in 1957. Since then both types are present regularly. Map No. 1 shows other two areas, one under sporadic outbreaks and the other free of the disease. Table No. VII describes the outbreaks verified from 1950 to 1965, totalling 813 outbreaks of both types - 585 corresponding to virus O and 228 to virus A. The years in which the greatest number of outbreaks of type O occurred were 1950, 1951, 1956, 1960 and 1962.

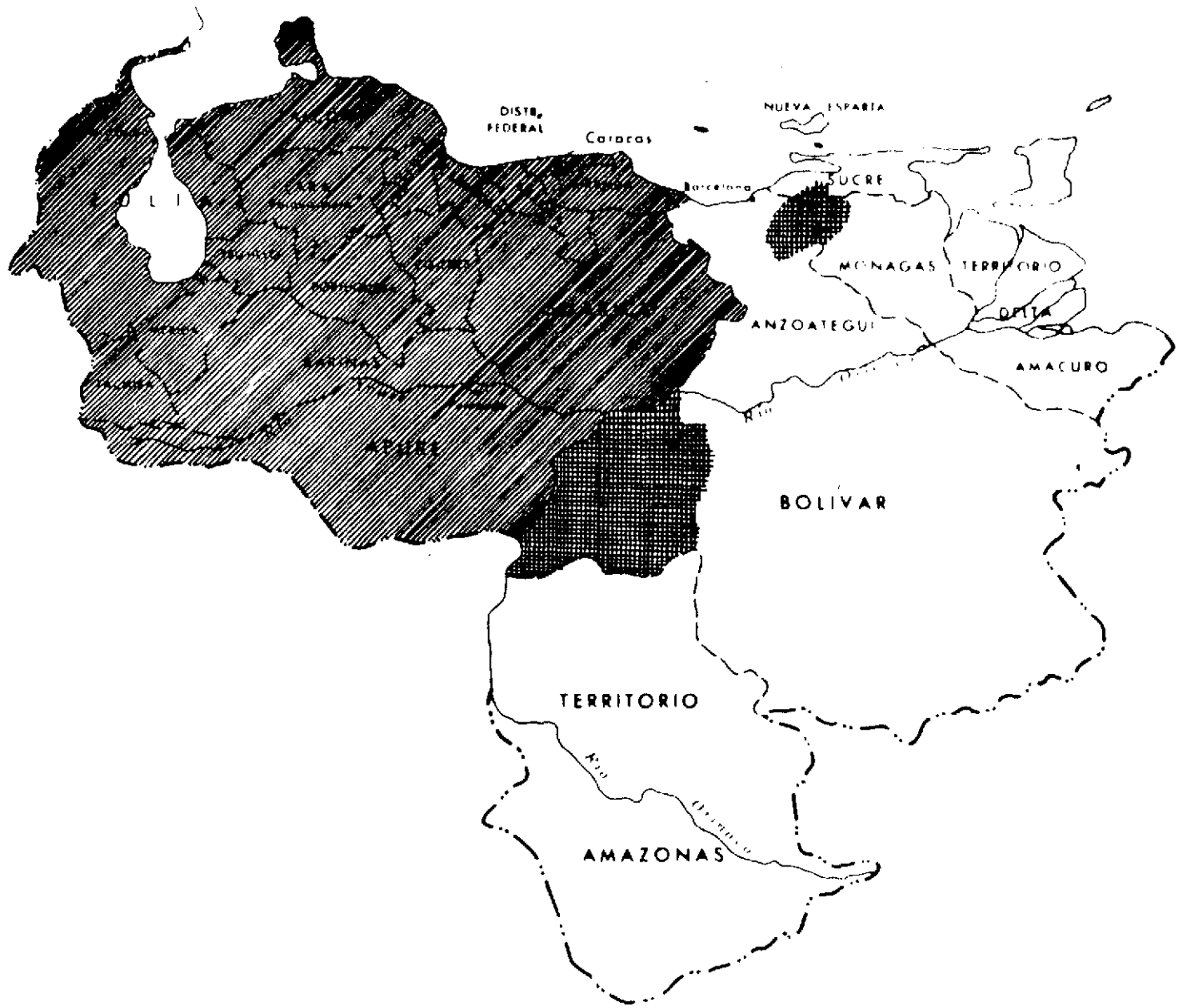
In regard to virus A Vallée, the years of highest incidence are 1957, when it spread all over the enzootic area, and 1962, when the new subtype A_{18} (Zulia) appeared.

