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PAN AMERICAN FOOT-AND-MOUTH DISEASE CENTER RESEARCH PROGRAM

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PAN AMERICAN FOOT-AND-MOUTH DISEASE CENTER RESEARCH PROGRAM

SUMMARY OF PROJECTS IN PROGRESS

Raul Casas Olascoaga
INTRODUCTION

The Center's research program is oriented toward solving the problems that arise during execution of the foot-and-mouth disease (FMD) prevention, control and eradication programs in the Americas.

The research policy has been oriented to knowledge of the disease behavior and to study the operative aspects in order to obtain information and technology for a better solution to the problem. There has been a permanent on-going concern in this activity that generates technological knowledge in order to be able to render creative and effective technical cooperation commensurate with the problems encountered.

Strategically, it is considered an open process, like an aperiodic information system activated and fed by the detection of problems that emerge as the programs are developed and whose solution was not possible with the present knowledge.

In summary, applied research is regarded as the backbone—in the essential meaning of the term—of technical cooperation for the countries.

Within this strategy, priority has been focused on a knowledge of the disease's basic aspects, which are: pathogenesis, methods of virus diagnosis and characterization, vaccine production and control, epidemiology and control of FMD with respect to rationalization of both strategic and operative decisions.

With this purpose in mind it has been necessary to adopt or incorporate a vast methodological arsenal about laboratory techniques.

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epidemiological surveillance, information systems, sampling surveys, time series, operational research, statistical inference, multivariated analysis, mathematical models, the theory of decisions and economic analysis.

The Center's research program can thus be divided into the following projects:

1. Diagnosis of vesicular diseases of livestock (Reference Center for the Americas)
2. Selection of FMD-vaccine strains
3. FMD-vaccine antigen production
4. FMD-vaccine adjuvants
5. Field application of oil-adjuvanted vaccines
6. Attenuated live virus vaccines
7. Quality control of FMD vaccine
8. Pathogenesis, transmission and reservoirs of FMD virus
9. Improvement of laboratory techniques for FMD research
10. Study of factors influencing FMD epidemiology
11. Operational analysis of FMD control measures
12. Models of FMD epidemiological processes
13. Study of production and yield losses in FMD-affected cattle
PROJECT 1. Diagnosis of vesicular diseases of livestock  
(Reference Center for the Americas)

Covers the identification and classification of agents causing vesicular disease in animals, and provision of reference biologics.

Since its founding the Center has given priority to the establishment of standardized diagnostic procedures on the Continent; a continuous research program has been directed towards adapting virological and serological techniques to make them practical for use by the national diagnostic laboratories. These developments resulted in the Center's being acknowledged as the FMD Reference Laboratory for the Americas and in the development of a hemispheric net of national laboratories for vesicular diseases diagnosis.

The FMD control campaigns in some areas of South America have unfortunately not always resulted in a solid herd immunity. In such partially immunized cattle populations, and also in epidemics when the virus comes into contact with thousands of animals, it is likely that virus strains develop with different antigenic characteristics. Consequently, a vaccine made with one FMD virus strain may be all right to use in a certain area but would not protect adequately in another region of the same country or in neighboring countries. Needless to say, this difference in strains complicates vaccine production and vaccine control.

Thus, to ensure vaccine effectiveness, a well-functioning monitoring system is needed to examine a high proportion of field strains in order to determine whether these emerging field strains are still covered by the immunogenic characteristics of the vaccine strains.

The close cooperation between the member countries and the Center has made it possible to identify the FMD virus subtypes present in the field, and to study their immunological relationship with the virus strains used in the production of vaccines.

More than 4888 suspected vesicular samples were examined by the National Diagnostic Laboratories in 1979-1980 (Table 1).

During that same period the Center received over 775 diagnostic samples from the countries (Table 2).

A large percentage of those samples originated from Brazil, the Center's host country. The samples from the other countries were studied mostly because they presented special antigenic characteristics or were causing particular problems in the field. This diagnostic coverage is favorably compared with many other parts of the world.
PROJECT 2. Selection of FMD-vaccine strains

The results of the first project are the basis for the second project dealing with the selection of FMD virus strains for vaccine production with good immunological relationships with strains acting in the field.

