

PREVALENCE OF ANTIBODIES AGAINST FOOT-AND-MOUTH DISEASE  
VIRUS-INFECTION-ASSOCIATED ANTIGEN (VIA) IN CATTLE  
OF THE PARAGUAYAN CHACO

Félix J. Rosenberg\*, Hernán Málaga Cruz\*, A. Alonso Fernández\*,  
Tomás Martínez\*\*, Aníbal Barreto\*\*

ABSTRACT

Sera from cattle in the Chaco region of Paraguay were tested for the presence of antibodies to virus-infection-associated (VIA) antigen. A sample of 1,890 cattle was selected, by a two-stage sampling procedure, from a total population of approximately 100,000 cattle.

The overall rate of positive cattle in the population was 0.26. The rate for cattle over 2 years old was 0.38; and for cattle under 2 years, 0.08.

The area was divided into six geo-political regions, according to the foot-and-mouth disease (FMD) history of each. The two regions which had had a severe FMD outbreak approximately two years previous to the study had positive rates of 0.34 and 0.40. The other four areas, where fewer cases of clinical disease had been reported, gave rates which varied between 0.16 and 0.24.

Significant differences were also found between populations of different sizes, although this difference was only noted in cattle over two years old. VIA antibodies in cattle under two years were probably due to colostrum, although the possibility of virus transmission from carrier cattle cannot be excluded.

The following variables should be taken into account when determining regional infection rates through serological studies: (a) period of time elapsed since last FMD occurrence; (b) age of animal population; (c) persistence of antibodies to VIA; and (d) number and location of cattle brought into the area.

INTRODUCTION

Campaigns against foot-and-mouth disease (FMD) form a major component of animal health programs in South America. It is now recognized that strategies for control of FMD must be based on the specific regional epidemiological characteristics of the disease (1). Although FMD information systems are functioning in most South American countries, occasional laboratory support is required for a more precise evaluation of the status of the disease in a certain region. The measurement of circulating neutralizing antibodies is not a reliable means of determining FMD status in most South American countries which have regular cattle vaccinations.

A non-structural antigen associated with FMD virus (FMDV) replication was described by Cowan and Graves in 1966 (2). In infected animals this virus-infection-associated antigen (VIA) induces antibodies which are similar in all of the various FMDV types and

\* Pan-American Foot-and-Mouth Disease Center, Caixa Postal 589-ZC-00, Rio de Janeiro, RJ, Brazil.

\*\* Servicio Nacional de Lucha contra la Fiebre Aftosa (SENALFA), Edificio Patria, 4º piso, Asunción, Paraguay.

subtypes. McVicar and Suttmoller (3) suggested the use of an agar-gel double diffusion test for the detection of VIA-antibodies in field surveys. In experiments, this test proved to be highly efficient in detecting and keeping track of infected animals, independent of the development of clinical signs of the disease (4).