Venezuela started a campaign against the disease in 1950. Vaccine production was initiated in 1951, by using an inactivated Waldmann vaccine (modified by Rosenbusch, Silvio Torres, Tellez Giron). Vaccinations reached 2,411,600 doses that year.

In June 1962 an epizootic outbreak of virus A Vallée affected the southern part of the State of Zulia. The information obtained seemed to indicate that it was the continuation of a focus found some months earlier in the Perija District of the same State. The characteristics of this outbreak showed that the Waldmann vaccine, produced until that time from the A Vallée Táchira strain, could not give an adequate protection against the new virus. A survey which covered 6,000 animals (vaccinated several times during the years 1961 and 1962) revealed that 79% of them contracted the disease, the percentage ranging from 44% to 94% according to different farms (35). This observation was confirmed by immunity tests done in the Centro de Investigaciones Veterinarias of Venezuela. Serological

VENEZUELA

0 50 100 150 200 KMS



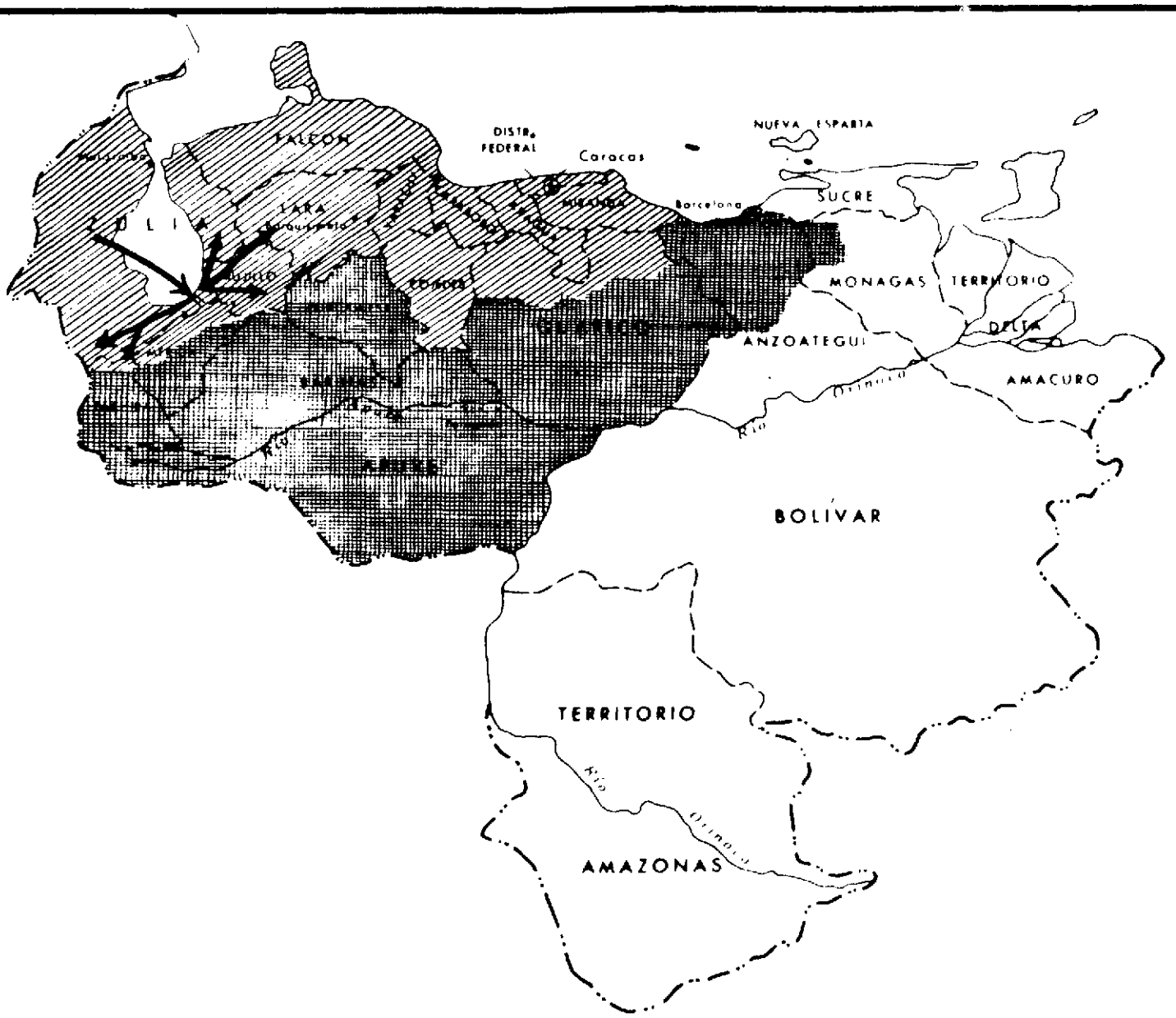
FOOT-AND-MOUTH DISEASE SITUATION - 1966

Zone	Incidence	Area - km^2	Cattle population
	Enzootic	372 800	5 384 585
	Sporadic	95 150	695 542
	Free	444 070	360 581

Map 1

VENEZUELA

0 50 100 150 200 KMS



FOOT-AND-MOUTH DISEASE VACCINATION CAMPAIGN - 1962/1963

Zone

Modified live virus vaccine.

Inactivated Waldmann vaccine.

Tendency of the diffusion of the type A virus, subtype Cuba (A_{18}).

studies carried out in the PAFMDC and in the CIV, Venezuela, demonstrated a wide difference between the strain of virus used in the production of the vaccine and the one present in the field. These results were confirmed by the World Reference Laboratory at Pirbright, England, and the new subtype was classified as A_{18} (Zulia).

Due to the danger of spreading the disease through the technics used for the preparation of the Waldmann type vaccine (inoculation of cattle in slaughter houses located in the center of the country), it was considered the possibility of using a modified-live-virus vaccine. Studies performed at the PAFMDC and at the CIV in Venezuela had shown promising results with modified strains through serial passages in rabbits and chicken embryos. The strain A Cruzeiro modified by Zahran (41, 42), Bernal (41, 42, 44) was elected for the first trials. A good protection was obtained with the use of this virus against the strain A_{18} (Zulia), together with an acceptable degree of pathogenicity. Details of these studies were given earlier.

