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RECENT ADVANCES IN FOOT-AND-MOUTH DISEASE VACCINES

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1. Introduction

Foot-and-mouth disease (FMD) is a worldwide problem. It exists in endemic form in Europe, Asia, Africa, and South America. North America, Central America and the Caribbean countries, Australia, and New Zealand have been free of this disease for many years or in some cases have never suffered from it. The causative virus has the property of propagating itself rapidly and thus creates very serious economic problems because of its impact on the meat and milk industries of the countries where it exists. The disease, wherever it occurs, is an obstacle to commerce.

It may be said without risk of contradiction that in one way or another the disease influences the price of almost all beef products on the world market. Besides the damage that foot-and-mouth disease does to the development of the cattle industry in affected countries, we must also take into account losses due to the upheaval in the normal commerce of importing and exporting animals, and animal and other agricultural products. This upheaval stems from the application of severe restrictive measures for control or eradication, or from decisions adopted by disease-free countries in relation to products from affected countries. We are thus dealing with a disease that in one way or another, harms all countries --afected or not--and directly interferes in their economic, social, and political lives.

Its inclusion among public health problems results from the fact that it is one of the most important causes of the scarcity of meat and milk protein in the nourishment of the American peoples, who need a greater amount of these products at more accessible prices.

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Some of the reasons for which foot-and-mouth disease is so serious and so difficult to control may be summarized as follows:

a. It is a highly contagious disease.

b. It affects all domestic cloven-footed animals and many wild species.

c. Many apparently healthy animals can be long-term carriers of the virus.

d. The virus can survive in the environment for a long time.

e. The virus is endowed with great plasticity, having the property of mutating very easily. This explains the fact that there are seven different immunologic types and more than 50 subtypes, some of which so differ immunologically among themselves that a vaccine prepared from one will not protect against some of the others. In endemic areas the virus mutates continuously and every few years several new subtypes are described, which requires frequent surveillance of the viruses that cause infection in comparison with those used in the production and control of vaccines.

2. Vaccines Against Foot-and-Mouth Disease

In some European countries the incidence of the disease has declined significantly in recent years. For the first time in recent decades, several have been free of foot-and-mouth disease for several consecutive years. Such a situation is possible due to vaccination combined with sanitary measures, including the sacrifice of affected animals. Nevertheless, the last measure is only possible when the incidence of the disease can be reduced to such a point that animal slaughter is economically feasible.

In countries where foot-and-mouth disease is endemic, control campaigns are based on the active immunization of the cattle population, together with a series of appropriate sanitary measures.

Vaccines used in FMD control and eradication campaigns should meet the following requirements: (1) they should be innocuous; (2) they should not

cause adverse reactions in vaccinated animals; (3) they should induce in vaccinated animals a solid and sufficiently lasting immunity against possible infections in the field; (4) they should have a wide antigenic spectrum; (5) they should be amenable to large-scale industrial production; and (6) their cost should be reasonable and their application practical.

Vaccines against foot-and-mouth disease may be divided into two groups: inactivated vaccines and modified live-virus vaccines. The latter are used only in certain countries that are not meat exporters. In the Americas, only Venezuela uses this type of vaccine in its campaigns. Meat-exporting countries cannot use them because of restrictions imposed by importing countries to avoid the possibility that meat products might contain modified virus.

The great majority of countries uses vaccines of the inactivated type, of which about 400,000,000 doses were produced in 1971 for use in various campaigns in this Hemisphere alone.

2.1 Modified Live-Virus Vaccines

Several types of modified live-virus vaccines were developed. One of of those that was used in large quantities, chiefly in certain African countries, was obtained through serial passage in mice. Another type that is being routinely used in Venezuela was developed after serial passage in one-day-old chicks or in embryonated eggs. The chief problems with this type of vaccine stem from the long time necessary to effect modification of strains and from the occurrence of a certain degree of pathogenicity. Among its advantages may be mentioned the stimulation of longer immunity and protection against a wider antigenic spectrum than is normally obtained with inactivated vaccines.

