

## THE RELEVANCE OF THE RESEARCH AND DEVELOPMENT PROGRAMS OF PANAFTOSA TO THE CONTROL AND ERADICATION OF FOOT-AND-MOUTH DISEASE IN THE AMERICAS

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**SUMMARY.** The contribution made by the Pan American Foot-and-Mouth Disease Center to our knowledge of vesicular diseases of animals and, in particular, foot-and-mouth disease, has assisted greatly the control programs initiated during the last four decades. This review examines the relevance of past research and explores future directions for research and development in relation to the control and eradication of the disease from the Americas.

The Pan American Foot-and-Mouth Disease Center (PANAFTOSA), located in Rio de Janeiro, Brazil, is part of the Pan American Health Organization/World Health Organization (PAHO/WHO). Since its creation in 1951 by the Organization of American States, with the support of the Pan American Sanitary Bureau and the Inter-American Institute for Cooperation in Agriculture, it has made significant contributions to the control of foot-and-mouth disease (FMD) in Latin America by virtue of its numerous activities. These include the role of Reference Laboratory for Vesicular Diseases for the Americas, involvement in technical cooperation and coordination of programs at the national and international levels aimed at the prevention, control and eradication of FMD in Latin America, FMD vaccine production, research and development. In 1968, PANAFTOSA came under the administrative control of PAHO.

In 1958, the Food and Agriculture Organization (FAO) and the World Reference Laboratory (WRL), Pirbright, U.K., officially recognized PANAFTOSA as the coordinating organization for FMD campaigns in South America and as the communication channel between the countries and the WRL. This status was accepted in 1960 by the Office International des Epizooties at its XXVIII General Session.

In 1969, the Ministers of Agriculture of the region attending the II Inter-American Meeting on FMD and other Zoonoses (RICAZ) in Brazil agreed, in the form of Resolution X, to recognize PANAFTOSA as the Regional Reference Laboratory of the Americas for FMD diagnosis (42). In 1979, the Ministers of Agriculture of the region, at the XII RICAZ Meeting in Curaçao, also agreed under Resolution V, to recognize PANAFTOSA as Regional Reference Laboratory for quality control of FMD vaccines (43). In 1992, the XIX Regular Meeting of the South American Commission for the Control of Foot-and-Mouth Disease (COSALFA), in Argentina, through Resolution V, appointed PANAFTOSA as the

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Reference Laboratory of the region for the epidemiological surveillance of vesicular diseases (45).

## **POLITICAL AND TECHNICAL DIRECTION**

Within the Americas, there are three international forums which are pivotal to the establishment of political and technical directives governing FMD prevention, control and eradication programs in the hemisphere. These are:

1. The Inter-American Meeting, at the Ministerial Level, on Animal Health (RIMSA, known as RICAZ prior to 1980) which takes place every two years to discuss animal health policies in the region. Typically (41), these meetings operate against a background of working documents provided by PAHO and resolutions from meetings such as COSALFA and COHEFA (see below) and culminate in resolutions by the representatives of the member countries. As a consequence, requests or recommendations may be made to PAHO or to member countries to implement or otherwise a course of action. RIMSA also analyzes the programs and budgets of the PAHO Centers for the next two years and makes appropriate recommendations to the Director of PAHO for future activities.

2. The Hemispheric Committee for the Eradication of Foot-and-Mouth Disease (COHEFA), which is formed by representatives of the public sector (Ministries of Agriculture) and private sector (producers) of six subregions of the Americas. This meets every two years at the same time as RIMSA but deals primarily with technical aspects of the programs for the prevention, control and eradication of FMD (44) in the Americas. COHEFA also oversees the financing of the Hemispheric Plan for the Eradication of FMD. Resolutions of COHEFA are channelled to the RIMSA meeting for further discussion and recommendation to member countries, COSALFA or PAHO, as appropriate.

3. COSALFA which consists of directors of animal health of the South American countries, meets annually and acts as an advisory group for

the Director of the Center, who serves as Secretary ex officio. The objectives of COSALFA are to promote, coordinate and evaluate national programs, subregional projects and border agreements regarding FMD in the countries of the region (26).