In December 1962 a total of 500,000 doses were administered to the major dairy cattle herds, which covered the central and western areas of the country (40)(Map No. 2). Later on, with more experience gained in the laboratory, it was found that in order to obtain a good immunity with virus from the 49th passage it would be necessary to inoculate more than $10^{7.0}$ $LD_{50\%}$/nbm, a requirement that would make very expensive the production of the vaccine using embryonated eggs. Further studies with earlier passages (Table No. IV) demonstrated the possibility of obtaining a good immunity with virus from the 36th and 37th passage level, with titer ranging between $10^{5.9}$ and $10^{6.7}$ LD_{50}/nbm per vaccinating dose.

Postvaccinal reactions with the passages 49th and 37th were studied by Villegas (40) and found to be respectively 0.81% (297/36,673) and 2.33% (99/4,243). The responses in general were of a mild nature.

The application of this vaccine took control of the new epizootic confining it to the State of Zulia, which has a cattle population of about 1,000,000 animals. Through mass vaccination in the enzootic area it was possible to prevent the disease from spreading to the rest of the country, with a livestock population of 5 million heads of cattle.

There have been very few outbreaks of type A Vallée since 1963 and subtype A_{18} has not been found since 1962 (Table VII).

The production of modified-live-virus vaccine with O Vallée Lara strain (modified by serial passages in 1-day old chicks and in embryonated eggs) was started late in 1964, by using the virus from passages 101-103. The use of this vaccine was followed by some postvaccinal reactions. While the percentages observed (0.3 to 1.5) proved to be rather low if related to the total vaccinated cattle, they appeared higher (± 30) than expected in some farms, especially among pure bred cattle. Therefore, after studies carried out at the CIV (which were previously described, Table V), a vaccine was applied in 1965 prepared with virus from the 162nd and 163rd passage levels in 1-day chicks. Since then no undesirable reactions have been found in vaccinated cattle (47).

