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# R E P O R T

of the

Session of the Research Group of the Standing Technical Committee

of the

European Commission for the Control of Foot-and-Mouth Disease

Rio de Janeiro, Brazil
15-18 October 1985

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# INTRODUCTION

A Session of the Research Group of the Standing Technical Committee of the European Commission for the Control of Foot-and-Mouth Disease was held at the Pan American Foot-and-Mouth Disease Center, Rio de Janeiro, Brazil, from 15 to 18 October 1985.

The invitation to hold this Session at the Pan American Foot-and-Mouth Disease Center (PAFMDC) was extended to the Group on behalf of the Pan American Health Organization by Dr. Raúl Casas Olascoaga, Director of the Center. It was later confirmed by Dr. Carlyle Guerra de Macedo, Director of the Pan American Health Organization (PAHO). This was the first Session of the Group held outside the European continent since 1967; when a Session was held at the Plum Island Animal Disease Center, U.S.A.

In the absence of the Chairman, Dr. J.G. van Bekkum, who was unable to attend, the Session was chaired by the Vice-Chairman, Dr. N. Mowat. The members of the Research Group present were: Dr. M. Eskildsen, Denmark, Dr. J. Leunen, Belgium, Dr. G.F. Panina, Italy, Dr. G. Wittmann, Federal Republic of Germany, Dr. G. Dannacher, France, Dr. E. Domingo, Spain, and Dr. D. Panjević, Yugoslavia.

The Session was also attended by observers from the Center, PAFMDC/PAHO, and from a number of South American member countries of COSALFA. The list of participants is given under Appendix 21.

Dr. P. Stouraitis and Ms. J. Raftery of the European Commission for the Control of Foot-and-Mouth Disease, provided the secretariat.

Dr. Casas welcomed the participants to the Center and wished them a successful meeting. He underlined the usefulness of this joint meeting as a forum for the exchange of information on the situation of FMD in Europe and South America particularly from the point of view of diagnosis, control and quality of vaccines, the fundamental aspects of the epidemiology of the disease, and the surveillance systems. He briefly outlined the national efforts in South America for the eradication of FMD which had commenced in the fifties with the support of the PAFMDC/PAHO and which by the seventies included national control programmes in progress in all the affected countries. To date eighty five percent of the South American cattle population are covered by these programmes.

He stated that inter-country coordination for FMD control had been consolidated through the meetings of the South American Commission for Foot-and-Mouth Disease Control (COSALFA) and by binational and trinational commissions established under international agreements as well as through workshops and seminars organized by the Center.

In concluding he stated that COSALFA at its IXth Regular Meeting had approved a "Policy and Strategy for Control of Foot-and-Mouth Disease in South America during the Decade 1981-1990".

Within this common framework several countries are restructuring their control programs and a number of countries have proposed measures for the eradication of FMD by the mid-nineties.

The agenda for the Session comprised the following items:

1. Evaluation of vaccine potency

2. Importance of strains of different subtypes

3. Items referred to the Research Group by the European Commission for the Control of Foot-and-Mouth Disease

   a) Necessity to keep cattle vaccinated with AOC European vaccine for challenge with field virus to assess the effectiveness of vaccines used;

   b) Harmonization of vaccine strains in Europe;

   c) Necessity to maintain seed virus stock and strains to be considered

4. Epidemiology of FMD in South America

5. Any other business

6. Conclusions and recommendations

ITEM 1 - EVALUATION OF VACCINE POTENCY

As an introduction to this item Dr. van Bekkum's paper reviewing potency testing since the beginning of FMD vaccine production, was presented by Dr. Mowat.

The current importance of vaccine potency testing was emphasized with reference to the special interest of the producer, the controller and the user, respectively.

As a generally accepted viewpoint, it was stated that as far as possible vaccines should be evaluated in the species for which they are destined. Thus, for vaccines to be used for cattle, testing in cattle is the ultimate aim. The classical cattle tests were reviewed and evaluated.

Since, however, routine use of cattle is not always possible various alternative test methods, e.g.: tests in guinea pigs and serological testing, were also evaluated with regard to advantages and limitations.

Two papers, from France and U.K., were presented giving a historical review of various aspects of FMD vaccine potency testing. The U.K. paper described in detail the $PD_{50}$ test as it was - and currently is - used at the AVRI (Appendix 2). The French paper gave a detailed description of the K-index method in cattle and its use not only in vaccine potency testing but also for studies of subtypes (Appendix 3).

Three papers, from Italy, Belgium and the Federal Republic of Germany, dealt with serological examination of sera from vaccinated cattle as an alternative to the challenge test (Appendices 4, 5 and 6). Although using different techniques and different methods, all papers confirmed the usefulness of such tests. Some of the results indicated that for certain virus strains further studies are needed for establishment of a significant correlation between antigen concentration of the vaccines and antibody response and percentage protection.

The presentation confirmed the valuable progress which has taken place over the years in the field of FMD vaccine potency testing. Nevertheless, the existing great variations in test systems from one laboratory to another makes the necessary comparisons between laboratories very difficult.

The discussion on vaccine potency testing also reflected the need for a standard vaccination strategy for calves with an unknown immunological status especially as regards residual maternal antibodies.

Three presentations by the Group from PANAFTOSA summarized the characteristics of the cattle population in South America. The number of vaccine producing plants has increased considerably in the cattle breeding countries of South America during the last years. More efficient vaccine control systems have been introduced by the National Control Authorities (Appendix 7).

Antigen is produced using either BHK cells or by the Frenkel method. Formaline and ethyleneimine are both used as inactivants. Most vaccines are formulated with an aluminium gel - saponin adjuvant, but the use of vaccine with oil adjuvants is gradually increasing. They are favored for application in areas where FMD is presumably endemic. Different methods are applied for vaccine control. A comparison of results of mouse protection assays, serum neutralization tests and challenge of cattle ($PD_{50}$) was presented which included about 10,000 cattle (Appendix 8).

It was shown that a vaccine with oil adjuvant that had been stored at $-70^{o}$ for four years maintained its potency. This may provide a method for developing reference FMD vaccines, stable for long periods of time (Appendix 9).

A computer simulation of a vaccine potency test showed the fluctuations of results of $PD_{50}$ tests, when they are carried out with a small number of animals (Appendix 10). The implications for the producer and the controller of FMD vaccines were discussed. Dr. Casas reviewed and summarized the position of South American countries and the Pan American Foot-and-Mouth Disease Center in relation to the subjects discussed in the first session. As far as vaccine potency testing is concerned, Dr. Casas mentioned that the $BPD_{50}$ is used as routine test. However analyzing the results of over 600 tests, it was noticed that due to its high variability about one in five tests do not show a clearcut dose/response relationship. Another constraint to this test is its high cost which does not allow for a trivalent control. Thus, some countries, including Argentina, Brazil and Colombia are developing the PG (Protection to foot generalization) test which is already being used for the routine quality control of oil adjuvanted vaccines. This test has shown to be more reliable and allows for complementary antibody testing for the virus types which have not been used for challenge. In this respect the Center (PAFMDC) and the countries have a very wide experience correlating challenge results with antibody titers measured both by neutralization and mouse protection tests. These methods, the PG and the antibody measurements, will continue to be used in the routine control of FMD vaccines. Mention was made of the control in guinea pigs ($PD_{50}$ and C Index). These tests are used for screening control of vaccines but not for official decision making on approval or rejection.

Finally, Dr. Casas referred to the use of oil adjuvanted vaccines in cattle. Because this vaccine has proven to elicit longer lasting immunity and good protection in young animals, its systematic use in cattle in

primary endemic areas of South America where the livestock management is very extensive is becoming an important tool for FMD control and eradication.

It was recommended that future efforts in this field should be directed towards inter-laboratory harmonization of standard methods especially as regards the alternative and more economical serological methods for testing the potency of vaccines.

ITEM 2 - CHARACTERIZATION OF STRAINS OF DIFFERENT SUBTYPES

As an introduction to this item a review of the evolution of FMD in Europe from 1951 to date was presented by the Secretary of the Commission. Emphasis was put on major epidemics in Europe and those outbreaks related with viruses exotic to Europe. Increasing systematic vaccination and severe restrictions on animal movements from infected areas reduced markedly the disease incidence. The importance of the buffer zone in southern Europe to prevent the introduction of viruses from Asia and Africa was stressed.

A presentation from Denmark, where a non-vaccination policy was implemented in 1977, reviewed the 1982-1983 outbreaks. Eradication was based on early diagnosis by CF and the more sensitive ELISA, on the immediate stamping out of all susceptible animals on affected farms and the application of strict control measures. Despite the obvious financial losses caused by these outbreaks, an economic study showed the advantage of the non-vaccination policy in Denmark (Appendix 11).

A presentation from Italy concerned the characterization of FMDV isolated in Italy during the 1984-1985 outbreaks. Results of CF tests confirmed that the virus A Modena/84 is highly related to the Italian vaccine reference strain A Parma/62. It is somewhat similar to A Bernbeuren/84, whereas a difference from A Allier/60 is evident. However, antibody elicited by A Parma/62 vaccine in bovines would appear to neutralize to the same extent the homologous virus and the other three strains (A Modena/84, A Bernbeuren/84 and A Allier/60) (Appendix 12).

A communication from Germany showed that the serological relationship between strains as measured in plaque reduction assays was different for guinea pig and bovine antisera. Guinea pig antisera may be very useful for strain identification, but they may be misleading in the evaluation of the protective value of a vaccine against a new FMDV isolate (Appendix 13).

Modern immunological, biophysical and biochemical techniques presently available for FMDV characterization were reviewed by the U.K. representative. The ELISA methods have proved very adequate for rapid diagnosis of FMD. This test allows examination of large numbers of samples in 1-2 days. Among other advantages, the ELISA will successfully type samples containing much less antigen than that required in CF tests (Appendix 14).

The use of monoclonal antibodies (MAbs) may provide a detailed antigenic profile of each strain of FMDV thereby making it possible to assess the antigenic relationships between vaccine strains and field isolates. Exchange of MAbs among laboratories is recommended.

Application of molecular biology techniques such as polyacrylamide gel electrophoresis, T1 oligonucleotide fingerprinting, isoelectrofocusing of viral proteins, and rapid sequencing methods, provide means of FMDV

strain characterization. The potential value of such analyses in epidemiological studies was illustrated. However, the significance of the correlation between the results of biochemical tests and the biological properties of virus strains remains to be determined.

A presentation from Spain showed the use of plasmid DNA to fingerprint defined regions of the FMDV genome (Appendix 15).

A presentation was made on FMDV strains of epidemiolgical importance in South America. A resume of the points of view expressed in the relevant Appendix (16) is given hereafter.'

The great capacity of FMDV for frequent mutation, plus the selective action affecting virus replication in field conditions, as well as its passage through different hosts, make it practically impossible for a strain to cause more than one epidemic wave.

Therefore, the list of subtypes and strains should be reviewed periodically to eliminate those that have not been identified in the field in recent years. At present the strains in South America are related to A24, A79, A32, C3 and $O_1$ viruses.

The initial concern regarding emerging strains should be to restrain their spread in the field. The viruses causing foci in the interepidemic periods should be carefully studied: their antigenic structure should be compared with that of the vaccine strains used in the region, and the immunological coverage of the sera from animals vaccinated and revaccinated with the vaccine strain should be determined against the field strain. This test can be complemented by challenge in cattle vaccinated and revaccinated with the vaccine strain.

When the degree of protection in cattle at four weeks post-revaccination is equal to or exceeds 75%, and the vaccination coverage in the regions is broad enough, then revaccination with the available vaccine and stringent control of animal movement will suffice to stop the spread of the new strain. The new strain should then be classified within the same subtype as the vaccine strain. When the coverage is less than 75% and the control of the foci is difficult, then the preparation and use of a specific monovalent vaccine should be considered.

Countries where FMD is controlled and where the susceptible population is still protected by systematic vaccination - such as Western Europe - or disease-free countries that possess vaccine banks, should be aware of the coverage of their vaccines against the epidemiologically important strains in the endemic countries. The mouse protection test with sera from cattle vaccinated and revaccinated with such vaccines is very suitable for this type of work. The Pan American Foot-and-Mouth Disease Center through the network of vesicular-disease diagnosis laboratories and the countries of the region have organized these studies.

A paper on the use of biochemical techniques for the characterization of FMD virus field strains was presented. Several examples of the application of fingerprints for the precise characterization of field viruses were presented. The most relevant was the identification of a virus C3 Indaial-BR/71 which escaped from a vaccine producing unit in Rio Grande do Sul, Brazil. In this case the T1 hydrolysate appeared to be indistinguishable from the vaccine strain leading to a very precise identification of the virus (Appendix 17).

The other relevant example of the use of this methodology was the characterization of the virus C Argentina/84. This virus appeared in the field in Argentina in 1984 causing a significant epizootic. The strain showed only a very limited degree of serological relationship (R=33) with respect to the prototype strain C3 Resende Brazil/55. Animals vaccinated with the prototype strain showed only a limited protection against this virus strain following primary vaccination.

T1 maps of this new field strain showed a high degree of homology with the virus C3 Resende (it has six additional spots and lacks 4 spots with respect to the prototype strain). In addition analysis of the T1 hydrolysates by means of one-D-gels showed that the field strain (C Argentina/84) had only one Poly (C) rich tract which is slightly larger than the largest poly (C) rich tract present in the prototype strain C3 Resende.

The close similarity of the T1 digest is in contrast with the serological identification by complement fixation test and cross protection experiments in cattle suggesting that this strain is a typical antigenic variant of virus C3 Resende.

Analysis of viral structural proteins by means of non-equilibrated electrofocusing gels (NEPHGE) showed a clear shift in the isoelectric point of capsid protein VP1 of virus C Argentina/84 with respect to the vaccine strains. Based on these observations the virus C Argentina/84 can be classified as an antigenic variant of virus C3 Resende. These data agree with studies made at the WRL, Pirbright, U.K.

Cross immunity tests have shown that animals vaccinated with virus C Argentina/84 were very well protected when challenged with homologous virus and with vaccine strains C3 Resende-Br/55 and C3 Indaial/71.

For these reasons it was decided to include this strain in the vaccine in Argentina. The virus was available as a monovalent vaccine in February 1985.

After incorporation of this new strain in the vaccine the incidence of the disease during 1985 dropped drastically.

ITEM 3 - ITEMS REFERRED TO THE RESEARCH GROUP BY THE EUROPEAN COMMISSION FOR THE CONTROL OF FOOT-AND-MOUTH DISEASE

(a) Necessity to keep cattle vaccinated with AOC European vaccines for challenge with field virus to assess the effectiveness of vaccines used.

The Research Group was in favour of a scheme whereby in each country groups of cattle should be identified which had received either a single dose of vaccine or had been revaccinated, in both cases with the currently produced vaccine. These animals would be available for transfer to the National Control Laboratory for the assessment by challenge infection of the level of protection likely to be given by the vaccine against any new important strain of FMDV occurring in the field.

The Group also emphasized the value of the scheme initiated by the International Association for Biological Standardization in which various FMD Institutes have undertaken to produce and when necessary distribute samples of antisera from cattle and guinea pigs immunized with commercially produced monovalent vaccines currently available. Sera from animals which received either a single dose of vaccine or were revaccinated are availabe.

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The Group also emphasized the value of the scheme initiated by the International Association for Biological Standardization in which various FMD Institutes have undertaken to produce and when necessary distribute samples of antisera from cattle and guinea pigs immunized with commercially produced monovalent vaccines currently available. Sera from animals which received either a single dose of vaccine or were revaccinated are availabe.

The Group strongly recommended national authorities to make use of these reagents in addition to the protection test mentioned above, in assessing the usefulness of existing vaccines against important new field strains.

(b) Harmonization of vaccine strains in Europe

The Group thought it premature at present to recommend an attempt to harmonize the vaccine strains currently in use in Europe. However, in view of the frequency and importance of outbreaks in recent years due to viruses of serotype A, the Group recommended that much more effort should be put into studies to define the antigenic relationships between currently used vaccine strains and as a second phase of these studies, the relationship between vaccine strains and recent European field strains of serotype A.

(c) Necessity to maintain seed virus stocks

The Group discussed the usefulness of the stock of exotic virus strains held at AVRI, Pirbright, as potential vaccine strains. It was agreed that it was very unlikely that these BHK adapted strains could be used by many vaccine manufacturers, since it was uncertain whether they would grow readily in the various lines of cells in industrial use and this factor applied particularly to the Frenkel culture system.

In addition since these strains had been selected and stored in 1970 it was probable that their relevance antigenically to strains presently in the field was small.

In view of the increased interest in Europe in the setting up of inactivated antigen banks for the rapid preparation of vaccine in case of emergency situations and the likelihood that a bank in Europe devoted to exotic strains of antigen would supercede the usefulness of the material stored at Pirbright, the Group thought it likely that the stored material could be discarded in the not too remote future. However, as a precaution against unforeseen events, it recommended that the stored material should be retained for one more year during which time it may become clearer that a European exotic antigen bank would eventually be set up.

FAO Collaborative Study

Following an invitation to participate in another phase of the Study (Phase VIII), favourable responses have been received from all the participants to the earlier phases. Sera from cattle given either a single dose of trivalent vaccine (01 Lausanne, C1 Vosges and A5 Allier) or from revaccinated animals will be made available by Rhône-Mérieux, France. Samples of sera together with freeze-dried viruses of serotype A, both homologous and heterologous to the A5 Allier in the vaccine will be distributed. The intention is for each laboratory to include these sera in their own routine tests and subsequently to provide data on their assessment of the neutralizing activity of the sera for comparison between laboratories.

ITEM 4 - EPIDEMIOLOGY OF FMD IN SOUTH AMERICA

A paper was presented on Vesicular Diseases, Epidemiological Surveillance and Information Systems established in the countries of South America to support FMD control programs (Appendix 18). In a first phase, from 1972 to 1978, the field implementation and training of personnel was accomplished. Starting in 1978, the analysis of the information was made

to have a better understanding of the epidemiology of FMD. Data was presented of the several indicators which have been developed to monitor the temporal and geographical behaviour of the disease and to evaluate the effectiveness of the control programs. Examples of Argentina, Brazil, Colombia and Uruguay were presented.

The Chairman congratulated the Center on the advances of the Epidemiological Surveillance System for Vesicular Diseases which have been established in South America and pointed out the importance of methodology for collecting, handling and using the information to orientate the FMD programs.

A paper entitled "Planning of FMD eradication: ecosystems and regional strategies" was presented by the epidemiologists of PAFMDC. Reference was made to the importance of considering the FMD problem as an infection rather than just clinical overt disease. After discussing the ecosystems and their corresponding livestock production forms as determinants of geographical behaviour of FMD in the South American countries, specific regional strategies for the eradication of FMD were proposed. Highest priority was given to extractive-extensive forms of production where the disease is endemic. A successful elimination of FMD virus from these areas should be achieved by means of the use of oil adjuvanted vaccines for official mass vaccination and the quarantine and revaccination of young steers in transit for fattening areas (Appendix 19).

Discussion in relation to this paper centered on the massive use of VIA antibody surveys for monitoring the reduction of herd infection and the possible interference of VIA antibodies elicited by repeated vaccinations. This was not considered to pose important problems. The role of FMD virus carriers in endemic areas was also analyzed. Specific details were also given on the methodology for the characterization of geographic clusters according to livestock and disease indicators and on the practical mechanics of quarantine and revaccination procedures for cattle exiting the breeding zones.

ITEM 5 - ANY OTHER BUSINESS

A paper was presented on oil adjuvanted FMD vaccine. It was stated that the PAFMDC has worked on oil adjuvanted FMD vaccine for cattle since 1968 in laboratory studies and field trials. The favourable results led to application of this vaccine in Government field projects in several countries in South America. The experience of application in larger groups of animals in FMD control programs was also very positive. Two programs in particular, in Argentina between 1977 and 1981 and in Brazil in 1980, were discussed in more detail. In both programs the morbidity rate in the oil adjuvanted vaccine group was only 13% of that in the control group with aluminum hydroxide vaccine. The oil adjuvanted vaccine therefore would be extremely useful for application in highly endemic FMD areas in South America (Appendix 20).

Although the present volume of production of oil adjuvanted vaccine is still small (about 20 million doses in Brazil), the vaccine production laboratories are more and more interested in this vaccine and the production volume is expected to increase considerably in the near future in Argentina and Brazil.

Dr. Panina enquired about the amount of antigen in oil vaccine. Dr. Abaracon stated that alhydrogel saponin vaccine containing three times the amount of the same antigens as oil vaccines gave similar immune responses

at 30 days post vaccination. However from two months after revaccination the oil adjuvanted vaccines clearly showed a more sustained high level of protection. Dr. Abaracon emphasized that in order to put the amount of antigen in the 2,5 ml required for a 5 ml vaccine dose, the antigen should be of high quality. A poor antigen will not substantially improve by concentration.

Dr. Strobbe reported on some difficulty to detect residual virus in oil adjuvanted vaccines using regular techniques. He indicated some problems with breaking the emulsion. According to Dr. Abaracon the Center (PAFMDC) has never encountered difficulties with the oil vaccine formulation. The first step is to test the antigens before emulsification but after formulation the emulsion can easily be broken by chloroform followed by centrifugation to separate the aqueous phase.

Place and date of next meeting

Dr. E. Domingo, Instituto de Biologia Molecular, Madrid, kindly invited the Group to hold its next meeting in Spain in October 1986; the exact date and location will be determined at a later stage.

This invitation was accepted by the Group and it was agreed that the following items should be included in the Agenda for discussion:

- immunity to FMD in young animals
- the application of monoclonal antibodies to the characterization of FMD virus strains
- prospects for new vaccines
- collaborative study (A5 virus)
- items referred by the Commission to the Research Group
- any other business

Closing remarks

In closing the Session, Dr. Mowat thanked all the speakers and contributors to the discussions. He also expressed gratitude on behalf of the Research Group to Dr. Casas Olascoaga and the staff of the Pan American Foot-and-Mouth Disease Center for the excellent arrangements which had been made for a very successful meeting and for their active and valuable contribution to the discussions.

Dr. Stouraitis, Secretary of the Commission, underlined the usefulness of this Session which had served as a forum for the exchange of scientific information on matters of common interest in the field of foot-and-mouth disease. He expressed the wish that this would further strengthen the valuable collaboration already existing between the FAO European Commission for the Control of Foot-and-Mouth Disease and the Pan American Foot-and-Mouth Disease Center. In thanking the Director and staff of the Center for their valuable assistance and generous hospitality, Dr. Stouraitis requested that the appreciation of the Commission be conveyed also to PAHO for having agreed to the holding of this joint meeting.

Appendix 1

# POTENCY TESTING OF FMD VACCINES

J.G. van Bekkum*

Since the beginning of FMD vaccine production innocuity and potency of vaccines have been major points of interest for producers, controllers and users.

Only inactivated FMD vaccines destined for the bovine species are considered as that is the main target animal in most of the world. A generally accepted viewpoint is that as far as possible vaccines should be evaluated in the species for which they are destined - thus for vaccines destined for cattle tests in cattle are the ultimate aim. As routine use of cattle for the purpose of vaccine potency testing is not always feasible alternative methods have been developed and studied over the years. The present standpoint is, that such tests may be acceptable as long as there is a satisfactory correlation between data obtained in the alternative system and the outcome of potency tests in cattle.

As indicated several different groups are interested in vaccine potency. We should however bear in mind that their lines of interest are not always in parallel. The producer's main interest is a not too expensive uniform product. It should be sufficiently potent but not excessively so i.e. it should meet the controllers criteria and pass his tests, i.e. non acceptance of only a small percentage is acceptable. For practical purposes a decision as to quality should be reached via tests which should not be too expensive or take too much time.

The controller is also interested in tests of short duration. His duty is to weed out vaccines that are of poor quality, "vaccines should protect" but he is not so much interested in further quantitative data.

Discrimination between "protected" and "unprotected" and clear cut results are the main points. Usually a challenge model bringing down 100% of susceptible controls is selected. The test method should preferably be standardized. The ideal, introduction of a standard vaccine to be included in all tests, is however not within our grasp at the moment nor is it likely to become available in the near future.

Although relatively stable antigens suitable for long term storage may now exist in various freezers, the problem of strain differences between vaccines will not be easy to overcome.

The third party involved is the user. In several European countries controller and user are both part of the same - governmental - organization. The State is at least as interested in quality as the private buyer of the vaccine because insufficient vaccine quality may result in huge financial losses, also for the State. The State therefore frequently prescribes vaccination, nonetheless controllers usually manage to take a rather abstract position.

The animal owner, i.e. the buyer of the vaccine is not much interested in short term protection. He wants his animals to be protected, at least until the next vaccination, preferably for a year. In practice exposure is not timed to take place two or three weeks after administration of the vaccine.

* Animal Virus Research Institute, Pirbright, U.K.

A single shot of vaccine usually produces a peak level of immunity after two to four weeks. Depending on the dose of antigen administered and type of adjuvant used, protection then weans. In four to six months it usually reaches a critical level in many animals.

Also, in the field no standard challenge is used. The amount of virus taken up by individual animals is variable. Probably in many instances the first animal infected in a herd is only exposed to a minimal amount of virus. In case of a take there is however a massive build-up and subsequent victims may be exposed to large quantities of virus and by different routes.

Different routes of exposure challenge the animal's defenses non-uniformly, and we know that protection against the development of generalized lesions is not the same as protection against a local challenge as in i.d.l. injection.

The concept of "protection against FMD" has shown a certain evolution over the past years. Initially protection against clinical disease was considered an acceptable advance. Mild clinical symptoms, such as the development of mouth lesions only were already a marked improvement in comparison with the fullblown picture seen before, which caused tremendous losses.

Nowadays, even the mildest possible clinical picture aleady constitutes a disease outbreak, with in many countries severe consequences, in Europe at least the loss of the animal, often destruction of the whole herd. Additional economical losses make even the mildest form of the disease outbreak unacceptable.

In addition we have recently come to the insight that exposure to the virus, not only in vaccinated animals, can result in reproduction of the virus without clinical disease. Such animals can disseminate the infection and therefore, what we really should be looking for in our vaccines, is protection not against some clinical symptoms but against infection.

The models that have been developed for evaluating FMD vaccine potency in cattle are largely based on outdated concepts of the infection's pathogenesis.

The original notion was that the virus entered the body mainly by the oral route. Primary multiplication was supposed to take place in the oral mucosa.

Experimental "standard" infections were therefore administered by applying a given quantity of virus to these tissues. Protection was measured by comparing the subsequent development of the disease with that in fully susceptible stock, experience soon indicating that appplication of a large enough quantity of virus to the oral mucosae in fully susceptible animals resulted in a high percentage of generalized infections.

We nowadays know that in the field the infection is usually first established in the upper respiratory tract virus and first multiplies in the nose and pharynx. Further dissemination then takes place via lymph to lymph nodes and then the bloodstream. However, some observations suggest that the most susceptible route might be the intrapulmonary one.

This new concept should have consequences for our challenge models.

The relationship between the results of short term laboratory trials in cattle and the quality of the same vaccines as manifested in the field, is not fully clear. There certainly is a positive correlation between the two kinds of observations. However there is also some uncertainty as to the minimum protection that is required in laboratory trials in order to obtain satisfactory results in the field.

In recent years field results in Europe have on the whole been highly satisfactory. Most cattle have however received multiple injections and challenge has been minimal. In addition, failure of a vaccine in the field can be due to factors other than insufficient potency. Consequently our field data do not really prove that our potency tests really only pass vaccines of sufficient quality. Under heavier exposure i.e. when no effective (and costly) veterinary police measures can be applied the requirements may be different.

A standpoint that has occasionally been defended i.e., 70% protection in a population will hold an epizootic may apply to some infectious diseases, such as rinderpest or rabies. Its applicability to FMD is however doubtful. In addition, what is protection.

This is the background against which we should view our discussions on potency testing of FMD vaccines, discussions which have been going on for some time and in which various international organisations such as OIE, FAO, IABS and WHO have been taking part.

What methods are then available or in use?

The bovine is the target animal and should therefore provide the reference system. All tests in cattle share a set of problems:

- the test animal is expensive and only tests in small numbers are routinely possible

- our animal material is heterogeneous - genetically, in condition and health, usually also in age.

In countries with regular vaccination programs test animals may have to be imported i.e. they may not be representative for the population for which the vaccine is destined.

The assay systems primarily use direct tests. Indirect tests have obvious advantages and find increasing application. The various correlations are still under investigation. The issue is always to get maximal information from a small number of animals. Quantitative data are preferable to only qualitative information.

Selection of the challenge virus is always a critical issue. it should be close to the expected field virus. In routine production control tests it is usually the production strain, maintained in cattle and applied in a standard dose.

In some instances, the national laboratorory issues the challenge strain; it may leave the choice of the production virus to the producer.

Variables that come back in all assay systems are intervals between vaccination and challenge, challenge dose, nature of the virus, length of the observation period after challenge.

Direct tests in cattle are:

- the i.d.l. challenge of a group of vaccinated cattle several weeks p.v., together with a group of susceptible controls. The latter should show generalized infections. A 70% protection level (against generalization) was usually considered an acceptable minimum. In this test immunity is challenged primarily at the level of oral mucosa with secondary exposure of the respiratory tract and systemic immunity.

- challenge of a (small) group of cattle by swabbing with a standard volume of virus suspension of a given titer. This method was intially introduced by Waldmann (no real titration was available at the time) and is still being used in the German Federal Republic (G.F.R.). Different, partly clinical, criteria can be evaluated such as fever, presence, extent or absence of oral lesions, extent of generalization and its rate of development. In the G.F.R. a statistical analysis of data from a large number of tests has been carried out and an evaluation scheme developed. The test aims at the elimination of inferior vaccines.

  This model again tests different aspects of the immune response. Dependent on the system of housing - control cattle in the same room as vaccinated cattle yes or no - non protected animals may add to the burder of infection.

- the $PD_{50}$, 50 protective dose, goes back to work by Rosenbusch in South America and by Henderson and Galloway in U.K. in 1952/3.

  The vaccine is administered in dilutions and vaccinated cattle are tested by i.d.l. injection of a fixed dose of virus. Protection is measured as absence of foot lesions. A quantity of vaccine protecting 50% of vaccinated cattle against generalization is estimated using some accepted statistical method such as Reed and Muench, Spearman=Karber or the probit method. "Partial" generalization is usually not taken into account, nor is the extent of the lesions in the oral mucosa.

Statistical calculations, including estimates of standard deviations and confidential limits are becoming standard practice. Different dilution intervals are in use for different virus strains and in the various laboratories. The number of animals per test is always small, five to eight per dilution. Differences of opinion still exist around the choice of the diluent. For emulsion vaccines, which are difficult to dilute, different volumes of vaccine may actually be injected. When aluminium hydroxyde is used as adjuvant vaccine dilution in a buffer yields a steeper dose-response curve (vaccine dilution versus percentage of protection) than dilution in adjuvant containing solution. A variant of the test uses one dose of diluted vaccine, the dilution being selected in such a way that at least 50% protection is required.

The $PD_{50}$ is difficult to standardize. Observed values have only a relative value due to the often wide confidential limits. Confidential limits are wide, especially when dilutions are made in adjuvant, and the endpoint determination is therefore unreliable.

Estimates for percentages of protection against generalization after administration of a given number of $PD_{50}$'s have been made by a number of authors. 10 $PD_{50}$ are generally expected to give over 95% of protection.

Vaccination with increased numbers of $PD_{50}$ not only results in a high level of protection at 2 to 4 weeks post vaccination but also lengthens duration of protection. The general pattern is accepted but detailed studies are scarce.

The method of the K-index was developed by Lucam and co-workers in France, based on the i.d.l. titration as described by Henderson.

A standard virus preparation is titrated, in tenfold dilutions, in four vaccinated and four control cattle three weeks after vaccination. Vaccine potency is evaluated from the endpoints of the two titrations. The difference between the two values, expressed as index K, is judged to give a reliable estimate of the level of protection, also against generalization. K values between 0.6 and 1.2 are reported to correlate with protection rates of 70% to 85%.

Protection against generalization as observed in the test is not taken into account, which according to some authors, is a weak point. Lack of reproducibility has also been reported by investigators using this test.

Other direct tests have been developed using animals other than the bovine, such as sheep, guinea pig and mouse. In addition, tests using immunological or physicochemical methods to estimate vaccine potency through antigen content have been reported. These latter will not be discussed in detail now.

The guinea pig challenge test has been used with some success, after a relationship between comparative results in cattle and guinea pigs had been established for given virus and guinea pig strains.

The guinea pig as a test animal could possibly be standardized internationally (there are marked differences between strains), but the absolute need to use a guinea pig adapted challenge virus plus the fact that a single additional passage of the virus in the guinea pig can markedly change virulence for the test animal constitute severe restrictions for this test.

Indirect methods, such as serological evaluation of the serum antibody response in the target animal have evident advantages. They obviate tests with virulent virus in cattle, reducing both the cost of the operation and the risks involved. A disadvantage is that only the humoral immune response is taken into account. Serum neutralizing antibodies are however considered to be the main factor in protection agains picornavirus infections. In addition to neutralization tests in cell culture, such tests in baby mice and serum protection tests in mice are being used.

Both tests probably could to some extent be standardized but we all know from experience that even if the identical ingredients down to the cells are used, the between laboratory differences in neutralization tests can still be highly significant. Inclusion of an international reference serum in all tests could help to facilitate interlaboratory comparison.

In general neutralization tests obtained in different laboratories show good correlation with protection data, especially if protection is read as absence of generalized lesions. Especially above a certain titre (slightly different per virus, test system, laboratory) there is a high probability of protection.

Appendix 2

# POTENCY TESTING OF FOOT AND MOUTH DISEASE VACCINE IN THE U.K.

G.N. Mowat*

## Introduction

The first work on the development of foot-and-mouth disease (FMD) vaccines was begun in Europe in the 1920's. However, it was only in the late 1930's that a product became available, albeit in limited amounts, for field use. This was the Schmidt-Waldmann vaccine (Schmidt, 1938, Waldmann & Kobe 1938).

Further developments for improved vaccines came from more efficient methods of producing viral antigen, notably the use of freshly harvested bovine tongue tissue (Frenkel, 1947) and subsequently the use of the continuous cell line BHK21 (MacPherson & Stoker, 1962, Mowat & Chapman, 1962). The introduction of the aziridine compounds such as acetylethyleneimine (AEI) by Brown & Crick (1959) resulted in safer vaccines since the infective component is totally inactivated by the use of these compounds.

The demand for increasingly large amounts of reliable vaccine for control programmes from the National Authorities and the desire of commercial and some State organizations to provide such a product resulted in the need to develop quality control procedures to ensure that vaccines were of an acceptable standard of efficacy.

Notable contributions in this field came from Henderson and his colleagues at Pirbright during the period from just before the war until the mid 1950's. They recognised the value of assessing vaccine directly in the target species, i.e. cattle, and the need to be able to assay infective virus by a test method of which the limits of precision could be estimated. The need for such an assay came from the requirement to quantify the viral content of vaccines and also the control of virus dosage in tests to assay vaccine potency by challenge. Much of this early work on the estimation of potency of vaccine in cattle was not published and it is only very recently that it has been brought together as a monograph by Sir William Henderson for publication (1985) following his receiving a Massey-Ferguson award for distinguished work in this field.

## Development of a potency test

The initial work at Pirbright was concerned with assessing the reaction of cattle to infection with the virus of foot-and-mouth disease.

In any challenge of immunity, whether by contact infection or by inoculation, the vaccinated cattle must be subjected to an exposure to the virus capable of consistently causing severe clinical disease in unvaccinated control cattle. The methods by which this could be achieved were extensively investigated and Table 1 shows experimental approaches used in tests of contact infection. A summary of this work is given in Table 2. It indicates that of 411 cattle used, only 349 (85 percent) developed full clinical disease. This is a reflection of the effects of various factors such as the relative insensitivity of route of infection,

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i.e. the very large amounts of virus needed to establish infection and, in some cases, the poor invasiveness or low virulence of the strain of virus employed.

Table 3 illustrates the routes of inoculation as a means of infection which were investigated. Again, wide differences in sensitivity of route were found, infection with some strains requiring approximately 250,000 times more virus to infect by the subcutaneous route as compared to intradermal inoculation of the tongue. This latter route was found to be consistently the most sensitive method for the initiation of infection. Of some 1,899 cattle involved in tests involving intradermal inoculation of the tongue, only 5 (0.3 percent) proved to be refractory to this route of inoculation. Also the time course of infection to generalised disease was shorter by this method as compared to contact infection - this is shown in Table 4. This was a significant advantage in the design of a practical assay system for assessing the immunity of vaccinated individuals.

In a series of five experiments comparison was made of the reaction of vaccinated cattle to infection either by contact exposure or by i/d inoculation of the tongue. Henderson & Galloway (unpublished results) came to the conclusion that complete protection following exposure by contact corresponded to complete protection against the development of secondary lesions. In all subsequent work to develop a reliable potency test the criterion of protection in challenge tests following i/d tongue inoculation has been the absence of secondary lesions.

In the early 1950's, the theory and statistical implications of biological assay systems was becoming better understood and comprehensive accounts of the design and analysis of such assays were published (Finney, 1952, Pizzi, 1950). The 50 percent end point test was favoured since under the right conditions the precision of the result could be calculated.

This approach to the titration of virus infectivity was already accepted (Henderson, 1949) and a similar approach to assessing vaccine was adopted. The limitations of a test based on quantal response was recognised and the inability to compare a test preparation against a standard vaccine in cattle for economic and other reasons was emphasized. The linear transformation of data from an essentially sigmoid dose response curve by probit analysis was adopted introducing a further refinement in the interpretation of the data.

## The $PD_{50}$ Test

The test design for the assay of vaccine potency as used in the U.K. for the last 20 years or so is summarised in Table 5. Of the various components of the test the one which has resulted in the most discussion is the method for preparing the test dilutions of the vaccine. It is considered by workers in the U.K. that an assay of vaccine potency should measure the immunogenic potential of the original and undiluted product. To achieve this only the viral antigen concentration should be varied to obtain an observable effect. In other words, the test should be based upon a design giving an antigen extinction limit. The test used in the U.K. is therefore one in which only the antigen is diluted out and this is done by diluting the original vaccine in a mock vaccine containing all the normal components except the viral antigen. Thus the adjuvant is available at normal concentrations at each dilution level to potentiate whatever immunogenic material is present.