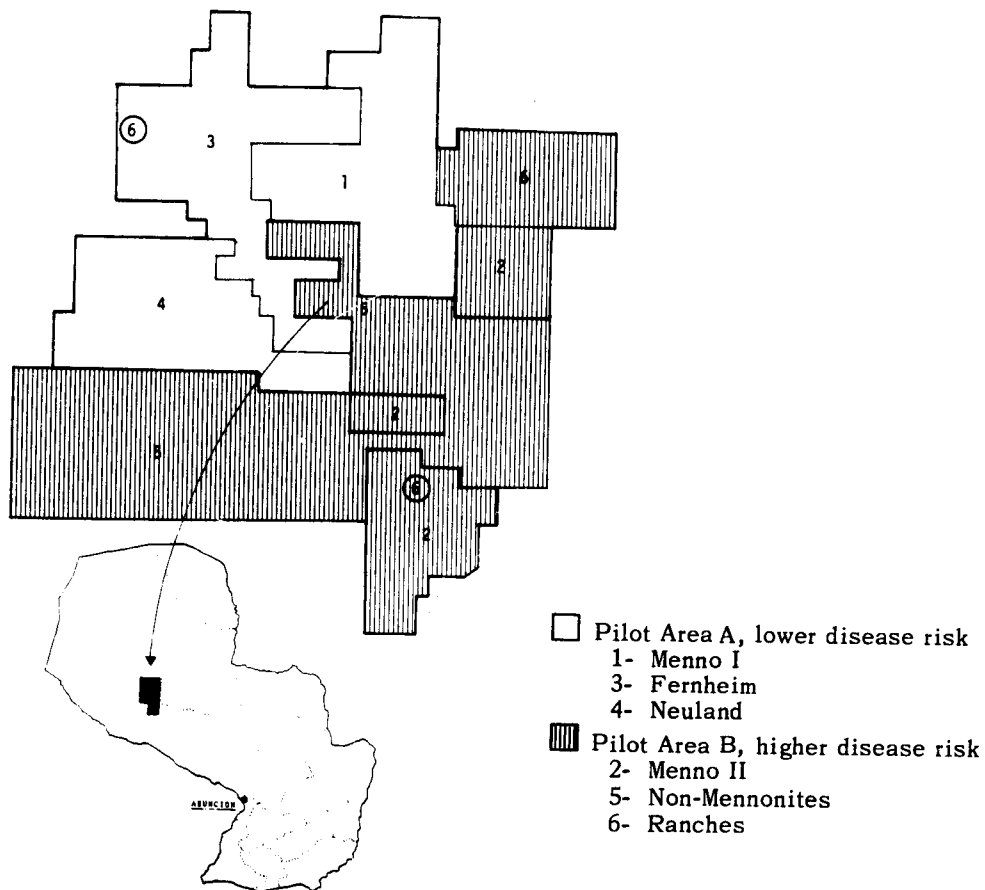
The present work evaluates the usefulness of the VIA test in detecting past FMDV infections in large cattle populations.

#### MATERIALS AND METHODS

The department of Boquerón, Chaco, Paraguay was selected for the survey (Map 1). In July 1973, the survey was carried out, at which time the cattle population was approximately 100,000 head, located in 151 villages and 3 large ranches; and owned by about 1,800 farmers.

In a retrospective survey made in August 1971, nearly 50% of the farmers in the area reported that the disease had never occurred

MAP 1 - *Distribution of the Geo-Political Regiones. Mennonite Colonies. 1973*



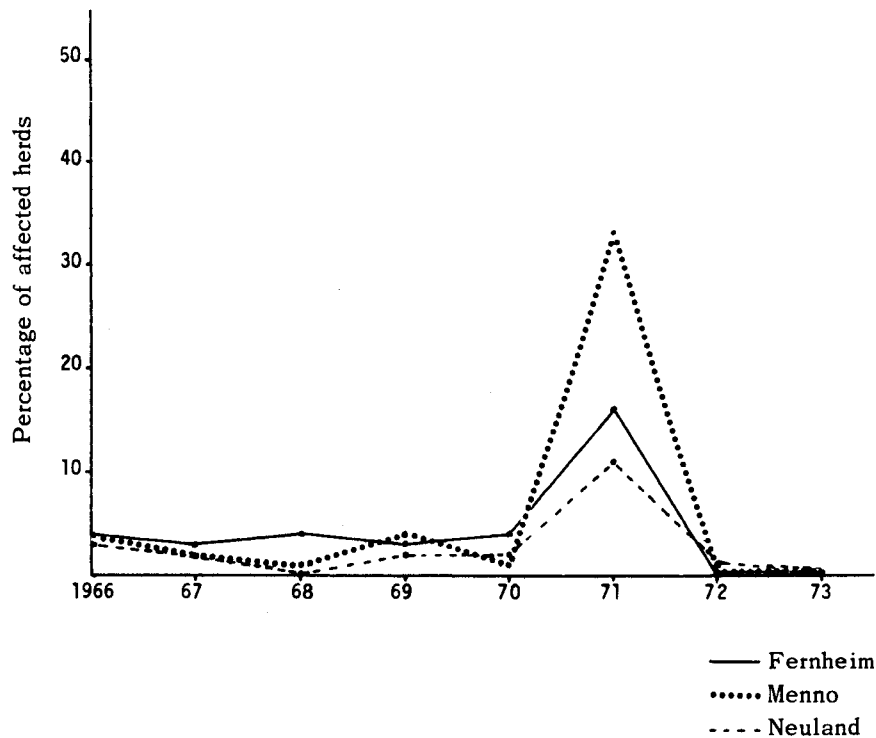
on their farms (Alvarez, unpublished data). Between 1966 and 1970, the yearly rate of affected farms appeared not to have exceeded 0.04 (Fig. 1).