In regard to immunity, vaccinations are being undertaken in Venezuela every six months. Field observations gathered from 1962, with an application of about 13.3 million doses of A Cruzeiro and ± 6.7 million of O Lara vaccines, as shown in Table VIII, sould indicate this to be an adequate rate of vaccination since it has been observed that in outbreaks occurring during this period animals vaccinated every 5 to 7 months have shown a good resistance to the disease.

TABLE VII

Annual distribution of foot and mouth disease outbreaks typed in Venezuela during 1950 1965

Year	Virus O	Virus A	Total
1950	38		38
1951	58	5	63
1952	21		21
1953	6	1	7
1954	5	6	11
1955	..	..	..
1956	53	10	63
1957	31	48	79
1958	28	12	40
1959	30	-	30
1960	72	4	76
1961	32	7	39
1962	94	88	182
1963	41	31	72
1964	43	12	55
1965	33	4	37
T o t a l	585	228	813

25/5/66

TABLE VIII

Live FMD Vaccines

Year	A	O	Total
1962	801,640	-	801,640
1963	3,663,445	-	3,663,445
1964	4,135,510	2,067,755	6,203,265
1965	4,716,355	4,716,355	9,432,710
	13,316,950	6,784,110	20,101,060

The most serious problem associated with the application of the modified-live-virus vaccine in cattle was the occurrence of anaphylactic shocks. Preliminary information from a survey covering 18,275 cattle revealed that the percentage of anaphylactic shocks was of 1.5% (Goic, 46). In several areas a considerable increase between the first and second doses of vaccine was observed. According to a study of 13 farms in the central region of the country, the incidence passed from 0.03% (2/5,739) to 2.1% (114/5,394) during the second vaccination - an increase of 70 times (Goic, 46).

Joint studies performed at the PAFMDC and CIV solved the problem by verifying that the anaphylactic reactions were caused by antibiotics used in the vaccine (penicillin and streptomycin) (Quiroz et al., 37). After vaccines started to be used without these antibiotics no further anaphylactic reactions were observed, although approximately 8.8 million doses of modified live A Cruzeiro virus vaccine, and 6.7 million of O Lara, were applied during 1964 and 1965.

Colombia

FMD is known in Colombia since 1950 when an epizootic of O Vallée virus, which affected Venezuela, moved out from the plains of Apure to the lowlands of Arauca, disseminating rapidly all over the country. Later on, in 1951, virus A Vallée was found in the Department of Valle del Cauca (District of Cali), from where it spread throughout the country. In 1963 the latter virus gave rise to an epizootic outbreak, whose consequences were more seriously felt in the Savanna of Bogotá.

In view of this situation, the Government of Colombia felt the need to test a live vaccine. The vaccine produced from the 49th passage of the A Cruzeiro in embryonated eggs at the PAFMDC was selected. To this effect an agreement was reached with the Pan American Sanitary Bureau in 1963.

The first step along these lines was to determine the pathogenicity and antigenicity of this vaccine in the Colombian environment. At the experimental farm of the Ministry of Agriculture, near the city of Bogotá, this avianized strain of type A Cruzeiro was applied to 32 highly susceptible bovines, from the Paramo of Soacha. Eleven of these animals showed lesions in the mouth and two of them lesions on the feet. In one case the lesions were of a vesicular nature, while in the others they were erosions or desquamations. At a glance all the animals appeared normal.

Twenty eight days after vaccination, 8 of the vaccinated cattle and 4 nonvaccinated controls were challenged with 2 x 10.000 ID_{50}/nbm of the local pathogenic virus. Seven of the vaccinated animals remained immune (7/8) while all the controls contracted the disease (4/4).

Since this potency test demonstrated that A Cruzeiro vaccine gives protection against the field strain of Colombia, controlled and limited applications of this vaccine were made in the Savanna of Bogotá, where there is a cattle population of 120,000, mainly Holstein dairy cattle.