From an extensive series of potency tests, Pay & Parker (1977) have computed from the regressions of the dose responses an overall median slope value of 2.0. From this they estimated that to obtain 70 percent protection, vaccines would have to have a $PD_{50}$ value of 1.9. To meet the O.I.E. Recommendations as amended in 1975, vaccines tested by this method should have a potency value such that its lower 95 percent confidence limit (one sided) is equal to or greater than 1.9. Similarly to obtain a protection level of 95 percent, vaccines should have a minimum observed value of 6.0. The minimum standard of acceptance for vaccines in the U.K. has therefore been set at 6 $PD_{50}$.

In attempting to measure the accuracy and precision of any particular assay based upon the use of small numbers of test subjects, the inherent biological variation in the method precludes against fine levels of precision. In using the probit transformation to analyse quantal data inevitiably the confidence limits, where they are calculable, are often extremely wide and in many cases the distribution of responses are such that a positive slope and confidence limits cannot be derived. In an assay involving eight subjects at each of three dilutions there are a possible 729 results. Of these only some 70 sets of results have a positive slope from which confidence limits can be calculated. A small section from the Probit Tables produced by the Wellcome Foundation Computer programme SY2F is shown in Table 6.

Results of Vaccine Potency Tests in the U.K.

The Animal Virus Research Institute, Pirbright, working with the method discussed above, has controlled vaccines submitted for inclusion in the U.K. Strategic Vaccine Reserve on behalf of the Ministry of Agriculture, Food & Fisheries.

The results of typical tests on vaccines of serotypes O, A and C are given in Tables 7, 8 and 9. The antigens for these vaccines were prepared by growth of the viruses in the industiral suspension culture of BHK21 cells. A previous report (Mowat, 1974) showed that vaccines with a $PD_{50}$ value well in excess of the minimum requirement could be obtained by this method of vaccine production and also that such vaccines could be stored for long periods without significant loss of potency. The results presented here confirm and extend the earlier observations on typical $PD_{50}$ values.

Experience both in the U.K. and in other countries producing FMD vaccines indicates that vaccines of serotype O will in general be of lower potency than those of serotypes A and C. Comparing the data in Tables 7 - 9 this fact is clearly evident. Of the 19 tests of type O reported here some three batches were of less than six $PD_{50}$. Potency can be among other things a function of how much antigen a manufacturer calculates he can economically afford to include in the formulation. Scanning the results it is clear that many of the batches must have contained high concentrations of antigen since their observed $PD_{50}$ values were considerably in excess of six $PD_{50}$. The series of results for the A and C vaccines indicate one batch out of 12 and one batch out of 14 respectively failed to meet the minimum standard. In many tests with both the A and C vaccines an end point in the assay was not obtained indicating values in excess of 112 $PD_{50}$.

Assessment of Protection following challenge

In the European Pharmacopoeia Commission document describing a recommended procedure for estimating the potency of FMD vaccines in cattle, the statement is made - "unprotected animals show lesions at sites other than

the tongue". Whilst the need for clear and unambiguous directions in the scoring of the lesions developing in test animals is obvious, this rather all or none interpretation of the responses to challenge may be too stringent.

Table 10 shows that in some tests animals are found which have an FMD lesion on a single foot and subsequent titration of their serum immediately prior to challenge reveals that they have antibody titres consistent with protection in other animals. That this effect is not a rare occurrence is indicated in Table 11. About 75 percent of the type O vaccines tested have given results which included one or more cattle in this category and about 50 percent of the tests with A and C vaccines were also affected.

One possible explanation for the phenomenon is that the lesion developing on a single foot after challenge by tongue inoculation, does not develop as the consequence of viraemia, but may be the result of external transfer of infective virus in saliva etc. from the virus rich mouth lesions to a small abrasion or wound in the interdigital cleft of a foot. In the test of batch 447 shown in Table 10, the unusual number of seven cattle all being affected may be related to the fact that they were all unusually large heavy animals confined by neck chains on cattle standings intended for small animals. A possible consequence of this may have been a higher than average number of minor injuries to the hind feet promoting infection from the heavily contaminated surroundings.

The fact that these cattle all had significant antibody titres appears to be consistent with this interpretation of the results.

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Table 1. I. Methods of infection of cattle
Exposure by Contact with Donors

(a) Strictly limited period - 2-4 hours.
(b) Contact for 24 hours.
(c) Continuous exposure with accumulation of infection -
    (i) Donor, vaccinates and controls together for 14 days.
    (ii) Donor and vaccinates - 14 days. Controls separate after 24 hours exposure.
(d) Continuous exposure with minimum accumulation of infection.
(e) Continuous exposure to individual donors.

Table 2. Time and extent of reaction of normal cattle exposed by various methods of contact to various strains of FMDV.

| No. of Cattle | % Reactors | No. of +ve sites | Day of 1st observed development of a lesion | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
| 349 | 95.1 | 5 | | 38 | 86 | 81 | 60 | 35 | 21 | 20 | 4 | 2 | - | - | - | - | 2 |
| 5 | 1.4 | 4 | | | | | | | 2 | | 1 | 1 | 1 | | | | |
| 2 | 0.5 | 3 | | | | | 1 | 1 | | | | | | | | | |
| 4 | 1.1 | 2 | | | | | | | | 2 | 1 | | | 1 | | | |
| 7 | 1.9 | 1 | | | | | | 1 | 1 | 1 | 1 | | 2 | | 1 | | |
| 367 | TOTALS | | 0 | 38 | 86 | 81 | 61 | 37 | 24 | 23 | 7 | 3 | 3 | 1 | 1 | 0 | 2 |
| | % | | | 10.4 | 23.4 | 22.1 | 16.6 | 10.1 | 6.5 | 6.3 | 1.9 | 0.8 | 0.8 | 0.3 | 0.3 | | 0.5 |

Data from 367 reactors out of a total of 411 cattle.

W. M. Henderson, 1985

Table 3. II. Methods of infection of cattle
Infection by inoculation of virus

Routes investigated:-

Intramuscular
Intranasal
"Frottement" of the tongue
Subcutaneous
Intracutaneous
Intravenous
Intradermal inoculation of the tongue

Table 4. Rate of development of secondary lesions following i/d tongue inoculation of normal cattle with FMDV.

| Day | Observation of 1st secondary lesion | | Observaton of last secondary lesion | |
|---|---|---|---|---|
| | No. of cattle | % | No. of cattle | % |
| 1 | 27 | 1.4 | 5 | 0.3 |
| 2 | 642 | 33.9 | 372 | 19.6 |
| 3 | 525 | 27.7 | 574 | 30.3 |
| 4 | 386 | 20.3 | 459 | 24.2 |
| 5 | 244 | 12.9 | 330 | 17.4 |
| 6 | 50 | 2.6 | 83 | 4.4 |
| 7 | 14 | 0.7 | 39 | 2.1 |
| 8 | 3 | 0.2 | 16 | 0.8 |
| 9 | 2 | 0.1 | 8 | 0.4 |
| 10 | | | 6 | 0.3 |
| 11 | | | | |
| 12 | | | | |
| 13 | 1 | 0.05 | 2 | 0.1 |

Data provided by 1,894 reactors.

W.M. Henderson, 1985

Table 5. Design of $PD_{50}$ Test for assay of vaccine potency

| | |
|---|---|
| Test animals:- | 18-24 month fully susceptible steers. |
| Vaccine Dilution range:- | 1:2 1:10 1:50 |
| Vaccine Diluent:- | Fully adjuvanted vaccine base. |
| No. of test animals:- | Groups of 8 at each vaccine dilution level. |
| Rate of inoculation:- | Subcutaneous. |
| Time of challenge:- | 21 days after vaccination |
| Route and dose of virus:- | Intradermal tongue, $10 \times 10^4$ cattle $ID_{50}$. |
| Observation time:- | 10 days. |
| Criterion of protection:- | Absence of secondary lesions. |
| End point calculation:- | Probit analysis. |

Table 6. The first 27 possible results from a total possible of 729 taken from the tables for probit analyses of quantal responses compiled by The Wellcome Foundation computer programme SY2F.

No. of cattle NOT protected

| 1:2 | 1:10 | 1:50 | TITRE | LIMITS | | SLOPE | S.E.B. |
|---|---|---|---|---|---|---|---|
| 0 | 0 | 0 | NON POSITIVE SLOPE | | | 0.9 | 0.00 |
| 0 | 0 | 1 | 102.9 | SLOPE NOT SIG. | | 3.7 | 11.89 |
| 0 | 0 | 2 | 71.5 | SLOPE NOT SIG. | | 4.3 | 11.88 |
| 0 | 0 | 3 | 58.2 | SLOPE NOT SIG. | | 4.9 | 11.88 |
| 0 | 0 | 4 | 50.0 | SLOPE NOT SIG. | | 5.3 | 11.88 |
| 0 | 0 | 5 | 44.0 | SLOPE NOT SIG. | | 5.8 | 11.88 |
| 0 | 0 | 6 | 39.0 | SLOPE NOT SIG. | | 6.3 | 11.88 |
| 0 | 0 | 7 | 34.2 | SLOPE NOT SIG. | | 7.0 | 11.89 |
| 0 | 0 | 8 | 22.4 | SLOPE NOT SIG. | | 10.6 | 16.78 |
| 0 | 1 | 0 | NON POSITIVE SLOPE | | | 0.0 | 0.80 |
| 0 | 1 | 1 | 1427.8 | SLOPE NOT SIG. | | 0.7 | 0.74 |
| 0 | 1 | 2 | 173.9 | SLOPE NOT SIG. | | 1.1 | 0.75 |
| 0 | 1 | 3 | 77.4 | SLOPE NOT SIG. | | 1.5 | 0.76 |
| 0 | 1 | 4 | 48.3 | 20.1 | 73497.2 | 1.8 | 0.84 |
| 0 | 1 | 5 | 35.2 | 15.7 | 278.6 | 2.2 | 0.90 |
| 0 | 1 | 6 | 27.7 | 12.4 | 81.3 | 2.6 | 1.00 |
| 0 | 1 | 7 | 22.4 | 10.8 | 47.2 | 3.3 | 1.12 |
| 0 | 1 | 8 | DATA DOES NOT CONVERGE | | | - | - |
| 0 | 2 | 0 | NON POSITIVE SLOPE | | | 0.0 | 1.12 |
| 0 | 2 | 1 | 2430.1 | SLOPE NOT SIG. | | 0.5 | 0.61 |
| 0 | 2 | 2 | 185.9 | SLOPE NOT SIG. | | 0.9 | 0.61 |
| 0 | 2 | 3 | 72.6 | SLOPE NOT SIG. | | 1.2 | 0.63 |
| 0 | 2 | 4 | 42.8 | 15.8 | 49927.3 | 1.5 | 0.66 |
| 0 | 2 | 5 | 30.0 | 12.5 | 273.8 | 1.8 | 0.70 |
| 0 | 2 | 6 | 23.0 | 10.3 | 73.9 | 2.2 | 0.75 |
| 0 | 2 | 7 | 18.4 | 8.4 | 42.3 | 2.7 | 0.92 |
| 0 | 2 | 8 | 12.8 | SLOPE NOT SIG. | | 6.3 | 11.75 |

Table 7. Results of potency tess on batches of type O (BFS 1860) vaccine for the U.K. Strategic Reserve.

| Batch No. | No. of cattle protected 1:2 | 1:10 | 1:50 | $PD_{50}$/dose | Confidence limits |
|---|---|---|---|---|---|
| 39 0 months | 3/4 | 3/4 | 2/4 | 79.0 | - |
| 12 months | 7/8 | 6/8 | 4/8 | 54.0 | non-calc. |
| 24 months | 8/8 | 6/8 | 3/8 | 30.0 | 12.5 - 273.8 |
| 48 | 8/8 | 6/8 | 1/8 | 18.4 | 8.4 - 42.3 |
| 65 | 7/8 | 6/8 | 3/8 | 29.6 | 8.7 - 8,104,613 |
| 69 | 8/8 | 7/8 | 6/8 | 173.9 | non-calc. |
| 92 | 8/8 | 4/8 | 2/8 | 15.8 | 6.3 - 52.9 |
| 111 | 8/8 | 4/8 | 6/8 | 136.1 | non-calc. |
| 184 | 7/8 | 2/8 | 0/8 | 5.4 | 2.4 - 11.9 |
| 193 | 6/8 | 5/8 | 1/8 | 9.8 | 1.8 - 49.5 |
| 213 | 4/8 | 3/8 | 0/8 | 2.8 | 0.0 - 8.1 |
| 291 | 5/8 | 3/8 | 3/8 | 5.8 | non-calc. |
| 295 | 5/8 | 5/8 | 2/8 | 9.9 | non-calc. |
| 301 | 7/8 | 5/8 | 2/8 | 16.1 | 5.0 - 136.0 |
| 355 | 6/8 | 7/8 | 3/8 | 41.5 | non-calc. |
| 370 | 8/8 | 6/8 | 3/8 | 30.0 | 12.5 - 273.8 |
| 416 | 7/8 | 6/8 | 5/8 | 164.8 | non-calc. |
| 447 | 7/8 | 5/8 | 5/8 | 112.4 | non-calc. |
| 536 | 6/8 | 5/8 | 2/8 | 13.2 | 0.7 - 5,817,751 |

Table 8. Results of potency tests on batches of type C (Noville) vaccine for the U.K. Strategic Reserve.

| Batch No. | No. of cattle protected 1:2 | 1:10 | 1:50 | $PD_{50}$/dose | Confidence Limits |
|---|---|---|---|---|---|
| 28 | 4/4 | 4/4 | 1/3 | 43 | non-calc. |
| 43 | 8/8 | 8/8 | 8/8 | >112 | non-calc. |
| 44 | 8/8 | 7/8 | 3/8 | 35.2 | 15.7 - 278.6 |
| 202 | 4/4 | 4/4 | 1/3 | 43 | non-calc. |
| 233 | 5/8 | 0/8 | 1/8 | 2.1 | 0.0 - 6.5 |
| 240 | 8/8 | 8/8 | 8/8 | >112 | non-calc. |
| 292 | 8/8 | 7/8 | 7/8 | >112 | non-calc. |
| 324 | 8/8 | 7/8 | 6/8 | 173.9 | non-calc. |
| 358 | 8/8 | 8/8 | 7/8 | 102.9 | non-calc. |
| 388 | 7/8 | 8/8 | 7/8 | >112 | non-calc. |
| 431 | 7/8 | 5/8 | 5/8 | 112.4 | non-calc. |
| 446 | 8/8 | 8/8 | 8/8 | >112 | non-calc. |
| 515 | 8/8 | 8/8 | 7/8 | 102.9 | non-calc. |

Table 9. Results of potency tests on batches of type A (Pando, 49-122, Cruzeiro 286-517) vaccine for the U.K. Strategic Reserve.

| Batch No. | No. of cattle protected 1:2 | 1:10 | 1:50 | $PD_{50}$/dose | Confidence Limits |
|---|---|---|---|---|---|
| 49 | 8/8 | 8/8 | 8/8 | ≥112 | non-calc. |
| 77 | 7/8 | 8/8 | 7/8 | ≥112 | non-calc. |
| 116 | 8/8 | 8/8 | 4/8 | 50 | non-calc. |
| 122 | 6/8 | 7/8 | 3/8 | 41.5 | non-calc. |
| 286 | 6/8 | 2/8 | 0/8 | 4.3 | 1.4 - 9.7 |
| 299 | 8/8 | 8/8 | 6/8 | 71.5 | non-calc. |
| 319 | 7/8 | 7/8 | 7/8 | >112? | non-calc. |
| 365 | 8/8 | 8/8 | 6/8 | 71.5 | non-calc. |
| 375 | 7/8 | 8/8 | 5/8 | 284.6 | non-calc. |
| 432 | 8/8 | 7/8 | 8/8 | ≥112 | non-calc. |
| 452 | 8/8 | 7/8 | 7/8 | ≥112 | non-calc. |
| 517 | 8/8 | 8/8 | 8/8 | ≥112 | non-calc. |

Table 10. Neutralizing antibody titres of cattle with an FMD lesion on a single foot after i/d tongue challenge.

Type O Vaccine

| Batch No. | Vaccine Dilution | Ab titre 21 days p.v. | Affected Limb | $PD_{50}$ |
|---|---|---|---|---|
| 69 | 1:2 | 2.71 | front | 174 |
| 447 | 1:2 | 2.1 | hind ) | |
| | 1:2 | 1.95 | hind ) | |
| | 1:10 | 2.95 | hind ) | 112 |
| | 1:50 | 1.95 | hind ) | |
| | 1:50 | 1.95 | hind ) | |
| | 1:50 | 1.80 | hind ) | |
| | 1:50 | 2.10 | hind ) | |

Table 11. Cattle showing lesions on a single foot after challenge by intradermal tongue inoculation.

Type O Vaccines

| Tests involved | Total No. Cattle | No. cattle 1 foot only | % | Vaccine dilutions 1:2 | 1:10 | 1:50 | Limb affected front | hind |
|---|---|---|---|---|---|---|---|---|
| 14/19 | 330 | 29 | 8.7 | 14 | 8 | 7 | 9 | 20 |

Type A Vaccines

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 6/12 | 144 | 8 | 5.5 | 2 | 3 | 3 | 5 | 3 |

Type C Vaccines

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 6/13 | 132 | 9 | 7.3 | 3 | 1 | 5 | 6 | 3 |

Appendix 3

# POTENCY TESTING OF FMD VACCINES BY K-INDEX METHOD

G. Dannacher and M. Fedida*

Many methods for potency testing foot-and-mouth disease vaccines have been proposed. Generally speaking these methods may be divided into two groups.

The first group includes methods in which vaccinated animals are tested for serum antibody determination by an appropriate serological test. For this particular purpose sera-neutralization is the most useful test. Antibody measurement is generally performed on cattle but other species (chicken, guinea pig, mouse) have been used in some particular testing methods. These methods are quantitative and less expensive but results must be correlated with those obtained by a method involving a challenge on naturally sensitive species.

The methods of the second group consist in vaccinating animals followed by a subsequent challenge with a virus fully pathogenic for the species concerned. Animals which do not present any local lesion at the inoculation point or any generalisation lesion are considered as protected. These methods are very expensive and therefore cheap laboratory animals are often used instead of bovines on the condition that results are correlated with those obtained on cattle which should always serve as a reference. The results of a challenge method may be expressed as a number on protected animals against number of challenged animals but some methods propose a quantitative determination of the challenge: 50 percent protective dose ($PD_{50}$) or K index.

The K index method was first described by LUCAM and FEDIDA (2, 4, 7) and is based on the observation that the higher the level of immunity of cattle, the bigger the dose of intradermolingually inoculated virus they can withstand without developing a primary lesion. In practice the result is obtained by a comparative titration of FMD virus on control and on vaccinated animals.

## Material and methods

1. Bovines

Three to eight year old cattle originating from the Department of Finistere (in which bovines are not vaccinated against FMD) are checked for absence of sero-neutralization antibodies. For each type of virus four controls and four vaccinated animals are used.

2. Vaccination

Four bovines are vaccinated according to the dose and the route proposed by the producer.

3. Challenge virus

Viruses required by the test are produced *in vivo* by intradermolingual route on cattle and titers must be at least $10^7$ $ID_{50}$/gramme. The same virus dilutions are used on vaccinated and control animals.

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4. Titration on control cattle

Titration is made according to the classical method of HENDERSON on four bovines using the virus dilutions from $10^{-4}$ to $10^{-7}$ and results of titration are obtained after 24 hours.

5. Titration on vaccinated cattle

The same titration is performed on vaccinated cattle three weeks after vaccination using virus dilutions from $10^{-3}$ to $10^{-6}$ and results of titration are obtained after 40 hours.

6. K index calculation

Titers on control and vaccinated cattle are calculated according to REED and MUENCH or SPRMAN-KAERBEN procedures and expressed in $ID_{50}$/0.1 ml. The mean virus infectivity titre T given by the ontrol cattle is compared with the mean virus infectivity titre V given by vaccinated cattle such as:

$$\log K \text{ index} = \log. T - \log. V$$

A vaccine is considered as passing the test when log. Index K is equal or superior to 1.2.

Results

1. Relevance of the test

At the period of the initial development of K index method it became evident that vaccines giving a log. K value between 0.6 and 1.2 were satisfactory in the field conditions provided by an FMD enzootic situation. A large scale experiment including more than 2,000 bovines permitted a more exact definition of the relationship between K index and the percentage of protection against podal lesions (2, 7). It could be shown that K index value of 1.2 corresponded to an 85 percent protection against generalisation.

2. Potency testing of vaccines

K index method has been used in France since 1960 for testing of FMD vaccines and in 1965 K index was proposed by the Control Authorities as the official method of testing for every batch of vaccine produced or imported into the country (5). At the same time C index method (challenge on guinea-pig) and S index method (sero-neutralization on bovine sera) were introduced in the official testing as screening methods (2.7).

3. Experimental applications of K index method

A. Negative or "hypersensibility" phase during FMD immunization

In the first period of FMD vaccine utilization it was sometimes considered that establishment of immunity was preceded by a negative phase during which vaccinated animals might be more sensitive to FMD virus. An experiment using K index showed that such a negative phase did not exist.

B. Time of establishment of immunity

Further experiments involving K index method demonstrated that immunity on vaccinated cattle appears between 7 and 20 days after vaccination according to the potency of vaccine, that is seven days for vaccines giving a K value of three or more, and 20 days for vaccines giving a K value of 1.5 or 2 (2).

C. Duration of cattle immunity

K index experiments were often used to establish the duration of immunity due to FMD vaccines (2, 6, 7). It could be shown that vaccines with K index of three furnished an immunity superior to

1.2 (K index) during three to five months while duration of immunity of vaccines with a K index of 1.2 did not exceed one month.

D. K index in subtype immunological studies

Homologous and heterologous K index challenge using three groups of bovines vaccinated with serial dilutions of vaccine have been carried out in different circumstances of isolation of viruses antigenically different from those present in the current French vaccine (1, 3). For example:

01 Lausanne versus $O_2$ Flandre
A24 Cruzeiro versus A Pando
A Greece 1969 versus A Allier
C Belgium 1969 versus C Loupoigne
A Greece 1972 versus A Allier
A Spain 1973 versus A Allier
A Morocco 1977 versus A Allier

In most cases vaccine against the new subtype was prepared to allow homologous and heterologous K index comparison of animals immunized with the new subtype.

In general, the antigenic differences demonstrated by serological cross reactions were confirmed by immunological results obtained in cattle and animals primovaccinated with usual French vaccines do not withstand a challenge with these new subtypes. In contrast, cattle vaccinated with two or more injections of usual vaccine perfectly withstand the new subtypes for a certain time after the last injection.

E. Other experiments

K index method has been used in other research points for example duration of storage of vaccine and development and assay of new type FMD vaccines.

Summary

K index determination is based on the tongue infectivity titration method and is defined as the difference between mean log., infectivity titre for fully susceptible control cattle, and for cattle previously vaccinated with the vaccine to be tested. The main advantage of the test is the need for only four cattle per subtype instead of 15 in the case of $PD_{50}$ method. Every batch of vaccine produced in France is submitted to official control of potency including if necessary K index method. The mean value of vaccines reaches K = 2.5.

In spite of the fact that only four cattle are involved in the test, K index method has proved to be very useful in experiments concerning bovine post-vaccination immunity and in immunological studies required by appearance of new subtypes.

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Appendix 4

# CORRELATION BETWEEN ANTIBODY LEVEL AND RESPONSE TO CHALLENGE IN FMD VACCINATED CATTLE RESULTS FOR "A" AND "C" TYPES

M. Amadori, S. Barei, M. Bugnetti, G.F. Panina*

The correlation between neutralizing antibody level and resistance to challenge with FMDV has been stressed by many research workers (1, 5, 7, 8, 9, 10); titration of serum-neutralizing or serum-protecting antibodies in sera of vaccinated cattle which might be employed to assess vaccine potency and efforts are under way to standardize the system.

In Italy, where compulsory annual vaccination of cattle is enforced, vaccine potency is checked by determining in cattle the number of $Pb_{50}$ and no modification of the present regulations is foreseen in the next future.

It has been possible to draw a few correlation data between antibody level and resistance to challenge from the results of serum neutralization tests in suckling mice, routinely carried out on sera of vaccinated cattle taken just prior to challenge, i.e. 20-21 days post vaccination in State Official potency tests. At the 1984 meeting of the Research Group, the results for "O" type were presented (2); the results for "A" and "C" types, are now given referring to the same batches of trivalent vaccine.

## Methods

Three groups of five FMD-seronegative cattle per virus type were vaccinated with a 5 ml dose of trivalent vaccine (O, A, C, Al-hydrogel + saponin), that was undiluted, diluted 1:4 and 1:16 in carbonate buffer, respectively. Twenty-one days later the animals were challenged intradermolingually with $10^4$ $ID_{50}$ of the homologous viruses, $A_5$ Parma '62 and $C_1$ Brescia '64. The number of $Pb_{50}$ derived from the number of animals against generalized FMD inside the groups, according to Litchfield's method and assuming that at a vaccine dilution 1:64 none would be protected. At about 20 days post-vaccination, serum samples were taken from all animals (unvaccinated controls included); they were inactivated at 56°C for 30' and frozen at - 30°C. A few weeks later they were used in serum neutralization tests in suckling mice, aged 3-5 days, against the homologous viruses. Sera were diluted in phosphate buffer M/90 in $\log_4$ scale. Each serum dilution (1 ml) was reacted with virus (500 $LD_{50}$/0.1 ml) in a 2 ml final volume at 37° for 1 hour. The serum-virus mixtures were then immediately chilled in an ice bath and a 0.1 ml dose of each was injected intraperitoneally into 7 suckling mice. The mortality inside the groups was scored during seven days and serum titres were calculated according to Reed and Muench. Titres were expressed as log 10 serum dilution.

## Results

In 12 potency tests in cattle the average number of observed $Pb_{50}$ was 20.17 for "A" type, 26.64 for "C" type and 19.24 (2) for "O" type. The difference between $Pb_{50}$ averages of "C" and "O" types is significant

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($P < 0.05$). The difference between $Pb_{50}$ averages of "C" and "A" types is signficant as well ($P < 0.05$).

The averages of serum neutralizing (SN) titres of 1/1, 1/4 and 1/16 dose injected cattle are listed in Table 1. As could be seen for "0" type (2), there is no significant difference between the averages of 1/1 and 1/4 dose injected cattle, both for "A" and "C" types. The mean difference between SN averages of 1/1 and 1/16 dose injected cattle is equal to 0.98 $\log_{10}$ for "A" type, with a high standard error of the mean difference (0.13 $\log_{10}$). The same difference is equal to 1.06 $\log_{10}$ for "C" type and standard error is even higher (0.21 $\log_{10}$).

No antigen dilution slope can be fitted to the above-mentioned data. However, a good correlation can be found between averages of SN titres of 1/1 dose injected cattle and $\log_{10} Pb_{50}$ for "A" type; by regression analysis we worked out the correspondent slope (see figure 1). The mean standard deviation of these SN averages is equal to 0.52 $\log_{10}$; 95 percent double-sided confidence limits for groups of five animals each are therefore equal to $\pm$ 0.65 $\log_{10}$ (see figure 1).

The same does not hold true for "C" type and, on the contrary, the averages of SN titres of 1/1 dose injected cattle and $\log_{10} Pb_{50}$ turn out to be inversely proportional dimensions; the correspondent slope is shown in figure 2. The mean standard deviation of these SN averages is equal to 0.54 $\log_{10}$; 95% double-sided confidence limits for groups of five animals each are therefore equal to $\pm$ 0.67 $\log_{10}$. However, $\log_{10} Pb_{50}$ is well related to percent degree of homology between SN averages of 1/1 and 1/16 dose injected cattle. Coefficient "r" of correlation is equal to about 0.85 (see figure 3).

These results can be better evaluated on the basis of the 146 S contents we routinely check in virus intended for vaccine production.

Mean 146 S contents from 1975 to 1983 per dose of trivalent vaccine were the following:

"0" type : ,ug 4.079
"A" type : ,ug 6.733
"C" type : ,ug 3.177

The average numbers of $Pb_{50}$ from 1975 to 1983 were the following:

"0" type : 18.05
"A" type : 21.62
"C" type : 27.82

From the above-mentioned data, an "immunogenicity index" can be derived, i.e. the reciprocal of the 146 S/$Pb_{50}$ ration of each virus type; results are listed below:

"0" type : 4.42
"A" type : 3.21
"C" type : 8.77

Discussion

The results we report herewith confirm and widen the present knowledge about the main efficacy parameters of FMD vaccines.

As for antibody titration, a lower response variability of cattle is observed for "A" and "C" types in comparison with "0" type; confidence limits of SN averages are anyway in the range we had previously reported (3). The variability of antibody response can be partly due to the "mouse" system used. By plaque titrating (1), confidence limits of SN averages would be actually narrower.

Our results confirm the minimal difference of SN response between undiluted and 1/4 diluted vaccines, at 20 days post vaccination, in the presence of vaccines of good efficacy.

The high standard error of the mean difference between SN averages of 1/1 and 1/16 dose injected cattle is probably due to considerable differences of immunizing 146 S contents in vaccines. In fact, our vaccines are not formulated on the basis of a fixed 146 S quantity; the main parameter in the formulation is virus infectivity for suckling mice, which is well related in our experience to immunogenicity in cattle (6). The "mouse system" could in fact reveal differences in the concentration of 146 S particles with a degraded protein coat, which normally increases in virus preparation as time elapses after the infection (4-6).

Results also confirm the different degree of immunogenicity of our vaccine strains, in good agreement with a previous report of ours (6). Thus, the correlation between average SN titre of 1/1 dose injected cattle and $\log_{10} Pb_{50}$ turns out to be inversely proportional to virus immunogenicity; in fact, coefficients "r" of correlation are equal to 0.50, 0.77 and -0.86 for "0", "A" and "C" types, respectively.

Double-sided, 95% confidence limits of these SN averages include all results of potency tests (see figures 1 and 2). The level of SN antibodies corresponding to 50% protection ($\log_{10} Pb_{50} = 0$) is in good agreement with previously reported data for "A" type (7).

Results for "C" type are particularly interesting; their noteworthy uncommon features have not yet been described to our knowledge. A suppressor effect of large amounts of 146 S antigen in trivalent vaccines could be envisaged on the basis of two remarks.

- the inverse relationship between average SN titre of 1/1 dose injected cattle and $\log_{10} Pb_{50}$;
- the good correlation between homology of SN titres of 1/1 and 1/16 dose injected cattle and $\log_{10} Pb_{50}$.

This suppressor effect could imply some consequences on resistance to challenge a few months post vaccination, as could be inferred from our previous experiments (3), even if a lower level of SN antibodies is required to resist challenge with FMDV type "c" (7).

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Table 1

| Batch | $Pb_{50}$ | Average $log_{10}$ SN 1/1 dose | SD $log_{10}$ SN 1/1 dose | Average $log_{10}$ SN 1/4 dose | SD $log_{10}$ SN 1/4 dose | Average $log_{10}$ SN 1/16 dose | SD $log_{10}$ SN 1/16 dose |
|---|---|---|---|---|---|---|---|
| 21 | A 7.9 | 2.748 | 0.608 | 2.136 | 1.045 | 1.333 | 0.715 |
| | C 13.7 | 4.344 | 0.425 | 3.244 | 0.633 | 2.402 | 0.621 |
| 26 | A 14.4 | 2.912 | 0.455 | 2.298 | 0.379 | 1.622 | 0.444 |
| | C 32 | - | - | - | - | - | - |
| 28 | A 13.7 | 2.932 | 0.357 | 3.327 | 0.578 | 1.938 | 0.207 |
| | C 32 | 3.024 | 0.581 | 2.720 | 0.715 | 2.674 | 0.517 |
| 30 | A 18 | 3.687 | 0.283 | 3.447 | 0.444 | 2.553 | 0.774 |
| | C 32 | 3.156 | 0.653 | 2.569 | 0.504 | 2.432 | 1.039 |
| 31 | A 13.7 | 2.792 | 0.379 | 3.144 | 0.217 | 1.916 | 0.673 |
| | C 23 | 3.482 | 0.324 | 3.352 | 0.498 | 1.958 | 0.234 |
| 35 | A 32 | 3.533 | 0.683 | 2.863 | 0.683 | 3.086 | 0.663 |
| | C 32 | 3.516 | 0.495 | 3.516 | 0.262 | 2.571 | 0.591 |
| 36 | A 23 | 3.474 | 0.493 | 3.128 | 0.580 | 2.299 | 0.626 |
| | C 18 | 3.584 | 0.365 | 2.897 | 0.427 | 1.988 | 0.618 |
| 38 | A 32 | 3.265 | 0.626 | 3.774 | 0.708 | 3.342 | 0.888 |
| | C 32 | 3.014 | 0.927 | 3.619 | 0.382 | 2.696 | 0.358 |
| 39 | A 14.4 | 2.898 | 0.896 | 2.735 | 0.396 | 1.502 | 0.900 |
| | C 18 | 3.603 | 0.387 | 2.868 | 0.481 | 1.600 | 0.383 |
| 40 | A 23 | 3.378 | 0.400 | 2.846 | 0.254 | 2.828 | 0.853 |
| | C 23 | 3.877 | 0.213 | 3.499 | 0.386 | 2.158 | 0.763 |
| 41 | A 18 | 3.516 | 0.849 | 2.602 | 0.249 | 2.259 | 0.802 |
| | C 32 | 3.071 | 0.749 | 3.157 | 0.609 | 2.880 | 0.407 |
| 42 | A 32 | 3.911 | 0.191 | 3.152 | 0.459 | 2.611 | 0.511 |
| | C 32 | 3.104 | 0.838 | 3.395 | 0.459 | 2.709 | 0.297 |

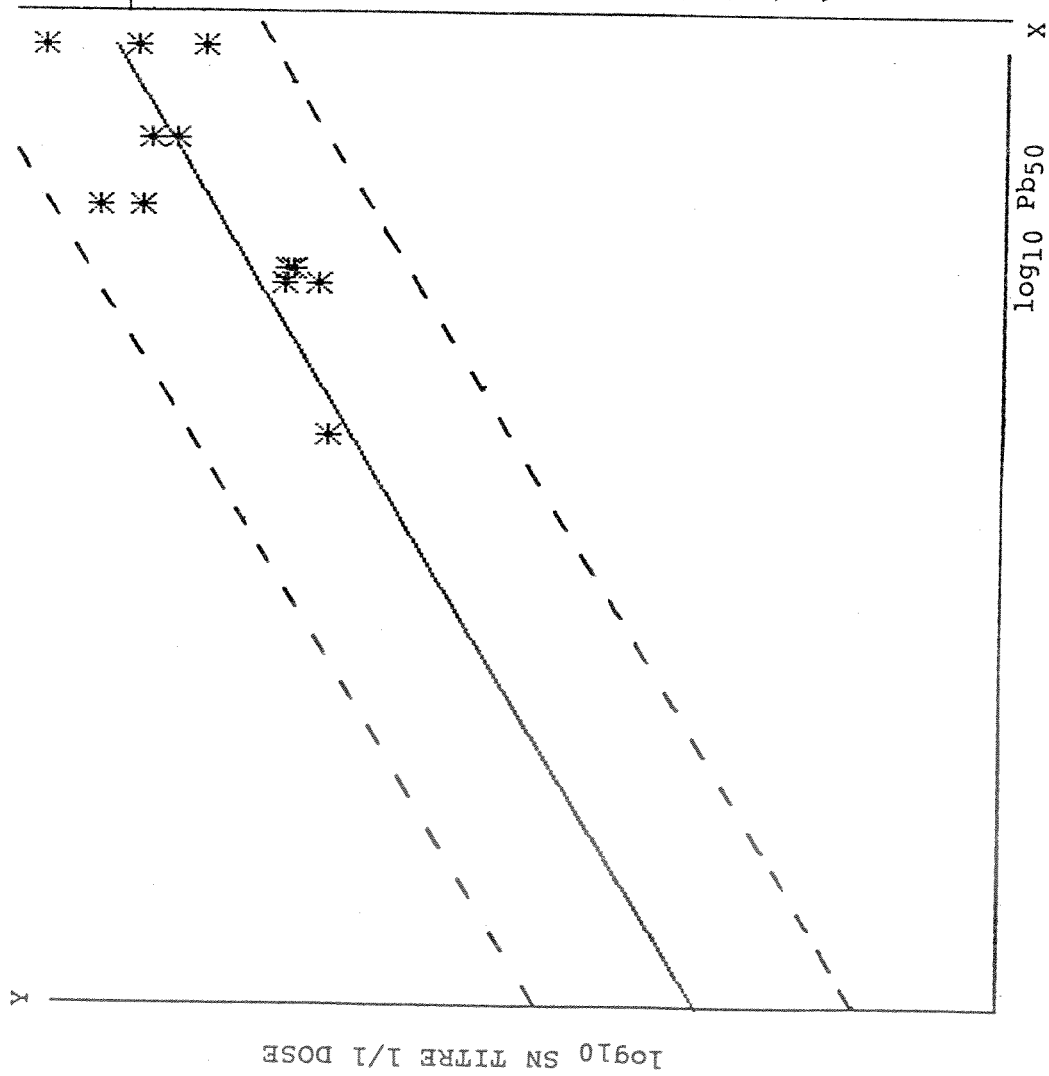
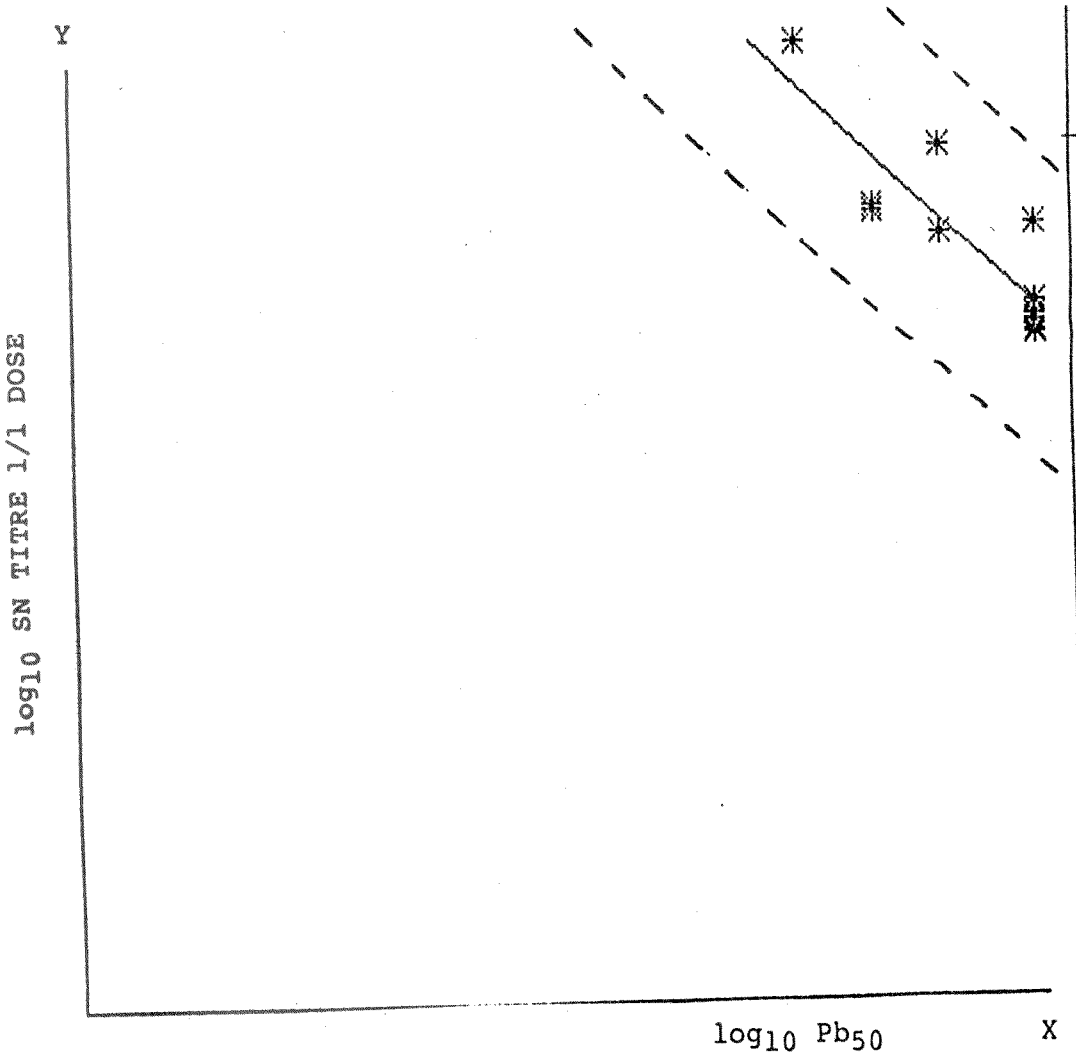



