SIXTEENTH MEETING OF THE
PAHO ADVISORY COMMITTEE ON MEDICAL RESEARCH

Washington, D.C.
11-15 July 1977

RESEARCH OF THE
PAN-AMERICAN FOOT-AND-MOUTH DISEASE CENTER
(A Brief Report)

The issue of this document does not constitute formal publication. It should not be reviewed, abstracted, or quoted without the consent of the Pan American Health Organization. The authors alone are responsible for statements expressed in signed papers.
Last year during the 15th Meeting of the Advisory Committee on Medical Research (ACMR), the Pan-American Foot-and-Mouth Disease Center presented an overview of problems related to foot-and-mouth disease (FMD) and research that could or should be done to help resolve some of these problems.

Economic implications of FMD

Little reliable data on physical or economical losses --such as the crippling effect of FMD on meat and milk production and restriction of international trade-- is available, even though this information is badly needed for planning FMD campaigns and for justification of the enormous expenditures for such campaigns.

The Center has started studies in this area; a project developed with the Government of Brazil and the Inter-American Development Bank (IDB) is now in the final stages of approval.

Although this study will last for several years, it can be expected to result in a better understanding of the economic consequences of the disease. The Center has been fortunate in receiving the assistance of FAO, which has made available an epidemiologist especially for this project. The results of preliminary studies already clearly show the complexity of the problem.

Another area of interest is the calculation vaccination campaign costs. The Center staff evaluated the Paraguayan campaign and showed that for each vaccination cycle the total cost per head of cattle was approximately US$ 0.20. Clearly, reducing number of vaccinations from 3 to 1 a year would mean a spectacular reduction in campaign costs. Such a reduction can be obtained for instance by the not vaccinating low risk areas as was done in certain areas of Paraguay or by the use of vaccines which induce a long lasting immunity such as oil adjuvanted vaccines.

Results from information systems presently in operation show some factors which determine increased risks of FMD, such as those related to cattle densities and livestock management. It is obvious that one will have to think increasingly in terms of regional ecosystems and that campaign strategies must be planned according to conditions in each of the systems. Specifically it will be important to think in terms of program cost/benefit and benefit/effectivity ratios. In this respect mathematical models may be useful tools.

*Prepared by Dr. Raúl Casas Olascoaga, Director, Pan-American Foot-and-Mouth Disease Center, Rio de Janeiro, Brazil
Epidemiological situation

The Center functions as the reference laboratory for the Americas and yearly receives for identification and typing as many as 250 samples from the infected countries and more than 300 samples from the free countries of Central America, Panama and the Caribbean.

Several of these isolates must be subjected to further serological and immunological studies to determine their potential threat to FMD campaigns. A speedy diagnosis of vesicular disease outbreaks in FMD-free countries is essential for the differential diagnosis of FMD and vesicular stomatitis virus (VSV). Should FMD be diagnosed, no time can be lost between the first observation of the disease and the implementation of eradication measures.

Unfortunately, the epidemiological situation of FMD in South America in general has been difficult during the past year. For example, virus type A has caused many outbreaks in Brazil, especially in the states of São Paulo and Rio Grande do Sul. The most troublesome aspect of these outbreaks is that most virus isolates are different from the vaccine strain and different among themselves. This situation is likely a result of the use of poor quality vaccines combined with a low vaccine coverage. Virus multiplying in poorly vaccinated cattle farms changes its antigenic characteristics while unvaccinated cattle will serve as amplifiers resulting in emerging variants and persistence of virus in the herd or livestock population.

Recently, the Center concluded a test in collaboration with the Brazilian Ministry of Agriculture, in which one group of cattle was vaccinated with the official vaccine strain (A Cruzeiro) and other groups with 2 of the field strains (A Bagé and A Venceslau).

The results (Table 1) showed that the vaccine strain in a potent vaccine gave a reasonable protection against A Bagé but not against A Venceslau. The 2 vaccines with A Bagé and A Venceslau only protected well against the homologous strain, although A Venceslau appeared to give a somewhat broader protective coverage. Work of this type has taken up a considerable portion of the Center's time and resources.
Antigen production

The Center has been able to produce consistently high quality antigens in its pilot vaccine plant, using BHK cells either in the roller bottle system or in suspension in vessels of up to 200 liters.

Some laboratories have reported problems using suspended cell cultures because the virus yields differed appreciably from the parent viruses. The virus produced therefore proved to be a poor vaccine antigen. So far the Center has not encountered such problems with its suspended BHK cell system and the South American strains used for vaccine production.

The Center has sufficient capacity for antigen production, should the need arise for a limited amount of emergency vaccine against an exotic type or new emerging subtype. However, since the plant was originally designed as a training facility only, deficiencies exist in the areas of mixing, holding and bottling terminated vaccines. Improvement of these aspects should receive serious consideration, since it is unlikely that present commercial or government production laboratories would be in a position to produce a sufficient amount of adequate emergency vaccine.