TABLE IX

Field trials in the Savanna of Bogotá with a type A (Cruzeiro) live virus vaccine (49th passage)

Year	Number of cattle	Pathogenicity	
		By individual observation	By detection of clinical cases in the field
1963	4,499	24/296 (8%)	4/4,499 (0.1%)
1964	4,727	50/295 (17%)	2/4,727 (0.05%)
1965	4,514	- -	0/4,514

Table IX summarizes the results of these tests, performed during 1963, 1964 and 1965. A rather high number of animals with mild lesions was found in the vaccinated cattle individually observed: 24/296 in 1963 and 50/295 in 1964. Clinical signs were observed only in a very low percentage of animals (0 to 0.1%).

It was observed that the most severe lesions occurred among young animals. Also interesting is the fact that in 1963 the vaccination carried out in a number of farms with no antecedents of the disease or previous exposure to inactivated vaccines, showed the greatest number of postvaccinal responses (21/149), representing the majority of reactions verified in that year (21/24). The laboratory studies of the corresponding sera corroborated this information, by determining that the antibody levels of 48 samples were very low.

Experiments have also been made in the Savanna of Bogotá with a vaccine prepared from a strain of virus O (Campos), modified through serial passages in embryonated eggs, at the 71st and 100th passage levels (Bernal et al., 44). The results obtained in these tests are summarized in Table X.

TABLE X

Field trials in the Savanna of Bogotá with a type O (Campos) avianized live virus vaccine

Passage number	Number of cattle	LD_{50}/per dose	Pathogenicity	Immunity
71	550	$10^{6.8}$-$10^{7.2}$	31/550 (5.6%)	8/10
100	396	$10^{6.3}$-$10^{7.0}$	0/396	-

After this preliminary and successful test, controlled and limited field trials were made.

These field trials were performed at the areas of Barranca Chica and Hacienda El Recreo, where vaccination against FMD had never been applied.

The results obtained are summarized in Table XI.

The total of animals found with reactions was 44. However, only 3 of those animals were indicated by the farmers. All other reactors were found only after careful inspection of the mouth and feet, being undetected by the farmers due to the absence of any significant salivation or lameness.

Remarkable is the high percentage of animals with lesions in the Barranca Chica area (43/312) when compared with El Recreo (1/126). The explanation for this must be the fact that the farms in the Barranca Chica area were stocked mainly with cattle from the FMD free Andean region. Moreover, the spread of the few outbreaks that have occurred in the area has been limited, because of the isolated localization of the pastures. At the farm El Recreo outbreaks of FMD have been reported a year previous to the vaccination. There were also 2 farms in the Barranca Chica area on which FMD type A was diagnosed a year previous to the vaccination. On those farms, again, no reactors with lesions were found, although the overall percentage for the area was as high as 12%.

Although theoretically the group of animals between 6 months and 1 year old must be without maternal or any other antibody, the animals vaccinated within this age group were the ones that showed the lowest rate of reactors 0/38. Unfortunately, the small number of calves that were actually inspected on the farms in the Barranca Chica area was too small to warrant a definite conclusion regarding the relatively insusceptibility of this group to the vaccine, compared to older cattle.

There was no drop in milk production, and no abortions or deaths occurred. Of all reacting cattle only three animals were indicated by the farmers as having lesions.

Ecuador

FMD, type A, was for the first time diagnosed in Ecuador in 1956. The disease had established itself in the low, humid coastal part of the country, so-called the Litoral. In this region there are approximately 600,000 head of cattle, mostly of a mixed zebu-criollo type.

The Andean part of Ecuador, where most of the country's high-grade cattle breeding regions are located, has been kept free from FMD because of strict quarantine and sanitary control measures, with the exception of two outbreaks with virus type O Vallée, in 1962 and 1965, and another with type A Vallée, also in 1965. The first occurred in the province of Carchi, introduced by cattle that came from Colombia. The second outbreak of type O occurred in the south provinces of Loja, Azuay and El Oro. The outbreak of type A Vallée was detected in the province of Carchi in the north of the country. These outbreaks were, however, quickly dominated by means of mass vaccination of the northern districts with inactivated vaccines and with modified-live-virus vaccine type O Vallée (Campos) in the south, completed with rigorous measures of control on cattle movements.

After the encouraging results obtained in Venezuela with modified-live-virus vaccines, the Government of Ecuador decided to use this type of vaccines as a base for the control of the FMD outbreaks.