**Figure 1.** Calculation of $\log_{10} Pb_{50}$ from $\log_{10}$ SN titre of 1/1 dose injected cattle. "A" type.

**Figure 2**. Calculation of $\log_{10}$ $Pb_{50}$ from $\log_{10}$ SN titre of 1/1 dose injected cattle. "C" type.




$Y = -2.68691x + 7.19527$
$\pm$ 0.67

| | | |
|---|---|---|
| X (y=0) | = | 2.67790 |
| Xm | = | 1.399818 |
| SDx | = | 0.134512 |
| Ym | = | 3.434091 |
| SDy | = | 0.417956 |
| r CORRELAT. | = | -0.8647264 |
| t on cor. | = | 5.165186 |
| D. Freedom | = | 9 |
| p < 0.001 | | |
| E.E.S. | = | 0.209916 |
| V res. | = | 0.048961 |
| V (Ym) | = | 0.004451 |
| V (m) | = | 0.270604 |
| V (q) | = | 0.534696 |

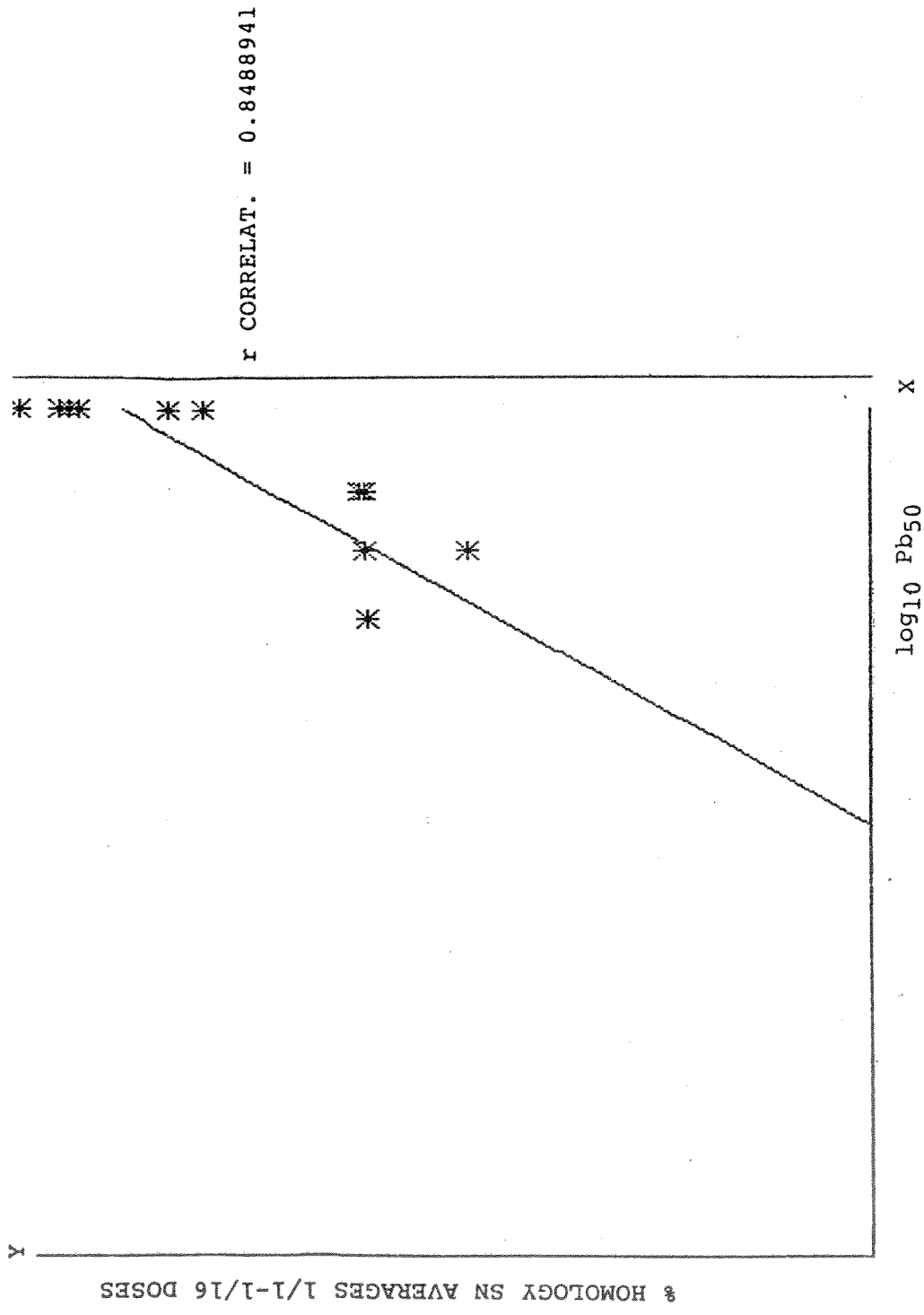



Figure 3. Correlation between % degree of homology of SN averages of 1/1 and 1/16 dose injected cattle and $\log_{10} Pb_{50}$. "C" type.

Appendix 5

# COMPARISON OF INDIVIDUAL AND POOLED SERA TECHNIQUES FOR THE CONTROL OF FOOD-AND-MOUTH DISEASE VACCINE

R. Strobbe*

## Introduction

The antibody response induced in cattle by foot-and-mouth disease vaccination is currently evaluated by seroneutralization in microtiter plates. Individual titration of sera and averaging of the titers is the usual method for vaccine control in test cattle. Titration of pooled sera is routinely used to monitor the antibody level in entire herds. Titrating pooled sera instead of averaging individual sera titers saves time and material, even for small groups of cattle. But it has been reported that the results obtained by these two methods were different.

In the present experiment, small groups of cattle were vaccinated, each with a separate vaccine lot, and the sera were titrated comparatively by the individual and the pooled sera procedures. The seroneutralization titers were calculated by three different modes. The variation within each test, and the variation and correlation between titers, depending on the titer calculation mode, were determined.

## Materials and Methods

### Vaccines

Thirteen trivalent O1-A5-C1 foot-and-mouth disease (FMD) vaccines were tested each on three Irish cattle. Each animal received two doses of vaccine subcutaneously, and one dose intradermolingually; as for typical vaccine safety testing. Blood samples for serological examination were taken at slaughter, ten days after vaccination.

### Seroneutralization

The serum antibody levels were assessed by the classical microneutralization method. Sera of each three vaccinated with the same vaccine, were titrated individually and after pooling. The sera were first diluted 1:2, and further 1:3 up to 1:4374. Each serum dilution was distributed in six wells. Specific O, A or C virus (100 ID50) was added to each well. Neutralization was allowed to take place one hour at 37°C. A SK6 cell suspension was added to reveal non-neutralized virus. The plates were examined daily under the microscope, for CPE, up to the stabilization of the neutralization end-point. Per test, there were 18 neutralization end-points for individual sera (I) and six for pooled sera (P). Per vaccine there were three P and three I tests, one for each virus type.

### Calculations

The vaccine seroneutralization (SN) titer per virus type and per serological technique, was calculted in three ways:

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a. Mean of the serum dilution factors
The neutralization end-points were expressed as serum dilution factors. The data were averaged per test. The mean serum dilution factor (m) of each test was considered as the vaccine titer.

b. Exponent of the mean
The mean of the serum dilution factors calculated in (a) was transformed in exponent of base 3. The exponent was considered as the vaccine SN titer.

c. Mean of the exponents
By this method, each of the neutralization end-points was transformed in exponent base 3. The mean of the 18 or 6 exponents was considered to represent the vaccine titer.

Statistics

Means, standard deviations and variation coefficients were computed for each of the titer calculation modes. The dispersion within test and the distribution of the titers in the vaccine series were expressed as variation coefficient. Correlation between paired data was expessed as r factor.

Results

The 13 FMD vaccine lots were administered each to three cattle. The induced neutralizing antibodies were titrated against O, A and C type virus, in the 13 pools and the 39 individual sera. Each vaccine was characterized, for each of the three virus types, by six neutralization end-points in the P tests, and by 18 points in the I tests. Of the total of 936 end-points, 234 were in P tests, and 702 in I tests.

The dispersion of the neutralization end-points within each test was of 26 percent on average, by the mean exponent (m(e)) calculation mode. By the arithmetical method, the average variation coefficient was about 70 percent. Dispersion was significantly lower in pooled sera tests than in individual sera tests (Table 1).

The vaccine titers by the pooled sera technique were on average four percent higher than the individual sera vaccine titers. The distribution shape of the vaccine titer series was narrower for pooled as for individual sera vaccine titers. Vaccine titers by the pooled sera technique were not identical to the individual sera titers, and the correlation varied from 0.08 to 0.76. But when computed on larger numbers of data, the correlation was of 0.60 to 0.73 (Table 2).

Comparison of the calculation modes showed that expressing the neutralization end-points as exponents reduced significantly the within test variation coefficient (Table 1).

The distribution shape of the vaccine titers was also influenced by the calculation method. Low and high titers were less different when exponents were used in the calculation (Table 2).

Ratio between pooled and individual sera vaccine titers was not much dependent of the calculation method. But correlation of paired vaccine titers was increased by the mean of the exponents (m(e)) method (Table 2).

The figures of vaccine titers calculated as serum dilution factors were quite larger than the titers represented by exponents. The ratios between m titers and e(m) or m(e) titers were therefore relatively high (Table 3). The correlation between arithmetical value and the correspondant exponent titer was rather low.

On the contrary, the exponent of the mean and the mean of the exponents titers correlated quite well, with r factors of 0.93 to 0.97. The e(m) method gave vaccine titers about six percent higher than the m(e) method.

Conclusions

a. Serological methods
The vaccine SN titers derived from the pooled sera tests were similar to those from individual sera tests, although not strictly identical. The 0.70 correlation appeared to be the consequence of the relatively high within test variability inherent to such type of techniques, rather than of a principle problem. The main advantage of the pooled sera method was the important reduction in time and material usage.

b. Calculation methods
The mean of the exponents method, most widely used although not sound from a mathematical point of view, provided results quite similar to the exponent of the mean method (e(m)). The use of exponents for the calculation of seroneutralization titers appeared to us as an unnecessary complication. Furthermore, when the base of the exponent is omitted, as is often the case, the comparison of results from different laboratories becomes impossible.

Therefore it seemed to us that the mean serum dilution factor was the easiest and the safest way to express the level of antibodies induced in cattle by a vaccine: the calculation is simple and sound, and there can be no doubt as to the meaning of the titer.

Table 1. - Dispersion of the neutralization end-points within test.

| Calculation method | Virus type | Averaged variation coefficients: Individual sera test n= | v% | Pooled sera test n= | v% |
|---|---|---|---|---|---|
| m | O | 18 | 67% | 6 | 39% |
| | A | 18 | 73% | 6 | 44% |
| | C | 18 | 70% | 6 | 43% |
| m(e) | O | 18 | 23% | 6 | 11% |
| | A | 18 | 30% | 6 | 18% |
| | C | 18 | 25% | 6 | 16% |

n= : number of neutralization end-points per test.
v% : mean variation coefficient in %, on 13 vaccine tests, of the neutralization end-points dispersion.

Table 2. - Comparison of vaccine SN titration by I and P tests.

| Calculation method | Virus type | Vaccine SN titers: n= | v%(I) | v%(P) | P/I | r(P,I) |
|---|---|---|---|---|---|---|
| m | O | 13 | 41% | 34% | 119% | 0.13 |
| | A | 13 | 44% | 36% | 109% | 0.18 |
| | C | 13 | 42% | 57% | 85% | 0.68 |
| | OAC | 39 | 62% | 55% | 104% | 0.60 |
| e(m) | O | 13 | 9% | 9% | 105% | 0.08 |
| | A | 13 | 15% | 14% | 104% | 0.11 |
| | C | 13 | 17% | 14% | 97% | 0.71 |
| | OAC | 39 | 19% | 18% | 102% | 0.70 |
| m(e) | O | 13 | 11% | 11% | 110% | 0.21 |
| | A | 13 | 16% | 16% | 109% | 0.25 |
| | C | 13 | 18% | 14% | 102% | 0.76 |
| | OAC | 39 | 21% | 19% | 107% | 0.73 |

n= : number of vaccine titers per series.
v%(I) : variation coefficient of the titer series (individual sera)
v%(P) : variation coefficient of the titer series (pooled sera)
P/I : ratio of P and I vaccine titers.
r(P,I): correlation factor of paired P and I vaccine titers.

Table 3. - Comparison of the 3 titer calculation modes.

| Virus type | n pairs of data | Compared calculation modes: m/e(m) ratio | m/e(m) r | m/m(e) ratio | m/m(e) r | e(m)/m(e) ratio | e(m)/m(e) r |
|---|---|---|---|---|---|---|---|
| O | 26 | 11.90 | 0.10 | 12.49 | 0.01 | 1.05 | 0.96 |
| A | 26 | 6.11 | 0.21 | 6.53 | 0.28 | 1.07 | 0.95 |
| C | 26 | 10.27 | 0.26 | 10.91 | 0.39 | 1.06 | 0.93 |
| OAC | 78 | 9.74 | 0.18 | 10.33 | 0.08 | 1.06 | 0.97 |

m : vaccine titer is the mean of the neutralization end-points expressed as serum dilution factors.
e(m) : vaccine titer is the exponent base 3 of m titer.
m(e) : vaccine titer is the mean of the neutralization end points expressed as exponents base 3 of the serum dilution factors.
ratio: mean ratio between paired vaccine titers.
r : correlation factor of the paired vaccine titers.

Appendix 6

# FURTHER STUDIES ON THE ASSESSMENT OF POTENCY OF FMD VACCINES BY MEANS OF ANTIBODY ASSAYS WITH SERA FROM VACCINATED CATTLE

R. Ahl and G. Wittmann*

In continuation of our programme to assess the potency of foot-and-mouth disease (FMD) vaccines by means of serum antibody neutralisation tests with sera from groups of primovaccinated cattle (Ahl et al., 1983) data from 30 vaccine batches were analysed. Each batch was tested in parallel in cattle according to the procedure given in the European Pharmacopeia (EP) and indirectly by plaque reduction assays of the individual sera.

The plaque reduction test in BHK-21-CT cells shows a low variation in repeated tests. The standard deviation for individual serum titrations is about 0.05 $\log_{10}$. The overall standard deviation of 0.25 $\log_{10}$ is therefore mainly due to the strong variation of the individual antibody response.

In our preliminary communication the relationship between log serum-titre and log $PD_{50}$ (log Pb) was presented as $\bar{y} = X' + 0.7$, where $\bar{y}$ is the mean antibody titre of a group of 5 animals, X' is log Pb, and 0.7 the log serum titre corresponding to 1 Pb (50% protection, C-value). The data accumulated so far indicated that, whereas this equation gave a good fit with sera from cattle vaccinated with 1:4 diluted vaccine, calculated Pb values from sera of animals vaccinated with undiluted vaccine deviated from direct challenge results. Based on the log difference between mean antibody titres from undiluted and 1:4 diluted vaccine from each batch a modified equation was established which has a slope of 0.73. No significant differences were found among the slopes for subtype $O_1$, $A_5$ and $C_1$.

From direct challenge tests and the calculated slope of 0.73 in the diagram log $SN_{90}$ vs. log Pb the C values were likewise recalculated. The 50% protection estimates for $O_1$, $A_5$ and $C_1$ in cattle vaccinated with undiluted vaccine were 0.75 ± 0.13 log, 0.67 ± 0.20 log, and 0.78 ± 0.18 log, respectively. C-values of sera from animals vaccinated with 1:4 diluted vaccine were 0.74 ± 0.16 log, 0.71 ± 0.23 log, and 0.82 ± 0.17 log, respectively, for $O_1$, $A_5$ and $C_1$. Because these values were not significantly different from each other, a common mean value of C = 0.75 is used, resulting now in the equation $\bar{y} = 0.73\ X' + 0.75$. This means that the mean serum titre of a group of cattle should increase 1.65-fold when the Pb-content of a vaccine is doubled (Figure 1).

The Pb values calculated from serum antibody titres were in fairly good agreement with results from direct challenge. However, the coefficient of correlation was still rather low, mainly because the range of log Pb values is short (from 0.4 to about 1.0 log) and vaccine batches with 8 or more Pb per dose protected all the animals in a potency test thus preventing an endpoint calculation.

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Unprotected animals in the challenge groups tested had, in general, very low serum titres. The geometric mean of these sera was 1:3, corresponding to a theoretical value of 18% protection. The mean serum titre of 1:3 is clearly below the minimum values corresponding to 3 Pb or 70% protection guaranteed required for a batch to pass.

A graphical representation similar to that given by Pay and Hingley (1984) of the relevant data is shown in Figure 2. A mean value of log $SN_{90}$ = 1.1 (1:12.5) is equivalent to 3 Pb for sera from animals vaccinated with undiluted vaccine, and a value of log $SN_{90}$ = 0.66 (1:4.6) for sera from 1:4 vaccinated animals, applying a slope of 2.4 for the relationship between probit of percent protection and log Pb (Steilmann et al., 1977; Terré et al., 1977).

This graph also shows the narrow range between Producer's score (PS) and Consumer's score (CS) for groups of 5 or 10 sera as calculated for trivalent vaccines due to the relatively small standard error. There is only a small difference between Passmark (PM) and Consumer's score.

From the equation $\bar{y}$ = 0.73 x log Pb + 0.75 in the diagram of log $SN_{90}$ vs. log Pb, and the slope b' = 2.4 in the relationship between probit of percent protection vs. log Pb, the equation for the relationship between probit and log $SN_{90}$ can be calculated as P = 3.29 x log $SN_{90}$ + 2.53. This equation indicates that under the conditions of potency testing according to the EP the level of protection in a cattle population is strongly increasing when the serum antibody level is raised (Figure 3). This is in accord with data from Sutmöller and Vieira (1980), but differs from results of Sutmöller et al. (1983) probably because a different cell system had been used in that trial.

Our value for the slope B' = 0.73 corresponds well to those reported earlier by Stellmann et al. (1968). The fact that for all three virus types tested a common equation can be applied indicates that the respective virus strains used in the laboratory tests are not different from those used for vaccine production and direct challenge. Likewise, our cell system proved to be stable now for more than a decade and thus forms a basis to compare data from many years. Nevertheless, the degree of variation in the individual antibody response to vaccination as well as the limited number of animals in potency tests makes it difficult to attach a $SN_{90}$ value to a distinct value of Pb per vaccine dose.

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Fig. 1

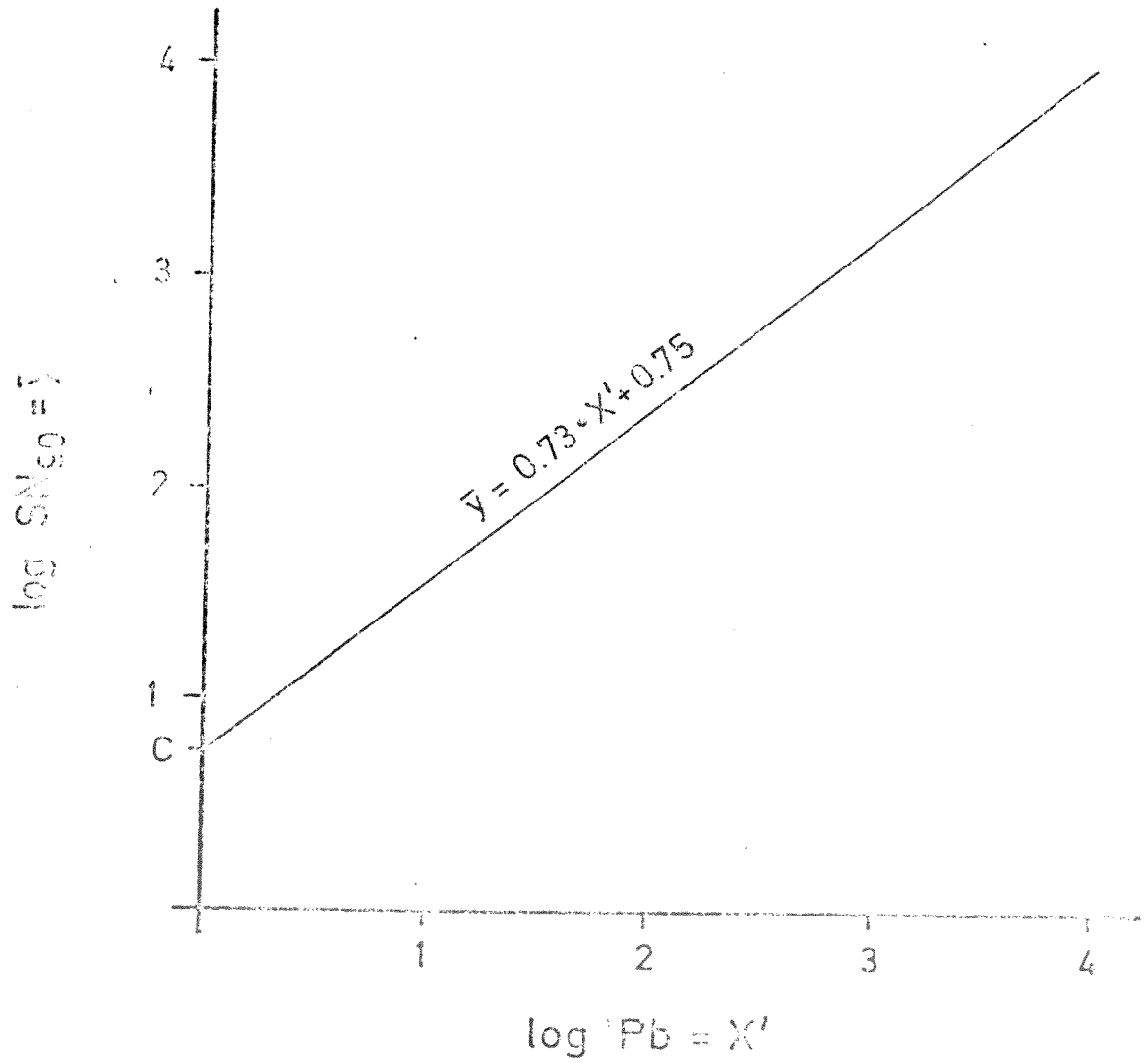


Fig. 2

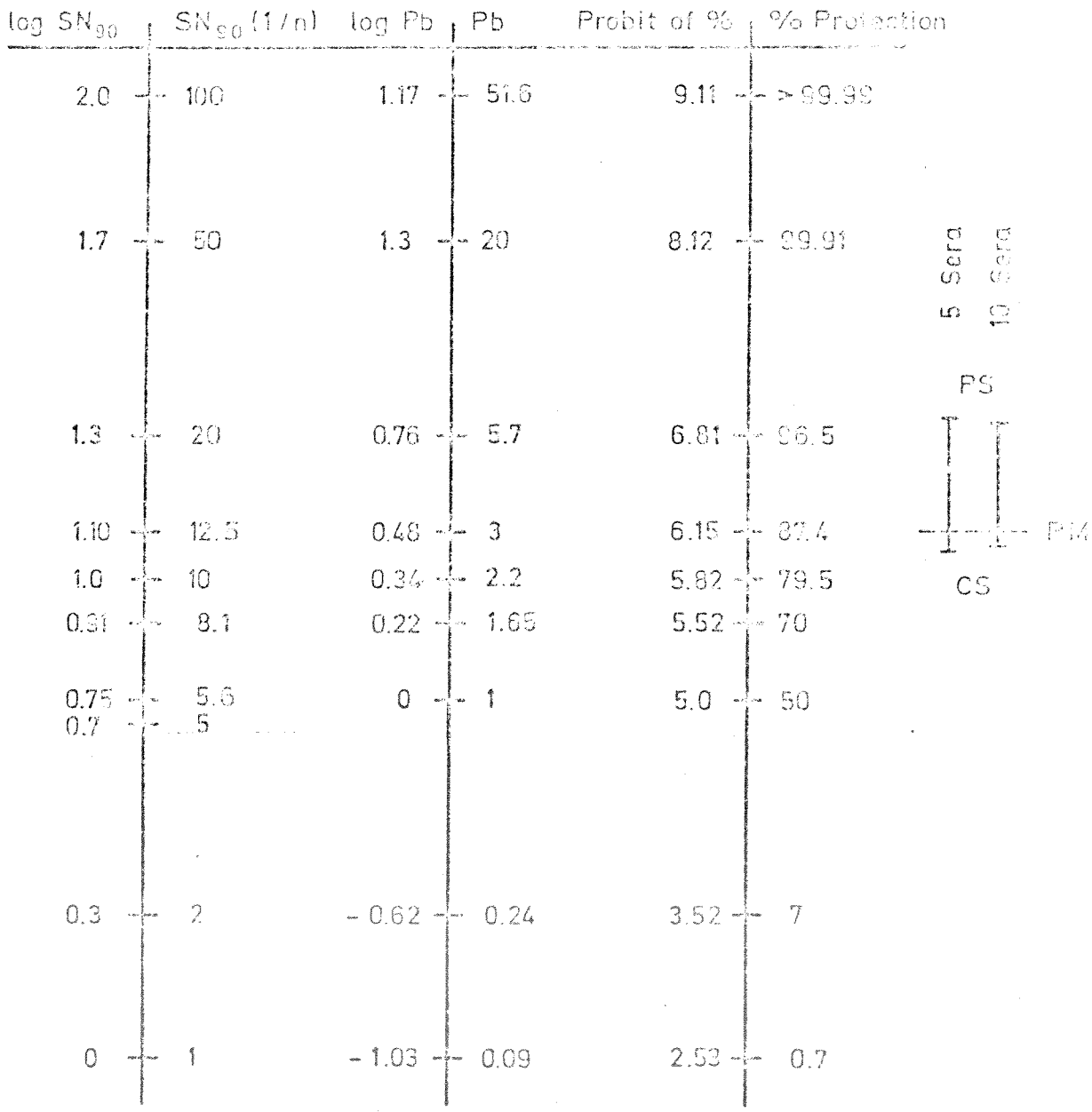
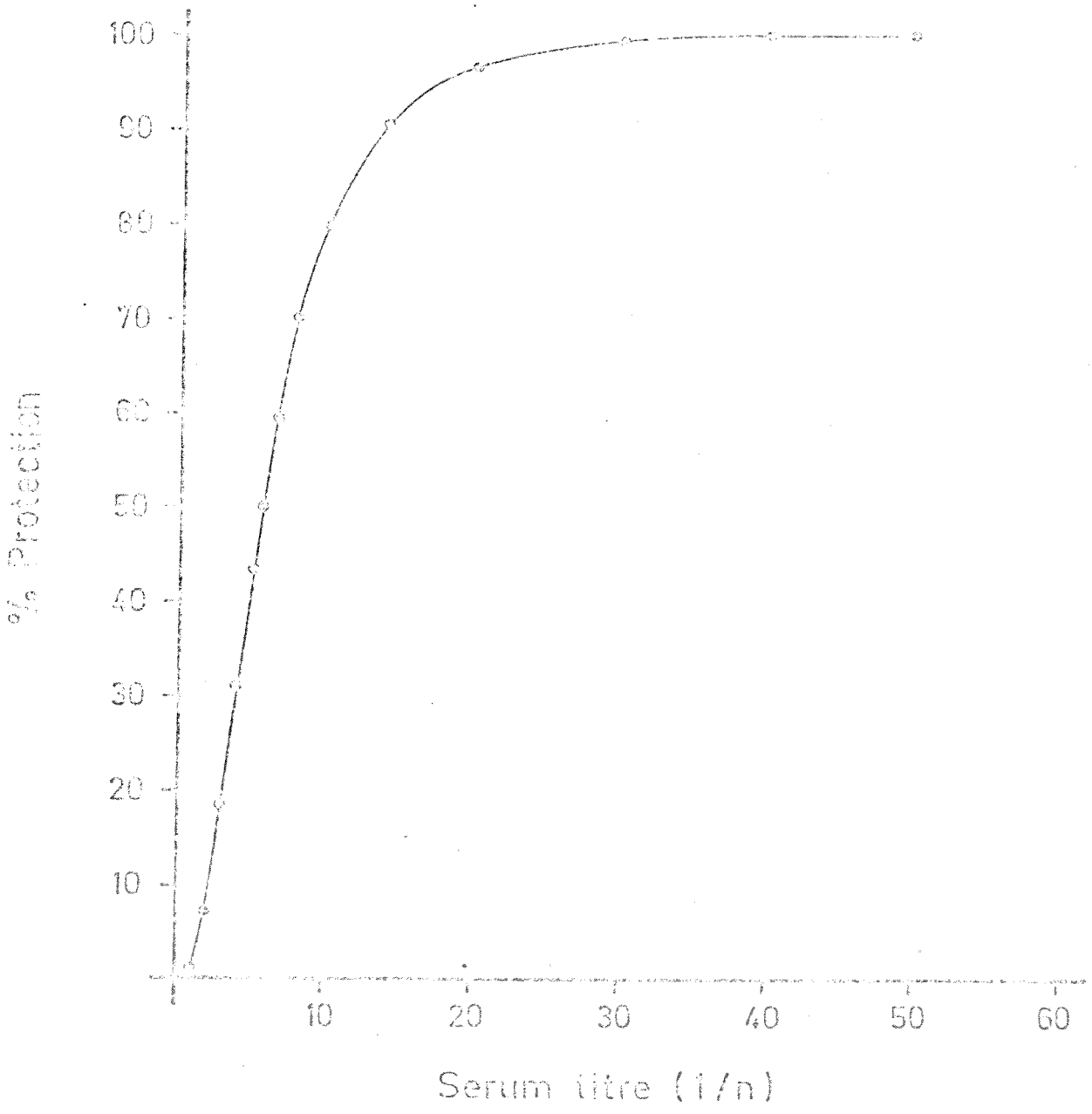

log $SN_{90}$
$SN_{90}$ (1/n)
log Pb
Pb
Probit of %
% Protection
2.0
100
1.17
51.6
9.11
> 99.99
1.7
50
1.3
20
8.12
99.91
1.3
20
0.76
5.7
6.81
96.5
1.10
12.5
0.48
3
6.15
83.4
1.0
10
0.34
2.2
5.82
79.5
0.91
8.1
0.22
1.65
5.52
70
0.75
5.6
0
1
5.0
50
0.7
5
0.3
2
- 0.62
0.24
3.52
7
0
1
- 1.03
0.09
2.53
0.7
5 Serg
10 Serg
PS
PM
CS

Fig. 3


% Protection
100
90
80
70
60
50
40
30
20
10
10
20
30
40
50
60
Serum titre (1/n)

Appendix 7

# PRODUCTION AND QUALITY CONTROL OF FOOT-AND-MOUTH DISEASE VACCINES IN SOUTH AMERICA

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In 1984 South America had a cattle population of 224 million, distributed in approximately 4,245,000 herds occupying an area of about 17 million square kms (4). This enormous population lives almost entirely on the open range. The animals are kept permanently outdoors and they are moved in accordance with the type of exploitation and commercialization.

Foot-and-mouth disease (FMD) is found in various countries of the region. Its frequency varies greatly from area to area, depending on the ecosystem in which it is located.

The economic importance of FMD and the losses it causes have led the governments of most of the South American countries to establish programs aimed to control and eradicate the disease. One of the major methods used to eliminate the disease has been massive immunization of the bovine population. This method has obliged the countries to create an important infrastructure to produce and control the FMD vaccines needed to meet the demands of the programs.

## Inactivated foot-and-mouth disease vaccine

### Antigen

The first FMD vaccine available in South America was prepared in 1948 by Santi Espiritu, a private Argentine laboratory, and in 1949, by the former Miguel C. Rubino Animal Biology Institute, since renamed the Miguel C. Rubino Veterinary Research Center of the Ministry of Agriculture of Uruguay. Both laboratories used the Waldmann method. In 1953 the Foot-and-Mouth Disease Institute of the Venezuelan Ministry of Agriculture and Livestock initiated production by the Waldmann method. And in 1954, the Cooper Laboratory in Uruguay produced the first vaccine by the Frenkel method.

In the 1950's and part of the 1960's the countries produced FMD vaccines according to the Waldmann and Frenkel methods. Brazil was the exception; for most of the 1960's and 70's it prepared a high percentage of vaccines with antigen obtained in newborn rabbits. Also in the 60's some private laboratories in Argentina produced limited quantities of vaccine using antigen obtained from newborn calves.

The 1970's brought large-scale production of antigen in cell cultures, mainly in BHK cell suspensions. About 350 million doses of

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vaccine are now annually prepared from antigen obtained in cell cultures, plus 75 million doses by the Frenkel method.

Inactivants

Several laboratories that use the Frenkel method for antigen production still use formalin as the inactivant.

Most of the laboratories mainly those that produce antigens in cell cultures, utilize first-order inactivants, of which binary ethylenimine (BEI) is the most preferred (3). Brazil and Colombia allow only first-order inactivants, whereas both inactivants are utilized in Argentina, Paraguay and Uruguay.

Adjuvants

The adjuvants most used in the production of the FMD vaccines are aluminum hydroxide (Columbia, Ecuador, Peru) and aluminum hydroxide with saponin (Argentina, Brazil, Paraguay and Uruguay). Approximately 20 million doses of trivalent vaccine are prepared with Freund's incomplete adjuvant as a primary emulsion (2) with regular inactivated antigen or with inactivated antigen concentrated on aluminum hydroxide (1).

Attenuated live virus vaccine (ALVV)

In the 1960's various countries, including South Africa, Brazil, England, Israel, Thailand and Venezuela, dedicated time and efforts into obtaining attenuated live virus FMD vaccines which were used experimentally. However, various problems arose to advise against the use of such vaccines: the difficulty of attenuation without loss of immunogenicity; varying degrees of sensitivity among the susceptible species; pathogenicity in pigs; difficulty in preserving the vaccines; virus neutralization after revaccination, induced by antibodies in previously vaccinated animals; the possibility of reversion to virulence, etc. Moreover, the fact that meat-importing countries objected to receiving meat from regions utilizing this type of vaccine strongly influenced the gradual abandonment of the experiments of FMD virus attenuation. Only Venezuela still utilized that method of vaccine production as a substitute for inactivated vaccine (7).

Needs and situation of foot-and-mouth disease vaccine in South America

The FMD programs in South America covered 184,663,200 cattle in 1984. Therefore, just to maintain this species suitably immunized throughout the year, utilizing aluminum hydroxide-saponin vaccines, would require approximately 550 million doses.

The ecological conditions in South America require the use of vaccines that confer the longest possible immunity. In this regard, the PAFMDC/PAHO have given considerable effort and technical support into developing an oil-adjuvanted vaccine for use in cattle. The use of vaccines providing longer lasting immunity and greater immunogenicity would mean a substantial economy in production, transportation, storage and cooling of vaccines, handling of herds, etc. The expenses of administering the vaccines would likewise be drastically reduced and would facilitate government involvement in special situations that might require official application of the vaccine. It would also lead to a more efficient surveillance, supervision and inspection, resulting in a more effective protection of the population in the program.

The aluminum hydroxide-saponin vaccines presently used in most of the countries must be administered every four months to obtain adequate and sustained immunization of the animal population. The costs of handling the vaccine are high and inspection is expensive and difficult due to the frequent vaccination cycles.

Evolution of the foot-and-mouth disease vaccine quality control in South America

The use of vaccines with bacterial contamination was allowed in the 1950's and part of the 60's provided that such contamination was not caused by proteolytic or pathogenic bacteria. The innocuity tests were not strict. There existed, therefore, the possibility of the presence of active virus that would not become inactivated by the residual action of the formalin, the only inactivant used at that time, during storage of the vaccine at $4^{\circ}C$.