Modified live-virus vaccines have been one of the contributions to the fight against the disease by the Pan American Foot-and-Mouth Disease Center, which has acquired considerable experience in this area over the years. At

present several modified FMD viral clones are still being investigated, and plaque selection is being studied. Markers for some of the modified virus clones are available, and the genetic characteristics and stability of some of these strains are known. Special attention is being given to problems resulting from the persistence of modified live virus in different organs and tissues of laboratory animals and bovines in order to clarify problems related to the multiplication of the virus in different organs, its persistence in primarily vaccinated and revaccinated animals, and possible differences in the viruses' persistence in animals with or without circulating antibodies.

Efforts are now being focused on investigating processes of rapid viral attenuation through mutations produced by chemical and physical agents. Investigations carried out with hydroxylamine demonstrated that this substance is capable of attenuating FMD virus. Nevertheless, extensive studies performed with a series of viruses that survived the treatment (after being purified to avoid reactivation of virulence, and passed in tissue culture) unfortunately demonstrated that they would lose their immunogenic capacity. Also, cold mutants, though they may be rapidly obtained, progressively and simultaneously lose their pathogenicity and immunogenic capacity.

Selection of clones with characteristic plaques is yielding more promising results. Using this technique, we now have some virus strains that are capable of inducing an acceptable immunity and at the same time are almost completely nonpathogenic for bovines. One of these viral clones, type A, subtype A_{24} Cruzeiro, has shown in laboratory experiments and some field trials that it does not induce pathogenicity even in highly susceptible animals and is capable of provoking an acceptable and lasting immunity, even against some other subtypes. The adaptation and modification of different samples of FMD virus are being carried out with these same techniques, and they could eventually be important epidemiologically.

2.2 Inactivated Vaccines

Inactivated vaccines have been used for more than 30 years. The first vaccine of this type against foot-and-mouth disease was developed in 1938 by Waldmann, who used as antigen the FMD virus extracted from the epithelium of fresh tongue lesions in artificially infected animals. The virus was adsorbed by aluminum hydroxide and inactivated with formalin. In reviewing the current problems in FMD vaccine production with inactivated antigen, we may analyze the following items separately: antigens, inactivants, and adjuvants.

2.2.1 Antigens

The large-scale production of antigen according to the Waldmann method presents serious problems, above all those of cost and the danger that the virus will migrate from places where animals are infected. A great advance in the technique of producing FMD antigen was the method developed in 1947 by Frenkel, which consisted of multiplying the virus in suspensions of bovine lingual epithelium explants. This method was easily adapted to the large-scale production of antigen and was rapidly adopted in the many countries where today it is used in vaccine production with good results. Only now, as a result of the development of modern tissue culture techniques and because of the difficulty of obtaining lingual epithelium in the quantities necessary to produce the vaccine needed in campaigns, the Frenkel method is being slowly replaced by other antigen production techniques that are more practical and economical.

The great strides of recent years in tissue culture techniques have been successfully adapted to the production of antigens for FMD vaccines. Several laboratories are producing FMD virus industrially in bovine, pig, and hamster kidney (BHK-21) cells cultivated in monolayers as well as in suspension. The thus multiplied virus maintains its immunogenic characteristics, and fears about the possible oncogenicity of some of these cell lines, such as BHK-21, appear to have been completely dismissed.

The production of FMD virus in newly born rabbits is used in some countries to a certain extent. Because the preparation of sufficient quantities of antigen requires the use of large numbers of newborn rabbits, it is necessary to resort to private breeders. Production thus depends on breeders for a feasible and uninterrupted supply--a negative factor. The virus multiplies particularly in the muscle tissue of the rabbits, and the preparation of the viral antigen will contain large quantities of rabbit specific protein in a suspension that may eventually interfere with the virus's inactivation and at the same time cause undesirable reactions in animal revaccination. Therefore, the preparation of the virus demands adequate purification in order to be usable in vaccine production.