In cooperation with the PAFMDC a series of steps were planned and executed to provide the necessary knowledge regarding both the production process and the use of the vaccine in the field. A potency test of the A Cruzeiro vaccine (49th passage) was performed in a small number of cattle, at the Foot-and-Mouth Disease Institute in Guayaquil, Ecuador. Twelve fully susceptible bullocks were vaccinated. Four other bullocks were left without vaccination in close contact with the vaccinated animals. None of the vaccinated animals showed lingual or feet lesions. Three weeks after vaccination all animals were inoculated intradermolingually with 4 x 10,000 LD_{50}/nbm of a pathogenic virus strain isolated in Ecuador. None of the vaccinated animals showed signs of generalization but the four contact animals presented generalized lesions of FMD.

In 1964 the vaccination was extended to the entire affected zone of Litoral, where 207,817 doses of vaccine were used with no information of unfavorable results.

68,850 doses of the same vaccine were used in 1965.

In the south of the country, 235,750 doses of a vaccine prepared from the 100th passage of virus type O Vallée (strain O Campos) in embryonated eggs were used. The only information available is that among 210 cattle examined, 57 reacted - all with benign lesions. It can be said, in conclusion, that the progress of the infection was prevented where the vaccine was applied.

TABLE XI

Live FMD vaccine (strain A Cruzeiro at the 49th passage level)

Farm	Vaccinated	Examined	Lesions	Lesions %	Clinical lesions
Barranca Chica					
Calves	356	18	-	-	
Heifers	567	77	11	14	
Cows	1,145	159	21	13	1
Bullocks	192	55	9	16	
Bulls	205	3	2	-	2
Subtotal	2,465	312	43	13.8	3
El Recreo					
Calves	126	20	-	-	
Heifers	377	14	1	0.7	
Cows	640	61	-		
Bullocks	166	24	-		
Bulls	43				
Subtotal	1,352	125	1	0.4	
T o t a l	3,817	437	44	10	3

Chile

The three classic virus types of FMD, O, A and C are found in Chile. The peculiar geography of the country and the characteristics of cattle distribution and movement make it possible to differentiate 4 epizootiologic areas.

In the north, between parallels 18 and 32, latitude south, the disease is sporadic, as a result of the scarce bovine population (about 100,000 animals) scattered throughout small valleys isolated by deserts.

In the central area, between parallels 33 and 42, FMD is enzootic. Epizootical outbreaks occur periodically and the disease spreads out rapidly all over the area where are concentrated most of the cattle herds of the country (2,663,200 animals out of a total of about 3,000,000).

Southward, between parallels 42 and 49, only sporadic outbreaks occur.

Between parallels 49 and 56 (Province of Magallanes) FMD has never been recorded, this area being considered free of the disease.

In the last quarter of 1962 an epizootic outbreak covered ten of the major cattle-breeding provinces of the country. Within the space of two months 15,800 cases were reported over a total of 23,113 for the whole country during that year.

In 1963 a marked decrease was registered, as a result of mass immunization of cattle performed during and after the outbreak, and of restrictions imposed upon cattle movement.

Early in 1963, the Ministry of Agriculture of Chile decided to give special attention to the problem of FMD. To a National Commission already established was given the responsibility of planning and implementing the FMD program in the country, in coordination with the Institute of Bacteriology.

Taking into account the potential interest for the country of modified-live-virus vaccines and in the light of the promising results already obtained in other countries, the Ministry of Agriculture decided to study the behavior of the avianized A Cruzeiro vaccine in Chile, in order to determine its pathogenicity and antigenicity characteristics for the local cattle.

The work was planned for the vaccine AE_{49} to be applied in approximately 5,000 cattle, completed within the following 3 weeks with inactivated O and C types vaccines, along with periodical bleedings in the space of a year to ascertain serological antibody level. Table XII show that 5,587 cattle were vaccinated with the avianized A Cruzeiro vaccine (49th passage in embryonated eggs) using $10^{-6.8}$ - $10^{-7.0}$ LD_{50}/nbm per dose.

Table XII summarizes the results of the individual pathogenicity control test in 670 animals. Vesicular lesions were found in 4.2% of the vaccinated bovines and desquamative lesions of different grade in 10%, both at the buccal cavity and at the interdigital spaces.