The potency controls in several countries were not very frequent and, in many cases, the immunogenicity of the vaccine was determined by the infectious and complement fixation titers obtained in the antigen suspensions prior to their inactivation.

In the 1970's emphasis was placed on the sterility and innocuity controls, which were complemented in several countries by the structure to conduct direct potency tests in cattle and indirect tests on guinea pigs or by determination of the antibody levels in the sera of vaccinated cattle. In this field important work was done by Chile and Uruguay in the early 1970's.

In the beginning, Argentina, Brazil, Paraguay, Peru and Uruguay adopted the quality-control methodology used in France (6). But later, based on the technical cooperation provided through the PAFMDC/PAHO, on their own observations, and on the requirements of the European standards, several countries gradually relinquished the Lucam test in favour of the 50 percent bovine protective dose ($BPD_{50}$), the protection against generalized foot lesions (PG), the 50 percent guinea pig protective dose ($GPPD_{50}$) and the serum protection test conducted with suckling mice (mouse protection test - MPT).

The direct tests in bovines ($BPD_{50}$ and PG) and the MPT were gradually expanded as the countries, lacking FMD-free areas from which to get susceptible cattle, created better systems for the selection of cattle coming from herds protected and free of FMD. Calves that had never been vaccinated were identified and selected in these herds. It had been previously established that the immunological response of such animals was comparable to that of cattle from disease-free areas. This fact was again demonstrated with $BPD_{50}$ tests conducted in Brazil and Colombia, using such selected animals. Argentina used bovines from Patagonia, an area free of foot-and-mouth disease.

Some countries have used indirect testing in guinea pigs (C index or $GPPD_{50}$) or the mouse protection and serum neutralization tests as alternative for the direct control tests using challenge in cattle.

A tone correlation between the direct test in cattle and the test in guinea pigs has never been determined. Generally, the vaccines rejected in guinea pigs are also rejected in cattle. However, a certain percentage of vaccines approved in guinea pigs are rejected when tested in cattle. The guinea pig test could therefore perhaps be useful when used as part of a screening process.

A good correlation has been found between the levels of circulating antibodies of vaccinated cattle and the results of virus challenge tests in cattle when the mouse protection or serum neutralization tests are used.

The expected percentage of protection (EPP) values were determined from tests conducted by the Pan American Foot-and-Mouth Disease Center in 1975, comparing circulating antibody levels and virus challenge results in more than 700 cattle. The EPP represent the percentage of probability that a vaccinated animal will be protected against infection with a given level of antibodies (5). In 1983 the EPPs and the results of virus challenge in groups of vaccinated cattle were compared. Considering the standard error of the mean of the EPP, the limits of confidence were defined and the test made more accurate (8).

Present situation of the quality control of foot-and-mouth disease vaccine

The quality control of FMD vaccines in Argentina, Brazil, Colombia, Paraguay and Uruguay is conducted by the official control laboratories, which are independent of the vaccine producing laboratories.

Quality control includes determination of sterility, innocuity and potency of all the vaccines produced. These controls are performed on each series of vaccine after it is completely bottled. Random samples are selected for testing by the official control services.

The vaccine is released after tests confirm that it is both sterile and innocuous and gives the protection indicated by the control services. This protection is measured by direct or indirect challenge against the strains homologous to those used in producing the vaccine.

1. Protection against generalized foot lesions (PG)

Sixteen cattle are vaccinated. At 20 to 31 days post-vaccination (DPV) they are challenged by intradermolingual inoculation of $10^{4,0}ID_{50}$. The vaccine is released for use if at least 12 animals are protected against generalized foot lesions.

2. 50 percent bovine protection dose ($BPD_{50}$)

A pure vaccine and vaccine diluted 1:3 and 1:9 in an inert diluent is normally used. Five cattle each are vaccinated with the pure vaccine and with the dilutions used. At 20-30 DPV each of the animals is challenged by intradermolingual inoculation of $10^{4,0}ID_{50}$. A reading is taken at 5-7 days postinoculation, the $BPD_{50}$ is calculated, and the vaccines providing a $BPD_{50}$ of three or higher are passed for release.

The current requirements were gradually established. In the case of Argentina, a level of 1.2 $BPD_{50}$ was first required in 1975. The level was subsequently raised to 1.6, then to 2.3 in 1977, and in January 1978, to the three $BPD_{50}$ now required.

This example is valid for all the methods and countries. There is currently no intention of raising the vaccine-approval levels. However, work is being developed to select the most reliable methods, to reduce their variability, and to improve vaccine handling techniques.

3. Circulating antibodies

In general, the tests most used with sera from vaccinated cattle are the mouse protection test and the serum neutralization test. Some

countries that use these vaccine control methods have established a serum protection index of 2.5 and a serum neutralization index of 1.5 and require a minimum of 75 percent protection of vaccinated animals as criterion for approval of the vaccine.

As criterion to evaluate vaccine potency, the PAFMDC/PAHO currently utilizes the EPP based on the mouse protection index (5). It has been established that the lower confidence limit of 95 percent should be equal to or greater than a protection of 75 percent. This method requires the use of at least 16 cattle per vaccine. However, the accuracy of the test increases as the number of test animals is increased.

The EPP's main advantage over the direct tests is that all the valencies of a vaccine can be tested in a single group of cattle, whereas the use of the PG and the $BPD_{50}$ make it economically unfeasible to obtain these data in all the series of vaccines produced. Argentina and Brazil, which use the PG and $BPD_{50}$ tests, manage to compare the vaccines only against a single valency. Also, when using the EPP, it is not necessary to inoculate the vaccinated cattle with live virus. This eliminates the high risks of virus escape from diseased animals. After the test is concluded they can be returned to the field, thus eliminating the inconvenience of sacrificing or destroying the animals. Commonly, cattle between 18-24 months of age are used in the PG, $BPD_{50}$ and EPP.

Conclusions

In the production of FMD vaccines in South America, the 1950's, 60's and part of the 70's were dedicated to creating an infrastructure of production laboratories capable of supplying sufficient quantities of FMD vaccine to meet the needs of the programs.

That infrastructure has developed significantly in both the private and official sectors. Most of the South American countries now have qualified FMD vaccine laboratories and advanced production technology.

Concomitantly, the infrastructure of the official control laboratories was established. The standards for the production laboratories and the vaccines they produce were determined. Quality controls that should be applied to the vaccines and the minimum requirements of the protection tests for approval of each series were also defined. The control methods, as well as the requirements, were systematically analyzed and updated at the international seminars on FMD vaccine quality control sponsored by the PAFMDC/PAHO in 1970, 1978, 1980 and 1983, in Uruguay, Argentina, Colombia and Paraguay, respectively.

The systematic application of the quality control of foot-and-mouth disease vaccines substantially improved the production and commercialization of a product providing greater potency and biological safety. The stricter requirements gradually applied in quality control generated a constant increase in the quality of the vaccines in Argentina, Brazil, Colombia, Paraguay and Uruguay.

At the same time production laboratories were remodelled or built with the capability to adjust to production systems using cell cultures and to the stricter potency requirements imposed by the official services.

At present the most important step is being taken which is to release systematically and dynamically the levels of vaccine production and quality control with the policies and strategies designed to control and eradicate the disease from the South American continent.

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Appendix 8

# CORRELATION BETWEEN 50% BOVINE PROTECTIVE DOSE ($BPD_{50}$) AND PERCENTAGE PROTECTION AGAINST FOOT GENERALIZATION (PG) IN FMDV VACCINE POTENCY

Yderzio L. Vianna Filho, Graciela Fernandez, Jose A. Ravison, Luis Durini and Albino A. Fernandez*

Summary

An analysis is made of the results of the 50 percent Bovine Protection Dose ($BPD_{50}$) and the protection against foot generalization (PG) as tests for the control of potency of FMD vaccines.

Data used were the results of 658 batches of vaccine tested, which amount to a total of one billion doses, and from 9,820 vaccinated and control bovines which were challenged.

The results indicated that the $BPD_{50}$ does not follow the expected theoretical model. There are 22 percent of the tests which cannot be interpreted because they do not follow the doses/effect relationship.

On the other hand, the correlation between $DPB_{50}$ and PG when the undiluted vaccine is used showed that this method is more reliable. The use of PG (80 percent) on a routine basis for the potency control of FMD vaccines is proposed.

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Appendix 9

# STORAGE OF INACTIVATED OIL ADJUVANTED FOOT AND MOUTH DISEASE VACCINE AT LOW TERMPERATURE

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Because of the instability of foot-and-mouth (FMD) vaccines, even under conditions of continuous refrigeration, it has been impossible to prepare a standard reference vaccine, which can be used for a prolonged period of time. The best approximation for such a standard vaccine with present technology, would probably be the formulation of a vaccine from purified known antigens, stored at very low temperatures. However, the required manipulation and formulation would always cast some doubts regarding its value as a reference reagent.

In April 1980, the Center stored a batch of about 3,000 doses of inactivated oil adjuvanted FMD vaccine at -70°C. This vaccine was prepared eight months earlier and had been kept at +4°C until the moment of freezing. Table 1 shows the infectivity and CF titers of the antigens. The vaccine was formulated according to the standard methods used at that time at the Center. Briefly, the antigens were inactivated with BEI (Bahnemann, 1975); the oily phase consisted of 90 percent Marcol 52 and 10 percent Arlacel A (Atlas); equal parts of the aqueous phase and the oily phase were emulsified by means of semi-industrial emulsification equipment developed by the Center in collaboration with a local firm. The results of vaccine potency tests at 30 and 180 days post vaccination are noted in Table 2. In addition, three months after formulation, the vaccine was used in 14 young cattle of 6 to 8 months old. The sera of these cattle were assayed by the mouse protection test (Cunha et al. 1957) 30, 90 and 180 days post vaccination (Table 3) with results expressed as the mean Expected Percentage of Protection (Gomes and Astudillo 1976, Sutmöller, Gomes and Astudillo, 1984). From cattle challenge test results and the serological data it is obvious that the vaccine was of excellent quality.

After a storage period of four years and three months at -70°C some of the vaccine was placed for 24 hours at +4°C and then used to vaccinate 22 cattle similar to those in the earlier test. The results of the mouse protection tests of sera collected 30, 90 and 180 days post vaccination also are shown in Table 3. These results demonstrate that oil adjuvanted FMD vaccine can be stored at -70°C without detectable loss of potency for a prolonged period of time. Such vaccine could serve as a reference vaccine for the purpose of standardization in vaccine control procedures.

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Table 1

Infectivity and complement fixation titers of the antigens used for the formulation of the inactivated FMD vaccine

| Antigens | $ID_{50}$/ml | C. F. titers |
|---|---|---|
| $O_1$ Campos | 7.5 | 1/20 |
| $A_{24}$ Cruzeiro | 8.2 | 1/18 |
| $C_3$ Resende | 8.2 | 1/22 |

Table 2

Cattle $PD_{50}$ vaccine potency test

| Dilution* | Number of cattle protected/vaccinated | |
|---|---|---|
| | 30 DPV** | 180 DPV |
| 1/1 | 5/5 | 5/6 |
| 1/4 | 5/5 | 6/6 |
| 1/16 | 5/5 | 4/5 |
| 1/64 | 4/4 | 3/6 |
| 1/256 | 2/5 | 1/6 |
| Controls | 0/2 | 0/2 |

*Diluted in emulsion without antigens

**DPV = Days post vaccination

Table 3

Mean Expected Percentage of Protection

| | Days post vaccination | | | | | |
|---|---|---|---|---|---|---|
| | 30 | 90 | 180 | 30 | 90 | 180 |
| | Vaccinated in 1979 | | | Vaccinated in 1984 | | |
| $O_1$ Campos | 95.8 | 85.5 | 53.9 | 98.2 | 95.4 | 76.5 |
| $A_{24}$ Cruzeiro | 94.3 | 94.8 | 87.5 | 98.6 | 97.0 | 91.7 |
| $C_3$ Resende | 97.7 | 78.2 | 62.9 | 97.5 | 92.8 | 75.1 |

Appendix 10

# COMPUTER SIMULATION OF FOOT-AND-MOUTH DISEASE VACCINE POTENCY TESTS

Paul Sutmoller*

The aim of an efficient vaccine quality control must be to discriminate between batches of vaccine of required potency and those of substandard potency: thus providing the livestock industry with an effective product while protecting the vaccine manufacturer against too many rejections of batches of adequate vaccine.

Presently several tests are in routine use for potency estimation of foot-and-mouth disease (FMD) vaccine (Centro Panamericano de Fiebre Aftosa, 1980). Such tests can either be done by the challenge of animals which are vaccinated with the complete or reduced vaccine dose or by measurement of antibodies induced by the vaccine. Vaccines can be tested in guinea pigs or in the species for which the vaccine is intended.

In the tests utilizing the complete vaccine dose it is common to expose the animals to a determined amount of virus 3 to 4 weeks after vaccination. Cattle are usually infected by the inoculation of virus into the tongue epithelium (Henderson, 1949) and pigs by inoculating the bulb of the heel (Burrows, 1986).

Antigen extinction tests can be performed in cattle, pigs or in guinea pigs. In this test groups of animals are injected with decreasing amounts of antigen and after a certain period, exposed to virus. The decreasing amount of antigen may be accomplished by diluting the vaccine in an inert substance such as buffer solution, by diluting the vaccine in the vaccine base, including any additive such as saponin, or by injecting decreasing amounts of vaccine.

In several papers FMD vaccine control methods have been reviewed with regard to technical problems encountered in their performance and the statistical interpretation and significance of their results (For instance de Leeuw, 1973, Pay, 1972, 1977, 1984, Hingley and Pay, 1984, Sutmoller, 1978). However, what may be an acceptable test method or sound statistical procedure for one group of workers is not always accepted by others. Scientific papers, presentations, on test methods or statistical discussions about vaccine control often are confusing and appear sometimes bewildering to those involved in writing vaccine control regulations or to those who must daily make the decisions in the control laboratory.

To demonstrate some of the problems of vaccine potency testing a set of simple computer simulation programs was developed. These programs demonstrate which role chance plays in the outcome of potency tests. This chance factor is of particular importance in view of the small number of animals used in routine FMD vaccine vaccine potency tests. The user of these simulation computer programs can change through keyborad commands the parameters of these tests such as group size, number of vaccine dilutions, dilution interval and percentage of protection per group and observe immediately the influence of the changes on the results of the tests.

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The programs

Vaccine potency tests produce "yes" or "no" answers: either the animal develops lesions or it remains healthy. The chances of a "yes" or "no" answer are determined by the percentage of protection of the vaccinated population. The computer is an ideal machine to simulate such a situation by generating a theoretical population of protected and non-protected animals for a given level of protection. The computer draws a random number between 1 and 100 for each animal and compares this with the level of protection. For instance, if the protection chosen for a vaccine is 70% and the random number is less than or equal to 70 the animal is protected. If the random number is more than 70 the animal is not protected.

Next we can give the computer other vaccine parameters such as the group size or the protection level of each dilution group as well as the dilution factor in the case of antigen extinction tests. We can repeat the same test many times over and compare the results from one test to another. Furthermore the computer can calculate endpoint titers for each of the vaccines and keep accumulative figures for repeated tests. In this way we are able to look at many test results which only differ because of random chance.

The programs are written for a microcomputer (TRS-80 Model III) and require a minimum configuration of 16K, one disk drive and a printer, but the programs can readily be adapted to other microcomputers.

When we start the programs the computer monitor shows:

VACCINE TEST SIMULATION
THE RESULT OF A VACCINE TEST DEPENDS ON:
- THE PROTECTIVE LEVEL INDUCED BY THE VACCINE
- THE NUMBER OF ANIMLS IN THE TEST
- THE VACCINE DILUTIONS USED
THESE VACCINE TEST SIMULATION PROGRAMS
PERMIT YOU TO VARY THESE FACTORS.

- EVEN IF ALL FACTORS ARE EQUAL YOU WILL OBTAIN DIFFERENT RESULTS FOR EACH TEST.
- THIS IS CAUSED BY SAMPLING VARIATION BECAUSE OF THE SMALL NUMBER OF ANIMALS IN THE TEST.
- THE SIMULATION PROGRAMS PERMIT YOU TO UNDERSTAND THE IMPORTANCE OF THIS SAMPLING VARIATION IN VACCINE TESTING.

The screen then shows the various programs from which we can choose:

1. PROTECT A PERCENTAGE OF A POPULATION
2. SELECT A PERCENTAGE OF PROTECTION AND GROUP SIZE
3. SELECT THE NUMBER OF VACCINES, GROUP SIZE AND PERCENTAGE OF PROTECTION
4. SAME AS 3, BUT WITH RESULTS TABULATED AS PROTECTED/TOTAL
5. SAME AS 4 FOR 100 TESTS WITH ACCUMULATED RESULTS
6. SELECT THE NUMBER OF VACCINES, GROUP SIZE AND PERCENTAGE OF PROTECTION OF FOUR VACCINE DILUTIONS

## 7. SAME AS 6 BUT WITH VARIABLE NUMBER OF DILUTIONS AND RESULTS EXPRESSED AS $PD_{50}$ VALUES

Programs: through 3 are designed to demonstrate the basis for the selection of animals from a population. Programs 4 and 5 simulate vaccine control tests with results expressed as the number of protected animals per group for each of the vaccines. Programs 6 and 7 simulate $PD_{50}$ vaccine potency tests.

### Running the programs

With program 1 the computer requests a percentage of protected animals and then proceeds to generate a group of 100 animals which are either protected or non-protected. The computer notes this on the screen of the monitor as a + or - for each animal respectively.

With program 2 we are requested to choose the level of protection and the number of animals to be selected from the population. The computer first generates the population according to the percentage of protection and then again randomly selects the required individuals from that population. The results can be seen in the printout of program 2.

Program 3 is an expansion of program 2 and allows us also to specify more than one group. The ramdom numbers are no longer presented. The output of program 3 is an example of what may appear on the screen of the monitor or on the computer printout.

So far these have been runs to show how the program selects the individuals of the groups with the result presented in terms of numbers of protected animals in each group.

In the next programs for each animal and each group these steps are repeated although not visible on the screen or printout.

If we run program 4 the results are presented in a more readable simplified form. Only the number of protected animals - the total of the group for each of the vaccines as shown in the printout of this program.

Program 5 was written for 100 tests in order to show and tabulate a large number of tests. A typical output of this program is shown in the printout of program 5. The tabulated figures show the frequency at which a certain number of protected animals per group occurred and the probability of the occurrence at a certain minimum requirement level (Passmark).

Finally we can turn to program 6 and 7 for $PD_{50}$ determinations. In these programs we are asked for the vaccine dilutions and their respective protection levels as well as the group size. The endpoints are estimated by means of probit analyses of the actual percentage of protection in each group of animals (not of the percentage of protection for the vaccine selected by the user!).

### Discussion

It can be seen from the runs of programs 2, 3, and 4 that when we specify a 70% protected population and randomly selected groups of 8 cattle, the number of protected animals varied from 3 - to 8 per group. Of course we could have done this selection process in one step by using

random selection directly, but setting up a vaccinated population with a given level of protection and then selecting the individuals of each group resembles or imitates the vaccine control process more closely.

Let us now consider the results of program 5. In this case we had a vaccine which protected a high percentage of the vaccinated animals (90%) and we used 8 cattle per test. In this run 8/8 cattle were protected 35 times and 7/8 were protected 38 times. Thus, if the minimum passing requirements would be 7/8 protected then only 75% of the batches would have passed. This obviously would be rejecting too many batches of vaccine which protected 90% of the population. It sounds reasonable therefore to set the minimum requirement at 6/8, in which case only some 4% of the vaccine batches would be rejected.

If the vaccine protects 70% of the population then in the order of 40% of the batches are likely to fail the test with a Passmark of 6/8. Vaccine manufacturers with this type of performance will soon be out of business. Thus a Passmark of 6/8 forces vaccine producers to make a vaccine which induces high levels of protection in the vaccinated population.

How would the 6/8 Passmark protect the livestock industry against the acceptance of too many batches of poor vaccines? With program 5 we showed also a run of a vaccines which only protect 50% of the cattle. It can be observed that in this run some 13% of the vaccines would be accepted with a Passmark of 6/8, which probably would not satisfy the livestock industry.

Further printouts of program 5 show what happens if we double the number of animls in the test. some 99% of the batches of vaccines which induce a protection level of 90% in the population will be approved with a Passmark of 12/16 protected. Doubling the number of cattle to test these good vaccines appears to offer a small advantage for the vaccine manufacturer. If we look at the 70% run it is obvious that with a Passmark of 12/16 we will weed out the poorer vaccines somewhat better. For instance this vaccine will have only a 47% chance to pass.

The decision to be made regarding the number of animals in the test is thus one of economics and compromise between the interest of the livestock industry and vaccine manufacturers. It appears that a reasonable compromise will be between 12 to 15 test animals per vaccine with Passmarks of 9/12 and 11/15, respectively.

A $PD_{50}$ test introduces additional factors such as the dilution set, the very small group size, usually not more than 5 animals, and the various calculation methods for the $PD_{50}$ value. A commonly used dilution set is the undiluted vaccine and the 1/3 and 1/9 dilutions of the vaccine in buffer.

Programs 6 and 7 simulate $PD_{50}$ tests in which the amount of antigen is decreased by diluting the vaccine. Diluting a vaccine with inert diluent can be simulated by choosing a dilution set of for instance 1/1, 1/3, 1/9. Dilution in vaccine base, which causes a rather flat dose response curve, can be simulated by using dilutions 1/4. 1/16, 1/64 and 1/256. We can choose the level of protection for each of the dilutions. For instance, a typical dilution set appears to be dilutions of 1/1,1/3, 1/9 with protection levels of 90%, 65% and 30%, respectively and 5 animals per dilution group. It can be seen that the $PD_{50}$ level of the 12 vaccines can range from 1,5 to 6,3. For one of the vaccines it was impossible to calculate the $PD_{50}$ in the absence of a dose response curve. In another

case, using this method of calculation, it was impossible to calculate the standard error (SE) as a consequence of very flat dose response curve. It can be observed that the flatter the curve, the larger the SE of the end-point. The real value for vaccines with protection level of 90%, 60% and 30% based on probits of these percentages is 4,4 with a SE of 0.39. It is thus obvious that the small number of animals in each dilution group leads to a large variation in the number of protected animals, particularly in the 60% and 30% groups, and to important deviations from this theoretical value. It is also quite clear that even a vaccine which induces a protection level of as high as 90% in the primo vaccinated population runs a high risk of not passing a 3 $PD_{50}$ value Passmark. No doubt, if the 15 animals used in the test would all have been vaccinated with the full vaccine dose for a protection test much more reliable estimation of the vaccine potency would have been obtained.

Finally, it must be remembered that the simulation programs presented here, assume a completely homogeneous animal population. In reality there is additional variation due to the heterogenicity of the animals.

So far, we only have considered the testing of vaccines against one strain of virus. Under the conditions of vaccine testing in South America it would be prohibitively expensive and practically impossible to use cattle in challenge tests for all the virus types in the vaccine. This limitation must be overcome by using additional techniques to guarantee the vaccine quality for the remaining valencies also. We believe that serological data obtained from sera of the test cattle prior to challenge would be a valuable adjunct for the estimation of the potency of these other valencies (Gomes and Astudillo, 1975, Sutmoller, 1980, 1983, Sutmoller and Freitas Costa, 1980). To obtain meaningful serological data however, at least 12 to 15 cattle must be vaccinated with the regular vaccine dose, which as indicated above, fits well with the number of cattle recommended for protection type challenge tests. In addition serological information on the homologous virus could serve to resolve some of the problems confronting the vaccine control authority in the case of the rejection of a vaccine, for instance, because of an animal with one foot lesion only.

At present the Panamerican Foot-and-Mouth Disease Center is collaborating with several countries of South America in the revision of their vaccine control procedures. These simulation programs have helped some of the regulatory staff as well as those involved directly with the testing of vaccines, to gain a better understanding of the random factors influencing the outcome of vaccine potency tests. A couple of hours at the computer keyboard provides a much greater comprehension of these factors, resulting in less "magic" of the Passmarks, sounder regulations and better protection of the livestock industry and vaccine manufacturers, as well as less wastage of expensive test animals. Finally we hope that in the near future cattle challenge will only be used for reference tests with a sufficient number of animals to yield statistically significant results and that the present routine challenge tests be replaced by alternative methods which guarantee vaccine quality, but minimize suffering of test animals and the risk of virus escape from the testing facilities.

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Abstract

This paper presents the results of computer programs to simulate Foot-and-Mouth Disease vaccine potency tests. The user can easily change the test parameters such as the number of test animals and the dilution factor of the vaccine. The programs allow the user to increase his comprehension of the importance of sampling variation in vaccine testing, due to the relatively small number of animals in the test.

Printout Program 2

PERCENTAGE OF PROTECTION 70 %

73 POS.
27 NEG.

GROUP SIZE 8
RANDOM NUMBERS 97 94 86 66 94 72 32 87 62
NUMBER OF PROTECTED ANIMALS 7

PERCENTAGE OF PROTECTION 70 %

70 POS.
30 NEG.

GROUP SIZE 8
RANDOM NUMBERS 69 22 56 26 71 34 71 6 27
NUMBER OF PROTECTED ANIMALS 4

Printout Program 3

NUMBER OF VACCINES 10
GROUP SIZE 8
PERCENTAGE OF PROTECTION 70 %

PROTECTED
8 / 8
5 / 8
6 / 8
5 / 8
6 / 8
4 / 8
6 / 8
6 / 8
5 / 8
6 / 8

Printout Program 4

NUMBER OF VACCINES 14
GROUP SIZE 8
PERCENTAGE OF PROTECTION 70 %

**** PROTECTED / TOTAL ****

| | | | | | | |
|---|---|---|---|---|---|---|
| 4 / 8 | 6 / 8 | 8 / 8 | 5 / 8 | 5 / 8 | 7 / 8 | 6 / 8 |
| 6 / 8 | 3 / 8 | 5 / 8 | 5 / 8 | 7 / 8 | 5 / 8 | 3 / 8 |

Printout Program 5

GROUP SIZE 8 / PERC. OF PROTECTION 90 %
NUMBER OF ANIMALS PROTECTED IN 100 TESTS

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 8 | 6 | 8 | 8 | 8 | 6 | 8 | 7 | 6 | 8 |
| 8 | 7 | 8 | 8 | 7 | 6 | 7 | 8 | 8 | 6 |
| 6 | 7 | 8 | 7 | 6 | 6 | 7 | 7 | 7 | 7 |
| 6 | 7 | 6 | 8 | 7 | 8 | 6 | 7 | 7 | 6 |
| 7 | 7 | 6 | 8 | 7 | 8 | 7 | 6 | 8 | 7 |
| 8 | 7 | 8 | 7 | 7 | 7 | 6 | 8 | 6 | 5 |
| 6 | 6 | 7 | 8 | 8 | 7 | 6 | 7 | 7 | 8 |
| 8 | 7 | 7 | 7 | 5 | 8 | 8 | 8 | 8 | 8 |
| 5 | 6 | 7 | 8 | 7 | 7 | 6 | 7 | 8 | 7 |
| 8 | 6 | 7 | 6 | 7 | 8 | 5 | 8 | 7 | 8 |

| NUMBER PROTECTED | FREQUENCY | MINUMUM REQUIREMENT | PROB. OF PASS | FAIL |
|---|---|---|---|---|
| 8 | 35 | 8 / 8 | 35 % | 65 % |
| 7 | 38 | 7 / 8 | 73 % | 27 % |
| 6 | 23 | 6 / 8 | 96 % | 4 % |
| 5 | 4 | 5 / 8 | 100 % | 0 % |

GROUP SIZE 8 / PERC. OF PROTECTION 70 %
NUMBER OF ANIMALS PROTECTED IN 100 TESTS

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 6 | 7 | 7 | 6 | 7 | 6 | 3 | 6 | 7 | 5 |
| 6 | 8 | 6 | 4 | 7 | 7 | 4 | 4 | 6 | 7 |
| 5 | 5 | 5 | 6 | 7 | 5 | 5 | 6 | 6 | 6 |
| 7 | 6 | 5 | 6 | 5 | 7 | 5 | 5 | 6 | 5 |
| 8 | 6 | 7 | 5 | 5 | 5 | 5 | 6 | 6 | 7 |
| 6 | 7 | 5 | 6 | 3 | 7 | 7 | 5 | 5 | 5 |
| 7 | 7 | 5 | 5 | 5 | 8 | 3 | 7 | 4 | 5 |
| 5 | 4 | 5 | 6 | 5 | 7 | 5 | 7 | 7 | 7 |
| 7 | 6 | 6 | 7 | 5 | 8 | 5 | 5 | 4 | 5 |
| 5 | 2 | 6 | 5 | 7 | 2 | 6 | 7 | 4 | 5 |

| NUMBER PROTECTED | FREQUENCY | MINUMUM REQUIREMENT | PROB. OF PASS | FAIL |
|---|---|---|---|---|
| 8 | 4 | 8 / 8 | 4 % | 96 % |
| 7 | 26 | 7 / 8 | 30 % | 70 % |
| 6 | 24 | 6 / 8 | 54 % | 46 % |
| 5 | 34 | 5 / 8 | 88 % | 12 % |
| 4 | 7 | 4 / 8 | 95 % | 5 % |
| 3 | 3 | 3 / 8 | 98 % | 2 % |
| 2 | 2 | 2 / 8 | 100 % | 0 % |

GROUP SIZE 8 / PERC. OF PROTECTION 50 %
NUMBER OF ANIMALS PROTECTED IN 100 TESTS

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 6 | 3 | 5 | 5 | 8 | 3 | 1 | 5 | 4 | 4 |
| 5 | 4 | 5 | 5 | 5 | 3 | 5 | 4 | 4 | 3 |
| 4 | 5 | 4 | 6 | 3 | 5 | 3 | 6 | 5 | 5 |
| 5 | 7 | 6 | 4 | 6 | 7 | 3 | 4 | 4 | 3 |
| 3 | 1 | 4 | 3 | 6 | 4 | 3 | 3 | 3 | 4 |
| 6 | 4 | 4 | 3 | 2 | 4 | 2 | 3 | 3 | 5 |
| 3 | 2 | 4 | 4 | 5 | 3 | 4 | 4 | 4 | 4 |
| 3 | 3 | 3 | 4 | 5 | 2 | 2 | 5 | 3 | 4 |
| 4 | 3 | 3 | 5 | 3 | 2 | 4 | 4 | 3 | 6 |
| 1 | 3 | 2 | 4 | 7 | 5 | 4 | 4 | 6 | 3 |

| NUMBER PROTECTED | FREQUENCY | MINUMUM REQUIREMENT | PROB. OF PASS | FAIL |
|---|---|---|---|---|
| 8 | 1 | 8 / 8 | 1 % | 99 % |
| 7 | 3 | 7 / 8 | 4 % | 96 % |
| 6 | 9 | 6 / 8 | 13 % | 87 % |
| 5 | 19 | 5 / 8 | 32 % | 68 % |
| 4 | 30 | 4 / 8 | 62 % | 38 % |
| 3 | 28 | 3 / 8 | 90 % | 10 % |
| 2 | 7 | 2 / 8 | 97 % | 3 % |
| 1 | 3 | 1 / 8 | 100 % | 0 % |

Printout Program 5

GROUP SIZE 16 / PERC. OF PROTECTION 90 %
NUMBER OF ANIMALS PROTECTED IN 100 TESTS

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 13 | 15 | 16 | 10 | 14 | 15 | 14 | 13 | 14 | 14 |
| 16 | 16 | 16 | 14 | 16 | 14 | 14 | 13 | 16 | 14 |
| 15 | 15 | 16 | 16 | 15 | 14 | 14 | 15 | 13 | 16 |
| 13 | 13 | 15 | 16 | 13 | 16 | 12 | 15 | 15 | 14 |
| 13 | 15 | 14 | 13 | 15 | 16 | 12 | 16 | 15 | 15 |
| 16 | 12 | 13 | 16 | 14 | 15 | 15 | 16 | 14 | 15 |
| 13 | 15 | 13 | 16 | 16 | 16 | 15 | 14 | 15 | 15 |
| 15 | 12 | 14 | 13 | 15 | 15 | 16 | 15 | 13 | 13 |
| 16 | 13 | 14 | 16 | 14 | 16 | 16 | 14 | 15 | 15 |
| 14 | 15 | 13 | 13 | 16 | 15 | 15 | 14 | 14 | 15 |

| NUMBER PROTECTED | FREQUENCY | MINUMUM REQUIREMENT | PROB. OF PASS | FAIL |
|---|---|---|---|---|
| 16 | 25 | 16 / 16 | 25 % | 75 % |
| 15 | 31 | 15 / 16 | 56 % | 44 % |
| 14 | 22 | 14 / 16 | 78 % | 22 % |
| 13 | 17 | 13 / 16 | 95 % | 5 % |
| 12 | 4 | 12 / 16 | 99 % | 1 % |
| 10 | 1 | 10 / 16 | 100 % | 0 % |

GROUP SIZE 16 / PERC. OF PROTECTION 70 %
NUMBER OF ANIMALS PROTECTED IN 100 TESTS

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 11 | 12 | 10 | 8 | 12 | 12 | 11 | 13 | 12 | 12 |
| 7 | 9 | 13 | 13 | 12 | 12 | 10 | 14 | 15 | 13 |
| 14 | 9 | 12 | 9 | 10 | 13 | 11 | 13 | 12 | 13 |
| 10 | 12 | 10 | 8 | 11 | 11 | 12 | 11 | 9 | 12 |
| 11 | 8 | 6 | 12 | 10 | 10 | 13 | 11 | 11 | 13 |
| 15 | 12 | 11 | 11 | 14 | 10 | 10 | 11 | 10 | 12 |
| 13 | 11 | 9 | 14 | 11 | 12 | 11 | 12 | 9 | 9 |
| 8 | 9 | 10 | 12 | 10 | 14 | 9 | 6 | 8 | 11 |
| 11 | 12 | 9 | 7 | 10 | 12 | 10 | 13 | 12 | 13 |
| 14 | 12 | 8 | 13 | 12 | 9 | 11 | 12 | 11 | 9 |

| NUMBER PROTECTED | FREQUENCY | MINUMUM REQUIREMENT | PROB. OF PASS | FAIL |
|---|---|---|---|---|
| 15 | 2 | 15 / 16 | 2 % | 98 % |
| 14 | 6 | 14 / 16 | 8 % | 92 % |
| 13 | 13 | 13 / 16 | 21 % | 79 % |
| 12 | 24 | 12 / 16 | 45 % | 55 % |
| 11 | 19 | 11 / 16 | 64 % | 36 % |
| 10 | 14 | 10 / 16 | 78 % | 22 % |
| 9 | 12 | 9 / 16 | 90 % | 10 % |
| 8 | 6 | 8 / 16 | 96 % | 4 % |
| 7 | 2 | 7 / 16 | 98 % | 2 % |
| 6 | 2 | 6 / 16 | 100 % | 0 % |

Printout Program 7

NUMBER OF VACCINES 12
GROUP SIZE 5
NUMBER OF DILUTIONS 3

| | | | | | |
|---|---|---|---|---|---|
| DILUTION 1:1 | | | PERCENTAGE OF PROTECTION | | 90 % |
| 5 / 5 | 5 / 5 | 4 / 5 | 5 / 5 | 5 / 5 | 5 / 5 |
| 5 / 5 | 5 / 5 | 4 / 5 | 5 / 5 | 5 / 5 | 4 / 5 |
| DILUTION 1:3 | | | PERCENTAGE OF PROTECTION | | 60 % |
| 1 / 5 | 5 / 5 | 3 / 5 | 2 / 5 | 0 / 5 | 1 / 5 |
| 1 / 5 | 3 / 5 | 3 / 5 | 2 / 5 | 5 / 5 | 3 / 5 |
| DILUTION 1:9 | | | PERCENTAGE OF PROTECTION | | 30 % |
| 3 / 5 | 1 / 5 | 1 / 5 | 1 / 5 | 1 / 5 | 0 / 5 |
| 2 / 5 | 1 / 5 | 1 / 5 | 1 / 5 | 0 / 5 | 0 / 5 |

| NO. | DILULTIONS | | |
|---|---|---|---|
| VACCINE | 1:1 | 1:3 | 1:9 |
| 1 | 5 | 1 | 3 |
| 2 | 5 | 5 | 1 |
| 3 | 4 | 3 | 1 |
| 4 | 5 | 2 | 1 |
| 5 | 5 | 0 | 1 |
| 6 | 5 | 1 | 0 |
| 7 | 5 | 1 | 2 |
| 8 | 5 | 3 | 1 |
| 9 | 4 | 3 | 1 |
| 10 | 5 | 2 | 1 |
| 11 | 5 | 5 | 0 |
| 12 | 4 | 3 | 0 |

| NO. | SLOPE | SE | Y-INT | SE | LOG PD50 | SE | DIL PD50 | SE |
|---|---|---|---|---|---|---|---|---|
| 1 | IMPOSSIBLE TO CALCULATE SLOPE | | | | | | | |
| 2 | -4.83 | -1.787 | 8.86 | -1.559 | 0.8 | 0.110 | 6.3 | 1.60 |
| 3 | -1.59 | -0.350 | 5.90 | -0.157 | 0.6 | 0.089 | 3.7 | 0.75 |
| 4 | -2.15 | -1.814 | 5.97 | -1.158 | 0.4 | 0.233 | 2.8 | 1.51 |
| 5 | -4.12 | -0.148 | 8.09 | -0.133 | 0.7 | 0.011 | 5.6 | 0.14 |
| 6 | -8.24 | -0.119 | 8.09 | -0.054 | 0.4 | 0.002 | 2.4 | 0.01 |
| 7 | -0.50 | -2.563 | 5.09 | -2.136 | 0.2 | 3.370 | 1.5 | 11.79 |
| 8 | -2.81 | -1.141 | 6.68 | -0.686 | 0.6 | 0.086 | 4.0 | 0.79 |
| 9 | -1.59 | -0.350 | 5.90 | -0.157 | 0.6 | 0.089 | 3.7 | 0.75 |
| 10 | -2.15 | -1.814 | 5.97 | -1.158 | 0.4 | 0.233 | 2.8 | 1.51 |
| 11 | IMPOSSIBLE TO CALCULATE SE | | | | | | | |
| 12 | -1.23 | -0.043 | 5.84 | -0.014 | 0.7 | 0.018 | 4.8 | 0.20 |

Appendix 11

# FOOT-AND-MOUTH DISEASE OUTBREAKS IN DENMARK 1982 AND 1983

## Diagnosis - Eradication - Economic consequences

M. Eskildsen & P. Have*

Foot-and-mouth disease (FMD) appeared in Denmark during the spring of 1982 after having been absent since 1970. Up to 1970 the Danish eradication and control programme had been based on the killing of all susceptible animals on affected farms and the use of ring zone vaccination. A general prophylactic annual vaccination of all cattle and sheep had never been practised, but prophylactic vaccination of herds within limited geographical areas and of animals destined for export was used until the early 1970s.