The research projects and technical activities of the Center are also evaluated by an ad hoc Scientific Advisory Committee made up of scientists of international repute who submit their conclusions and recommendations to the Director of PAHO.

## **OPERATIONAL BASIS OF PANAFTOSA**

Much of the work of PANAFTOSA is conveniently divided into two areas. The first of these is that of Field Services, which interacts closely with member countries and provides support in many ways, including operation of the vesicular disease continental surveillance system, consultant and advisory services and technical assistance on different aspects of the control programs, and training and information.

The second area is that of Laboratory Services, encompassing research, development and scientific services. This area in turn is underpinned by essential support services such as those provided by the small and large animal experimental groups and the tissue culture laboratory. In certain respects, the Laboratory Services are remote from the customers, the veterinary authorities of the member countries, but this does not in any way minimize the importance of its work. The following section, in which the scientific achievements of the Center are reviewed, attempts to show the crucial role that research and development have played and will continue to play in the diagnosis and control of FMD in the Americas.

## **ACHIEVEMENTS AND FUTURE ACTIVITIES OF THE LABORATORY SERVICES**

Staff of the research laboratories of the Center have contributed to our knowledge of a wide

variety of topics in vesicular diseases of animals, with particular reference to the diagnosis and control of FMD. A catalogue of the Center's publications from 1952 to 1993 has recently become available and provides a useful summary of the scientific activities of PANAFTOSA over four decades (25). Through close interaction and collaboration with the diagnosis and vaccine production laboratories, many of the developments at the research level have been transferred effectively to practical applications.

As stated previously, PANAFTOSA has the role of Reference Laboratory for the Americas and, accordingly, one of its major functions is to analyze samples submitted by member countries. These may be sera for use in antibody assays or tissue samples/vesicular fluid for virus detection assays. In the last decade alone, the diagnostic laboratory has processed 38,582 samples and clearly plays a vital role in the vesicular disease surveillance service of the Center. Because of the need for a comprehensive knowledge of the strains of FMD circulating in the field and aspects such as their relationship with vaccine strains, workers in this sector have been particularly active in the serological characterization of FMD strains occurring in Latin America using a range of techniques (6,8,21,28,34). Since the mid-eighties, basic characterization studies have been supplemented by molecular biological techniques including oligonucleotide mapping and nucleic acid sequencing (21,23), as well as the selective application of monoclonal antibodies developed against major strains of FMD (11). It is anticipated that the high definition which can be achieved with the more modern serological and molecular biological techniques will make routine diagnosis in the future even more reliable than at present. Equally, more investigative molecular epidemiology will be expected to play an increasingly important role as the numbers of FMD outbreaks are reduced by the effective implementation of control programs.

Research into FMD vaccines has always been a dominant feature of the activities of PANAFTOSA (40). Early studies which examined the susceptibility of different cells were followed by the adoption and exploitation of virus

production in both the Frenkel system and BHK-21 cells (4,38,39). During this period, there were also extensive studies made with attenuated forms of the virus because of the considerable successes achieved with other attenuated virus vaccines (35). However, the numerous problems associated with the use of attenuated FMD vaccines led to the exclusive development and use of inactivated preparations. In this respect, the Center was at the forefront and made invaluable contributions in the areas of inactivation kinetics and adjuvancy. The deficiencies of formaldehyde inactivation were soon recognized by the FMD world and the problem of obtaining the preferred inactivant, acetyleneimine, prompted the evaluation of alternatives of which binary ethyleneimine (BEI) was the most successful (18,19). BEI is a very effective inactivant provided it is used correctly and a colorimetric procedure was developed for monitoring the synthesis of the reagent immediately prior to its use at the industrial level. BEI is now used almost exclusively throughout the world in the production of FMD vaccines.