The comprehensive picture which the Center obtained with regard to the distribution of types, subtypes and strains of FMD virus on the Continent, places it in an excellent position to provide the countries with information on the coverage of the strains used for vaccine production and those active in the field (Table 3). Selected strains were tested at the Center's vaccine plant for their growth and stability characteristics.

The Center continues to follow the established routine of testing emerging field strains with significant complement fixing differences by the mouse protection and neutralization tests. For this purpose the Center maintains a serum bank with a collection of sera from cattle vaccinated with the different strains in South America. The emerging field strains are tested against selected sera from this serum bank. As a rule both vaccination and revaccination sera are included in the test, since it often is observed that revaccination adequately increases the immunological response to newly emerging strains.

In accordance with the results of these tests and the epidemiological situation, the Center can rapidly prepare several lots of emergency vaccines.

PROJECT 3. FMD-vaccine antigen production

In the Center's pilot vaccine production plant it is determined the optimum conditions for antigen production and means for antigen purification, concentration and stabilization.

The production of inactivated FMD vaccines at the Center started on a research scale with the production of Frenkel type cultures in surviving bovine tongue epithelium in 1957. This experience enabled the Center to provide assistance in this technique to private and official vaccine production laboratories on the Continent, with particular emphasis on Argentina, Bolivia, Brazil, Ecuador and Peru.

Rabbit adapted virus strains were also used as attenuated live-virus vaccines, but in Brazil private production laboratories used the lapinized virus to produce inactivated vaccines. The Center investigated the production of such vaccines in its pilot plant and showed that although
adequate vaccines could, in principle, be prepared by that method, it would be difficult to produce a satisfactory industrial product consistently.

The Center's present research efforts are directed towards the production, purification and concentration of antigen using baby hamster cell cultures (BHK) adapted to growth in suspension but with cells still adhering to the glass. Such cells can be used in suspension tanks and in rolling bottles; it has been shown that in both types of cultures more immunogenic antigens can be produced consistently.

Studies were started with the cooperation of the Plum Island Animal Disease Center to determine the immunogenicity of vaccines prepared from antigens which previously were concentrated by ultrafiltration and stored as concentrates.

Originally FMD vaccines were inactivated by the combined action of heat and formal. It was shown, however, that this inactivation does not follow first order kinetics and because of the "tail effect", residual infectivity may exist in the vaccines.

In Europe, where formal inactivation is still used in the major vaccine production laboratories, several outbreaks during the last decade were traced back to the use of incompletely inactivated vaccines.

The Center has been instrumental in the use of inactivants with first-order inactivation kinetics for the vaccines used in the FMD campaigns now in progress in South America.

Workers at the Center developed a method based on FMD antigen inactivation with so-called binary ethyleneimine (BEI) which is cheaper and safer than AEI. BEI is easier to use and is considerably less toxic, which makes it very attractive as an inactivant for industrial vaccine production.

BEI has been used successfully at the Center for several years and is presently being used in both private and official industrial vaccine production. As examples can be mentioned the "Instituto de Zoonosis e Investigacion Pecuaria", Lima, Peru; several Brazilian production laboratories such as the "Instituto Vallee" in Uberlandia, Minas Gerais, the "Instituto Rio-grandense da Febre Aftosa" in Porto Alegre, Rio Grande do Sul, "PFIZER" and "Wellcome do Brasil", Sao Paulo, SP; "Wellcome" in Montevideo, Uruguay, etc.

BEI can also be used for the inactivation of viruses in biological materials, and has facilitated international exchange of test reagents. More recently BEI has been used at the Pan American Zoonoses Center to inactivate rabies virus growth in BHK cells for vaccine production.
PROJECT 4. FMD-vaccine adjuvants

The fourth project deals with the development of a high-potency vaccine for cattle and pigs, with long-lasting immunity and no undesirable side effects.

The Center started joint laboratory experiments with the Plum Island Animal Disease Center in 1968, using mineral oil as an adjuvant in an attempt to develop a vaccine which would induce a longer-lasting immunity. The vaccine consists of an emulsion of the aqueous phase containing the antigens and incomplete Freund's adjuvant. This work has been very successful. At present, this oil-adjuvanted FMD vaccine can protect cattle for longer periods than has been possible with the aluminum hydroxide vaccine and saponin. Particularly, after revaccination, these vaccines induce a prolonged solid protection.