At the end of 1976 the time was found appropriate for the introduction of a non-vaccination policy, and since January 1977 the use of FMD vaccines has been prohibited. There are obvious advantages connected with a non-vaccination policy, e.g. as regards disease control and export prospects. However, as a result of this policy the whole national population of cloven-hoofed animals will be highly susceptible to FMD, and consequently, the risk of secondary spreading will be extremely high once the infection has been introduced into the country. The Danish non-vaccination policy was challenged in connection with the outbreaks in March-May 1982 and in January 1983 comprising 22 infected herds and one single case, respectively. The disease was eradicated on the basis of an early diagnosis, immediate implementation of stamping out and other strict combat and control measures, without the use of vaccination.

The present paper gives a summarized description of the main features of this work with special emphasis on the laboratory diagnosis (including ELISA), eradication procedures and economic consequences.

## Laboratory Diagnosis

### Primary diagnosis

One of the most important preconditions for a successful outcome of the non-vaccination strategy was considered to be the recognition of the disease as early as possible after introduction of the infection into the individual herd. In this respect the herd owner and the field veterinarian were important "key persons" supported by the district veterinary officer and the diagnostic laboratory. Generally speaking, an early confirmative laboratory diagnosis is of special importance in case of a first time introduction of the disease into the country. In order to meet the demands for rapidity and safety the field material was always transported by courier directly from the affected herd to the laboratory at Lindholm, where it was usually received within a few hours after collection. The traditional laboratory methods for the primary FMD diagnosis, i.e. a complement fixation (CF) test (Traub & Möhlmann, 1943) and inoculation of primary calf and pig kidney cell cultures, were supplemented with an enzyme-linked immunosorbent assay (ELISA). This technique, which has been described in detail by Have et al. (1984), is a double sandwich ELISA in microplates with the following reaction steps:

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1. Adsorption of rabbit anti-FMD-virus IgG for one hour at 37°C. The IgG was diluted 1:1000 in carbonate buffer pH 9.6.

2. Reaction with virus (test sample) for one hour at 37°C.

3. Reaction with hyperimmune guinea pig IgG of the same type as used in (1) for 30 min. at 37°C. The guinea pig IgG was diluted 1:1000 in PBS-0.05% Tween 20 containing 10% normal bovine serum and 5% normal rabbit serum.

4. Reaction with peroxidase-conjugated antibody to guinea pig IgG for 30 min. at 37°C. The conjugate was diluted 1:1000 in the same buffer as in (3).

5. Enzyme reaction with the substrate O-phenylenediamine in citrate-phosphate buffer, pH 5.0, for 15 min. and termination of the reaction with 1 mol/l $H_2SO_4$.

All reagents and samples were dispensed in 100 ul volumen, and each of the steps 1-4 was followed by washing 3 times with PBS-0.05% Tween 20. Washing after step 4 included a final short rinse in citrate-phosphate buffer, pH 5.0. Plates were either read visually or measured at 492 nm in a multiscan spectrophotometer.

The rabbit sera used to establish the double sandwich test for types O, A and C had neutralizing titres of 256-1024 and did not exhibit cross-neutralizing activity. When preparations of 146 S particles of each type were titrated in type O, A and C ELISA, a considerable cross-reactivity was observed for all three virus types. Similarly, minor cross-reactions were seen, when the ELISA was used on FMD field samples. Detailed studies of type specificity in the ELISA using both 146 S particles and corresponding 12 S subunits revealed that 12 S subunits titrate approximately 4 times more efficiently than 146 S particles (Fig. 1a), and the higher reactivity of the 12 S subunits could be attributed to heterologous reactions (Fig. 2a-b). However, even with the 12 S subunits there was a clear typing with all three types in spite of the cross-reactions (Fig. 4a-c). The cross-reactions could be eliminated by addition of heterologous hyperimmune serum to the titration buffers resulting in a corresponding reduction in reactivity (Fig 1b).

Thus in the described ELISA sensitivity and specificity are to some extent complementary features, i.e. maximum sensitivity and type specificity cannot be achieved within the same diagnostic test. As to the primary diagnosis of FMD, sensitivity should be given priority and, in particular, the test should not be limited to detection of 146 S particles since these can be expected to constitute only a minor fraction of the antigens in samples collected under adverse field conditions.

Field samples were examined simultaneously for the virus types O, A and C using the same tissue suspensions for ELISA as for CF except that the samples for ELISA were not heat treated. The ELISA was found to be a useful supplement to CF being much more sensitive and requiring less material than the CF test. These advantages of the ELISA were illustrated in connection with the examination of field material from the single FMD case in January 1983.

The material initially received from the herd in question was sparse and not quite typical for FMD. The CF results on this material were inclusive as were also the observations in the inoculated cell cultures. However, a positive type O reaction was obtained with the ELISA a few

hours after receipt of this material. The following day classical FMD symptoms were observed in the herd, and additional material was now found positive in both CF and ELISA. The sensitivity of the ELISA compared to CF has been examined in parallel titrations of epithelial samples of virus types O, A and C (Fig. 5a-c). If, arbitrarily, values above 5 times background (hyperimmune serum without antigen and/or normal serum plus antigen) are considered significant, it follows that the ELISA is approximately 500 times more sensitive than the CF test.

From March 1982 until January 1983, 21 Danish field samples of type O were examined by CF and ELISA. Of these samples 19 were positive in both tests, whereas 2 samples were positive in ELISA only. One of these samples was confirmed by CF after passage in primary bovine cell cultures. The other one was verified by CF after submission of additional material.

A large number of FMD virus negative samples has been examined by the two tests, or when only small amounts were available, by ELISA and inoculation of cell cultures. This material includes samples containing BVD virus and herpes mammilitis virus as well as samples most probably originating from various physical injuries. None of these samples gave significant reactions in any of the tests, which for the ELISA means absorbance values within 2-fold of control values.

The high sensitivity of the ELISA makes this test especially useful when only small amounts of field material are available. Finally, the higher sensitivity of the ELISA allows a stronger and more confident attitude towards rejection of suspect - but non-FMD - cases, especially if the samples submitted are of vesicular origin.

Serological examinations

A reversed sandwich ELISA (Have & Holm Jensen, 1983) was used in parallel to a two-dimensional micro-neutralization test for comprehensive serological surveys as part of epidemiological examinations.

Furthermore this test system was used for the characterization of virus isolates in addition to the CF test and the two-dimensional micro-neutralization test (Table 1).

Eradication

One of the key words during the whole eradication period was - speed -, in particular with regard to the work related to:

- the FMD diagnosis
- depopulation of affected farms immediately after FMD was diagnosed
- a "freeze" on movement of animals, animal products etc. within certain defined areas.

Depopulation

Once the FMD diagnosis had been confirmed in the first case the decision to depopulate a herd was based on:

- typical symptoms only, or
- clinical symptoms and an established contact to a previous case, or
- clinical symptoms confirmed by a laboratory diagnosis

The depopulation was supervised by a district veterinary officer, and the work was carried out in close cooperation with the local police. All susceptible animals of the holding were killed on the spot without delay, and the animals were killed in the following order of priority:

i animals showing clinical symptoms of FMD
ii pigs without symptoms of FMD
iii cattle without symptoms of FMD

The total number of animals killed and buried on the 22 affected farms in 1982 and in connection with the single case in 1983 was 4,194 and 258, respectively. In addition 29 animals were killed as "high risk animals" being placed on farms adjacent to a positive case or suspected of having been exposed to FMD virus. All substances in farm buildings likely to be contaminated, such as milk, feeding stuffs, manure etc., were immediately destroyed and buried or treated in such a way as to ensure the destruction of any FMD virus. Cleansing and disinfection (2 percent NaOH) of the buildings used for housing the animals and their surroundings were commenced during depopulation or as soon as the depopulation had taken place.

The burial site was always chosen with the help of the farmer and the local municipal engineer (environmental officer) in order to avoid damage to the system of drains, water reservoirs etc. The sites chosen were all located on fields adjacent to buildings of the infected farm.

Area control measures

1. A cordon sanitaire, usually with a radius of about 250 metres, was established by the police around a farm as soon as FMD was diagnosed. Animals, animal products, animal feed, waste and utensils could not be moved from the cordon sanitaire, and the movement of persons to and from the area was subject to authorization by the police. The specific measures enforced in the cordon sanitaire area were maintained for about 21 days. Restrictions, which remained in force within the area after 21 days, were:

   i the restrictions described below for the control area as these restrictions were kept in force for at least 28 days after depopulation,

   ii restrictions with regard to restocking of the affected farm.

2. A control area with a radius of at least 10 kilometres was established around all of the depopulated herds. The following measures were applied within this area:

   - the movement of cloven-hoofed animals from one farm to another and out of the area was prohibited,

   - it was prohibited for any visitor, including cattle and pig dealers, production recording technicians etc., to enter farms with cloven-hoofed animals,

   - artificial insemination was prohibited,

   - it was prohibited to remove animal feed (hay, straw etc.) and manure from a farm,

   - it was prohibited to carry out public milk control, mastitis control and to collect milk samples for milk payment schemes,

- it was prohibited for milk truck drivers to enter barns and during milk collection they had to wear waterproof boots, coats and overtrousers. The protective clothing was disinfected at each farm,

- pipes and tubes on milk trucks used for milk collection were disinfected at each farm, and air-outlets on the milk truck tanks were covered with cloth wetted with disinfectant, or air-outlets were connected by tubes to the air-intake of the engine of the truck, .

- only veterinarians were permitted to visit the barns with cloven-hoofed animals,

- restrictions on slaughterings.

3. In order to reduce the risk of spreading the disease, livestock markets, sales, shows and all other gatherings of cloven-hoofed animals including common grazing on summer pastures were prohibited in all parts of the country.

Cleansing and disinfection procedures at depopulated farms

In order to prevent recrudescent outbreaks when depopulated farms had been restocked, all buildings on FMD affected farms underwent complete cleansing and disinfection. This work was supervised by a district veterinary officer. The major steps during the cleansing and disinfection process were:

A. Disinfection I:

Contaminated areas, buildings, equipment etc. were sprayed with a solution of sodium hydroxide during depolpulation or immediately after completion of the depopulation.

B. Cleansing:

The cleansing operation included a thorough cleaning of all buildings, equipment, and surrounding areas. All animal products, manure, urine, and animal feed except silage with a pH below 4.0 and grain, which had undergone a treatment with propionic acid, were destroyed before removal from the farm.

C. Disinfection II:

Cleaned farm buildings, slurry tanks, ventilation systems, milking machines etc. underwent disinfection with an appropriate disinfectant.

D. Testing of Facilities:

Samples of dust, silage and propionic acid treated grain were collected at farms about 10 days after the completion of disinfection II. The samples (environmental samples) were forwarded to the institute at Lindholm, where the material was fed to susceptible calves kept in isolation units. The calves were inspected daily for 10 days and furthermore tested for antibodies to FMD-virus. All examinations were negative as regards FMD.

C. Restocking:

Owners of depopulated herds were permitted to restock 8 weeks after approval of disinfection II (including a negative result of the feeding of calves with environment material).

The eradication procedures including case reports and epidemiological observations etc. are described in detail in two reports published by the Danish Veterinary Service (1982 and 1983).

Economic consequences

The costs and losses in connection with the 1982-outbreak have been estimated as follows (Stougaard, 1985):

1. Public expenditure (compensation, destruction, burial, disinfection, etc.) 26 mill.Dkr.

2. Costs and losses to agriculture:

| | | |
|---|---|---|
| Directly: | (Loss on return of products and change of production methods at the slaughterhouses, etc.) | |
| | Pig slaughterhouses | 60 mill.Dkr. |
| | Cattle slaughterhouses | 6 mill.Dkr. |
| Indirectly: | (Estimated losses on the discontinuation of export to third countries and reduction of prices in the EEC) | |
| | Pig sector | 320 mill.Dkr. |
| | Cattle sector | 75 mill.Dkr. |

Total costs and losses at the FMD outbreak in 1982: 487 mill.Dkr.

Furthermore the economic consequences of various methods used to combat FMD were studied by Stougaard (1985) on the basis of the above-mentioned costs in 1982. The following three methods were compared:

A. Non-vaccination with destruction and burying of all susceptible animals on affected farms.

B. Ring-zonal vaccination in an area around the outbreak of the disease combined with destruction and burial of infected herds.

C. Annual, preventive vaccination of the entire cattle population.

For a comparison between costs/losses using the various methods of combating the disease it is naturally of decisive importance how often the country is expected to be affected by FMD, if annual vaccination is not used.

If it is assumed that Denmark will experience an outbreak corresponding to the 1982-outbreak every tenth year the total expenditure and loss over this period would be:

A. Combating without vaccination (1982-outbreak) 487 mill.Dkr.

B. Combating by ring-zonal vaccination (Costs include vaccination expenditure and estimated export losses over a five-year period). 1424 mill.Dkr.

C. Annual preventive vaccination (Costs include annual 2820 mill.Dkr. vaccination expenditure and estimated export losses over the ten-year period).

The comparison clearly demonstrates the economic advantages of a non-vaccination policy.

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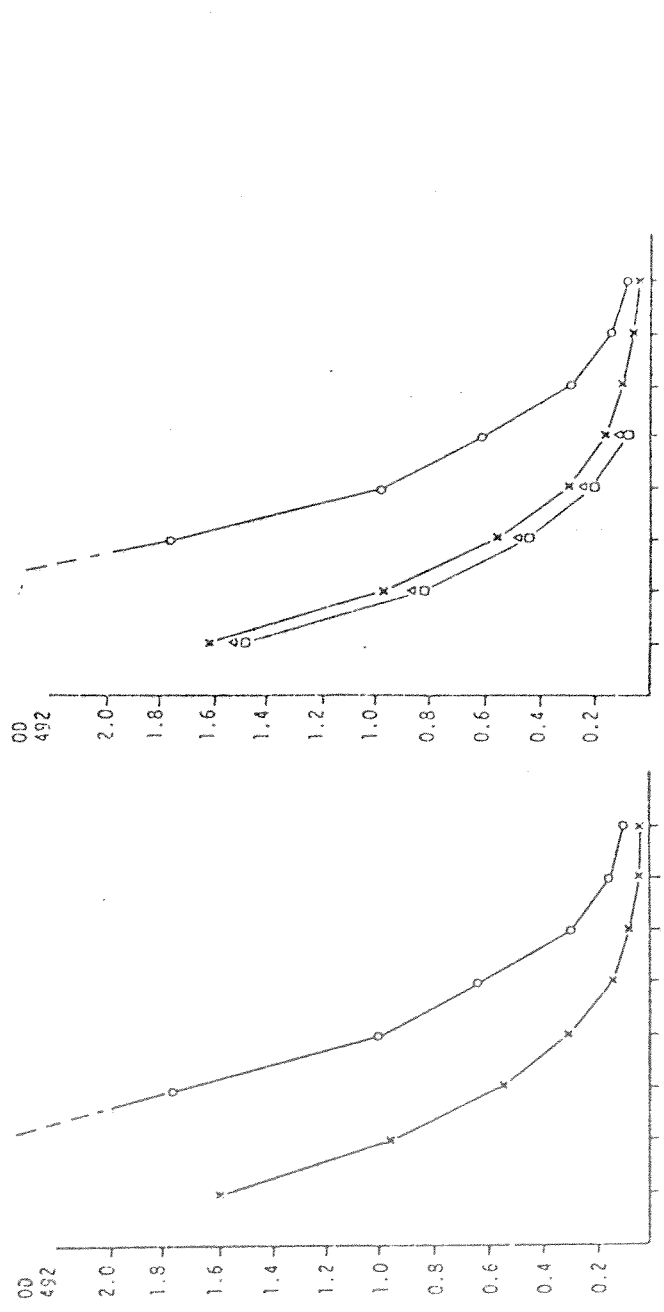


Fig. 1a-b. Titration of $O_1$BFS virus in sandwich ELISA using hyperimmune guinea pig IgG type O as catching antibody (step 1) and rabbit antiserum type $O_1$BFS (step 3). a: titration of $O_1$BFS 146 S particles (x——x) and 12 S subunits (o——o) with rabbit antiserum and conjugate diluted in buffer containing normal guinea pig serum. b: titration of 146 S particles (Δ——Δ) and 12 S subunits (□——□) from (a) with rabbit antiserum and conjugate diluted in buffer containing 2.5% of each of guinea pig hyperimmune serum type A and C. The curves from (a) are included for comparison. The first dilution contained 125 ng/ml of 146 S particles or the equivalent amount of 12 S subunits. (Have et al. 1984).

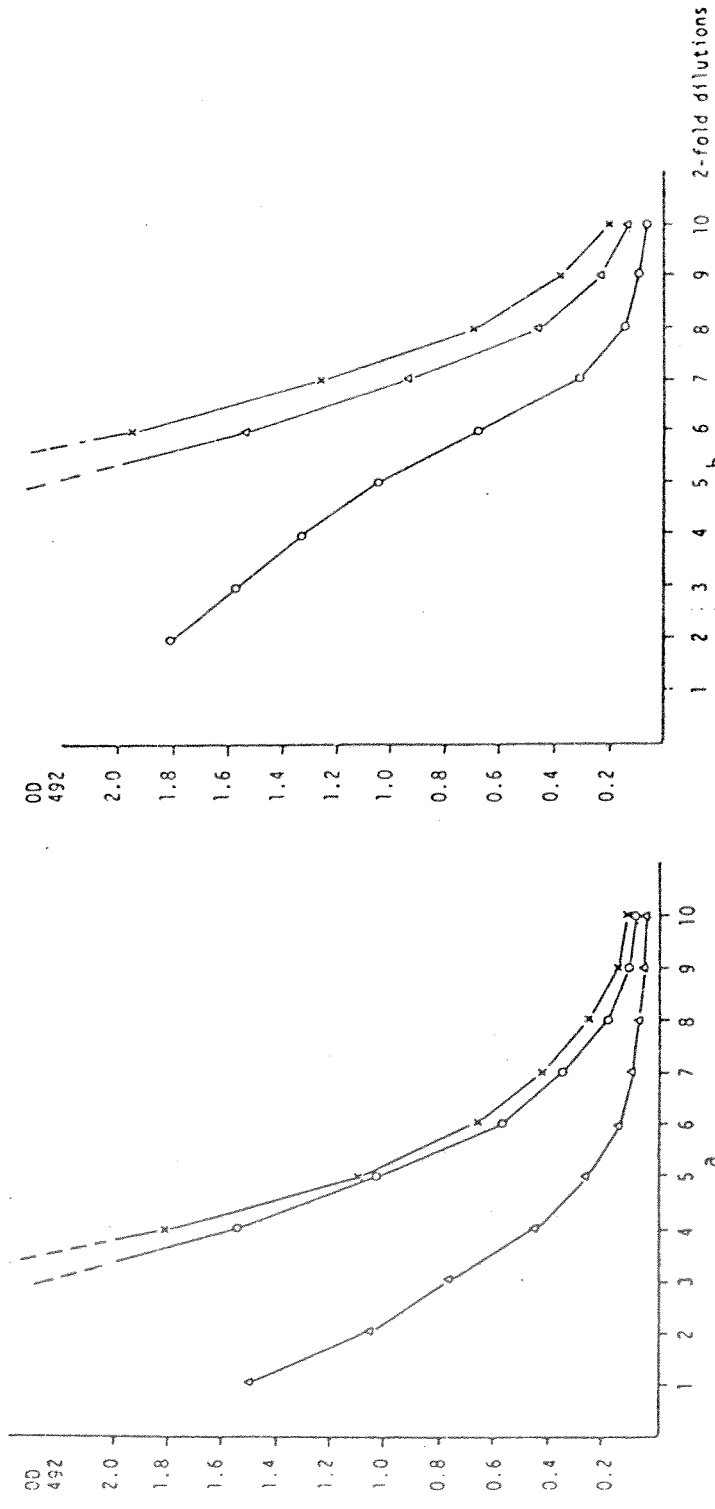


Fig. 2a-b. Titration of $O_1$BFS 146 S particles (a) and 12 S subunits (b) in the same sandwich as in fig.1. Rabbit $O_1$BFS antiserum diluted in buffer containing normal guinea pig serum (x——x) or hyperimmun guinea pig serum type A and C (o——o). Rabbit C antiserum diluted in buffer with normal guinea pig serum (Δ——Δ). The first dilution contained 2 ug/ml of 146 S particles or the equivalent amount of 12 S subunits. (Have et al. 1984).

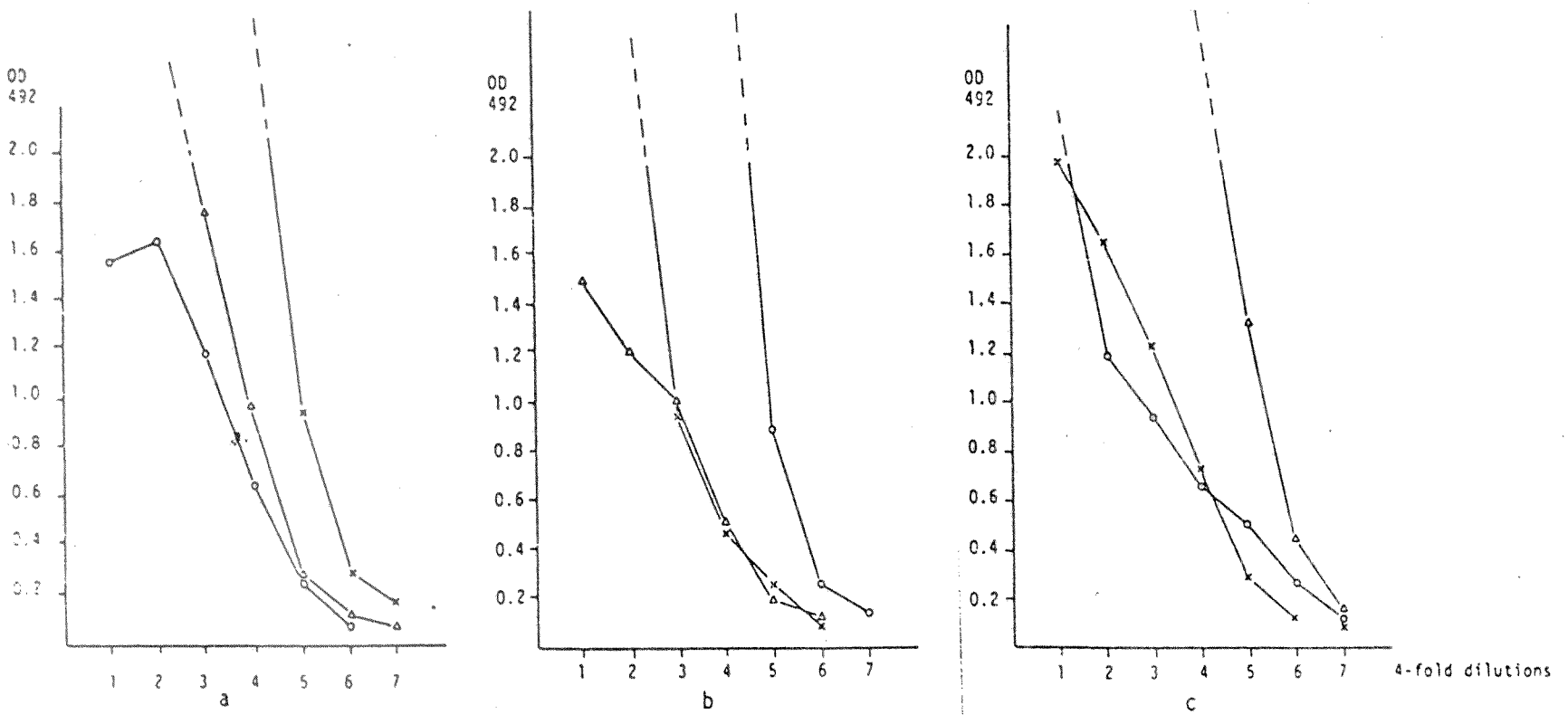


Fig. 4a-c. Parallel titrations of 12 S preparations of sucrose gradient purified 146 S particles of $O_1$ (a), $A_{10}$ (b) and C (c) virus in type O, A and C sandwich ELISA using rabbit IgG as catching antibody (step 1) and guinea pig IgG in step 3. x——x type O ELISA, o——o type A ELISA, Δ——Δ type C ELISA. (Have et al. 1984).

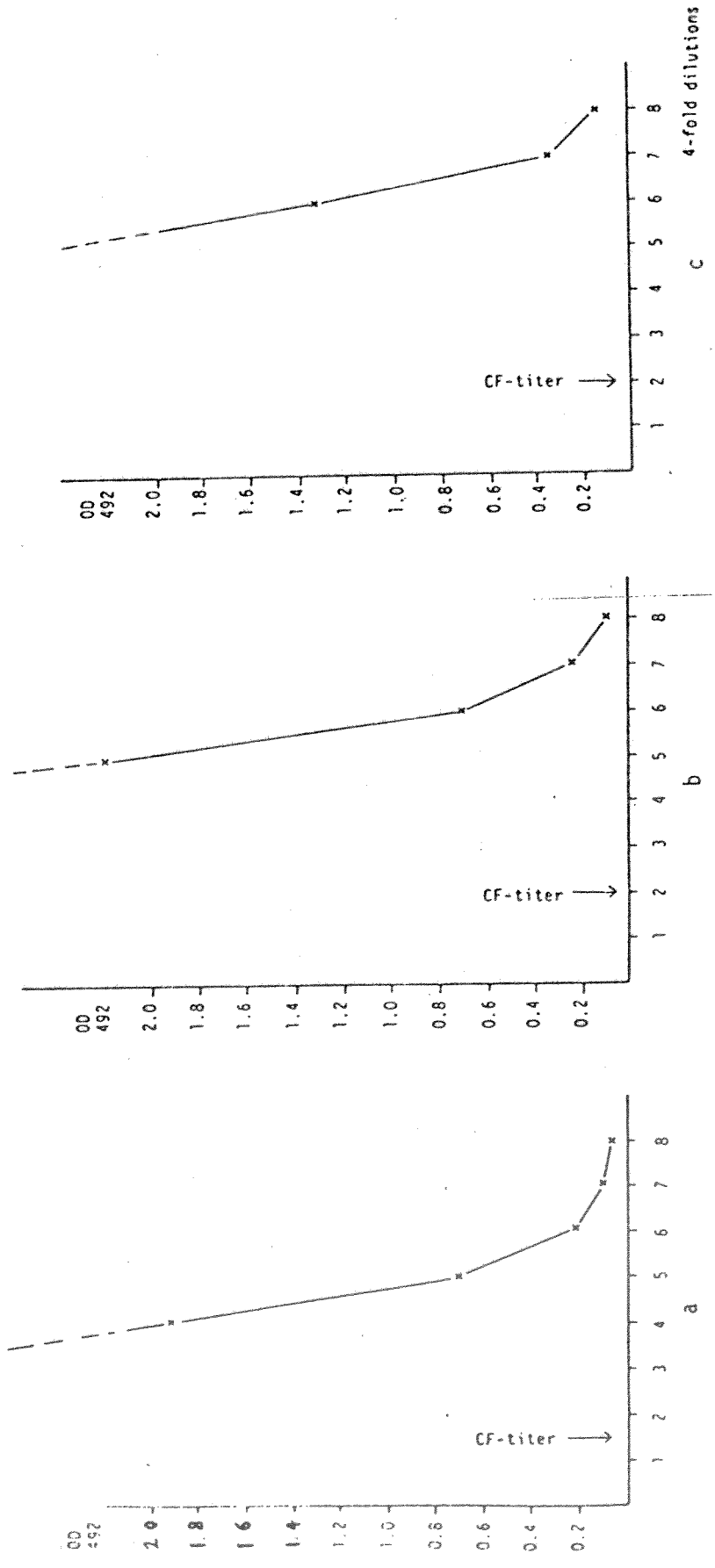


Fig. 5a-c. Comparison of the CF test and ELISA. Epithelial suspensions of $O_1$ (a) $A_5$ (b) and C (c) were examined by CF (2-fold dilutions, heat-inactivated) and ELISA (4-fold dilutions). The arrow indicates CF titre. The ELISA was performed with homologous antisera using rabbit IgG as catching antibody. (Have et al. 1984).

Table 1. 'r'-values obtained with a quantitative CF test, ELISA and a two-dimensional micro-neutralisation test (MNT) using guinea pig immune and hyperimmune sera.

| VIRUS | O Funen 82 | O Funen 83 | $O_1$BFS |
|---|---|---|---|
| SERUM | CF/ELISA/MNT | CF/ELISA/MNT | CF/ELISA/MNT |
| O Funen 82 | 1.0/ 1.0/ 1.0 | 0.53/0.66/0.63 | 0.82/0.87/0.40 |
| O Funen 83 | 0.58/0.43/0.40 | 1.0/ 1.0/ 1.0 | 0.62/0.27/0.16 |
| $O_1$BFS | 0.85/0.81/1.32 | 0.69/0.33/1.26 | 1.0/ 1.0/ 1.0 |

Appendix 12

# CHARACTERIZATION OF THE FMD VIRUS ISOLATED IN ITALY DURING 1984-1985

G.F. Panina, S. Barei, F. De Simone,
M. Bugnetti, E. Brocchi, M. Amadori

## Introduction

FMD appeared in Italy during the winter 1984-1985, after being absent since June 1981.

From the first outbreak in Northern Italy (region Emilia, province of Modena) observed on 26 November 1984, the disease spread to other regions, in Northern, Central and Southern Italy.

The virus was identified as type "A". Serological tests have shown a strict similarity between the new isolate and the Italian reference strain of type $A_5$, named A Parma 1962. This virus was responsible for a large number of outbreaks in Italy in the years before the policy of compulsory vaccination was adopted. Viruses strictly similar to A Parma 1962 have been isolated from five other outbreaks during the last 15 years in the following Italian regions: Piemonte (April 1969), Lombardia (May 1971), Puglia (November 1971), Lombardia (February 1973), Veneto (December 1980), Emilia (June 1981).

The epidemiological investigation has not revealed the source of infection of the first FMD outbreak which occurred in Modena in 1984.

Factors which apparently could have contributed to the appearance of the disease are:

- Modena is the centre of very active animal marketing and movement of animals is very intensive. The province is rich in industrial slaughterhouses which slaughter and transform tons of animals, arriving from many European countries.
- Many fattening farms are present in the region. Serological survey has shown that many of such animals had no antibody, because they had not been vaccinated or in any case had not been properly vaccinated.
- Many calves and some sheep present in the area had been recently imported from other countries not only of Western but also of Eastern Europe.
- Considering that an outbreak due to the same virus occurred in 1981 in the same area, one could suppose a survival of the 1981 virus in cloven-hoofed animals.

## Characterization of the isolates

Preliminary results of the tests performed in Brescia were reported at an *ad hoc* consultive meeting held last April at the OIE headquarters in Paris.

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A more complete picture of the available data is summarized in the enclosed tables and figures.

Table 1. Italian a isolates 1984. serological relationships by CF test.

| Serum* | Virus | | "r" value |
|---|---|---|---|
| A Parma 1962 | A Modena | 1984 (bovine) | 1.2 |
| | A Perugia | 1984 " | 1.0 |
| | A Salerno | 1984 " | 1.0 |
| | A Cuneo | 1984 " | 0.97 |
| | A Rieti | 1984 " | 1.0 |
| | A Ragusa | 1984 " | 1.0 |
| | A Castiglione | 1984 " | 1.16 |
| | A Bagnolo Mella | 1984 (swine) | 1.11 |
| A Modena 1984 | A Parma | 1962 | 0.96 |

*Guinea pig hyperimmune serum.

Comment

Figures show that FMD strains isolated in 1984 in Northern, Central and Southern Italy, in bovines and swine, are highly related with each other and with the Italian $A_5$ reference strain "A Parma 1962"

Table 2. Serological relationships among the isolate A Modena 84 and other A viruses isolated in Europe (CF test).

"r" values

| Serum* \ Virus | A Parma 62 | A Modena 84 | A Sicily 77 | A Bernbeuren 84 | A Allier 60 |
|---|---|---|---|---|---|
| A Parma 62 | 1 | 1.11 | 0.27 | 0.83 | 0.4 |
| A Modena 84 | 0.83 | 1 | 0.17 | 0.77 | 0.5 |
| A Sicily 77 | 0.23 | 0.27 | 1 | 0.27 | 0.11 |
| A Bernbeuren 84** | 0.61 | 0.67 | 0.15 | 1 | 0.35 |
| A Allier 60 | 0.6 | 0.4 | 0.2 | 0.4 | 1 |

* Guinea pig hiperimmune sera.
** Kindly furnished by Tübingen Institute.

"R" values

| | A Parma 62 | A Modena 84 | A Sicily 77 | A Bernbeuren 84 | A Allier 60 |
|---|---|---|---|---|---|
| A Parma 62 | 100 | | | | |
| A Modena 84 | 95 | 100 | | | |
| A Sicily 77 | 25 | 21 | 100 | | |
| A Bernbeuren 84 | 71 | 71 | 20 | 100 | |
| A Allier 60 | 49 | 44 | 14 | 45 | 100 |

Comment

Figures confirm that A Modena 84 virus is closely related to the Italian reference strain A Parma 1962. It is somewhat similar to A Bernbeuren 84, whereas a difference from A Allier 60 is evident. A remarkable difference can be noted also for A Sicily 77, which in a previous serological investigation had been characterized as South-American A type virus.

Table 3. Relationships between A Parma 62 and A Modena 84. Neutralization index on IBRS-2 cells.

| Serum | Virus | N.I. |
|---|---|---|
| A Parma 1962* | A Parma 1962 | 3.5 |
| | A Modena 1984 | 3.8 |
| A Modena 1984** | A Modena 1984 | 1.1 |
| | A Parma 1962 | 1.6 |

* Convalescent bovine serum.

** Guinea pig immune sera.

N.I. Neutralization index.

Comment

Results confirm the similarity between the two isolates.

Table 4. Relationships between A Parma 62 and A Modena 84. Serum protection index in mice.

| Serum | Virus | S.P.I. ($log_{10}$) |
|---|---|---|
| A Parma 1962* | A Parma 1962 | 2.14 |
| | A Modena 1984 | 2.00 |
| A Modena 1984** | A Modena 1984 | 2.14 |
| | A Parma 1962 | 2.00 |

* Convalescent bovine serum.

** Guinea pig immune serum.

S.P.I. Serum protection index.

Comment

Results confirm the similarity between the two isolates.

Table 5. Potency test in cattle of FMD vaccine (OAC) against the homologous virus A Parma 62 and the new isolate A Modena 84

| Vaccine virus | Challenge virus | |
|---|---|---|
| | A Parma 1962 | A Modena 1984 |
| A Parma 1962 | 32 $Pb_{50}$ | 32 $Pb_{50}$ |

Note. The vaccine was produced by Istituto Zooprofilattico of Brescia for the Italian vaccination campaign 1984/85. The potency tests were performed at Istituto Zooprofilattico of Perugia by Istituto Superiore di Sanita of Rome, on Danish cattle, free of FMD antibodies.

Table 6. SN averages of sera of cattle vaccinated (21 days) with different doses of FMD vaccine (OAC), produced by Istituto Zooprofilattico of Brescia. Neutralization tests on IBRS-2 cell using the homologous strain A Parma 62 and other A strains isolated in Europe.

| Serum \ Virus | A Parma 62 | A Modena 84 | A Bernbeuren 84 | A Allier 60 | A Sicily 77 | A Italy 75 |
|---|---|---|---|---|---|---|
| 1/1 dose injected cattle* | 30.6 | 32 | 25.2 | 27.8 | 6.5 | 4.75 |
| 1/4 dose injected cattle* | 17.4 | 18.4 | 16.8 | 19.8 | 3.8 | 2.2 |
| 1/16 dose injected cattle* | 9.8 | 9.6 | 8.4 | 10.6 | 0 | 0 |

* Five animals per vaccine dose.