The Center is also known for its pioneering work on adjuvants for FMD vaccines, with particular reference to oil-based preparations (1,2,3,5,14,15,16,17,32,33,36,40). Numerous publications covering the quality and duration of immunity of cattle vaccinated with different preparations have culminated in the production by PANAFTOSA of oil adjuvanted FMD vaccines because of their superiority over classical aluminum hydroxide/saponin formulations. This type of vaccine is also gaining wider acceptance worldwide for the same reasons. Oil-based vaccines have also been developed for pigs. The justification for research aimed at vaccine improvement is simple. Vaccines against FMD, and particularly the aluminum hydroxide/saponin preparations, have to be applied regularly and comprehensively in order to achieve high and durable levels of herd immunity. Both economic and logistic considerations make the development of even better FMD vaccines a high priority and the Center will maintain its commitment within this area both with respect to improved adjuvants and delivery systems as well as investigations into recombinant vaccines. The

latter have the considerable advantage of freedom from potential contamination with live virus, a problem identified particularly in Europe following the reduction of outbreaks of disease due to contaminated meat and livestock (20).

In common with several other FMD laboratories, staff of the Center are also in the process of establishing a significant reserve (bank) of concentrated, inactivated FMD antigens which are stored at very low temperatures. These antigens will be reserved for formulation into vaccine in emergencies where conventional supplies are unavailable. Such antigen banks are particularly relevant, therefore, to the hemispheric plan for the eradication of FMD because of the generation of extensive disease-free areas and the corresponding loss of incentive for commerce and government laboratories to continue FMD vaccine production.

Potency and safety testing of vaccines made by both the Center's facility and by production units elsewhere has also represented a major area of work. The mouse protection test was developed by staff as a less costly alternative to live virus challenge testing in cattle. Similar studies have examined other approaches, including measurement of serum neutralizing antibodies and their correlation with potency in cattle (9,12,13,27,29,31,48,49). In this respect, the Center has a significant advantage over other FMD laboratories because of the very large number of sera and quantity of cattle potency data available to it through its role in Latin America. Safety of FMD vaccines with particular respect to allergic responses in cattle has also formed the basis of studies by staff of the Center (24).

A continuing concern among animal health authorities world-wide is the issue of persistence of FMD in cattle following recovery from infection or field challenge of vaccinated livestock, and numerous studies at the Center (30,46,47) and elsewhere have addressed the key questions. In fact, there is very little evidence of transmission from 'carrier' animals to fully susceptible livestock despite considerable attempts to demonstrate this experimentally. Nevertheless, a finite risk is perceived by the animal health authorities and the detection of persistence in cattle remains an impor-

tant issue. Classically this has been done, and will continue to be done for the foreseeable future, by the culture of probang fluids with susceptible cells.

Other approaches have been developed at PANAFTOSA to augment the infectivity assays including the measurement of antibody titres against one of the non-structural proteins of the virus, the so-called VIA antigen (7,10). This test gives some indication of whether an animal has been vaccinated and/or infected. Like all serological techniques, however, the VIA test has its limitations and is just one of an array of tests done by workers at the Center to assess the immune status of an animal. A new test, currently under evaluation, examines antibody levels against all of the non-structural proteins of the virus (22,37). The individual proteins, which are purified from recombinant bacteria, are electrophoresed in polyacrylamide gels and then blotted onto nitrocellulose strips. The strips are then soaked in a very small volume of cattle sera, followed by anti-species antibodies and positively staining bands detected by classical ELISA conjugates and substrates. In view of the overall goal to eradicate FMD from the hemisphere by a combination of vaccination and zoosanitary measures, this key area will assume even greater importance in order to discriminate between virus-infected and virus-free livestock. International acceptance of such tests will have profound implications for the export from Latin America of both carcasses and livestock.

## CONCLUSIONS

This brief review of the role of the Laboratory Services of PANAFTOSA is not intended to be comprehensive but rather to highlight the past and present research contributions by staff of the Center in the overall scheme to control and eradicate FMD in the Americas. For the future, the research programme of the Center is expected to be highly flexible and dynamic to meet both the needs of the Field Services of PANAFTOSA and those of the FMD affected countries. The different epidemiological situations which will arise as a consequence of the progressive eradication of this highly

contagious disease will pose new questions and provide the stimulus for new approaches for diagnosis and control. The role of the Laboratory Services of PANAFTOSA will be crucial in the eradication of FMD and the maintenance of the disease-free state.

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