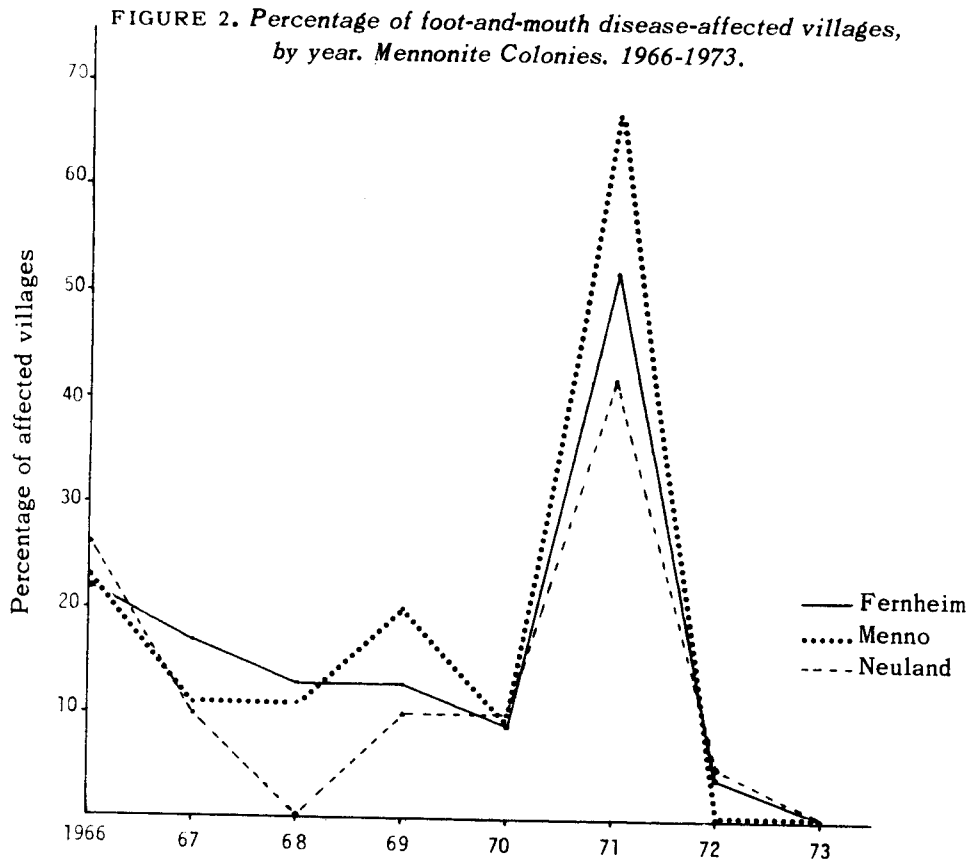
An epidemic caused by FMDV subtype O<sub>1</sub> began in May 1971. In August, when the epidemic peaked, cattle in about half the villages and a quarter of the farms were affected by the disease (Figs. 1 and 2).

Until this outbreak there had been no organized FMD control program, although farmers would occasionally vaccinate their cattle.