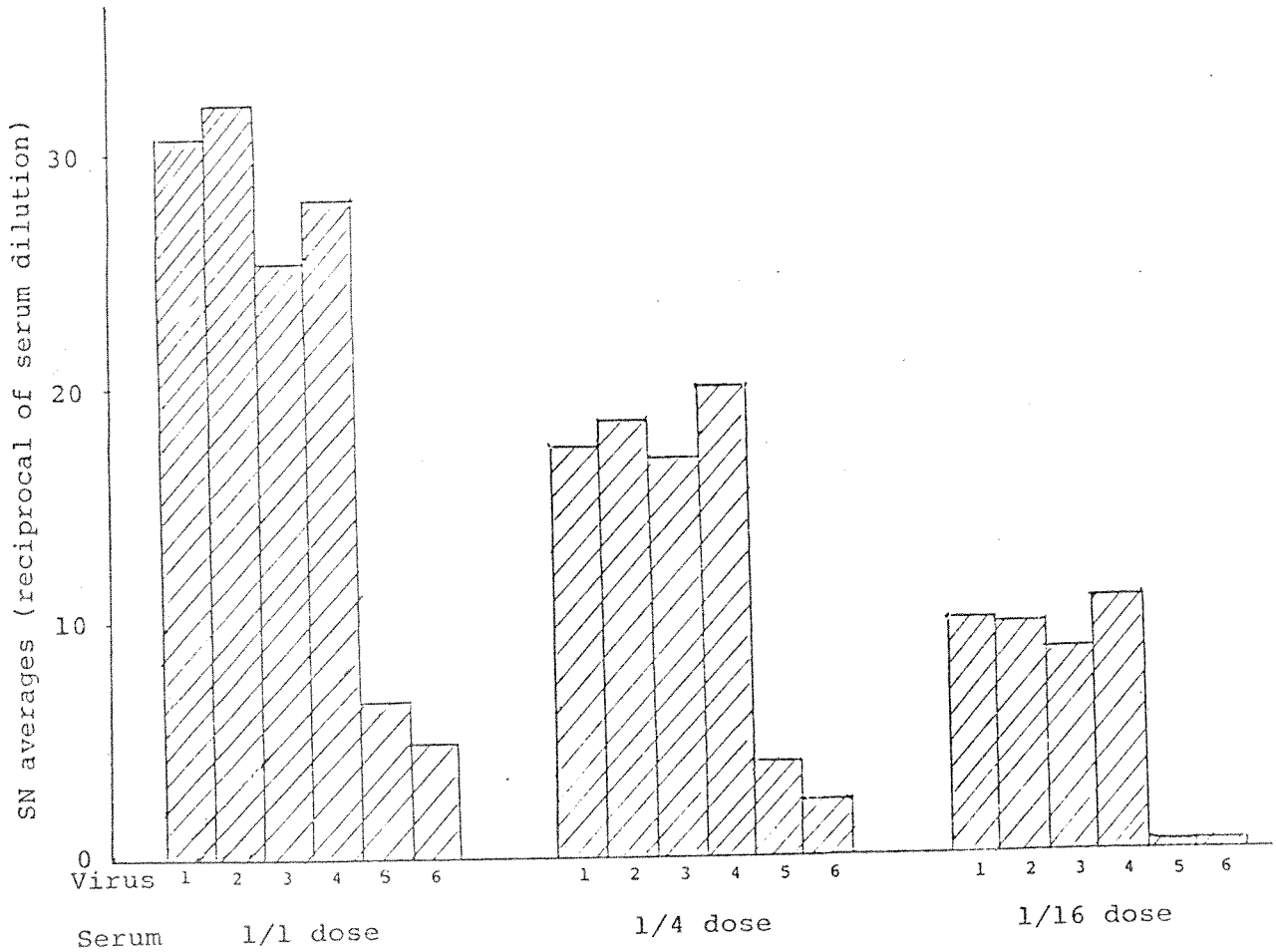
Comment

The Student t test on SN averages shows that the differences among:

- A Parma 62 and A Modena 84, A Bernbeuren 84, A Allier 60 are not significant ($P > 0.05$);
- A Parma 62 and A Sicily 77, A Italy 75 are significant ($P < 0.05$)

Previous serological investigations have characterized the isolates A Sicily 77 and A Italy 75 as South American A type viruses.

Figure 1. Graph of data of Table 6



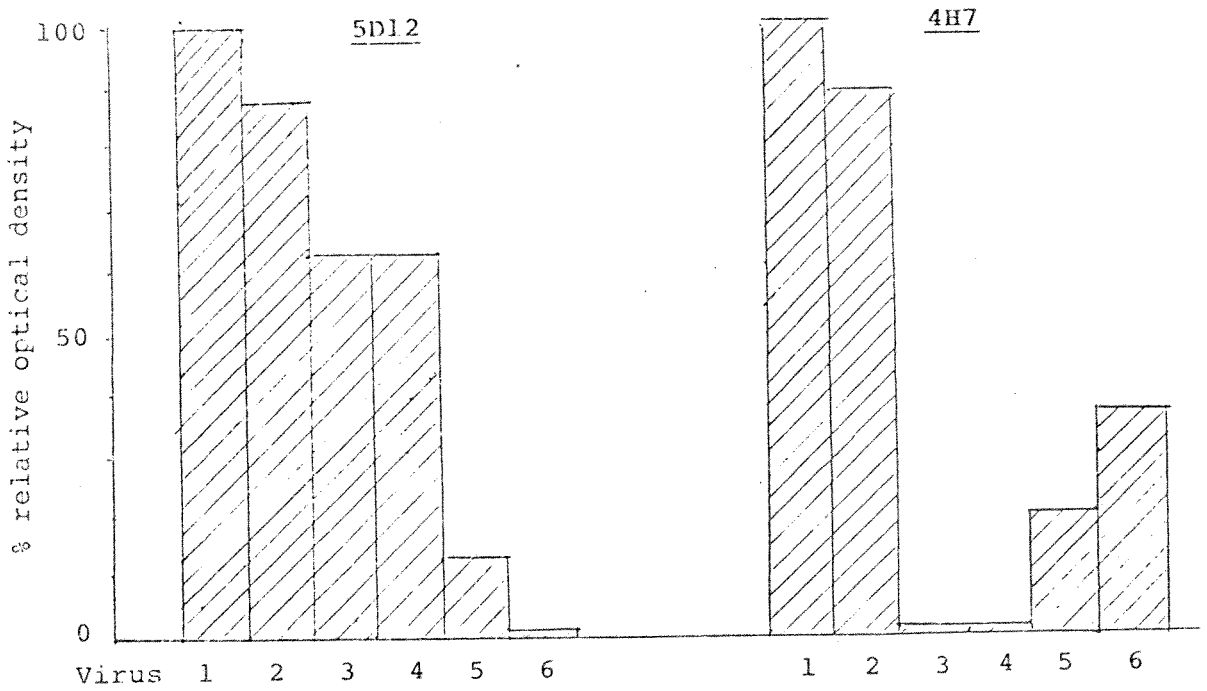

1 = A Parma 62 2 = A Modena 84 3 = A Bernbeuren 84
4 = A Allier 60 5 = A Sicily 77 6 = A Italy 75

Comment

Antibodies elicited by A Parma 62 neutralize to the same extent the homologous virus and the strains A Modena 84, A Bernbeuren 84, and A Allier 60.

Low reactivity was found with A Sicily 77 and A Italy 75.

Figure 2 Characterizaton of some FMD viruses of type A isolated in Europe. ELISA (sandwich) using the monoclonal antibodies 5D12 and 4H7.




1 = A Parma 62 2 = A Modena 84 3 = A Bernbeuren 84
4 = A Allier 60 5 = A Sicily 77 6 = Italy 75

Comment

A Parma 62 and A Modena 84 are similar and differ from A Bernbeuren 84 and A Allier 60. The latter ones are very much alike, as shown by an identical pattern of reaction with two MAbs used in the test.

The above mentioned results are preliminary in that they must be confirmed using a larger panel of MAbs.

Monoclonal antibodies used in the test were produced against A Parma 62. They have the following characteristics:

5D12: MAb directed against a trypsin-sensitive, SDS-resistant determinant.

4H7: Mab directed against a trypsin-resistant, SDS-liable determinant.

Both neutralize in vivo and in vitro and protect in mice.

Appendix 13

# COMPARISON OF STRAIN SPECIFICITY OF ANTISERA TO FOOT-AND-MOUTH DISEASE VIRUS FROM CATTLE AND GUINEA-PIGS IN NEUTRALISING TESTS

Reinhard Ahl*

Guinea-pig antisera are frequently used for subtyping and strain differentiation of foot-and-mouth disease (FMD) virus isolates by complement fixation or neutralizion tests when antisera from cattle are not available. The information obtained from serological analysis gives an estimate of the relationship of an isolate to virus strains used for vaccine production and may influence the choice of a vaccine in case of emergency vaccination (Rweyemamu et al., 1977; Pereira, 1977).

It has been concluded that guinea-pig antisera demonstrate the same subtype or strain specificity in neutralization tests as bovine antisera, at least those obtained from vaccinated animals (Rweyemamu et al., 1984).

We show in this communication that the serological relationship between virus strains as measured in plaque reduction assays is in some cases different for guinea-pig and bovine antisera.

Guinea-pig sera used were from groups of at least four animals hyperimmunized with purified virus in Freund's complete adjuvant. Bovine antisera were mainly from individual convalescent animals. Bovine sera to $O_1$ Basel, $O_1$ BFS, and $A_{10}$ Holland were from vaccinated cattle. The bovine antiserum to $A_{10}$ Holland was a pool of eight selected individual sera received from the Central Veterinary Institute, Lelystad. These animals had been vaccinated three times in the course of the annual lifestock vaccination and had a mean homologous neutralization titre of 1:1100 in plaque reduction tests. The $A_{10}$ virus available for immunizing guinea-pigs was from the Animal Virus Research Institute, Pirbright and was therefore named $A_{10}$ Pirbright.

Tables 1 and 2 demonstrate that antisera from cattle and guinea-pig to FMD virus strains of type O and A can differ significantly in the reaction with heterologous virus strains or subtypes. This is shown for convalescent bovine serum as well as for serum from vaccinated animals, especially for that to subtype $A_{10}$.

In these tables the log differences and the corresponding r-values are shown. In most cases heterologous antibody titres deviate more from the respective homologous serum titre with guinea pig antisera than with bovine sera. Virus $O_1$ Fyn (Denmark, 1983) had lower r-values with bovine serum to four different $O_1$ strains.

Among type A virus strains, A Aachen (1976) and $A_{24}$ Bilbao which are closely related isolates showed a bigger difference in log serum titres and smaller r-values to three $A_5$ strains with bovine antiserum than with guinea pig antiserum.

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The data suggest that apparently in a number of virus strains guinea-pigs recognize other antigenic determinants on FMD virus particles than cattle. The results indicate that although guinea-pig antisera may be very useful for strain identification they may be misleading in the evaluation of the protective value of a vaccine against a new virus isolate from the field.

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Table 1: Comparison of strain specificity of antisera to FMDV from cattle and guinea-pigs: Subtype $O_1$ strains

| Virus strain | Immune serum | Difference in -log $SN_{90}$ | | r - Values | |
|---|---|---|---|---|---|
| | | bovine serum | guinea-pig serum | bovine serum | guinea-pig serum |
| $O_1$ Kaufbeuren | $O_1$ Basel (1965) | -0.04 | 0.31 | 1.10 | 0.49 |
| $O_1$ BFS, SP[1)] | - " - | 0.33 | 0.74 | 0.47 | 0.18 |
| $O_1$ BFS, SP | $O_1$ Kaufbeuren | -0.09 | 0.25 | 1.23 | 0.56 |
| $O_1$ Fyn (1983) | - " - | 0.33 | -0.06 | 0.47 | 1.15 |
| $O_1$ Fyn (1983) | $O_1$ BFS, SP | 0.49 | 0.16 | 0.32 | 0.69 |
| $O_1$ Fyn (1983) | $O_1$ Laus.-Lyon | 0.62 | 0.34 | 0.24 | 0.46 |
| $O_1$ Denmark(1982) | - " - | 0.08 | 0.28 | 0.83 | 0.52 |
| $O_1$ Kaufbeuren | $O_1$ Murchin(1982) | -0.08 | 0.41 | 1.20 | 0.39 |
| $O_1$ BFS, SP | - " - | -0.03 | 0.67 | 1.07 | 0.21 |
| $O_1$ Laus.-Lyon | - " - | 0.11 | 0.70 | 0.78 | 0.20 |
| $O_1$ Denmark(1982) | - " - | -0.04 | 0.28 | 1.10 | 0.52 |
| $O_1$ Fyn (1983) | - " - | 1.01 | 0.44 | 0.10 | 0.36 |
| $O_1$ BFS, SP | $O_1$ Denmark(1982) | 0.01 | 0.45 | 1.02 | 0.35 |
| $O_1$ Laus.-Lyon,SP | - " - | 0.07 | 0.30 | 0.85 | 0.50 |
| $O_1$ Kaufbeuren | $O_1$ Fyn (1983) | 0.31 | 0.85 | 0.49 | 0.14 |
| $O_1$ Denmark, 1982 | - " - | 0.57 | 0.76 | 0.27 | 0.17 |

1) SP = small plaque mutant

Table 2: Comparison of strain specificity of antisera to FMDV from cattle and guinea-pigs: Type A strains

| Virus strain | Immune serum | Difference in -log $SN_{90}$ | | r - Values | |
|---|---|---|---|---|---|
| | | bovine serum | guinea-pig serum | bovine serum | guinea-pig serum |
| $A_4$ West Germany | $A_5$ Allier | 0.16 | 1.18 | 0.69 | 0.07 |
| $A_{10}$ Pirbright | - " - | 0.57 | 0.87 | 0.27 | 0.13 |
| $A_{24}$ Bilbao | - " - | 0.87 | 0.56 | 0.13 | 0.28 |
| $A_4$ West Germany | $A_5$ Westerwald | -0.34 | 1.28 | 2.19 | 0.05 |
| $A_{10}$ Pirbright | - " - | 0.84 | 1.48 | 0.14 | 0.033 |
| A Aachen (1976) | - " - | 2.05 | 1.58 | 0.009 | 0.026 |
| $A_{24}$ Bilbao | - " - | 1.82 | 1.19 | 0.015 | 0.065 |
| $A_4$ West Germany | $A_5$ Bernbeuren | 0.46 | 1.63 | 0.35 | 0.023 |
| A Aachen | - " - | 1.29-1.64[1)] | 0.92 | 0.05-0.02 | 0.12 |
| $A_{24}$ Bilbao | - " - | 0.68-1.09[1)] | 0.48 | 0.21-0.08 | 0.33 |
| $A_4$ West Germany | $A_{10}$ Holland | 0.35 | 2.02 | 0.45 | 0.010 |
| $A_5$ Westerwald | - " - | 0.79 | 1.58 | 0.16 | 0.026 |
| $A_5$ Bernbeuren | - " - | 0.84 | 2.08 | 0.14 | 0.008 |
| $A_5$ Müllheim | - " - | 0.75 | 2.45 | 0.18 | 0.004 |
| $A_5$ Spain (1983) | - " - | 0.81 | 1.86 | 0.15 | 0.014 |
| $A_5$ Modena (1984) | - " - | 0.83 | 1.82 | 0.15 | 0.015 |
| A Tunesia (1982) | - " - | 1.11 | 2.89 | 0.078 | 0.001 |
| A Aachen | - " - | 0.99 | 1.66 | 0.10 | 0.022 |
| $A_{24}$ Bilbao | - " - | 0.83 | 1.75 | 0.15 | 0.018 |

1) 2 individual sera, not pooled

Appendix 14

# RECENT APPROACHES TO THE CHARACTERISATION OF STRAINS OF FOOT-AND-MOUTH DISEASE VIRUS

C. Hamblin, J.R. Crowther, D. Baber, N.J. Knowles and D. McCahon*

## Introduction

During the last five years, investigations for the characterisation of strains of foot-and-mouth disease virus (FMDV) have benefited from the application of several new methods. These were developed originally for work outside the field of FMD research, never-theless their introduction has significantly increased the sensitivity of detection and the level of discrimination at which strains of virus or viral components can be compared. Advances in molecular biology and the biochemistry of viruses have resulted in methods in which the proteins of the capsid or the nucleic acid constituents of the genome can be separated and subsequently analysed. The use of highly specific reagents such as monoclonal antibodies in the newer serological methods e.g. ELISA can conveniently provide more information on the identity, specificity and possible origin of viruses than was possible with the older methods such as complement fixation and virus neutralization. The purpose of this paper is to bring together examples of these newer methods as applied to investigations with various strains of FMD virus.

## A. Immunological tests

### I. Diagnosis of foot-and-mouth disease virus by ELISA

The direct complement fixation test (CF) has been used routinely for many years in the World Reference Laboratory (WRL) at the A.V.R.I., Pirbright. Between 1979 and 1985, 1,490 epithelium samples were received for typing. Some of these samples were inadequate in terms of low amount and poor condition (pH, contamination, etc.) on receipt. Although virus was isolated after tissue culture passage from 1,027 of these samples, only 552 (54%) contained sufficient material for direct typing by CF tests. One hundred and forty (25%) of the 552 gave type specific results, a further 102 (19%) were anti-complementary and the remaining 310 (56%) were inconclusive or gave negative results.

A rapid ELISA is now available for typing FMDV. The test is type specific and considerably more sensitive than the current CF test. The assay (Figure 1) is based on the method described by (Hamblin et al 1984) but uses rabbit anti-FMDV antisera bound to the solid phase plates. Bovine proteins are often present in the virus antigen suspensions used to generate the rabbit and guinea pig antisera for typing purposes and therefore the antisera produced contain anti-bovine as well as anti-viral activity. However it can readily be blocked by the incorporation of bovine serum in the diluent.

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Epithelium samples have been examined using both ELISA and CF. The results of this comparison are given in Table 1. In no case did the routine CF test type any sample which was not typed by ELISA. A few samples which gave positive typing results by ELISA and were negative by CF did not contain demonstrable levels of infectious virus, however, in each case the virus type detected was the same as that causing the outbreak. Figure 2 shows the typing results which were recorded for three of the epithelial samples received at the WRL.

The ELISA has several advantages over the "traditional" CF test.

1. The assay specifically types samples containing live or inactivated virus and/or 12S viral sub-units.

2. Pro- and anti-complementary effects are irrelevant in the ELISA.

3. The assay is considerably more sensitive and will successfully type samples containing 20-100 times less FMDV antigen than is required in the CF test.

4. Heterologous and non-specific reactions are of low order, making unequivocal reading of the results of an ELISA assay relatively easy.

5. The ELISA uses less reagents than the CF test, e.g. a yield of 60 ml and 12 ml of serum from rabbits and guinea pigs respectively is average and using such sera at their optimal dilution in the ELISA, it can be estimated that the serum from one rabbit and ten guinea pigs for each virus type would be sufficient to last 2,800 years at the current rate of testing in the WRL, AVRI. Thus standardised reagents may be easily established for all typing laboratories in the world for the forseeable future.

## II. Antibody assay by ELISA

Virus neutralization (VN) has been the accepted test for the quantification of antibodies against FMDV for many years. The test is theoreticlly sensitive, specific and relatively simple to perform, although it requires tissue culture cells which vary in sensitivity or fail to grow because of contamination, poor growth conditions or toxic substances in the sample under test. The assay also requires three days incubation before end-points are read.

A rapid liquid-phase-amplified ELISA has been developed for the quantification of anti-FMDV antibodies and for the screening of sera from animals before import or export. The assay is similar to the typing ELISA described previously but test serum dilutions are incubated in the presence of a constant dose of clarified tissue culture virus in a carrier plate (Figure 3). Unreacted antigenic sites on the virus in the serum/virus mixtures are then detected by the ELISA.

Large numbers of sera have been examined against each of the seven immunologically distinct virus types to determine the correlation between ELISA and VN test results. The results shown in Figure 4 were obtained for FMDV types A and SAT 1 and are typical of those obtained for positive and negative sera to each of the virus types studied.

The advantages of the ELISA are:-

1. Tissue culture cells are NOT required and the assay is unaffected by factors which influence cell growth and sensitivity to virus.

2. The assay can be made in one day for samll numbers of sera, large numbers require two days.

3. Each test has an in-built control from which the antibody titres are calculated, tests should not therefore need to be repeated.

4. Since the ELISA will function with inactivated virus, it can be used in laboratories normally restricted in the use of live virus as for VN tests.

5. The correlation between the VN test and ELISA is good and individual antibody titres are of similar order, making the interpretation of results easy and acceptable to workers only used to VN titres.

III. Use of monoclonal antibodies in subtyping

The production and characterisation of monoclonal antibodies (mabs) against FMD virus has been done at several laboratories internationally. As an example of their use in subtyping, a panel of mabs from Brescia has been used in conjunction with the ELISA. The basis of the method is similar to that shown in Figure 3 but using monoclonal antibody as the specific antibody.

The particular mabs in this study have been used since most of them neutralize virus infectivity both in vitro and in vivo. Three separate antigenic sites have been characterised on type O Swiss (Lausanne) FMDV using this panel. Hence, heterologous virus is being compared on epitopes which are neutralizable. The test dilution of the mabs is determined by titration of homologous systems (virus and mab) by the method described above where mab dilution ranges replace the mab single dilution. The dilution of mab showing the last reading on the plateau maximum (where the antigen is saturated by antibody) is used in the test proper. The test is controlled in terms of assessing how much of each virus is being measured by the addition of a polyclonal mouse serum instead of mab for each antigen tested. Such a serum is highly cross-reactive for the Type O viruses and the colour obtained for each antigen should be the same, this is made clearer if the results are examined. Table 2 shows some raw data for the examination of seven viruses on a single plate. Tables 3 and 4 show how data are processed to obtain antigen profiles (epitope comparisons). Table 5 shows processed data for a larger number of viruses and Figure 5 shows some of these data in diagrammatic form.

Conclusions

1. Viruses are being compared by using mabs which define neutralizable epitopes, therefore the comparisons may be pertinent to protective antibodies induced in animals against these epitopes. Thus the data may offer a real comparison of the relevance of serological differneces between vaccine and field strains.

2. The test is rapid. Subtyping can be performed on any material e.g. epithelial samples, tissue culture virus, using previously prepared and characterised mabs. Thus results are available on the same day as the sample arrives, provided that there is

enough virus to be typed by the ELISA described in this paper. This means that an adequate service could be provided for the subtyping of FMDV. Strong support for the preparation and characterisation of mabs against all vaccine strain viruses is recommended. It will take about three years to obtain the panels of mabs and investigate the performance of these against all the isolates of FMDV at the AVRI, Pirbright. An internationally funded research project should be considered immediately in order to facilitate this.

3. Reproducible differences in the epitopes expressed by the type O viruses examined are found. Situations where no specific epitope expression is found are common. The number of shared epitopes also varies. Situations where lower percentage reactions for the binding of mabs as compared to the homologous reaction remain to be elucidated using experiments in the laboratory with field isolates and with mutants from the homologous virus generated by mab pressure.

B. Biochemical examination of foot-and-mouth disease virus strains

This method of investigation is illustrated by studies on the viruses causing the recent outbreaks of FMD in Northern and Southern Italy and Sicily. By the analysis of the virus capsid structural polypeptides and the viral RNA's we have shown the outbreak viruses to be closely related to each other and to the current type A Italian vaccine strain A5/Parma/Itl 62. Comparative studies described here and elsewhere indicate that FMD virus is extremely unlikely to survive unaltered in the field for 22 years. In all the field isolates examined in the World Reference Laboratory, the subtype was demonstrated as being A5, with no serological evidence that any significant antigenic change had occurred. To characterise these isolates more precisely we have used two independent biochemical methods; polyacrylamide gel electrophoresis (PAGE) and $T_1$ RNase oligonucleotide mapping. By these methods we have found evidence that early outbreaks were caused by the same virus and that this strain is indistinguisable from the A5 Parma strain of virus isolated in 1962, but is clearly different to other strains of type A.

I. Polyacrylamide Gel electrophoresis

PAGE in a SDS buffer system separates virus structural proteins according to their size. By the use of this technique it has been demonstrated that the four virus proteins present, VP1, VP2, VP3, and VP4, consistently take up the same position relative to each other in gels and this holds true for both virus type and subtype. Virus samples received as epithelium were passaged once in primary bovine thyroid cells and then once or twice in BHK cells and labelled with radioactive 35S-methionine. The labelled virus was concentrated, purified and denatured and finally layered on a stacking gel (acrylamide 30:0.8 bis acrylamide). The resolution gel was 10% (acrylamide 30:0.8 bis) containing 8M urea and was run at 40 volts for 19 hours. Figure 6 illustrates the results obtained with Type A strains of epidemiological importance. A Bernbeuren, Bavaria, W. Germany, 1984; $A_5$ Allier, France, 1960; A Modena, Italy, 1980; $A_5$ Parma, Italy, 1962; $A_5$ Gorizia, Italy, 1960.

Comparison of these seven isolates was made on the position of the structural polypeptides VP1, VP2 and VP3. Isolate A Bernbeuren clearly shows a difference in the position of VP2 in comparison with

the other isolates, which all show a characteristic $A_5$ pattern, however some minor size difference exists in VP2 in A Modena when closely compared to $A_5$ Allier and $A_5$ Gorizia.

II. $T_1$ oligonucleotide mapping

Ribonuclease T.1 fingerprinting has been used for the identification and differentiation of isolates of many different families of viruses. It has found application in studying the epidemiology of diseases and has proved valuable in the rapid identification of specific outbreak viruses. The fractionation of the oligonucleotides by two-dimentional electrophoresis is firstly performed by separating the oligonucleotides according to their charge and then according to their size in the second dimension.

Figure 7 shows RNase fingerprints prepared from virus RNAs obtained from RNA extracted from 32P labelled virus particles. The virus isolates were grown as described previously except that the virus maintenance medium was tris buffer pH 7.6 (phosphate free) containing 100 uCi 32 $PO_4/ml^{-1}$.

The viruses examined were:-

A = A Modena/Itl 1/84
B = $A_5$/Parma/Itl 62
C = $A_5$/Allier/Fra 60
D = A/Arg 79
E = A/Modena-$A_5$ Parma mixture.

The $A_5$ Parma and A/Modena strains examined in this study were identical, however, $A_5$/Allier was shown to be considerably different in large areas of the maps with an even greater dissimilarity to A/Arg 79. Confirmation of the close relationship between $A_5$/Modena and $A_5$/Parma was provided by analysing a mixture (panel E) which was indistinguishable from the two viruses on their own and showed no extra oligonucleotide.

III. Electrofocussing of virus induced proteins

Electrofocussing separates proteins according to their iso-electric point and this is determined by the individual pKs of the ionizable groups. A mutation in the RNA that results in the substitution of an amino acid of a different charge will change the iso-electric point of the protein. Using electrofocussing we have readily detected differences between FMD virus strains (McCahon et al, 1982) and it has proved to be a useful and rapid method of identifying strains and detecting changes in virus isolates. We have used the method to study the evolution of FMD viruses in carrier animals, in unvaccinated susceptible animals and most recently from the Italian outbreaks in partially immune animals.

The details of the methods have been published previously (King et al, 1981). Essentially the virus (preferably an epithelium sample) is grown for the minimum possible number of passages in primary bovine thyroid cells and then labelled in the same cells after the addition of a radioactive labelled amino acid (35S methionine). The cell extract is then analysed on electrofocussing gels and a typical result is shown in Figure 8 for some of the Italian virus isolates. The initial isolate and three of the early isolates were indistinguishable from the $A_5$ Parma vaccine strain which is consistent with

the $T_1$ oligonucleotide mapping of their RNA's The other isolates examined were changed from the "original" and it is possible to draw a likely evolutionary relationship between the viruses based on the electrofocussing results (Figure 9), which is consistent with the temporal and geographical relationships of the isolates, i.e. generally mutants were found in the same province as their presumptive parent and at the same time or after the isolation of the parent, never before. The pattern of a rapidly evolving virus resembles that seen with sequential isolates from carrier animals (types O, A and SAT2, unpublished results) and is quite unlike observations on viruses from outbreaks in fully susceptible animals. We have studied three separte outbreaks of the latter type, U.K. 1967-68; U.K. 1981 and Denmark 1982 (unpublished data and King et al, 1981). In each case, little consistent change was observed in the virus during the course of the outbreak despite passage in different hosts and under different conditions. All three type O outbreaks occurred in fully susceptible animals (unvaccinated) whereas the carrier animals and a high percentage of the Italian animals were vaccinated. Therefore we conclude that the apparently rapid evolution observed in the Italian outbreak is related to the presence of antibody in the animal population. It will be interesting to see if the examination of the later isolates from this outbreak show a continuing evolution of virus proteins.

The potential value of the biochemical analyses of viruses in epidemiological studies is apparent from the illustrations given here and other similar work on FMD viruses in other outbreaks. The relevance of the differences and the actual link between biochemical and serological tests remains to be evaluated.

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Fig. 1 Diagrammatic representation of the components in an ELISA for typing Foot-and-Mouth Disease virus.

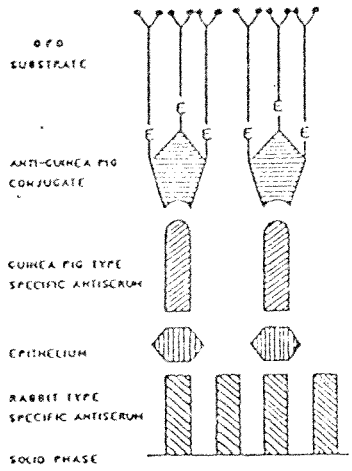



TABLE 1

COMPARATIVE FMDV TYPING RESULTS BY CF AND ELISA

| | DIRECT CF | % | DIRECT ELISA | % |
|---|---|---|---|---|
| POSITIVE TYPING | 16 | 23 | 52 | 75 |
| ANTICOMPLEMENTARY | 12 | 17 | | |
| NEGATIVE OR INCONCLUSIVE | 41 | 60 | 17 | 25 |
| TOTAL | 69 | 100 | 69 | 100 |

Fig. 2

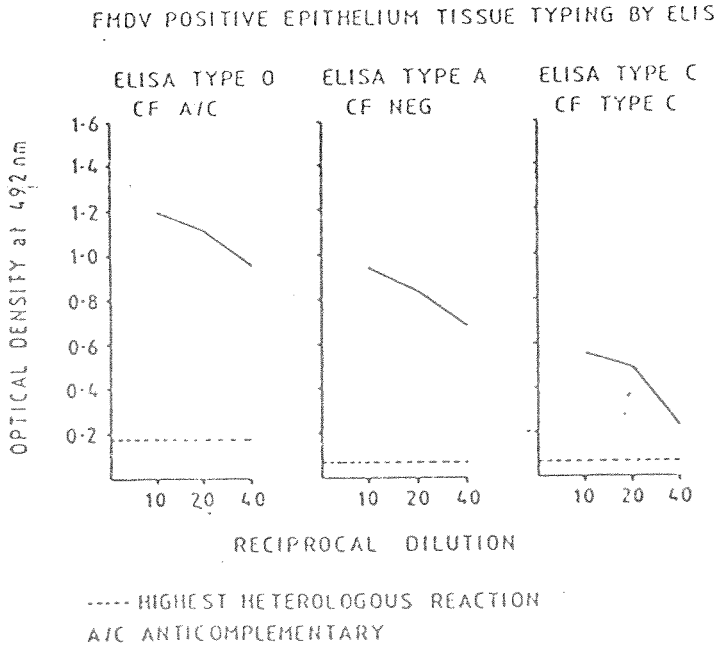


TABLE 2 The observed data from 7 type O viruses reacted in an ELISA with a panel of 8 monoclonal antibodies and a polyclonal serum

MAB (ABSORBANCE VALUES FROM SPECTROPHOTOMETER AT 492NM)

| VIRUS | B2 | D9 | C6 | C8 | C9 | G4 | G5 | A8 | POLY |
|---|---|---|---|---|---|---|---|---|---|
| $O_1$ Campos | .86 | 1.08 | .55 | .54 | .72 | .39 | .10 | .60 | 1.48 |
| Moz 1/72 | .42 | .75 | .32 | .33 | .33 | .30 | .05 | .21 | 1.41 |
| $O_1$ Lausanne | .90 | 1.07 | .85 | .54 | 1.02 | .78 | .77 | .52 | 1.35 |
| OV1 | .18 | .86 | .46 | .67 | .77 | .04 | .07 | .69 | 1.36 |
| Tun 3/75 | .83 | 1.11 | .24 | .40 | .75 | .37 | .00 | .47 | 1.40 |
| $O_5$ India 62 | 1.09 | 1.43 | .55 | .79 | 1.06 | .56 | .26 | .71 | 1.61 |
| Swiss | .78 | 1.03 | .78 | .53 | .94 | .84 | .96 | .79 | 1.37 |

POLY = mouse anti type O FMDV polyclonal serum.

TABLE 3 Data from Table 2 expressed as a percentage from the ratio Mab/Polyclonal

PERCENTAGE MAB A/POLYCLONAL A (for each sample tested)

| VIRUS | Mab B2 | D9 | C6 | C8 | C9 | G4 | G5 | A8 |
|---|---|---|---|---|---|---|---|---|
| $O_1$ Campos | 58 | 72 | 37 | 36 | 49 | 26 | 7 | 41 |
| Moz 1/72 | 36 | 65 | 28 | 29 | 29 | 26 | 4 | 18 |
| $O_1$ Lausanne | 67 | 79 | 63 | 40 | 76 | 58 | 57 | 39 |
| OV1 | 13 | 64 | 34 | 50 | 57 | 3 | 5 | 51 |
| Tun 3/75 | 59 | 79 | 17 | 29 | 53 | 26 | 0 | 34 |
| $O_5$ India 62 | 67 | 89 | 34 | 49 | 65 | 35 | 16 | 44 |
| $O_1$ Swiss 65 | 58 | 75 | 57 | 39 | 69 | 63 | 71 | 58 |

TABLE 4 Data from Table 3 for each virus strain expressed as a percentage in relation to the results with the $O_1$ Swiss strain

PERCENTAGE RELATIVE TO SWISS % VALUE FOR EACH MAB

| VIRUS | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| $O_1$ Campos | 100 | 96 | 65 | 92 | 71 | 42 | 10 | 71 |
| Moz 1/72 | 62 | 87 | 48 | 74 | 42 | 42 | 6 | 31 |
| O1 Lausanne | 115 | 105 | 110 | 102 | 110 | 93 | 85 | 67 |
| OV1 | 22 | 85 | 60 | 128 | 83 | 5 | 8 | 88 |
| Tun 3/75 | 101 | 105 | 29 | 74 | 76 | 0 | 0 | 59 |
| $O_5$ India 62 | 115 | 118 | 60 | 125 | 94 | 25 | 23 | 76 |
| $O_1$ Swiss 65 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |

TABLE 2 The observed data from 7 type O viruses reacted in an ELISA with a panel of 8 monoclonal antibodies and a polyclonal serum

MAB (ABSORBANCE VALUES FROM SPECTROPHOTOMETER AT 492NM)

| VIRUS | B2 | D9 | C6 | C8 | C9 | G4 | G5 | A8 | POLY |
|---|---|---|---|---|---|---|---|---|---|
| $O_1$ Campos | .86 | 1.08 | .55 | .54 | .72 | .39 | .10 | .60 | 1.48 |
| Moz 1/72 | .42 | .75 | .32 | .33 | .33 | .30 | .05 | .21 | 1.41 |
| $O_1$ Lausanne | .90 | 1.07 | .85 | .54 | 1.02 | .78 | .77 | .52 | 1.35 |
| OV1 | .18 | .86 | .46 | .67 | .77 | .04 | .07 | .69 | 1.36 |
| Tun 3/75 | .83 | 1.11 | .24 | .40 | .75 | .37 | .00 | .47 | 1.40 |
| $O_5$ India 62 | 1.09 | 1.43 | .55 | .79 | 1.06 | .56 | .26 | .71 | 1.61 |
| Swiss | .78 | 1.03 | .78 | .53 | .94 | .84 | .96 | .79 | 1.37 |

POLY = mouse anti type O FMDV polyclonal serum.