Aluminum-hydroxide is presently used as the adjuvant. In most cases, saponin is added to enhance the immunizing effect of the vaccine. These vaccines are administered to cattle at 4-month intervals, but are unsatisfactory for use in swine.

Oil-adjuvanted vaccines have been shown to be satisfactory for use in swine. A double-emulsion vaccine is being developed at the Center. It consists of an emulsion of the aqueous antigens, reemulsified in a second phase. These vaccines have been given intraperitoneally to 2-month old pigs which were then protected until slaughter 4 months later. Breeding stock can be protected by an intramuscular injection. Here again, revaccination induces a prolonged solid immunity.

PROJECT 5. Field application of oil-adjuvanted vaccines

Their advantages and eventual disadvantages are being tested in large-scale field application. At present more than half a million cattle have been included in these field trials with vaccines produced by the Center.

The first field trials began in 1972 with some 3000 cattle in the south of Brazil in the Municipality of Bage, Rio Grande do Sul. After an initial evaluation period the pilot and demonstration program rapidly expanded. The Center's pilot production plant was adapted within the limitations of budget and facilities; it now has a production capacity of slightly over 1 million doses of trivalent oil-adjuvanted vaccine a year. This vaccine is used in the pilot and demonstration areas in different countries. The semi-industrial production scale also lets us train professionals and helps us develop procedures and equipment which are readily adaptable to large-scale industrial production. The pilot production plant is a very important instrument for transference of technology and training activities.
These projects presently involve about 500,000 cattle in Argentina, Bolivia, Brazil, Colombia, Paraguay, Peru and Uruguay. Different climate and geographical conditions, different cattle management situations and cattle of different breeds are included. In revaccinated adult cattle the vaccine is used on an annual basis. Young cattle are vaccinated twice a year until two years of age.

The results can be summarized as follows:

The Center's pilot vaccine plant has demonstrated that high-quality, oil-adjuvanted vaccines can be consistently produced on a semi-industrial scale.

The use of oil vaccines has been well received by the livestock industry in field programs in Argentina, Bolivia, Brazil, Peru and Uruguay, and no undesirable secondary effects have been registered.

The immunological and epidemiological evaluations made at laboratory level in cattle, swine and guinea pigs as well as in field demonstration programs in several thousands of cattle have been very satisfactory even in areas with epidemic occurrence of FMD.

A high degree of population immunity can be obtained which brings about a reduction in the number of annual revaccinations. Special syringes or equipment are not necessary for the application of the standard vaccine formulation.

It is believed that once these highly efficient oil-adjuvanted vaccines become readily available to the national programs for extensive and strategic application will represent a very important tool for the control of the disease.

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**PROJECT 6. Attenuated live virus vaccines**

A few words about the use of modified live virus vaccines in South America.

The Center played a very active role in the development of attenuated FMD virus vaccines; that endeavor was a very promising adjunct to the disease control measures in the early sixties, when the inactivated vaccines were available only in limited amounts of rather mediocre quality. The attenuation of the virus was obtained by serial passage of the virus in foreign host systems such as embryonated eggs or cell cultures combined with cloning of the resulting progeny.
Several field tests of the vaccine were made in Brazil, Chile, Colombia, Ecuador and Venezuela. The first reported outbreak of FMD in Guyana was controlled by the use of attenuated-virus vaccine produced at the Center. This vaccine is still being used in the FMD control programs in Venezuela.

Until recently the Veterinary Research Institute in Maracay, Venezuela, and the Center performed joint experiments to explore the oral or intranasal routes of the attenuated virus vaccine.

However, with the advances made in the area of inactivated vaccines, particularly after the very successful development of immunogenic oil-adjuvanted vaccines, the work on attenuated live virus vaccines has gradually been de-emphasized and presently has come to a virtual stop.

### PROJECT 7. Quality control of FMD vaccine

In order to provide the livestock industry with adequate vaccines, the Center has dedicated a proportion of its resources to the development of FMD vaccine control procedures and acts as Reference Center for the Americas for the quality control of FMD vaccines.