Progress has been made at the Center in antigen production using slaughterhouse serum from which the antibodies are removed by precipitation with polyethylene glycol (PEG).

By using this "poor man's fetal calf serum" for cell growth in suspension tanks, the cells can be infected without change of medium, causing a considerable saving while practically doubling the plant's production capabilities. Presently tests on several hundred cattle are in progress using vaccines produced by this method, to determine if the higher-than-normal residual serum components in the vaccine may create any undesirable sensitivity in cattle. Similarly
treated serum is now routinely used for regular cell culture work with excellent results, and it is hoped that this serum will eliminate in the near future the need for special antibody-free donor cattle.

Oil vaccines

The Center is rapidly expanding its oil adjuvanted vaccine research, which can be divided into 3 main areas:

1. Application of the "standard" simple water-in-oil-emulsion vaccine in larger number of cattle. Field trials are being carried out in the States of Rio de Janeiro, Rio Grande do Sul and Uruguay, covering a total of some 50,000 head of cattle which will increase next year to about 100,000 head. The objective of these trials is to determine if with larger scale use undesirable side effects such as local or systemic reactions may occur. These trials also serve to determine the acceptability of the vaccination procedures and schedule to the farm community and to evaluate herd immunity.

The oil vaccine, when applied intramuscularly, produces no visible local reactions; that route is now used routinely with the Center's oil adjuvanted vaccines. Subcutaneous application of the vaccine may cause swelling at the inoculation site, but it should be remembered that the aluminum hydroxide-saponin vaccine often produces an equal or greater local tissue reaction.

To date, no problems of anaphylactic or hypersensitivity reactions to the oil vaccine have occurred.

2. Determination of the characteristics and parameters of single and double emulsions which give the best immunological response. These vaccine characteristics include stability, dispersion of the particles, viscosity, etc.; the bioengineer must have this information and specifications in order to choose the necessary emulsification equipment and design for vaccine production plants. The Center is also investigating other emulsifiers besides one now in use and plans to expand these studies to include mineral oils that can be produced locally.

3. Vaccine control - Potency control oil adjuvanted vaccines present a number of special problems, such as the most convenient laboratory animal to use as a test system, the best moment of challenge and the most efficient means of
diluting the antigen. Recently an experiment was made in collaboration with the Uruguayan Ministry of Agriculture to correlate the number of protective guinea pig doses and the number of protective doses in cattle. Table 2 summarizes the guinea pig results. It can be seen that 0.25 ml of the vaccine intramuscularly contained 45 or more protective doses against generalization at 30 days. The number of protective doses was higher when measured at 60 or 90 days after vaccination. From this test we have concluded that the guinea pig PD$_{50}$ is probably an excellent way to test the potency of oil adjuvanted vaccines. The results of the cattle tests are listed in Table 3. For the interpretation of these data it is necessary to note that the majority of the non protected animals only had a small lesion on one foot. Each group had 4 contact and 4 tongue inoculated cattle and the contact group showed more lesion than the inoculated ones. It can be seen that the vaccine contained approximately 40 PD$_{50}$ for each type of virus.

The Instituto Nacional de Tecnología Agropecuaria (INTA) of Argentina has been testing for several years a vaccine containing not only Arlacel as an emulsifier in the oily phase but also Tween 40 in the aqueous phase. This has resulted in an emulsion of lower viscosity that the Center's standard oil vaccine, an obvious advantage. Argentine scientists have used the Center's technical facilities to formulate 30,000 doses of this vaccine for use in the field. Comparative potency and shelf-life tests between this INTA vaccine and standard Center oil vaccine, as well as an aluminum hydroxide vaccine with the same antigens, are being carried out in collaborative studies between the Center and Argentina. The results of guinea pig tests and challenge of cattle in Argentina 30 days after vaccination showed excellent protection against all 3 types of virus. Only 1 of the cattle challenged with virus type O developed foot lesions. The results of the mouse protection tests are shown in Table 4 (zero/months storage). The same table summarizes the results of the shelf-life tests. To date, several oil vaccines prepared by the Center have shown no deterioration after prolonged storage periods.

Attenuated virus vaccine

Last year's ACMR report to the Director of PAHO reads:
"that there should be an expanded program to find an effective vaccine and that the Director of the PAFMDC should be requested to present proposals with a budget for the development of a live attenuated vaccine for FMD".

As a result of discussions between Dr. Sabin and officials of the IDB a small group of experts met to discuss the feasibility of an expanded research program. This meeting took place under the auspices of the IDB on August 23, 1976, in Washington and was attended by Dr. Sabin, representatives of IDB and PAFMDC; the Plum Island Animal Disease Center (PIADC), NY, USA; the Animal Virus Research Institute (AVRI), Pirbright, UK; the National Institute of Allergy and Infectious Disease, NIH, Bethesda, USA; and the PAFMDC, Rio de Janeiro, Brazil.