TABLE 3 Data from Table 2 expressed as a percentage from the ratio Mab/Polyclonal

PERCENTAGE MAB A/POLYCLONAL A (for each sample tested)

| | Mab | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | B2 | D9 | C6 | C8 | C9 | G4 | G5 | A8 |
| VIRUS | | | | | | | | |
| $O_1$ Campos | 58 | 72 | 37 | 36 | 49 | 26 | 7 | 41 |
| Moz 1/72 | 36 | 65 | 28 | 29 | 29 | 26 | 4 | 18 |
| $O_1$ Lausanne | 67 | 79 | 63 | 40 | 76 | 58 | 57 | 39 |
| OV1 | 13 | 64 | 34 | 50 | 57 | 3 | 5 | 51 |
| Tun 3/75 | 59 | 79 | 17 | 29 | 53 | 26 | 0 | 34 |
| $O_5$ India 62 | 67 | 89 | 34 | 49 | 65 | 35 | 16 | 44 |
| $O_1$ Swiss 65 | 58 | 75 | 57 | 39 | 69 | 63 | 71 | 58 |

TABLE 4 Data from Table 3 for each virus strain expressed as a percentage in relation to the results with the $O_1$ Swiss strain

PERCENTAGE RELATIVE TO SWISS % VALUE FOR EACH MAB

| VIRUS | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| $O_1$ Campos | 100 | 96 | 65 | 92 | 71 | 42 | 10 | 71 |
| Moz 1/72 | 62 | 87 | 48 | 74 | 42 | 42 | 6 | 31 |
| O1 Lausanne | 115 | 105 | 110 | 102 | 110 | 93 | 85 | 67 |
| OV1 | 22 | 85 | 60 | 128 | 83 | 5 | 8 | 88 |
| Tun 3/75 | 101 | 105 | 29 | 74 | 76 | 0 | 0 | 59 |
| $O_5$ India 62 | 115 | 118 | 60 | 125 | 94 | 25 | 23 | 76 |
| $O_1$ Swiss 65 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |

TABLE 5 Results obtained from ELISA's with a wide range of type OI strains expressed as a percentage of activity in relation to the results with the $O_1$ Swiss strain

| | PANEL OF MABS | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| VIRUS | B2 | D9 | C6 | C8 | C9 | G4 | G5 | A8 |
| $O_1$ CAMPOS | 100 | 100 | 65 | 90 | 70 | 40 | 10 | 70 |
| MOZ 1/72 | 60 | 90 | 50 | 75 | 40 | 40 | 5 | 30 |
| ZAM 6/76 | 15 | 25 | 5 | 5 | 10 | 0 | 0 | 15 |
| $O_5$ INDIA | 100 | 100 | 60 | 100 | 95 | 25 | 20 | 75 |
| $O_2$ BRESCIA | 95 | 100 | 90 | 100 | 90 | 10 | 10 | 75 |
| $O_1$ SWISS (1965) | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| TUN 1/75 | 100 | 100 | 30 | 75 | 80 | 0 | 0 | 60 |
| FRA 2/81 | 100 | 100 | 70 | 90 | 80 | 40 | 5 | 50 |
| AUSTRIA/81 | 100 | 100 | 100 | 100 | 100 | 40 | 30 | 45 |
| MALAWI 1/75 | 80 | 100 | 50 | 85 | 50 | 45 | 15 | 45 |
| UKG 18/81 | 100 | 100 | 100 | 100 | 100 | 90 | 90 | 65 |
| YEM 12/74 | 90 | 100 | 65 | 80 | 100 | 10 | 5 | 30 |
| ANG 1/75 | 100 | 100 | 95 | 90 | 60 | 100 | 15 | 50 |
| $O_1$ LAUSANNE | 100 | 100 | 100 | 100 | 100 | 95 | 85 | 70 |
| DEN 1/82 | 100 | 100 | 75 | 100 | 70 | 45 | 20 | 50 |
| DEN 2/83 | 90 | 80 | 80 | 80 | 80 | 65 | 70 | 60 |
| UGA 12/76 | 40 | 55 | 15 | 45 | 20 | 25 | 5 | 55 |
| BFS 1860 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 75 |
| OV1 | 25 | 90 | 35 | 85 | 70 | 0 | 0 | 90 |
| HOL 1/84 | 70 | 80 | 65 | 60 | 60 | 60 | 40 | 55 |
| HOL 2/84 | 35 | 45 | 20 | 20 | 25 | 30 | 0 | 20 |
| HOL 3/84 | 20 | 25 | 10 | 15 | 20 | 5 | 0 | 5 |
| HOL 4/84 | 10 | 5 | 10 | 15 | 15 | 0 | 0 | 0 |
| HOL 5/84 | 40 | 45 | 26 | 30 | 20 | 25 | 0 | 25 |
| HOL 6/84 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| HOL 7/84 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| HOL 8/84 | 65 | 80 | 40 | 45 | 35 | 40 | 0 | 50 |
| HOL 9/84 | 85 | 90 | 50 | 55 | 40 | 50 | 0 | 60 |
| KEN 101/80 | 55 | 80 | 35 | 45 | 35 | 10 | 0 | 25 |
| KEN 113/83 | 55 | 60 | 20 | 20 | 25 | 5 | 0 | 50 |
| KEN 70/77 | 60 | 60 | 0 | 65 | 0 | 0 | 0 | 25 |
| KEN 120/64 | 65 | 80 | 20 | 85 | 55 | 0 | 0 | 100 |
| KEN 146/81 | 70 | 85 | 30 | 100 | 65 | 10 | 0 | 70 |
| KEN 77/78 | 70 | 75 | 0 | 95 | 0 | 35 | 0 | 50 |
| BFS 1848 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 75 |

Fig. 3 Diagrammatic representation of an ELISA for FMD antibody detection and assay

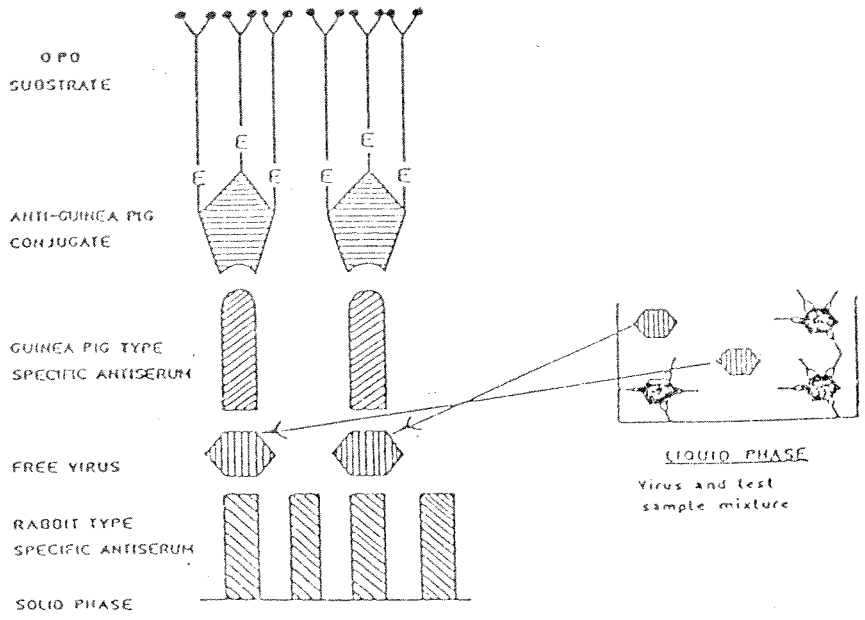



Fig. 4 Relationship of antibody titres of sera assayed in both the virus neutralisation test and ELISA

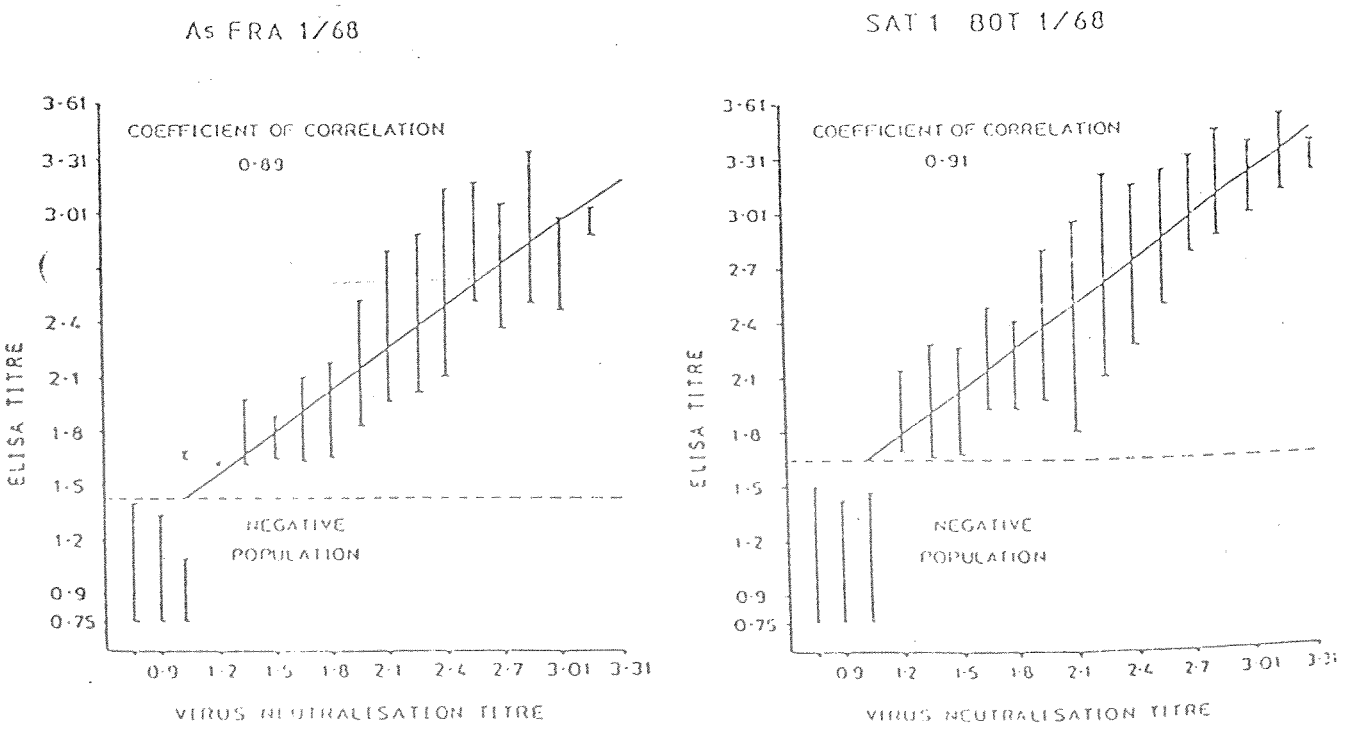


Fig. 5 The data from Table 5 presented as a series of histogram patterns.

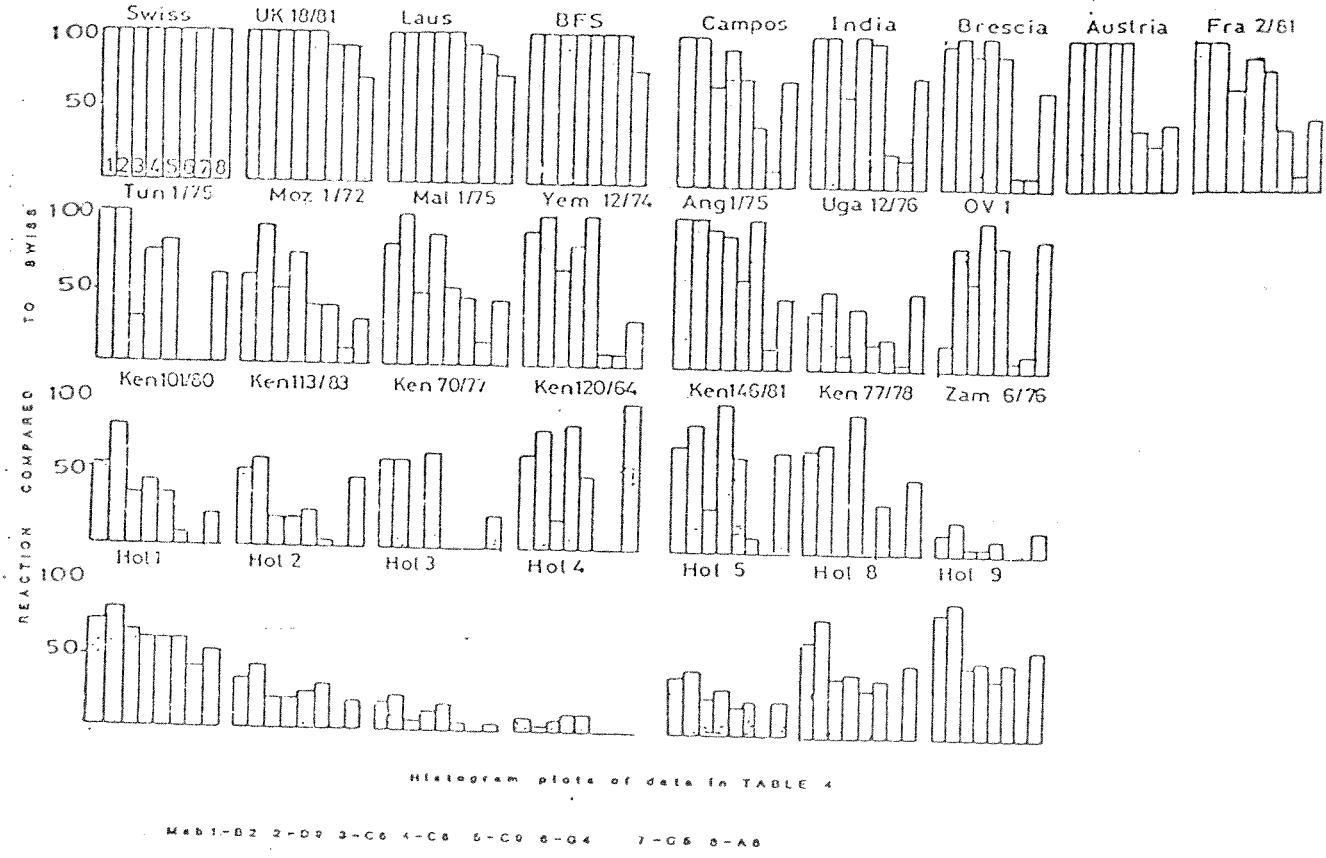
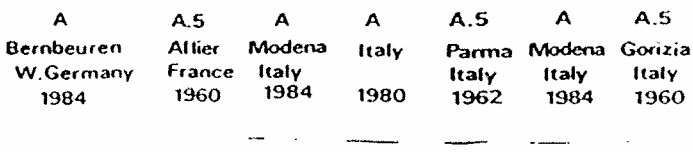


Fig. 6 PAGE analyses of the structural polypeptides of FMD type A viruses.



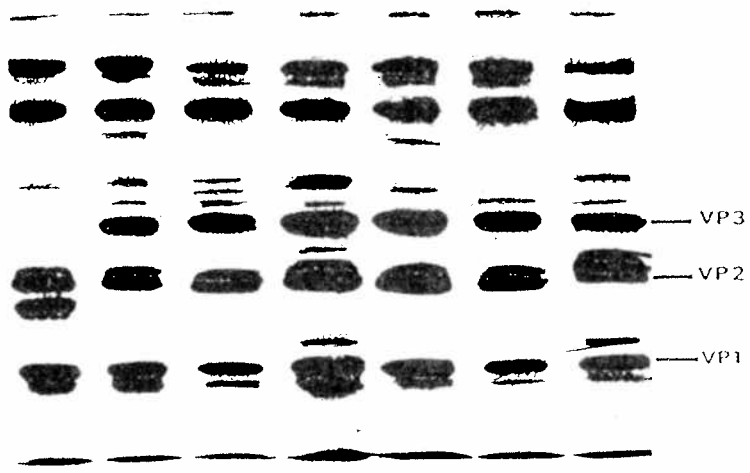




Fig. 7 $T_1$ oligonucleotide maps of FMD type A viruses

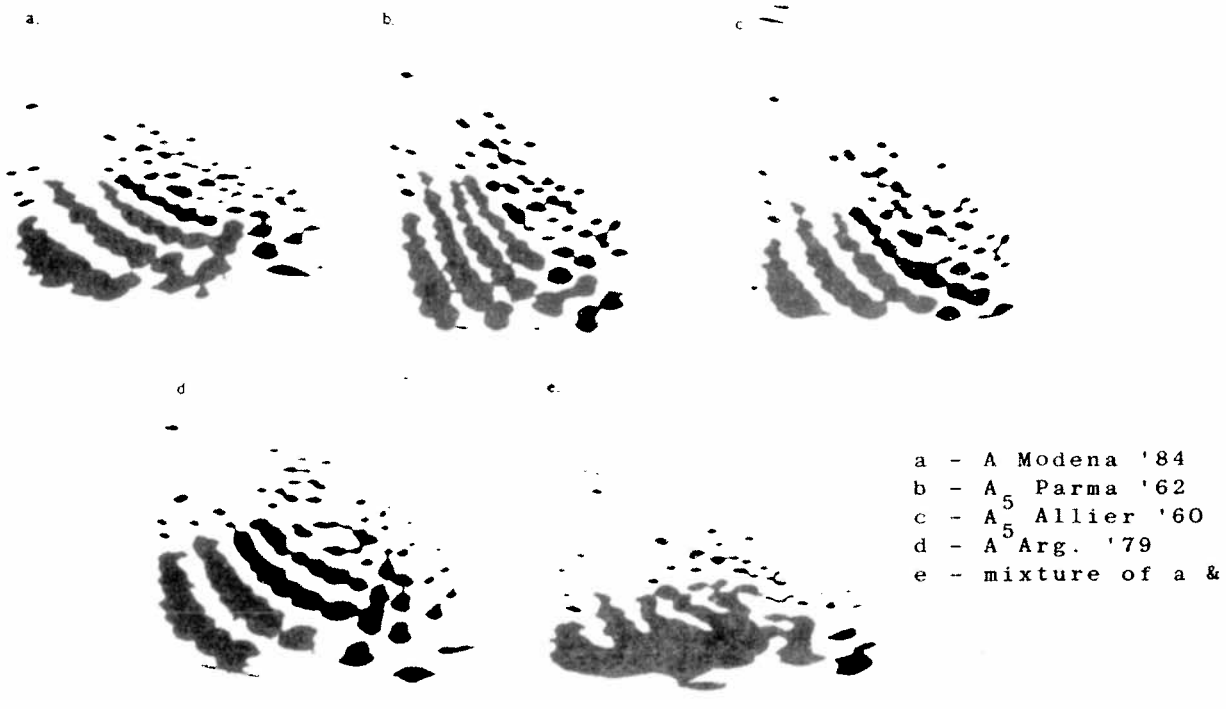


Fig.8. ELECTROFOCUSSING ANALYSIS OF THE ITALIAN TYPE A ISOLATES 1984-85.

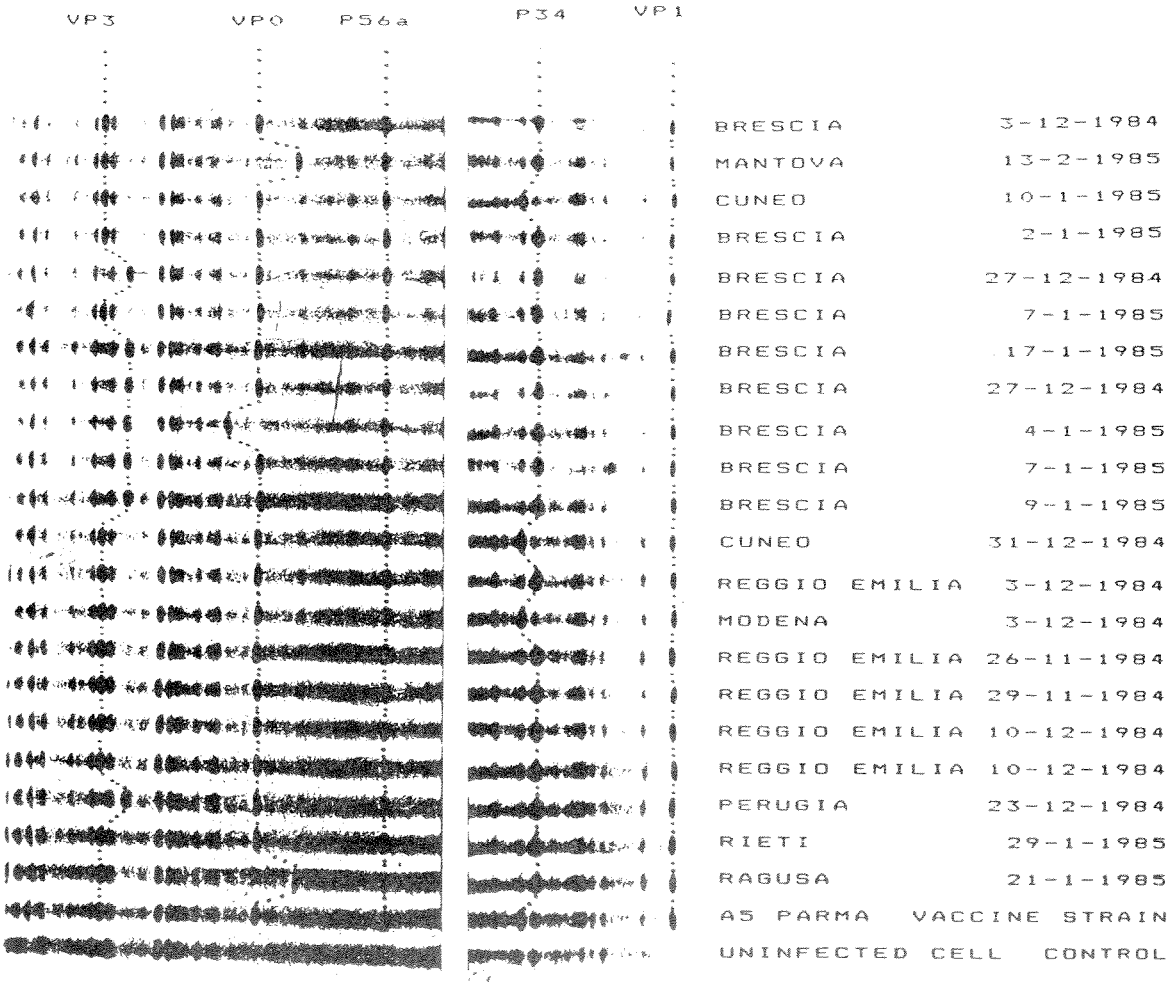
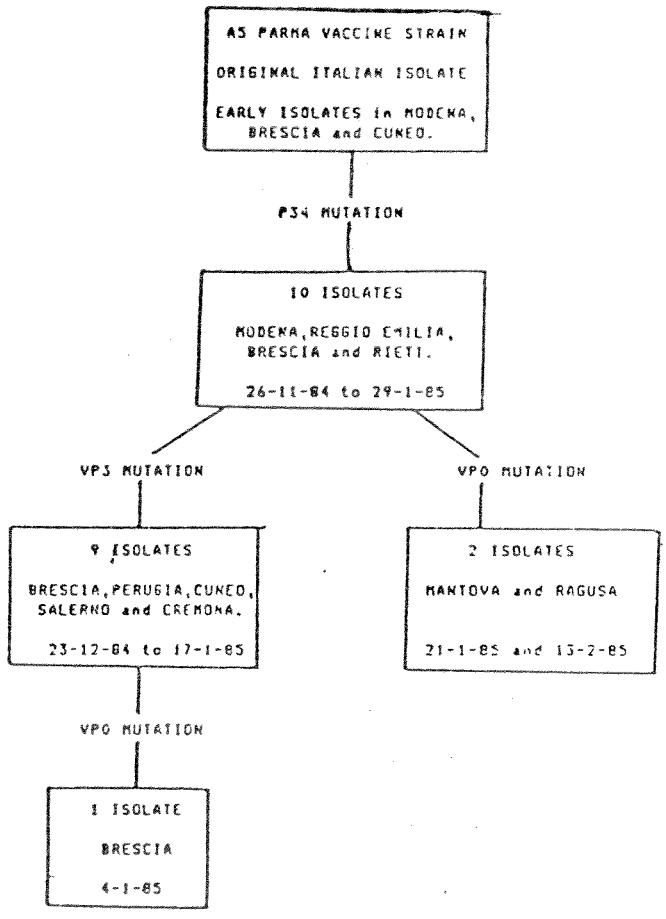


Fig.9 A POSSIBLE EVOLUTIONARY PATHWAY FOR THE ITALIAN TYPE A ISOLATES.



Appendix 5

# METHOD OF RNA ANALYSIS AND ITS APPLICATION TO THE SURVEY OF FMDV TYPE $C_1$ IN SPAIN (1970-1981)

F. Sobrino*, J.C. de la Torre, M. Dávilla, J. Ortín and E. Domingo**

Plasmids including cDNA fragments copied from RNA of FMDV are used to hybridize ($p^{32}$)RNA obtained either from virions or from infected cells. The procedure involves production of 32p-labelled FMDV RNA, extraction of the RNA and hybridization to denatured plasmid DNA bound to nitrocellulose filters. Then the hybrids are treated with a limited amount of $T_1$ RNase so as to trim the RNA tails from the hybrids and, finally, the hybridized RNA is eluted, digested to completion with $T_1$ RNase and subject to two dimensional polyacrilamide electrophoresis (de Wachter and Fiers. 1972). The conditions of the hybridization, of the T1 RNase digestion and of the oligonucleotide fingerprinting are included in the paper by de la Torre et al. (1985).

Advantages of this method are: (1) Purified FMDV RNA is not required. Identical fingerprints have been obtained using as starting material RNA extracted either from purified virions, crude preparations of virions or infected cells. (2) Plasmids may be constructed to include defined cDNA fragments, corresponding to genomic segments of interest.

The length of the cloned cDNA in the plasmid chosen for hybridization determines the complexity of the RNA to be fingerprinted. For a plasmid spanning 223 nucleotides encoding the carboxyterminal end (aminoacid 111 to 185) of capsid protein VP1, (thought to include antigenic determinants involved in virus neutralization) 51% of the nucleotides represented in the plasmid is sampled (Fig. 1). (3) The analysis may be extended to any cloned FMDV genome segments, even those for which no oligonucleotide primers are available for use in rapid sequencing methods. (4) Comparisons between viral strains can be carried out in one to two weeks, and they may complement serological methods either for diagnostic purposes or for epidemiological surveys. (5) The selection of FMDV cDNA segments corresponding to regions with a different degree of variation [i.e. the replicase P61 coding segment is four times more conserved than the capsid VP1 coding segment (Martinez-Salas et al. 1985)] will allow more adequate epidemiological surveys of the virus during outbreaks. DNAs representing variable regions will be useful to trace the spread of virus within localized geographical areas - i.e. between farms - and DNAs representing more conserved regions will be useful to compare viruses from widely different areas - i.e. within a country or from different countries.

Disadvantages of the method are: (1) Nucleotide sequences and corresponding aminoacid sequences are not deduced from $T_1$ oligonucleotide fingerprints. (2) A limited proportion of genomic sequences is subject to analysis, and thus (3) Similarity in $T_1$ oligonucleotide fingerprints does not imply similarity in phenotypic (i.e. antigenic) behaviour. (4) Labelling of RNA is needed.

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Some conclusions can be drawn from the analysis of FMDV type $C_1$ isolated in Spain in the period 1970-1981. (1) A close genetic relationship between C-S8 (isolated in 1970 and used as vaccine strain in Spain) and C-S10, the 1979 isolate. (2) Rapid genetic variation in the period 1979-1981 of maximal epizootic activity. (3) The confirmation of the extensive variability of FMDV, with mutations even among the three coetaneous isolates analysed. This is consistent with the known high mutability of RNA viral genomes (Holland et al. 1982).

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Appendix 16

# UPDATING OF FOOT-AND-MOUTH DISEASE VIRUS STRAINS OF EPIDEMIOLOGICAL IMPORTANCE IN SOUTH AMERICA

Alonso Fernandez, A.[1], Casas Olascoaga, R.[1], Astudillo, V.M.[1], Sondahl, M.S.[1], Gomes, I.[1], Vianna Filho, Y.L.[2]

Foot-and-mouth disease (FMD) is produced by a virus of the Picornaviridae family. Due to its physicochemical properties the virus is classified in the Aphtovirus genus.

Seven immunological serotypes (O, A, C, SAT1, SAT2, SAT3 and Asia 1) have been identified within the Aphtovirus genus which do not give cross protection between each other.

Due to their high mutation capability, each serotype includes various subtypes that are characterized by immunological differences among themselves. The subtypes are represented by strains isolated from foci, outbreaks or epidemics, which have similar antigenic and immunogenic characteristics. To prevent errors in identification, the denomination of a strain must include the serotype, the virus subtype, the place and the year of isolation.

## Variability of the virus in the endemic areas

The great capability of variation of FMD virus is due to the high rate of mutation (7), the genetic recombination (5) and the selective action of antibody on virus replication in partially immune animals (4). These processes cause changes in the sequence of the nucleotides, which induce modifications in the amino acids of the capsid polypeptides.

The changes in the nucleotides can be detected by biochemical techniques like RNase $T_1$ resistent maps (2). But the modifications of the sequence of the amino acids, when located in the antigenic determinants, are detected by antigenic and immunogenic tests (1, 6, 8) or by the sequencing of the amino acids of the viral protein (3).

The changes in antigenicity and immunogenicity of the viruses in the field are of utmost importance for the FMD control programs, since the degree of protection of the vaccinated population depends on the quality of the vaccine applied and on the homology between the vaccine strain and the field strain. These changes also have a repercussion on the diagnosis and on the production and control of the vaccine.

Because of high contagiousness of FMD and high capacity of variability of the virus, the success of the control programs depends initially on the efficiency of the epidemiological surveillance in detecting the foci, collecting samples and blocking the spread of the disease. This aspect is of vital importance in the countries having intensive livestock exploita-

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tion methods and much animal movement, which enormously facilitate propagation of the virus. The diagnosis laboratory, jointly with the quality control laboratory, must determine coverage of the vaccine strains, and the potency of the vaccines.

The antigenic characterization studies of the field virus and the analysis of the vaccine strain coverage against the epidemiologically important field viruses, must be available to other countries that vaccinate systematically and that have vaccine banks.

History of the virus strains identified in South America from 1950 to 1984

Retrospective studies of the FMD field viruses identified in South American countries have shown that in the Southern Cone the predominant viruses in the 1950's could be classified among the subtypes $O_1$, $A_{24}$ and $C_3$. In the Andean countries, the viruses belonged to the $O_1$ and $A_5$ subtypes. Peru was an exception because it frequently imported bovine meat and by-products from Argentina and Colombia, which led to the identification of viruses from the two regions.

During the 1950's, FMD vaccine in the Southern Cone of South America was produced with the Waldmann method which led to the emergence of very different virus strains when partially immune cattle at slaughterhouses were inoculated to produce antigen. Thus subytpes $O_8$, $A_{13}$, $A_{16}$ and $A_{17}$ were identified in Brazil and $A_{19}$ in Argentina. The isolation of those viruses was related to the sites of virus handling and vaccine production (slaughterhouses and laboratories).

The exchange of strains among laboratories led to the accidental introduction of subtype $A_{10}$ into Argentina. That subtype was used in Holland for vaccine preparation. Strains $C_2$ and $A_{30}$ caused epidemics in the 1940's and 50's in Rio Grande do Sul (Brazil) and in Uruguay, respectively.

Strains of the subtypes $O_3$, $O_8$, $A_{10}$, $A_{13}$, $A_{16}$, $A_{17}$, $A_{19}$, $A_{25}$, $A_{30}$ and $C_2$ have totally disappeared and have not caused foci or any epidemic outbreaks since 1963.

In the 1960's, when the network of vesicular disease diagnosis laboratories was consolidated in South America (Fig. 1), several countries initiated FMD control programs based on systematic vaccination of the cattle population. That network of laboratories led to a striking improvement in epidemiological surveillance and in the characterization of viruses occurring in the field. At that time the control programs still suffered from several shortcomings due to their inexperience. In some countries, therefore, epidemics were caused by virus strains very similar to those used in vaccine production. For example, the $A_{18}$ subtype was isolated in Venezuela in 1962. The disease was eliminated by massive field application of an attenuated live-virus vaccine of $A_{24}$ Cruzeiro and, in 1969, of subtype $A_{32}$ which still persists. Strains of the $A_{26}$ and $A_{29}$ subtypes, similar to $A_{24}$, were identified in Peru. Throughout Colombia the existence of viruses similar to those of subtype $A_5$ has been recorded. Those viruses were classified among the $A_{27}$ subtype. In 1969 the plains of Bogota were affected by virus $A_{31}$. It was eliminated by strict control of foci and strategic revaccination with homologous monovalent vaccine, thus preventing its spread into other areas of the country.

With respect to type C virus strain, Argentina and Paraguay were affected in 1969 by an epidemic caused by a strain antigenically not much different from subtype $C_3$. The World Reference Laboratory (WRL) classified the Argentine strain as subtype $C_5$ and the strain found in Paraguay

as $C_3$. A focus caused by $C_4$ Tierra del Fuego virus occurred in 1966 in Argentine Patagonia; it was different from those previously identified in the Southern Cone. Epidemiological data on that episode indicate that carrier cattle were possibly responsible for the focus.

The 1970's were characterized by the consolidation of the vaccine-production and control laboratories, first in Uruguay and Chile, then in Argentina, Brazil, Colombia and Paraguay. The consistent production of vaccines and their official control led to improved immunization of the cattle population in the programs. Nevertheless, strains not very different from those used in vaccine preparation were isolated. For example, $C_3$ Indaial affected southern Brazil from 1971 to 1974, and Argentina and Uruguay in 1974-75. The A Venscelau and A Bagé strains were isolated in 1976 in Brazil. The latter strain affected cattle in Rio Grande do Sul before spreading into Argentina and Uruguay. During its process of evolution it originated A Argentina/79 and A Brazil/79. Because those two strains are very similar and also similar to A Venceslau and A Bagé, they are included in a single group.

The 1980's are characterized by better structured programs both in the field and in the laboratories. This factor is evident in the effort developed against the O RS-Brazil/80 strain, which is rather different from the $O_1$ Campos virus vaccine (Table 1). The O RS-Br/80 strain produced a severe epidemic in Rio Grande do Sul, Brazil, in 1980, but quick identification of the virus and the adoption of efficient epidemiological surveillance blocked its spreading into Uruguay and Argentina.

In 1981, virus strains A Argentina/81, A Brazil/81 and A Uruguay/81 emerged. Those strains are believed to be the end effect of the epidemic caused by the A Argentina/79 - Brazil/79 strain. Those type A virus strains isolated in 1981 did not spread due to the epidemiological measures put into effect on that occasion.

The incidence of type C virus increased in Argentina in 1983 until it caused an epidemic in 1984. The epidemic was controlled by the use of a homologous monovalent vaccine jointly with the polyvalent vaccine. On that occasion the spread of the disease into the neighbouring countries was likewise restrained, except for the occurrence of foci in the Bolivian Chaco area controlled by perifocal vaccination.

The A São Carlos virus was identified in 1984 in the state of São Paulo, Brazil (Tables 2 & 3). It was controlled through intensified epidemiological surveillance and strategic vaccination with classic A Cruzeiro and A Venceslau vaccine. The virus has not been identified since February 1985.

## Virus strains of current interest identified in South America

Detailed analysis of the antigenic and immunogenic characteristics of the viruses identified in South America (Tables 4, 5, 6) leads to the conclusion that the currently predominant strains should be classed in the $O_1$, $A_{24}$, $A_{32}$, and $C_3$ subtypes. Additionally, the A Argentina-Brazil/79 and $C^2$ Argentina/84 strains should be included (Table 7), pending their classification.

## Classification of the foot-and-mouth disease

At the Internatinal Symposium on Variants and Immunity, held in Lyon, France, in 1967, the WRL set down the standards for classifying FMD viruses. The standards were based on obtaining the serological relation-

ships by means of the complement fixation test (1, 8). In view of the difficulty of applying those standards, due to the existence of many subtypes each with groups of various strains, the 1976 Internatinal Symposium on Variants and Immunity proposed to analyze only the relationship (r) against the different existing subtypes. When the value of both r was below 0.25, the strain under study was held to be a new subtype. Furthermore, it was proposed that only the epidemiologically important strains should be classified (6). The existing proposals have not resolved the problem, for no new subtypes have been identified since 1970 when virus $A_{32}$ was identified.

The Pan American Foot-and-Mouth Disease Center as the Reference Laboratory for the Americas and the network of national diagnosis laboratories have jointly sought to resolve the situation for the countries of the region by applying the following procedure:

1. Preparation of the virus strain collection

- Identify the existing strains in the field and those handled by the official and private laboratories in the region.

- Designate the representative strain of each recognized subtype.

- Prepare antigens and hyperimmune sera from the virus strains listed in the two previous points, for $CF_{50}$ and ELISA tests.

- Prepare virulent virus suspensions for potency tests.

- Compile RNase $T_1$ resistant maps (one and two dimensional "fingerprinting").

- Keep "Serum Bank" of sera from cattle vaccinated and revaccinated with vaccines prepared with the various vaccine strains used in South-America up-to-date.

- Establish the serological relationships.

- Determine the immunological coverage of the vaccine strains by means of the serum protection (mouse protection test) and/or serum neutralization tests, with sera from vaccinated and revaccinated cattle (Serum Bank), and by direct testing.

2. Identification of new strains

- Collect samples of all the field foci.

- Characterize the strains antigenically by subtyping.

- Obtain the $r_1$ values against the standard strains used in vaccine preparation.

- Establish the immunological coverage of the "Serum Bank" sera from cattle vaccinated and revaccinated with the vaccine strains used in the region.

- Epidemiological information.

- When the strain persists in the field, it is included in the strain collection and all the work stated in point 1 above is performed. If the strain used in the vaccine provides protection at revaccination equal to or greater than 75%, it will be included in the subtype to which the vaccine strain belongs.

## Conclusions

The great capacity of FMD virus for frequent mutation, plus the selective action affecting virus replication in field conditions, as well as its passage through different hosts, make it practically impossible for a strain to cause more than one epidemic wave. Therefore, the list of subtypes and strains should be reviewed periodically to eliminate those that have not been identified in the field in recent years.

The intial concern regarding emerging strains should be to restrain their spread in the field. The viruses causing foci in the interepedimic periods should be carefully studied: their antigenic structure should be compared with that of the vaccine strains used in the region, and the immunological coverage of the sera from animals vaccinated and revaccinated with the vaccine strain should be determined against the field strain. This test can be complemented by tests of protection against development of foot lesions in cattle vaccinated and revaccinated with the vaccine strain.

When the degree of protection in cattle at four weeks post-revaccination is equal to or exceeds 75%, and the vaccination coverage in the region is broad enough, then revaccination with the available vaccine and stringent control of animal movement will suffice to stop the spread of the new strain. The new strain should then be classified within the same subtype as the vaccine strain. When the coverage is less than 75% and the control of foci is difficult, then the preparation and use of a specific monovalent vaccine should be considered.

Countries where FMD is controlled and where the susceptible population is still protected by systematic vaccination - such as Western Europe- or disease- free countries that possess vaccine banks, should be aware of the coverage of their vaccines against the epidemiologically important strains in the endemic countries. The serum protection (mouse protection) tests with sera from cattle vaccinated and revaccinated with their vaccines are very suitable for this type of work. The Pan American Foot-and-Mouth Disease Center through the network of vesicular-disease diagnosis laboratories and the countries of the region have organized those studies.

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TABLE 1. Serological relationships (r), protection against foot generalization (PG), mouse protection indexes (MPI) and expected percentage of protection (EPP) in cattle revaccinated with strain $O_1$ Campos challenged against homologous virus and O RS-Br/80

| Challenge strains | Hyper.serum $O_1$ Campos r | $O_1$ Campos vaccine | | |
|---|---|---|---|---|
| | | PG | Mean MPI | EPP* |
| $O_1$ Campos-Br/58 | 1.00 | 16/16 | 3.62 | 87.7 |
| O RS-Br/80 | 0.34 | 6/16 | 2.40 | 72.5 |

*Low limit of confidence, expected percentage of protection.
Gomes, I. & Astudillo V. Bol. Centr. Panam.Fiebre Aftosa 17-18: 9-16, 1975.

TABLE 2. Serological relationships (r) and expected percentage of protection (EPP) obtained from hyperimmune sera and from cattle vaccinated and revaccinated with $A_{24}$ Cruzeiro-Br/55 and A Venceslau-Br/76 vaccines challenged against A São Carlos-Br/84

| Challenge strains | r - Hyper.serum | | EPP - 30 DPV | | EPP - 30 DPR | |
|---|---|---|---|---|---|---|
| | $A_{24}$Cruz. | A Venc. | $A_{24}$Cruz. | A Venc. | $A_{24}$Cruz. | A Venc. |
| $A_{24}$Cruz.-Br/55 | 1.00 | - | 69.7 | - | 99 | - |
| A Venc.-Br/76 | - | 1.00 | - | 96.1 | - | 98.7 |
| A S.Carlos-Br/84 | 0.31 | 0.05 | ≤44.6 | ≤61.0 | 73.2 | ≤47.9 |

TABLE 3. Protection against foot generalization (PG) in cattle vaccinated and revaccinated with $A_{24}$ Cruzeiro-Br/55 and A Venceslau-Br/76, challenged against A Venceslau-Br/76 and A São Carlos-Br/84

| Challenge strains | Cattle protected/used | |
|---|---|---|
| | 30 DPV | 21 DPR |
| A Venceslau-Br/76 | 14/16 | 18/18 |
| A São Carlos-Br/84 | 3/16 | 11/18 |

TABLE 4. Foot-and-mouth disease virus type O of historical or current interest, identified in South America. 1950-1984

| Subtype | Strain | Year | | |
|---|---|---|---|---|
| | | Classification WRL | Identification 1st | Identification Last |
| $O_1$ | $O_1$ Campos-Br/58 | 1967 | 1958 | 1984 |
| $O_3$ | $O_3$ Venezuela/50 | 1956 | 1950 | 1958 |
| $O_8$ | $O_8$ Bahia-Br/60 | 1962 | 1960 | 1962 |
| O* | O* Rio Grande do Sul-Br/80 | * | 1980 | 1980 |

*It was not sent to the World Reference Laboratory (WRL).