At a farm, on the very day of vaccination 4 heifers and 3 cows out of a total of 110 and 171 respectively showed visible edematous swellings on the face, vulva and perineum which disappeared within a few hours without having been treated. These reactions were considered as anaphylactic shocks of a mild character.

Two cows aborted between the 4th and the 6th day after vaccination. Both animals were in the seventh month of pregnancy and the clinic examination performed did not indicate lesions attributable to the vaccine.

The effect of the vaccination on milk production could not be analized since the vaccination was applied during the time in which the cows ceased to produce milk.

At the Ministry of Agriculture experimental farm facilities in the commune of Frutillar, Department of Puerto Varas, Province of Llanquihue, this vaccine was applied intramuscularly in a dose of $10^{6.7}$ LD_{50}/nbm to a group of 20 1-2 year old Holstein steers. Ten steers of same age and characteristics were kept together to serve as contacts.

These animals came from the island of Chiloé (southern area) and could be considered as having no previous contact with FMD.

All the animals were observed every day during the subsequent 10 days. Out of the 20 vaccinated animals only two showed vesicular lesions in the mouth and on the feet. The ten contacts remained unaffected.

Twelve vaccinated animals, excluding those which had shown vesicular lesions, along with 3 contacts selected at random and 5 bovines brought in especially from Chiloé to serve as controls were subject to the efficacy test 3 weeks after vaccination. Prior to inoculating the virus all the animals were bled to ascertain the respective antibody level. Following this the animals were given intradermolingually 10,000 ID_{50}/nbm of type A virus (strain 208 Chile) in four sites.

10 out of the 12 vaccinated animals (10/12) were protected against experimental infection.

In view of the effectiveness of the vaccine against the Chilean strain of virus A it was decided to make a field application on herds of the Province of Llanquihue. The Departments of Puerto Varas, Maullín and Llanquihue were chosen to this effect since they had 90.2% of the total cattle population of the province. An information survey was carried out on 49 farms. 29 were selected, trying to keep the percentual distribution of animal categories corresponding to the province.

As much as 78% of the area of these farms is utilized for livestock, the permanent dairy exploitation (87%) prevailing over the seasonal one. All the cattle are Holsteins with its red and white variety. 5% are pure-bred registered animals.

British Guiana

In September 1961 an outbreak of FMD (type A Vallée) occurred at the Savanna of Rupununi, probably originating from the Brazilian border, where the disease appeared in July of that year.

An application of 10,000 doses of inactivated vaccine was performed around the affected area, and 5,000 cattle were vaccinated with a modified-live-virus vaccine, (24th passage of strain A Cruzeiro, in embryonated eggs) within the central zone of the outbreak. This was decided after a preliminary application of the same vaccine in 20 animals did not show any undesirable postvaccinal effects. 800 of the 5,000 vaccinated animals were individually observed, but no reactions were detected. It seems that a good immunity was induced in the vaccinated cattle since the spread of the disease was halted.

Another outbreak appeared in January 1962 in a nonvaccinated herd where 10 out of 300 cattle were sick. The same modified-live-virus vaccine was immediately used in all animals of that farm, and from that date on it seems that there was no spreading of the disease since no new outbreaks have been reported up to now.

TABLE XII

Numerical and percentual distribution of lesions presented in animals vaccinated with the avianized A Cruzeiro (49th passage) vaccine in relation to the total of animals examined. Province of Llanquihue, Chile - 1964.

Category	Total Vaccinated	Total Examined	Lesions					
			Vesicular		Desquamatives		Total Lesions	%
			Total	%	Total	%		
Calves	819	140	5	3.5	13	9.2	18	14.2
Heifers	1,568	159	12	7.5	20	12.5	32	20.1
Cows	2,733	329	7	2.1	30	9.1	37	11.2
Steers	287	28	4	14.2	4	14.2	8	28.5
Bullocks	108	12	-	-	-	-	-	-
Bulls	72	2	-	-	1	-	1	-
T o t a l	5,587	670	28	4.1	68	10.1	96	14.3