Concurrently with the development of vaccine production methods, the Center has directed its research efforts toward the development of vaccine control procedures which are compatible with the infrastructure of the Animal Health Services in South America. Direct and indirect assay methods for vaccine control were found to be suitable and correlations between the results of the various test systems were established.

Comparing the results of mouse protection test and neutralization test of sera from vaccinated cattle with the results of the challenge of such cattle, allowed the establishment of expected percentage of protection for the values of each of these test systems.

The validity (sensitivity, specificity and predictive capability) of the antibodies serological measurement tests (serum protection index, neutralizing dose titer 50%, microneutralization titer) was studied in relation to the protection afforded.

Statistical quality control techniques have been adopted that can be applied to the quality control of vaccines and measurements.

### PROJECT 8. Pathogenesis, transmission and reservoirs of FMD virus

Deals with research on pathogenesis, transmission and reservoirs of FMD virus, to provide a basis for understanding disease control and prevention measures.

The Center's work on FMD pathogenesis has provided further knowledge on virus-host interaction and provided better means of dealing with the disease.
The Center started original research in the area of bovine virus carriers in 1964. Its efforts resulted in the important finding that cattle could maintain infectious in their pharyngeal mucosa virus for many months.

The publication of that discovery, highly controversial at that time, triggered a wealth of research at the main FMD research institutes around the world. The Center subsequently published various papers on the subject and became internationally recognized for its work related to the development of testing procedures which decreased the risk of disease transmission by animal movements from one country to another. The work resulted in the development of an infrastructure and procedures that allowed the safe commercialization of breeding cattle from Brazil to Venezuela and Colombia, from Europe to Canada, the USA and Japan, and among other countries with different epidemiological situation.

At present the Center is engaged in a project for long-term virus isolation from cattle at slaughter. It is believed that such a survey could indicate virus activity in large areas and provide an early warning system against the emergence of new strains.

Research on virus-host interaction has been directed to determine the factors influencing the outcome of vaccine potency tests. The results of these studies have contributed to a better understanding of the infectious process during a potency test and thus to a better standardization of procedures and interpretation of results.

For epidemiological surveys the Center extensively uses the so-called VIA-test which determines the presence or absence of antibodies to virus-infection-associated (VIA) antigen. This technique was first introduced at the Center in the early seventies. It was shown that the results of this test, when interpreted correctly, taking into consideration age distribution of the animals and other factors, is a most valuable adjunct to epidemiological surveys. Since then, researchers at the Center have improved and simplified the large-scale production of this antigen and developed a more sensitive method for the detection of VIA antibodies. This method has also been used to improve the serological diagnosis of vesicular stomatitis.

PROJECT 9. Improvement of laboratory techniques for FMD research

The main objective is to adapt laboratory techniques for FMD research to the conditions existing in South America to fulfill the needs of the national FMD campaigns.

Regarding diagnostic techniques studies have been made to adapt the agar-gel double diffusion, microneutralization and counterimmunoelectrophoresis tests for the diagnosis of vesicular stomatitis virus. Improvements have also been made for the preparation of reagents such as hyperimmune sera and antigens.
The Center has collaborated in the United Nations Food and Agriculture Organization (FAO) international project for the standardization of laboratory methods in FMD research.

At this time a study is underway to standardize the methods and procedures for vaccine quality control used in the laboratories responsible for this activity in South America.

Based on statistical analysis studies have been made to establish the correlation between levels of antibodies measured by different methods and protection against virus challenge of vaccinated animals. Statistical analysis studies have also been made to determine the reproducibility, sensitivity and specificity of the different tests used in FMD research.

**PROJECT 10. Study of factors influencing FMD epidemiology**

The objective is to determine factors that influence FMD epidemiology in South America and also aims to characterize the FMD regional ecosystems.

The epidemiological surveillance of FMD has demanded a great effort in order to develop a methodology appropriate for the integration and coordination of a surveillance system comprising various animal and human diseases within the socio-economic conditions of Latin America.

The Center has actively cooperated with the countries on epidemiological research to improve the output, feedback and utilization of the surveillance systems. Thus, great progress was made in the knowledge about the regional behavior of FMD on the Continent.