In January 1972, when the last cases of FMD occurred, the Paraguayan National Service for the Control of Foot-and-Mouth Disease (SENALFA) began two pilot programs for the eradication of the disease in the study area. In both the lower disease risk area (Area A) and the higher disease risk area (Area B), cattle were vaccinated 3 times during the year. After December 1972, no further FMD vaccinations were performed. A commercial vaccine produced in tissue culture and inactivated with acetyleneimine (AEI) was used.

FIGURE 1. *Percentage of foot-and-mouth disease-affected herds, by year. Mennonite Colonies. 1966-1973.*





#### *Selection of the sample*

A cluster sample in two stages (5) was selected. Due to the particular sociological pattern of the area, villages were taken as primary sampling units instead of cattle farms. Two variables were used to stratify the sample selection: (a) geographical distribution of the cattle population, and (b) age of cattle (Table 1).

Area A (lower disease risk) was composed of three groups of Mennonite villages: Menno I (No. 1), Fernheim (No. 3), and Neuland (No. 4). Area B (higher disease risk) consisted of two groups of villages, Menno II (No. 2), the non-Mennonite villages (No. 5), and a final group of large ranches (No. 6).

Cattle were divided by age into those over and under 2 years.

#### *Serological tests*

Blood samples were collected in July 1973, approximately 18 months after the last recorded case of FMD, and again in November 1973 from calves less than 2 years old. Sera were kept at 4° C for 3-10 days and then stored at -20° C until tested.

The agar-gel double diffusion test (3) was used for the detection of VIA-antibodies. The antigen was prepared as reported by Alonso Fernández and Sondahl (6).

## RESULTS

#### *Prevalence of antibodies to VIA*

The overall population rate of positive cattle (Table 2) was  $0.26 \pm 0.02$  within 95% confidence limits. Rates in the age division over

two years ( $0.38 \pm 0.02$ , 95%) were significantly higher than those in the younger division ( $0.08 \pm 0.03$ , 95%). The differences between the two age divisions were significant for all areas. The rate of positive cattle in groups 2 and 5 (0.40 and 0.34) was higher than in the remaining areas which ranged between 0.16 and 0.24; and the differences were significant in both age divisions.

In order to determine the association between clinical disease and prevalence rates of antibodies to VIA, the results were analyzed according to whether or not the villages had reported clinical disease in the 1971 survey. A significant association existed be-

tween the reporting of clinical disease in the village and the prevalence rates ( $X^2 = 35.4$ ,  $P < 0.001$ ) (Table 3). The  $X^2$  test was significant for both age divisions (under 2 years = 12.88; over 2 years = 28.47).

The results of the prevalence study were also analyzed in terms of the size of animal population in sampled villages, dividing them into those with less than 350 cattle, with 350-950 cattle, and with more than 950 cattle. As can be seen in Table 4, for cattle over 2 years old, the population group of over 950 animals had a significantly higher percentage of positives than the two groups of smaller populations ( $X^2 = 10.7$ ;  $P < 0.01$ ).

TABLE 1. Stratification of sampled cattle. Mennonite Colonies, 1973.

Pilot area	Village group	Population			Sample		
		Villages	Farms	Cattle	Villages	Cattle	
						<2 years	>2 years
A	1 (Menno I)	65	690	28,715	12	189	285
A	3 (Fernheim)	31	432	20,320	9	142	210
A	4 (Neuland)	27	217	13,731	7	112	168
B	2 (Menno II)	19	265	16,329	8	118	194
B	5 (Non-Mennonites)	9	232	18,805	9	128	196
B	6 (Ranches)	3	3	10,000	3	54	94
Totals		154	1,839	107,900	48	743	1,147

TABLE 2. Number and prevalence rates of positive animals to VIA according to geographical distribution and age divisions. Mennonite Colonies, 1973.