The participants at this meeting noted the new prospects for development of active attenuated FMD vaccines, in view of new knowledge available concerning the pathogenesis and spread of FMD. They also noted that a well organized inter-institutional and interdisciplinary research program would be an efficient way to develop such a vaccine, since it would make use of the specific conditions and expertise existing in the various institutions.

Subsequently, a meeting of a larger group was held in Washington, March, 1977 with representatives of the PIADC, AVRI, Instituto de Investigaciones Veterinarias (IVV), Venezuela, the Center and officials of the IDB, resulting in a valuable exchange of information and ideas.

Serological surveys

The VIA (virus-infection-associated-antigen) test discriminates between animals that have acquired neutralizing antibodies from vaccine only and those which have become infected. Several surveys made in various countries (Brazil, Chile, Colombia and Paraguay) are providing basic information on field of this test. In Bolivia a VIA antibody survey was made in sheep and combined with studies of neutralizing antibodies. This study provided highly important epidemiological data since sheep in that area are not vaccinated, thus allowing for determination of sensitivity of the VIA test.

Some doubt still existed as to whether a low percentage of VIA positive animals could be due to false positive
reactions. To date the only truly negative group of animals was tested at the Plum Island Animal Disease Center; it is possible that in the tropics other related viruses producing VIA positive animals would exist. Several thousand of sera from cattle in El Salvador and Panama received for VSV antibodies testing at the Center, were also tested for VIA antibodies. All were negative. Further sera from Central America will be tested as they become available, but results to date have strengthened reliability of positive test results.
Table 1

Expected percentages of protection* of cattle 30 days after vaccination with 3 strains of FMDV type A and tested against the same strains in the mouse protection test

<table>
<thead>
<tr>
<th>Vaccine**</th>
<th>Challenge virus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cruzeiro</td>
</tr>
<tr>
<td>Vaccine</td>
<td>1 ml</td>
</tr>
<tr>
<td>A Cruzeiro</td>
<td>86</td>
</tr>
<tr>
<td>A Bagé</td>
<td>67</td>
</tr>
<tr>
<td>A Venceslau</td>
<td>46</td>
</tr>
</tbody>
</table>

* Calculated from mouse protection indices according to Gomes and Astudillo Bull. CPFA 17-18: 9-16, 1975.

** 12 cattle per group

(--) Being tested
Table 2

Guinea pig PD$_{50}$* of oil adjuvanted FMD vaccine**

<table>
<thead>
<tr>
<th>Virus</th>
<th>Days post vaccination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30</td>
</tr>
<tr>
<td>$O_1$</td>
<td>45</td>
</tr>
<tr>
<td>$A_{24}$</td>
<td>&gt;80</td>
</tr>
<tr>
<td>$C_3$</td>
<td>56</td>
</tr>
</tbody>
</table>

* Against 1000 DI$_{50}$ intraplantar

** 0.25 ml vaccine dose intramuscular
Table 3

Protection of cattle* at challenge with FMDV type O₁

<table>
<thead>
<tr>
<th>Vaccine Dilution</th>
<th>Days post vaccination</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30</td>
<td>60</td>
</tr>
<tr>
<td>1/10</td>
<td>7/8</td>
<td>7/8</td>
</tr>
<tr>
<td>1/40</td>
<td>5/8</td>
<td>5/8</td>
</tr>
<tr>
<td>1/160</td>
<td>4/8</td>
<td>4/8</td>
</tr>
</tbody>
</table>

* In each group 4 IDL, 4 contacts and 4 controls; all control cattle generalized
Table 4

Expected percentages of protection* of cattle 30 days after vaccination with vaccines before and after storage for 4 months at 4°C

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Storage period</th>
<th>Challenge virus</th>
<th>Challenge virus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 month**</td>
<td>4 months***</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Virus: 01 A24 C3</td>
<td>O1 A24 C3</td>
<td></td>
</tr>
<tr>
<td>Aluminum hydroxide</td>
<td>93 92 98</td>
<td>94 99 84</td>
<td></td>
</tr>
<tr>
<td>Oil Adjuvant CPFA</td>
<td>97 97 99</td>
<td>99 99 95</td>
<td></td>
</tr>
<tr>
<td>Oil Adjuvant INTA</td>
<td>90 92 96</td>
<td>72 87 62</td>
<td></td>
</tr>
</tbody>
</table>

* Calculated from mouse protection indices according to Gomes and Astudillo. Bull. CPFA 17-18: 9-16, 1975

** 8 cattle per group

*** 12 cattle per group