TABLE XIII

FMD Modified-Live-Virus Vaccination

Year	Virus/Modification	Vaccinated cattle	Reactors	
			Ind.(3)	General(4)
	Monovalent Vaccines			
1956/65	0.111- RAB(1)	26,685	103/1,484	7/1,159
1963/65	052-65 E.E.(2)	4,917	85/508	27/1,083
1961/62	A24. E.E.	669	19/103	-
1962/64	A49. E.E.	11,422	35/592	-
1963/64	C67 - RAB	2,699	50/419	-
	Polyvalent Vaccines			
1964	A49-E.E. 0111-RAB	2,309	14/113	3/919
1965	A49-E.E. C67-RAB	7,255	33/730	0/1,133
1965	A49-E.E. 0106 E.E. C67-RAB	6,489	31/446	5/5,440

(1) - 0111 - virus type and number of passages
RAB - modification in rabbits

(2) - E.E. - modification in embryonated eggs

(3) - Individual - All the cattle were carefully examined to detect reactions in the mouth, feet, etc.

(4) - General - Field observation to detect any animal which could be clinically affected.

Brazil

This country -in which the headquarters of the PAFMDC are located- has an area corresponding to 48% of the total area of South America. Its cattle population consists of about 80,000,000, among which the three classic types of FMD viruses are enzootically encountered. Field applications of modified-live-virus vaccines, in experimental scale, have been carried out since 1956.

Table XIII gives the results of the application in cattle of mono- and polyvalent vaccines in regard to the pathogenicity. These data have been collected through careful examination of groups of vaccinated animals as well as through observation of the animals to detect only those presenting clinical symptoms of the disease.

A wide variation in postvaccinal responses has been noticed concerning the same vaccine, a fact that can be explained by the variants existing among the cattle populations utilized, such as race, age and immunity condition, among others. A noteworthy observation is that apparently the polyvalent vaccination does not substantially increases the number of cattle showing reactions. In other words, there appears to be no cumulative pathogenicity. Cunha et al. (19) and Palacios et al. (35) found that the efficacy of polyvalent modified-live-virus vaccines is not inferior to the one conferred by the respective monovalent vaccines.

Observations made in a small number of young cattle during a natural outbreak of FMD in one of the farms in which the previous experiments were performed, showed a clear antigenic difference between an O strain modified in rabbits and in chick embryos. After nine months, the first one protected 40% (5/13) and the second one 70% (9/13) of the inoculated animals. The latter level of immunity was similar to the one observed in a group of cattle that have contracted the disease nine months earlier (15/24).

The study of modified-live-virus markers will permit us to understand the behavior of the virus in relation to the problems of carriers. Finally, it is necessary to study the application of these vaccines on other species of animals such as swine, in which a high degree of pathogenicity exists, a factor that has been completely overcome in cattle.

Summary

The current level of research on the development of modified-live-virus vaccines indicates a promising future in their application on the control of FMD in South America. These vaccines have already solved some problems, such as those encountered in British Guiana, Venezuela and Ecuador, although some of the modified virus strains utilized still possess a limited degree of pathogenicity for cattle. Another important consideration is that FMD outbreaks were never observed to have been caused by the application of modified-live-virus vaccines. In British Guiana and Ecuador vaccination measures were terminated after the disease was under control. In Venezuela, the type virus A Vallée, subtype A_{18}, which caused the 1962 outbreak, has never been encountered since the vaccination was applied, as previously described.

The available information points out the possibility that certain strains may protect cattle against the attack of different subtypes, as in the case of the A Cruzeiro strain used in Venezuela to control the A_{18} (Zulia) virus. Revaccinations seem to produce a better and longer immunity which lasts for more than 6 months. The application of polyvalent vaccines has revealed neither a cumulative pathogenicity nor a weaker immunity. An additional advantage brought about by modified-live-virus vaccines lies in the possibility of an almost unlimited production, while the production of inactivated vaccines has well defined limitations.

The above does not imply that all problems concerning modified-live-virus vaccine have yet been solved. For example, there are still some aspects to be clearly defined regarding the immunity given to young cattle, which is often insufficient. This leads us to further investigate the most effective cycle of vaccination in order to improve immunity. We also need to improve our knowledge on the survival of modified virus in the different tissues from inoculated animals. This would eventually result in a reevaluation of the policies followed by the meat exporting countries, which are not in a position to use now modified-live-virus vaccines in their campaigns because of import limitations imposed by some of the importing countries.

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