Village group	A g e				Total	
	<2 years		>2 years		No.	Rate*
	No.	Rate	No.	Rate		
1	9	0.05	106	0.37	115	$0.24 \pm 0.04$
2	17	0.14	107	0.55	124	$0.40 \pm 0.05$
3	5	0.04	66	0.31	71	$0.20 \pm 0.04$
4	5	0.04	40	0.24	45	$0.16 \pm 0.04$
5	24	0.19	85	0.43	109	$0.34 \pm 0.05$
6	2	0.04	31	0.33	33	$0.22 \pm 0.07$
Total	62	$0.08 \pm 0.03$	435	$0.38 \pm 0.02$	497	$0.26 \pm 0.02$

\*95% confidence limits.

TABLE 3. Number and percentage of positive and negative animals to VIA according to age and past FMD clinical experience\*. Mennonite Colonies, 1973.

	Under 2 years				Over 2 years				Total			
	Positive		Negative		Positive		Negative		Positive		Negative	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
With FMD	34	9	348	91	258	43	337	57	292	30	685	70
Without FMD	4	2	231	98	92	26	263	74	96	16	494	84
Total	38	6	579	94	350	37	600	63	388	25	1179	75

\*Information on clinical history of FMD in village group 5 was not available.

TABLE 4. Number and percentage of positive and negative animals to VIA by age and size of the bovine population in the villages. Mennonite Colonies, 1973.

Cattle population in the village	Under 2 years				Over 2 years			
	Positive		Negative		Positive		Negative	
	No.	%	No.	%	No.	%	No.	%
<350	12	8	147	92	85	36	149	64
350 - 950	30	10	276	90	158	33	317	67
>950	20	7	260	93	192	44	245	56
Total	62	8	683	92	435	38	711	62

#### DISCUSSION

Since the serological survey was made in July 1973, 18 months after the epidemic, some positive animals could be expected in cattle under 2 years of age. Of the 8 percent positive in this age group (Table 2), many were not yet six months old at the time of bleeding. It is likely that these calves acquired their antibodies to VIA passively; since by November 1973 no VIA was detected in any animals of less than 2 years in 4 of the 6 vil-

lages where VIA-positive calves had been identified 4 months earlier. Some of the positive calves in the other 2 villages could also be accounted for through infection by contact with animals in neighboring areas. Eight calves, 10-18 months old at the time of the survey, had no history of contact with known diseased cattle, but had persisting antibodies to VIA. This could indicate virus transmission from subclinical cases or adult carriers.

Thus, when interpreting results of serological surveys for VIA antibodies, it should be recognized that young animals may have maternal antibodies, although occasional virus transmission from FMDV carriers to newborn calves or older animals may also have occurred. This latter possibility has not yet been proven experimentally.

The AEI-inactivated vaccine used in both pilot areas probably did not cause an increase in the number of positive cattle, since it has a low probability of residual live virus (7).

Village groups 2 and 5 (Area B, lower disease risk) both had higher percentages of positive cattle than the other four groups. Group 6, although also a part of Area B, had a relatively low rate of cattle positive to the VIA reaction, due to the heavy influx of cattle from Area A.

Highly significant differences found between villages groups with and without disease during the 1971 outbreak further support the hypothesis that the differences between the rates of positive cattle are due to varying disease risks. Special reference should also be made to the 26% positive adults in the non-affected villages. These animals may have become affected at the end of the epidemic, although this was not observed (Kaethler, personal communication); they may have had sub-clinical infections, as sometimes occurs even with non-vaccinated cattle (4); or they may have been brought from neighboring infected villages. The last is the most probable, given the frequent and unrecorded movement of cattle within the Mennonite colonies, especially into and out of the large ranches (Group 6). This observation agrees with the significantly higher prevalence rates noted in the larger villages.