Extensive work has been performed on the development of laboratory and field indicators of disease occurrence and of administrative elements affecting the progress of the programs. Those indicators are being utilized by the countries for their progress reports and for occasional epidemiological research projects.

Characterization of the disease's epidemiological behavior is made through: (a) determining and ranking the risk levels to which the livestock population is exposed; (b) identification and consideration of the factors associated with the risk of FMD occurrence; (c) synthesis of the interactions among the ecological, economic or social factors associated with FMD occurrence.

The following are several of the main results obtained:

Characterization of FMD epidemiological behavior in regional ecosystems; areas are recognized where FMD reveals a high or regular persistence
and assumes the character of endemic regions (high or medium risk). Other areas appear with sporadic or scarce occurrence and take on the character of paraendemic occasional areas (low risk). And finally, no-risk areas have been detected (risk is nil).

There have been identified associations between FMD occurrence and factors originating in the livestock activity, such as: (a) type of activity - the beef cattle areas have higher risk levels than the areas where dairy cattle are predominant. The risk level in the beef cattle regions is higher in the fattening or finishing areas; (b) density - a higher FMD occurrence risk level has been noticed in areas having a greater cattle concentration; (c) movements - periodic temporal changes have been detected in FMD occurrence risk levels during periods of high cattle mobilization, whether for slaughter, commercialization or due to livestock raising cycles. This temporal change in the risk status becomes evident in the regions that receive the incoming cattle; (d) population structure - it has been noticed that the younger age ranges show the higher risk levels (in calves, steers).

In the endemic regions it is possible to distinguish areas of stable levels with medium or slightly high risk levels, from other areas where it is evident seasonally in endemisms (high risk).

The identification of the economic determinants acting on the livestock production structures and related to the FMD behavior has allowed the formulation of hypothetical models about the relationship between the livestock production structures and FMD status.

Large-scale studies have been conducted to evaluate the prevalent infection in livestock populations and a methodology has been developed for screening the immune status of cattle population, classifying it with respect to critical antibodies levels elected in an optimized way, minimizing the risk of errors in classification.

**PROJECT 11. Operational analysis of FMD control measures**

Covers the studies of vaccination costs and evaluates the effectiveness and the costs of FMD vaccination alternatives. This project also includes the cost-benefit analysis of control programs.

The development of pilot programs in cooperation with the member countries has enabled the Center to advise on decision-making at the strategic and operational levels. Mathematical models are currently being studied.

One of the areas of support research which the Center considers of utmost importance for the future development of more efficient animal production procedures involves the socio-economic analysis of animal health and disease determinants. This project undertakes to establish regional
health profiles which might offer a global overview of particular problems affecting production.

Studies have been made on the costs of FMD vaccination and the different components' importance in the unit cost of vaccination (per bovine vaccinated) has been characterized. The relatively important weight of the vaccine price has been noted and the inverse ratio between the unit cost of vaccination and the size of the livestock population to be vaccinated has been determined. A cost accounting system was proposed for the FMD vaccination activity to upgrade the FMD control programs' efficiency through improved utilization of resources.

The cost effectiveness study was conducted with the objective of facilitating the decision-making task of the FMD control programs' executive levels. This is the case of the cost-effectiveness study of different FMD vaccination procedures.

Strategic alternatives for FMD control in paraendemic regions have been assessed. Great feasibility has been found for those alternatives that place a higher relative importance on controlling the entry of infection sources into the region, as compared to those which base their effectiveness on massive and systematic livestock vaccination only.

The level of attention has been studied, with special concern for the ratio between veterinarians and the quantity of livestock and livestock establishments in the region, at the attention demand levels and service time-lapse intervals.

PROJECT 12. Models of FMD epidemiological processes

This project deals with the disease's spatial dispersion and temporal recurrence, the correlation between causal factors and FMD behavior and mathematical models that characterize its behavior.

Indicators that express the risk of FMD occurrence have been prepared and multivariated types of statistical techniques have been applied in order to weigh the importance of factors associated with FMD occurrence as well as to identify homogeneous clusters with regard to risk situations, and to classify different statuses of endemicity that enable the regional ecosystem to be profiled objectively.