There are very few laboratory and field reports about vaccines produced with this antigen. The Pan American Foot-and-Mouth Disease Center is carrying out a research program to study methods of preparing the antigen and its immunizing capacity. Preliminary results obtained to date indicate that it is possible to produce an acceptable vaccine using antigen multiplied in the neonatal rabbit. These investigations will terminate at the end of the present year and should lead to better understanding and evaluation of vaccines produced with this type of antigen.

Analyzing the three principal methods of producing antigen for inactivated FMD vaccines--bovine lingual epithelium, tissue culture, and neonatal rabbit--we can foresee that the first and last will continue to be used for a time only. The advantages of tissue culture techniques, especially those employing methods of culture in suspension, are such that it can be predicted they will be applied ever increasingly. Although it is a more sophisticated and technically more difficult method, the tissue culture technique allows laboratories to remain less dependent on other sources for their supply of biologic material. Cell culture material of greater uniformity and less contamination can be produced continuously, and its production may be increased to desired levels and at reasonable cost. With this method we can easily achieve the necessary quantity of

vaccine to carry out the various national campaigns that are being undertaken in the Hemisphere.

Several investigators are studying methods of purifying and concentrating FMD virus for vaccine production. Antigen purification seems to offer several advantages over the use of crude viruses, especially those obtained from heterologous cells or cell lines. In some cases the vaccines prepared with unpurified antigen, obtained in cell culture, have caused allergic reactions in revaccinated bovines. The incidence of these allergies seems to be reduced if the antigen used in vaccines is purified. The other advantages are related to the greater ease of identifying and quantifying intact particles.

Using purified virus, the antigenic mass of even the immunologically poorest types can be increased more easily, without increasing the dose of the vaccine. This fact is of great importance because it is known that there is a considerable difference in immunogenic capacity between viruses used in vaccine production. Nevertheless, purified and concentrated antigens will not be widely used until an economical production method is established. Among those being investigated should be mentioned ammonium sulfate precipitation, differential centrifugation, saccharose gradient centrifugation, treatment with sodium desoxycholate, and polyethylene glycol precipitation.

It may be said in summary that our chief problems in the use of purified virus are technical ones in the application of the method to large-scale production, the product's cost, and the risk of product degradation and particle aggregation.

2.2.2 Inactivants

Formalin was the first inactivant used in the production of vaccines against foot-and-mouth disease. Vallée, in 1926, was the first investigator to use it. The mechanism of viral inactivation by formalin showed that the rate of inactivation changes quite frequently, which makes it unusually

difficult to determine when inactivation ends. The time necessary for complete inactivation is very long, which may affect the immunogenic capacity of the particles. To lessen this problem the adsorption of the antigen on aluminum hydroxide is performed before formalin inactivation in many instances, but it is known that even with this procedure not all the antigen is inactivated in a definite period and often it is very difficult to detect the small quantities of virus that still remain infectious.

It is for this reason that other FMD virus-inactivating agents have been studied, for example, beta-propylactone, glyceraldehyde, and acetyleneimine (AEI). The first-grade inactivation that is obtained with these substances, their lesser harm to the immunizing agent, and the good results from field experience with vaccines using inactivants such as these, lead us to believe that their use will be more widespread in the future.

Tests of innocuousness are normally carried out in cattle and lactating mice. The use of bovines, though they are highly susceptible animals, has the economic disadvantage that only a small number of animals can be employed to test each batch of vaccine. This fact limits the volume of the sample and greatly increases the possibility of statistical error. In South America where the disease exists, on the other hand, it is difficult to find susceptible animals because the area is endemic. The use of lactating mice therefore becomes more feasible. Tests using cell culture techniques have recently been described. These tests allow the analysis of larger volumes of vaccine and at the same time considerably increase the safety factor.