#### CONCLUSIONS

VIA antibody surveys in large cattle populations can contribute effectively to epidemiological diagnoses of FMD infections in

areas of South America where control programs are being developed. In order to best utilize the results of such a survey, however, the following parameters should also be considered:

##### 1. *Age of the animal population*

Very young animals may carry passive antibodies, although this does not necessarily imply active virus replication. Selecting cattle to be surveyed by age groups is critical for the detection of the last FMD occurrence in the area. The possibility of virus transmission from FMDV carriers to newborn calves or older animals, as Hedger (8) and Augé *et al.* (unpublished data) have suggested, should be studied further.

##### 2. *Persistence of VIA antibodies*

A previous study (4) reported that VIA antibodies lasted for at least 15 months post-infection in 5 of 12 infected cattle, regardless of whether or not the animals had developed clinical disease. In the present study, approximately 40% of the animals were positive to the VIA test, even though some of the animals in the Pilot Area may not have been exposed to the virus during the 1971 epidemic. We may assume, then, that antibodies to the VIA persisted much longer in the Chaco study than in the previous one. The cause of this continuing presence of VIA antibodies may lie in differences due to FMD virus strains, the small amounts of polymerase used as a booster in the vaccines, or the frequency of infection. Although in the present survey the population was considered to have had a single infection, it should be kept in mind, when surveying for VIA antibodies in areas without history of the disease, that frequent infections with different types of FMDV will evoke a longer-lasting response.

##### 3. *Cattle movement*

Cattle in South America often travel relatively long distances for commercialization.

Any serological survey should therefore include reliable information on cattle turnover and sites of origin of the cattle introduced into the area. Animals coming from areas of high disease incidence will increase the VIA antibody prevalence rate of the population as a whole, without necessarily reflecting actual infection rates in the native cattle.

Surveys for VIA antibody can play an important role in determining the infection rates of large vaccinated cattle populations and may be a useful tool for retrospective epidemiological surveillance. Repeated yearly surveys can be a particularly reliable means of monitoring the progress of FMD control programs.

#### REFERENCES

1. ROSENBERG, F.J.; GOIĆ M., R.  
Programas de control y prevención de la fiebre aftosa en las Américas. *Bltm Centro Panamericano Fiebre Aftosa* 12: 1-22, 1973.
2. COWAN, K.M.; GRAVES, J.H.  
A third antigenic component associated with foot-and-mouth disease infection. *Virology* 30 (4): 528-540, 1966.
3. McVICAR, J.W.; SUTMOLLER, P.  
Foot-and-mouth disease: The agar gel diffusion precipitation test for antibody to virus-infection-associated (VIA) antigen as a tool for epizootiologic surveys. *Am. J. Epidemiol.* 92 (4): 273-278, 1970.
4. ALONSO FERNANDEZ, A.; AUGÉ DE MELLO, P.; GOMES, I.; ROSENBERG, F.J.  
The use of virus-infection-associated antigen (VIA) in the detection of cattle exposed to foot-and-mouth disease virus. *Bltm Centro Panamericano Fiebre Aftosa* 17-18: 17-22, 1975.
5. HENSEN, N.H.; HURWITZ, W.N.; MEDOW, W.G.  
*In "Sample surveys methods and theory"*. Vol. 1, J. Wiley Ed. N. York, 1970.
6. ALONSO FERNANDEZ, A.; SONDAHL, M.S.  
Preparación y concentración de los antígenos 140 S, 12 S y VIA del virus de la fiebre aftosa. *Bltm Centro Panamericano Fiebre Aftosa* 17-18: 1-9, 1975.
7. GRAVES, J.H.; ARLINGHAUS, R.B.  
Acetyleneimine in the preparation of inactivated foot-and-mouth disease vaccines. *Proceedings 71 Am. Meeting US Livestock Sanitary Assn.* pp.396-403, 1967.
8. HEDGER, R.S.  
The isolation and characterization of foot-and-mouth disease virus from clinically normal herds in Botswana. *J. Hyg., Camb.* 66 (1): 27-36, 1968.