Epidemiological and mathematical models have also been developed to understand the mechanisms of most important interaction responsible for FMD behavior.
PROJECT 13. Study of production and yield losses in FMD affected cattle

This project is funded by the Inter-American Development Bank and the Ministry of Agriculture of Brazil and aims at the assessment of losses caused by FMD in cattle.

This project undertakes to research the losses caused by FMD: physical losses in bovines and economic losses to the livestock property. The basic study methodology is the "control-case" method and is being developed on beef cattle farms in Rio Grande do Sul and Sao Paulo and in dairy cattle in Rio de Janeiro and Sao Paulo.

Fifty (50) properties (control-cases) are included in the beef cattle study; the disease's influence on weight gain in a sampling of 1,638 bovines is assessed by weighing the individual animals weekly for ten weeks after the outbreak and then monthly for a year's time.

In addition to the weight gain study, it is also monitor the following statistics monthly: births, abortions, and deaths, as well as income and expenses of the properties included in the project.

Thirty-eight (38) properties (control-cases) are involved in the dairy cattle study. Out of the total population of 2,450 animals, the milk production from 1,474 cows and calving heifers is individually measured on a weekly basis for 10 weeks immediately following an outbreak, and then monthly for a minimum of one year.

On these properties a monthly record is also kept of births, deaths, abortions, dry cows, rejected animals, mastitis occurrence, income and expenses.

The project was initiated in April 1979 and is scheduled to end in August 1981; the data compiled are presently being processed and analyzed.

FINAL REMARKS

Finally, a few words concerning the Center's place in the FMD research programs in South America and other continents.

The Center participates in cooperative studies with several international research centers and organizations such as the World FMD Reference Center, Pirbright, England, and the Plum Island Animal Disease Center, USA. Presently is participating in the FAO project for the "Standardization of Laboratory Techniques for FMD Research".

Since the early years of its existence, the Center has actively promoted FMD research in the countries of the Region through cooperative or joint projects. Some early examples were: (a) the program for the development of attenuated live-virus vaccines, conducted jointly with the Veterinary Research Institute in Maracay, Venezuela, in the fifties and
sixties; (b) the work with the joint Argentine-USA Commission, on the survival of FMD virus in meat and meat products, and (c) the serological survey on Tierra del Fuego Island.

It is important to make emphasis on the studies which have been carried out in the countries within the last decade on the epidemiology of FMD based on the information and epidemiological surveillance systems organized by the Center at the national and continental level.

During the last 5 years the Center has called upon the national research institutes for assistance in conducting experiments, particularly, those involving susceptible cattle for short and long-term vaccine evaluation experiments. The authorities of Argentina, Brazil and Uruguay have responded with great enthusiasm and have provided hundreds of cattle, made their facilities and personnel available for virus challenge, and collected serum samples and other valuable materials and information.

There is little doubt that without this invaluable help the Center would not have been able to reach the present status in the development of oil-adjuvanted vaccine and the vaccine control methods. More important however, is the gradual shifting of research responsibilities to the national research institutions with cooperation from the Center. Although important progress is being made in this respect, the Center still maintains and conducts the great bulk of research on FMD prevention and control problems. At any rate, the Center's efficient and continuously updating of training and technical assistance programs could hardly be conceivable without an adequate support research structure at the Center.

Research is the channel that constantly nourishes technical cooperation and the transfer of technology to the national programs.
TABLE 1. FREQUENCY OF DIAGNOSES OF TYPES OF FOOT-AND-MOUTH DISEASE AND VESICULAR STOMATITIS VIRUSES. SOUTH AMERICA 1979-1980

<table>
<thead>
<tr>
<th>Country</th>
<th>Foot-and-Mouth Disease</th>
<th>Vesicular Stomatitis</th>
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<td><strong>Total</strong></td>
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## TABLE 2. EPITHELIUM SAMPLES RECEIVED FROM SOUTH AMERICA 1979-1980

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<th>C</th>
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<tr>
<td>Venezuela</td>
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### TABLE 3. FOOT-AND-MOUTH DISEASE VIRUS IDENTIFIED BY COUNTRY.

**SOUTH AMERICA 1980**

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<td>-</td>
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**Source:** Diagnostic and Reference Laboratory of the PAFMDC and monthly reports of the countries