2.2.3 Adjuvants

The type of adjuvant used in the production of FMD vaccines seems to be important. The first was aluminum hydroxide, introduced in 1936 by Schmidt. It continued to be widely used inasmuch as it has certain advantages over other adjuvants. Its preparation is easy and economical, and is

often done in the same laboratory that produces vaccines. Aluminum hydroxide is very well tolerated by animals and does not cause problems at the inoculation site. Saponin was recently added to aluminum hydroxide to improve the immunity conferred by vaccines against foot-and-mouth disease.

The action of an oil adjuvant derived from mineral oils of pharmaceutical quality and Arlacel A has been studied recently. In some cases Tween 80 has been used to increase the emulsion's stability. FMD vaccines with oil adjuvant have given good protection in laboratory experiments with bovines and piglets. It is an adjuvant that seems to be well tolerated by bovines, but in pigs it causes quite serious local reactions at the injection site. Studies carried out by the Pan American Foot-and-Mouth Disease Center in collaboration with the Plum Island Animal Disease Laboratory in the United States show that a good immunity can be obtained in primary vaccinated bovines until the sixth month after vaccination. Revaccinated bovines showed an acceptable protection until a year after revaccination. Since it seems possible to reduce the number of annual vaccinations from three to two and thereby derive considerable economic benefits from campaigns against foot-and-mouth disease in the Hemisphere, extensive field studies of these vaccines are being carried out in collaboration with the Government of Brazil. But since the completion of these experiments must be awaited and the technical requirements for the large-scale production of vaccines with oil adjuvant have not yet been determined, it appears that this type of vaccine will not be available for widespread application for some time.

Several laboratories are also investigating other adjuvants, among them DEAE-dextran, various polyionic substances, and polynucleotide complexes. According to some investigators, DEAE-dextran is a useful adjuvant in the vaccination of piglets. This substance still causes general reactions of some severity in these animals. The Pan American Foot-and-Mouth Disease Center is investigating the potentiation of the immune response with polynucleic polycationic acid complexes. Preliminary results seem to indicate that the addition of DEAE-dextran to Poly (IC) after the

formation of the double chain produces a substance that appreciably increases the formation of antibodies against foot-and-mouth disease. From preliminary experiments in swine we can conclude that protamine is superior to DEAE-dextran when it becomes a complex with Poly (IC).

The experiments are in their initial phase and at the moment it is difficult to predict whether they are going to have practical application to industrial vaccine production.

2.3 Quality Control

The final aspect that we wish to deal with is quality control of FMD vaccines, which we consider of major importance. Tests of quality or potency should be carried out in all vaccine lots produced. There are several tests that allow the evaluation of a vaccine's quality with sufficient precision. The test giving the most exact results is that carried out in bovines to determine the median protective dose. Others use techniques capable of determining the quantity of specific circulating antibodies, measured in tissue culture or in mice. The most recent indirect test is that performed in guinea pigs. Because of the large number of vaccines now being produced for use in the various national campaigns underway in South America, it is difficult to conduct quality tests in bovines of all lots produced. Inasmuch as a large number of bovines susceptible to the disease does not exist in South America because the areas free of the disease are very few, the possibility of using this species in quality control tests is reduced still further. For these reasons the Pan American Foot-and-Mouth Disease Center is focusing its attention on the investigation and perfection of indirect techniques for evaluating the quality of vaccines against foot-and-mouth disease. Tests in the guinea pig--an animal easily obtained in the Hemisphere--that with some modifications use the method developed by Lucam will be one of the methods of choice for the routine control of these products. For greater efficacy in campaigns now under way, only vaccines that have passed a potency test in an official control laboratory should be used in the field.

In conclusion, it may be said that several laboratories in different countries have made important contributions in recent years toward the development of more potent and safer FMD vaccines. We may say on the basis of our present knowledge that the groundwork has been laid for producing satisfactory vaccines that may be successfully used in campaigns against foot-and-mouth disease.