TABLE 5. Foot-and-mouth disease virus type C of historical or current interest, identified in South America. 1950-1984

| Subtype | Strain | Year | | |
|---|---|---|---|---|
| | | Classification WRL | Identification 1st | Identification Last |
| $C_2$ | $C_2$ Pando-Urug/44 | 1969 | 1944 | 1974 |
| $C_3$ | $C_3$ Resende-Br/55 | 1969 | 1955 | 1984 |
| $C_3$ | $C_3$ Paraguay/69 | 1969 | 1969 | 1969 |
| $C_4$ | $C_4$ Tierra del Fuego-Arg/66 | 1969 | 1966 | 1966 |
| $C_5$ | $C_5$ Argentina/69 | 1969 | 1969 | 1974 |
| C* | C* Argentina/84 | * | 1983 | 1984 |

*Pending classification by the World Reference Laboratory (WRL).

TABLE 6. Foot-and-mouth disease virus type A of historical or current interest, identified in South America. 1950-1984

| Subtype | Strain | Year | | |
|---|---|---|---|---|
| | | Classification WRL | Identification 1st | Identification Last |
| $A_{10}$ | $A_{10}$ Argentina/61 | 1961 | 1961 | 1961 |
| $A_{13}$ | $A_{13}$ Santos-Br/58 | 1962 | 1958 | 1958 |
| $A_{16}$ | $A_{16}$ Belém-Br/59 | 1964 | 1959 | 1960 |
| $A_{17}$ | $A_{17}$ Guarulhos-Br/59 | 1964 | 1959 | 1962 |
| $A_{18}$ | $A_{18}$ Zulia-Ven/62 | 1964 | 1962 | 1963 |
| $A_{19}$ | $A_{19}$ Suipacha-Arg/62 | 1964 | 1963 | 1963 |
| $A_{24}$ | $A_{24}$ Cruzeiro-Br/55 | 1967 | 1955 | 1984 |
| $A_{25}$ | $A_{25}$ Argentina/59 | 1967 | 1959 | 1964 |
| $A_{26}$ | $A_{26}$ Argentina/66 | 1967 | 1963 | 1967 |
| $A_{27}$ | $A_{27}$ Colombia/67 | 1967 | 1967 | 1983 |
| $A_{29}$ | $A_{29}$ Peru/69 | 1970 | 1969 | 1970 |
| $A_{30}$ | $A_{30}$ Uruguay/45 | 1970 | 1945 | 1960 |
| $A_{31}$ | $A_{31}$ Colombia/69 | 1970 | 1969 | 1970 |
| $A_{32}$ | $A_{32}$ Venezuela/70 | 1970 | 1969 | 1984 |
| A* | A* Argentina/76 | * | 1976 | 1976 |
| A** | A** Ecuador/75 | ** | 1975 | 1978 |
| A** | A** Venceslau-Br/76 | ** | 1976 | 1984 |
| A** | A** Bagé-Br/76 | ** | 1976 | 1984 |
| A** | A** Argentina/79 | ** | 1979 | 1984 |
| A** | A** Brasil/79 | ** | 1979 | 1984 |
| A** | A** Argentina/81 | ** | 1981 | 1982 |
| A** | A** Brasil/81 | ** | 1981 | 1981 |
| A* | A* São Carlos-Br/84 | * | 1984 | 1984 |

*It was not sent to the World Reference Laboratory (WRL).

**Pending classification by the WRL.

TABLE 7. Foot-and-mouth disease virus strains and subtypes epidemiologically important at present, identified in South America. 1984

| Country | Subtypes and strains | | | | | |
|---|---|---|---|---|---|---|
| | $O_1$ | $A_{24}$ | $A_{32}$ | A Arg/79 Br/79 | $C_3$ | C Arg/84 |
| Argentina | x | - | - | x | x | x |
| Bolivia | x | x | - | - | x | - |
| Brazil | x | x | - | x | x | - |
| Colombia | x | x | - | - | - | - |
| Ecuador | x | x | - | - | - | - |
| Paraguay | x | x | - | - | x | - |
| Peru | - | x | - | - | - | - |
| Uruguay | x | - | - | - | x | - |
| Venezuela | x | x | x | - | - | - |

Appendix 17

# BIOCHEMICAL AND SEROLOGICAL CHARACTERIZATION OF DIFFERENT APHTOVIRUS STRAINS RECOVERED FROM THE FIELD IN SOUTH AMERICA

I.E. Bergmann[1], P. Augé de Mello[2], A. Alonso Fernández[2], E.A. Scodeller[1], R. Casas Olascoaga [2], J.L. La Torre[1]

Unitl recently classical serological techniques were the only ones used to follow the epidemiology of outbreaks of foot-and-mouth-disease (FMD) occurring in South America (1). The high mutation rate of the foot-and-mouth disease virus (FMDV) genome requires the application of highly sensitive techniques in order to make it feasible to establish the possible source of outbreaks as well as to have a better understanding of the molecular basis of virus variability (2, 3, 4, 5) and the epidemiology of FMD which is of critical importance for the eradication of the disease.

In a previous publication we reported the application of RNA fingerprinting (RNase $T_1$ maps one and two dimensional gels) in combination with serological techniques for the characterization of the prototype strains used for vaccine production or as reference strains in South America (P. Augé de Mello et al., submitted for publication). The results obtained constitute the basis for a data bank containing available information about the genomes of strains of aphtovirus prevalent in this continent and can be used as an adjunct to serological and immunological information.

These data are currently being used in South American countries to: (a) complement other tests in the updating of vaccine strains; (b) assess the genetic stability of strains during vaccine production; (c) establish possible vaccine origin of field outbreaks and (d) monitor the origin, behaviour and fate of new strains in the field.

In the present communication we extend our studies to the analysis of several relevant strains of FMDV serotypes O, A and C isolated at different times in the field in different regions of South America.

Like cardioviruses, FMDV contains a polyribocytidylic acid tract near the 5' end of the genome (6, 7). The length of this segment is significantly different for each serotype as well as for each subtype and strain (8) and therefore has an additional value for distinguishing better different isolates (9). The data clearly show the relevance of applying these biochemical techniques in combination with serological ones for a rapid and precise identification of field isolates. Therefore they provide a basis for defining rapidly the possible source of an FMDV outbreak such as already shown by King et al. (10).

It is clear that the biochemical analysis combined with serological identification of strains is a good approach for the characterization of viruses with techniques which can be rapidly applied to the study of field isolation.

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In addition to the characterization of isolated cases we are at present studying more defined epidemiological models including several chronologically related isolates from the same field outbreak.

These models in which a large amount of field isolates are studied within a short period of time could be very relevant to understand the epidemiological behaviour of viruses and will provide excellent models to study the biological and biochemical basis of virus variability in the field.

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Appendix 18

# EPIDEMIOLOGICAL SURVEILLANCE OF FMD IN SOUTH AMERICA

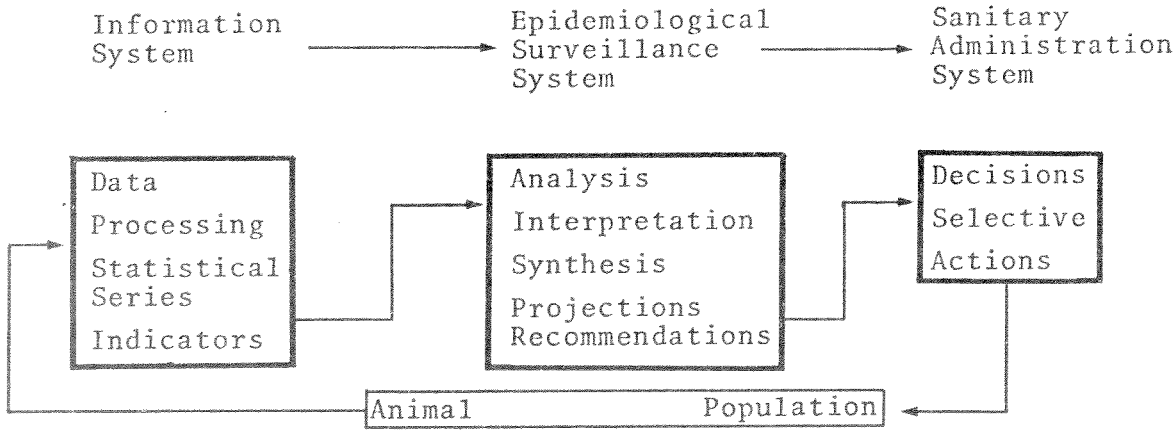
Vincente M. Astudillo*

## Introduction

We live today in a highly dynamic world, one that is subject to permanent, on-going change. That dynamism is very evident when the direct goal of our endeavor is the very complex environment, which undergoes even more change spawned by man's constant transforming action. Such is the case in the agriculture environment where animal production is developed, and where the "negative" interferences on it occur. Generically, we call them diseases.

The need to capture and become aware of changes and their determinants has led the animal health field to develop sensing and intelligence-gathering systems that can assist in selecting the proper corrective sanitary actions.

## DISEASE CONTROL FEEDBACK SYSTEM




Epidemiological surveillance is by nature an information-based discipline. Through it the animal health field is being enriched by the adoption of modern decision systems, processes directed toward goals through information or processes controlled by feedback. The introduction of this modern methodology in other fields of human endeavor has greatly boosted productivity and spurred efficacy in the attainment of proposed objectives.

In the area of foot-and-mouth disease control, the passive phase of epidemiological surveillance formerly restricted to simple mechanisms of

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notificatoin and recording of data is progressively giving way to a more dynamic surveillance phase that integrates the information specifically for FMD-control action.

Experiences involving the use of the data provided by the Continental System of Surveillance of Vesicular Diseases in Cattle, especially in South America, in the FMD-control programmme, are presented below as examples.

The application of the information to the efforts of the control programmes envisages two aspects: strategic surveillance, which is related to the analysis of epidemiological situations in a large physical environment and over the long term; and, conjunctural surveillance, involved with the analysis of temporary localized situations that should be quickly resolved. The following are among the main experiences of what we have termed strategic surveillance:

1. Identification of the FMD ecosystems

The study of the spatial behavior is basic for veterinarians of the FMD control programmes. Much can be learned about the epidemiology of this disease by characterizing its patterns and having them represented on maps. Very often hypotheses are formulated on disease distribution related to the spatial behavior of ecological, livestock and economic factors.

We can characterize the disease's behavior in terms of the disease's persistence (monthly) in grid squares on a map. Thus we might classify a persistent stage of the disease in a grid square as endemic and an unpersistent stage as rare or occasional.

The presence of the disease and its sequence in time in a geographic unit is valid in studying the persistence as a manifestation of endemism. By grouping geographic units where the behavior is similar, one can identify and delimit regions or ecosystems where in the virus' mode of occurrence profiles a characteristic pattern.

The persistent occurrence of affected herds, over a chronological series of months/years in a geographic unit, is evaluated by the indicator $\%\Omega_0$. Conceptually, it may be regarded as an approximate indicator of endemism.

The identification of geographic clusters is based on the representation of the disease's occurrence in space, which must be related to the way in which it is transmitted, the environmental factors that determine it, and the livestock production characteristics that are associated with a lesser or greater risk of occurrence.

Figure 1 shows the results of applying this indicator to the geographic grid squares in Brazil, using the FMD records for the 1978-1984 period.

Table 1 summarizes the situation, showing that the primary endemic ecosystem in Brazil's Central-West region has an arithmetic mean of 11% for $\Omega_0$ (grid squares lined or striped in Figure 1). The livestock density is low, due to the poor natural pastures; the steer-to-cow ratio is less than 0.50 (there are many cows) and the herds tend to be large. From the standpoint of production forms the primary endemic ecosystem is a region where unfinished young cattle are extensively bred.

FIG. 1 - PERSISTENCE OF FMD IN BRAZIL (% $\Omega_0$)

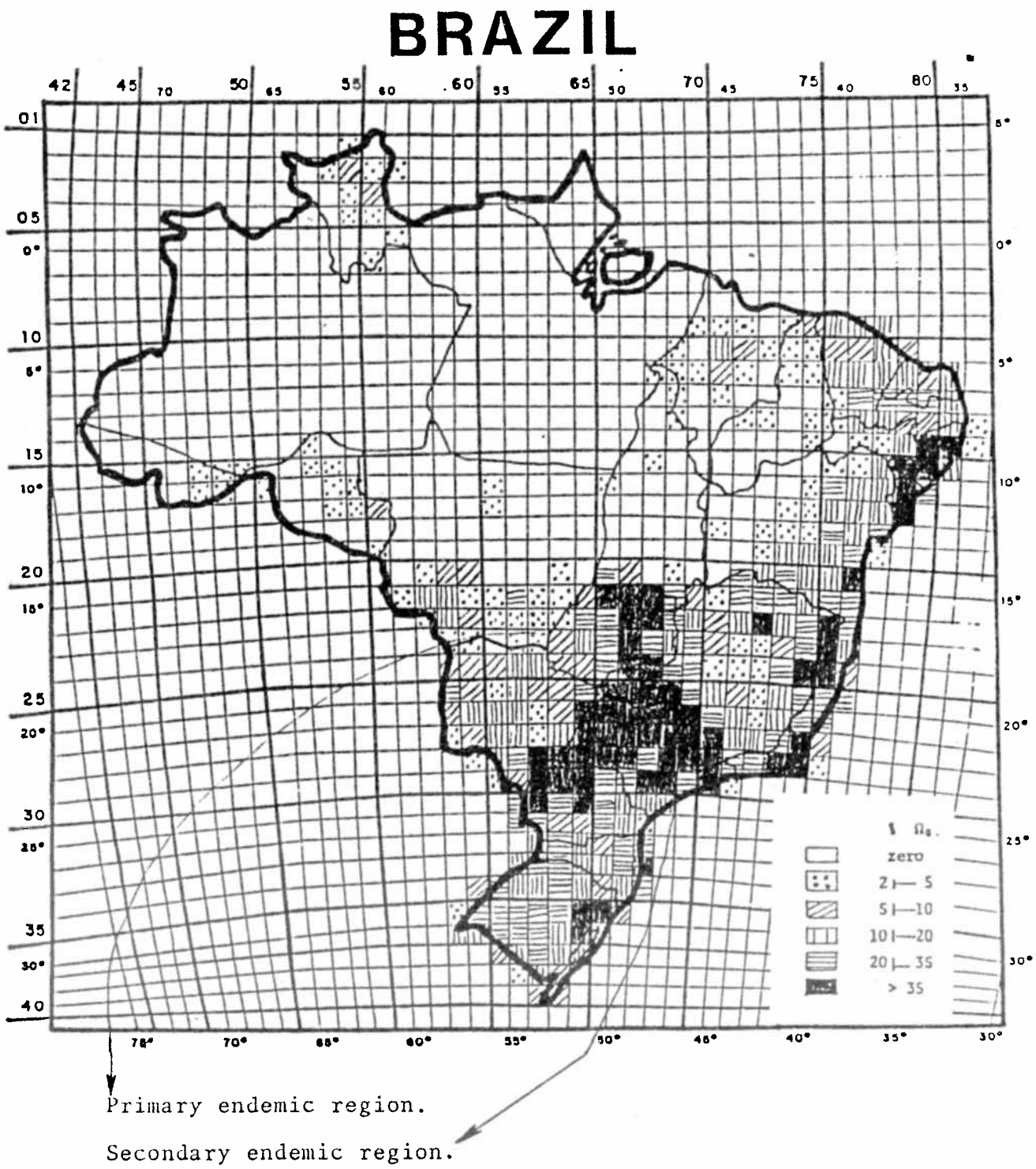


Table 1 also shows that the secondary endemic ecosystem in Brazil's Central-West indicates an arithmetic mean of 47% for $\int_0$ (solid black grid squares in figure 1). Livestock density approaches or exceeds one animal per hectare, which indicates regions having a high capacity to support livestock and has cultivated pasturelands. The steer-to-cow ratio is close to two, and greater than two, likewise common of regions dedicated to fattening young steers. The herd size averages smaller than in the primary ecosystems where land values are lower.

The cattle currents move from the primary endemic extractive region toward the secondary endemic fattening region.

TABLE 1. EMD ECOSYSTEMS. CENTRAL WEST REGION OF BRAZIL

FMD ECOSYSTEM PRIMARY ENDEMIC ($\%\int_0$ =11)

- Extensive extractive breeding region

| Munic./State | Index of livestock production forms | | |
|---|---|---|---|
| | Cattle Density | Steer/ Cow | Herd size (heads) |
| Corumba/MS | 0.27 | 0.46 | 1.649 |
| P. Murtinho/MS | 0.28 | 0.45 | 1.013 |
| Aquidauana/MS | 0.34 | 0.48 | 1.046 |
| Caceres/MT | 0.17 | 0.25 | 226 |
| Pacone/MT | 0.29 | 0.48 | 474 |

FMD ECOSYSTEM SECONDARY ENDEMIC ($\%\int_0$ =47)

- Fattening region

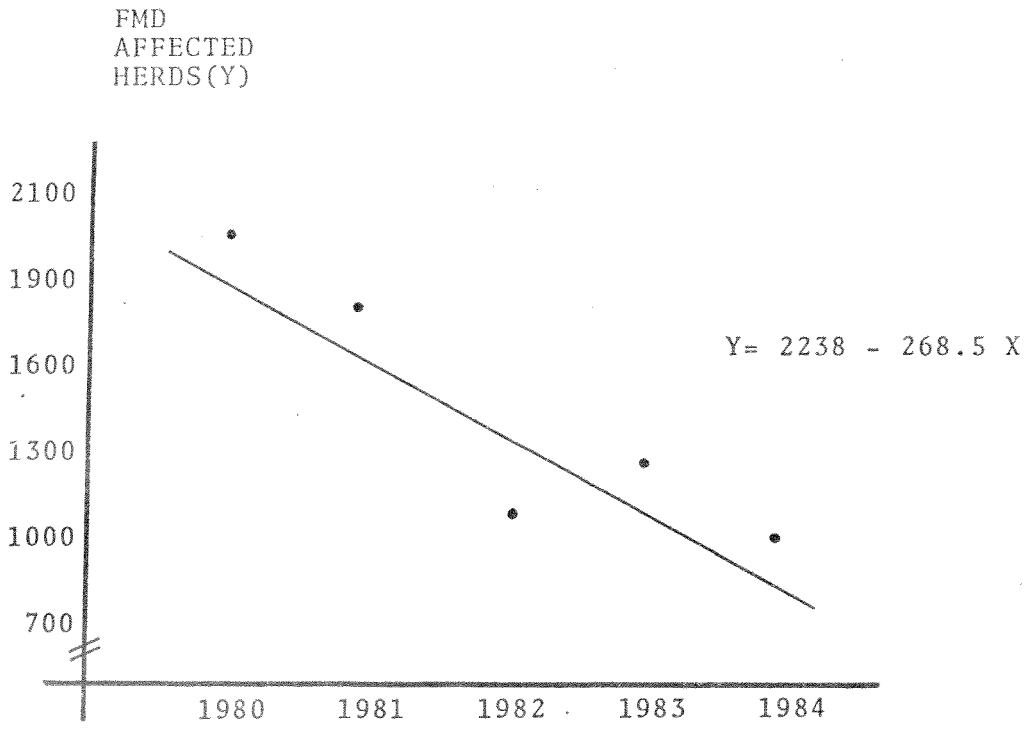
| | | | |
|---|---|---|---|
| Castilho/SP | 0.79 | 3.51 | 375 |
| Aracatuba/SP | 0.91 | 1.99 | 264 |
| S. Vitoria/MG | 0.99 | 1.91 | 405 |
| Ipiacú/MG | 1.15 | 4.38 | 354 |
| Carapó/MG | 0.90 | 1.55 | 420 |

2. Evaluation of the FMD tendency

Annual trends are long-term changes where, in addition to short-term ups and downs, the curve either climbs or drops more or less steadily over an extended period of time, usually years.

Figure 2 shows the FMD tendency in recent years. The values reflecting herds affected were higher in the preceding ten-year period.

FIG. 2 - FMD TREND. SOUTH AMERICA




Moreover, on the basis of the information acquired through the weekly grid square reports over a year, it is possible to evaluate modificatons in disease occurrence in the South American countries. The countries are represented on a grid map subdivided into 1700 $1^{o}$ x $1^{o}$ squares.

Table 2 shows that the persistence of vesicular diseases in South America showed an evident downtrend in the historical period 1978 - 1984. Disease presence for more than 15 weeks, or from 8 to 15 weeks per year, has clearly declined, especially since 1980. The trend reflects the overall decline in FMD presence.

TABLE 2. DISTRIBUTION OF GRID SQUARES ACCORDING TO THE NUMBER OF WEEKS WITH VESICULAR DISEASE OCCURRENCE: SOUTH AMERICA, 1978-1984.

| YEAR | NO. OF WEEKS WITH VESICULAR DISEASE PRESENCE PER YEAR | | |
|---|---|---|---|
| | 1 - 7 | 8 - 15 | > 15 |
| 1978 | 249 | 110 | 73 |
| 1979 | 285 | 123 | 74 |
| 1980 | 313 | 101 | 77 |
| 1981 | 314 | 83 | 47 |
| 1982 | 313 | 51 | 27 |
| 1983 | 319 | 52 | 25 |
| 1984 | 374 | 51 | 36 |

In relation to experience with conjunctural epidemiological surveillance, the following examples are of interest:

3. Detection of modifications in the weekly and/or monthly patterns of presence.

The weekly distribution of the grid squares that indicate the presence of Vesicular Diseases can be represented. The affected map squares are considered regardless of the number of affected herds or cases. The comparative situation in Colombia, up to week 37 in 1984 and 1985, was as follows:

| WEEK RANK | NO. GRID SQUARES | | WEEK RANK | NO. GRID SQUARES | |
|---|---|---|---|---|---|
| 1 | 6 | 8 | 20 | 0 | 7 |
| 2 | 7 | 10 | 21 | 6 | 8 |
| 3 | 9 | 10 | 22 | 8 | 9 |
| 4 | 11 | 14 | 23 | 8 | 6 |
| 5 | 13 | 11 | 24 | 10 | 12 |
| 6 | 15 | 13 | 25 | 11 | 7 |
| 7 | 15 | 20 | 26 | 17 | 4 |
| 8 | 13 | 7 | 27 | 15 | 6 |
| 9 | 13 | 10 | 28 | 28 | 9 |
| 10 | 8 | 8 | 29 | 23 | 6 |
| 11 | 12 | 7 | 30 | 26 | 9 |
| 12 | 17 | 13 | 31 | 34 | 15 |
| 13 | 10 | 10 | 32 | 28 | 16 |
| 14 | 2 | 3 | 33 | 32 | 11 |
| 15 | 9 | 8 | 34 | 29 | 16 |
| 16 | 12 | 7 | 35 | 35 | 18 |
| 17 | 8 | 10 | 36 | 22 | 15 |
| 18 | 11 | 9 | 37 | 30 | 8 |
| 19 | 5 | 12 | | | |

The data reveal that the frequency of grid squares affected in Colombia in 1985 was noticeably higher after the 26th week; 372 grid squares had been affected up to the 37th week in 1984, but 558 grid squares were affected in 1985.

The frequency of vesicular diseases (VD) affected herds in 1985 in Colombia reached epidemic proportions in June, July and August, as indicated in Table 3.

The number of herds affected by type A FMD virus exceeded the monthly forecasts after May, 1985.

TABLE 3. AFFECTED HERDS. VESICULAR DISEASES AND FMD TYPE A. COLOMBIA, 1985.

| MONTHS | TOTAL VD | | FMDV TYPE "A" | |
|---|---|---|---|---|
| | FORECAST** | OBSERVED | FORECAST** | OBSERVED |
| January | 117 | 98 | 17 | 11 |
| February | 123 | 81 | 9 | 9 |
| March | 85 | 80 | 8 | 2 |
| April | 84 | 52 | 7 | 1 |
| May | 62 | 39 | 5 | 8* |
| June | 87 | 155* | 4 | 43* |
| July | 164 | 370* | 8 | 96* |
| August | 156 | 300* | 13 | 71* |

* Equal or above expected levels

** Calculation made by PANAFTOSA using chronological series of the country.

4. Monitoring the FMD sporadic and free areas.

The computer enables us to maintain an active observation of the free and sporadic grid squares. This further allows us to issue an alarm when those squares become affected. Such a mechanism is used with weekly FMD reports from Argentina when grid squares near the border with Chile (FMD-free), from the 67° longitude westward, are affected. A warning can also be issued when FMD occurs in squares that were unaffected for a certain period.

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Appendix 19

# PLANNING OF FMD ERADICATION: ECOSYSTEMS AND REGIONAL STRATEGIES

Felix J. Rosenberg*

The following key words summarize the differences between the traditional set up of Foot-and Mouth Disease Campaigns in South America and what might be considered a modern socio-economic information based planning:

| | Problem | Cause | Means | Decision |
|---|---|---|---|---|
| Traditional | Disease | Virus | Vaccines | Technical? |
| Present | Infection | Ecological/ Economical | Regional strategies | Political |

## A. The Problem

The differences between infection and disease have been extensively discussed at this meeting. Three major subjects will be pointed out: 1st, FMD is detected, considered and recorded only as an overt disease. Therefore strategically more attention is paid to its control where clinical outbreaks are more widespread and individual signs more severe; 2nd, the role of subclinical infection and transmission of virus even in well vaccinated animals is not considered; and 3rd, from the economical and political points of view a meat exporting country will obtain maximum benefits when the country is declared free, i.e., no vaccination is needed because a virus no longer exists. In this respect one outbreak, 100 outbreaks or just subclinical presence of the virus makes no difference.

## B. The Cause

The ecological determinants of virus maintenance have been described previously (Rosenberg, 1977; Rosenberg et al., 1979). Four ecosystems were considered: endemic (primary endemic), epiendemic (secondary endemic), paraendemic (sporadic) and virus-free. The endemic ecosystem (Fig. 1) is to be considered of first and highest priority if FMD is to be eliminated because it is there where the virus is maintained and from where sources of infection and susceptible young animals derive. As opposed to the traditional overt disease phenomena as a basis for the establishment of priorities, the endemic ecosystems are characterized by the continuous, slow virus transmission, generally subclinically, or at least without being easily noticeable. In order for endemic virus transmission to take place, some specific cattle management conditions must be present: very large cattle breeding farms with a high percentage of adult virus carrier animals, a slow input of susceptible calves through non-seasonal low birth rates, low animal density (avoiding massive virus exposures), low virus transmission conditions given by scarce technology, wild

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animals, etc. These conditions are met in what is called extrative-extensive cattle breeding farms, typically distributed in N.E. Argentina; S.W. Paraguay; S.W. of Rio Grande do Sul, Mato Grosso Pantanal, N.E. of Minas Gerais and S.E. of Bahia in Brazil; the "Llanos" region in Venezuela and Colombia, and N and E of Bolivia.

The epidemiologic conditions prevailing in these areas will impact in the epiendemic (secondary endemic) ecosystems (Fig. 2) where, besides the potential existence of mechanisms for endemic maintenance of virus, the replacement of immune animals (steers finished for slaughter) by younger animals (mostly susceptible or acute sources of infection) creates the classical conditions for seasonal epidemics. This ecosystem is obviously determined by the entreprenurial fattening farms located in the best grazing areas of the continent including the "Wet Pampa" in Argentina, south of Rio Grande do Sul, Central States of Brazil and the Savanah in Colombia as the most representative areas.

In the remaining areas, the characteristics of cattle management are not suited for epidemiological mechanisms allowing for maintenance of the virus, i.e. the disease, if introduced, is self limiting because of the small size of farms, the reduced movement of cattle, the predominance of adult cattle, etc. or even, in those areas where the entreprenurial milk farms predominate. Here the high cattle density and the milk handling create optimal conditions for massive, short term exposure of most of the cattle, thus creating a very high "natural" herd immunity which will tend to self eliminate the virus (Fig. 3).

The various livestock production forms and the ecosystems by them determined (Obiaga et al, 1979) are geographically characterized through the use of a matrix composed by objective livestock indicators such as steer/cow ratio; various cattle and other species densities according to grazing, agricultural or total surfaces; distribution of herd-size; cattle movements, etc.; economical indicators including average price of the land, number and type of manpower employed and specific technologic investments, and disease recurrence indicators (Astudillo, 1978).

C. The Means

Once the geographical determination of FMD has thus been characterized specific regional strategies are established accordingly. Briefly, the extensive-extractive breeding regions, holding endemic ecosystems will become of highest priority. Two major objectives are sought: initially, to prevent the exiting of either susceptible or infected young animals potentially involved in the appearance of FMD outbreaks in the fattening areas. This is achieved through the quarantine and revaccination of all young steers gathered for fattening movements. This operation takes place at the farm of origin five to seven days prior to the actual mobilization. Next, measures must be programmed to eliminate the endemic virus. This can only be achieved through a high coverage of repeated vaccinations with high quality vaccine. Because of the difficulties for cattle management in these areas, oil adjuvanted vaccines, which offer a longer lasting immunity, must be used. It is desirable that this vaccination be handled exclusively by government services. The reduction of endemicity is to be monitored through yearly VIA antibody surveys, emphasizing the decay of prevalence in the less than two years of age category.

The epiendemic/fattening regions are economically and epidemiologically dependent on the regular inflow of young steers from the previous regions. This is why the planning of FMD eradication is here also secondary to the previously mentioned strategies. Vaccination as the most

important preventive tool within these regions is easily and willingly achieved by the farmers who are well aware of the losses which FMD produces in high density fattening herds. The major modification with respect to the official animal health service relates to the need for broad quarantines in the event of FMD outbreaks in order to ensure that the disease will not be widely distributed through animal movements, thus reducing the risk of secondary outbreaks.

In the remaining regions (paraendemic ecosystems), emphasis is put on reducing the private cost of animal care. If an adequate transit control can be implemented for the relatively low number of incoming animals, regular vaccinations with commercial aluminum hydroxide vaccines could be reduced from three to one per year. This one single vaccination accompanied by a well organized disease monitoring system will ensure fast control of outbreaks with ring vaccinations eliciting booster immune answers.

D. The Decision

As early as 1973, the Center critically reviewed the existing FMD control programs in South America and proposed that alternative regionally oriented strategies be investigated (Rosenberg and Goic, 1973). Two alternative strategies were actually evaluated in Paraguay (Rosenberg and Astudillo, 1976). Since then various countries established an FMD framework of reference with the assistance of the PAFMDC characterizing disease ecosystems and predominant forms of livestock production. This methodology is being systematically taught in the courses in which the Center's staff takes part.

Finally, animal health officers in the South American countries became aware of the economic burden caused by both, the disease and its control and of the technical possibilities of FMD eradication. The economic crisis helped a lot to try to rationalize costs and to establish priorities.

A plan considering in detail all aspects discussed is to be started nationwide at the beginning of 1986 in Argentina. Brazil and Uruguay are also quite ready to enforce a similar project. It is believed that by the end of the 80's, this approach to FMD control and eradication will start a new era in the animal health programming in the whole continent.

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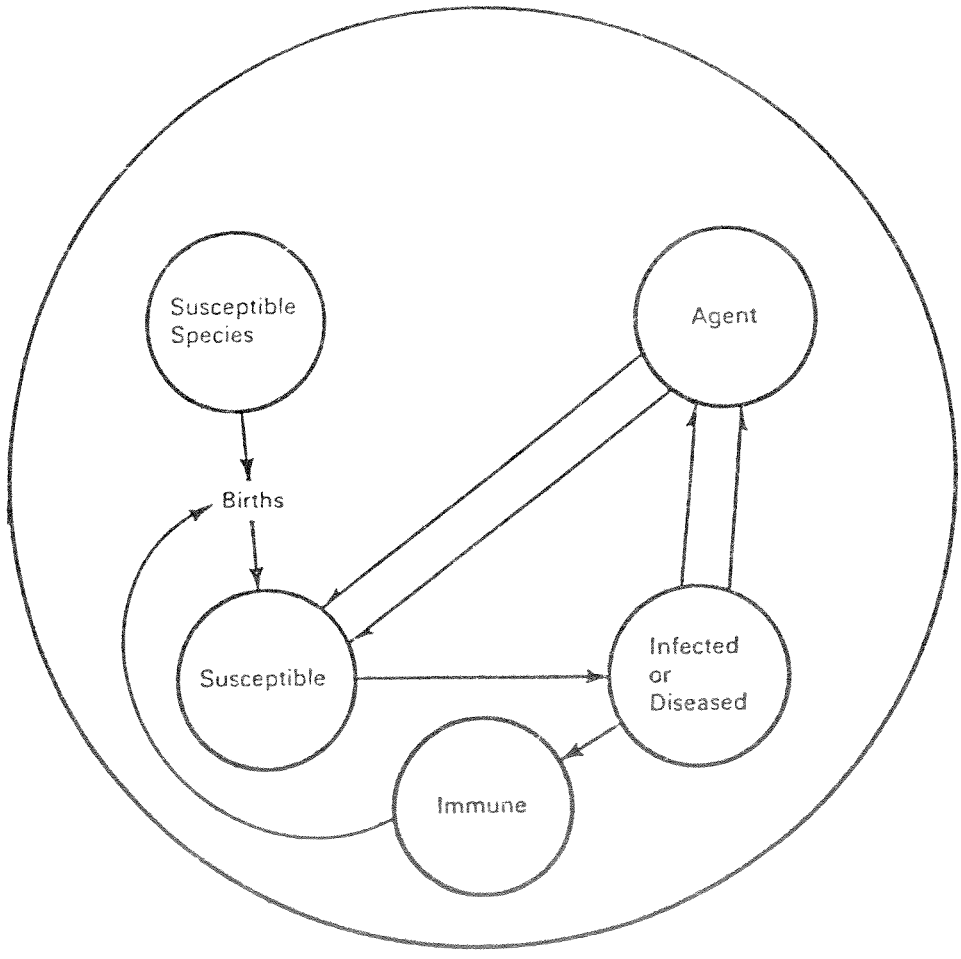


Figure 1: Endemic Ecosystems: the ecosystem contains all elements necessary to maintain the disease: the agent, the susceptible and an adequate transmission mechanism.

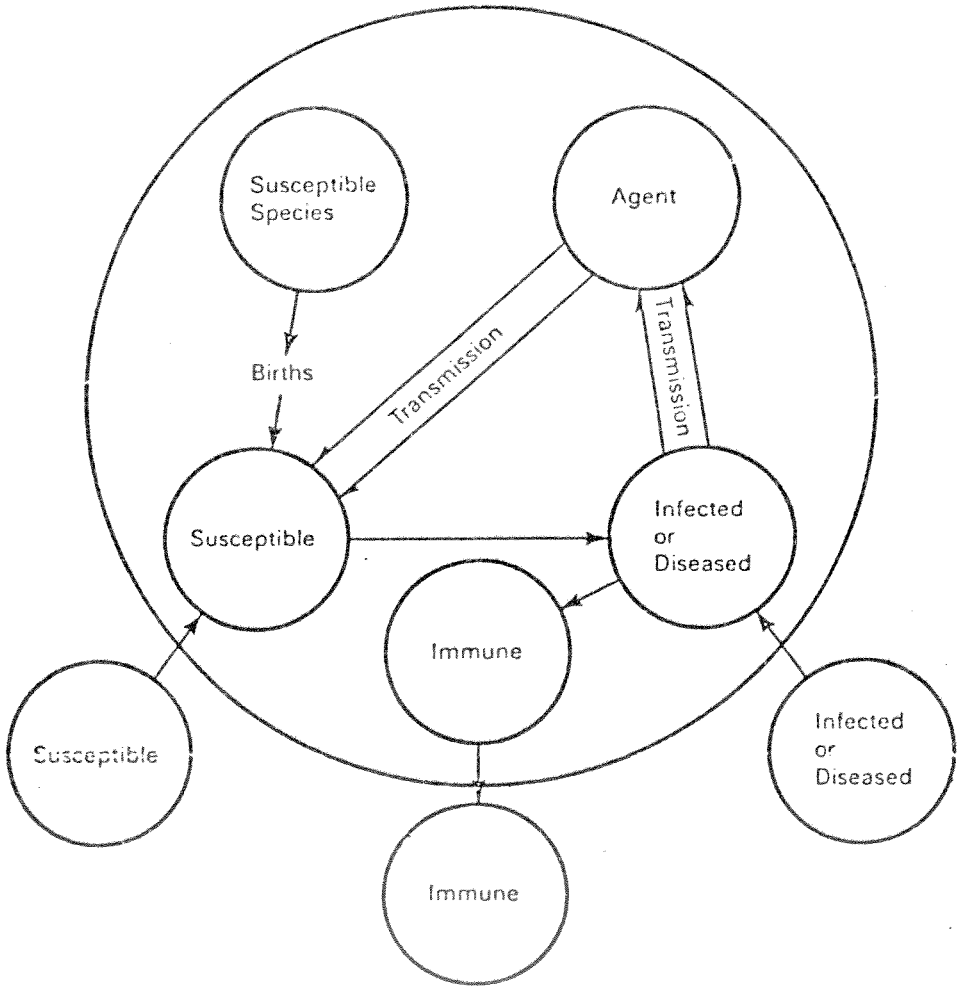


Figure 2: Epiendemic Ecosystem (secondary endemic): the ecosystem contains all elements necessary to maintain the disease. In addition there is an exchange (generally seasonal) and susceptible or infection sources (inputs) of immunes (exists) which tends to increase the number of cases.

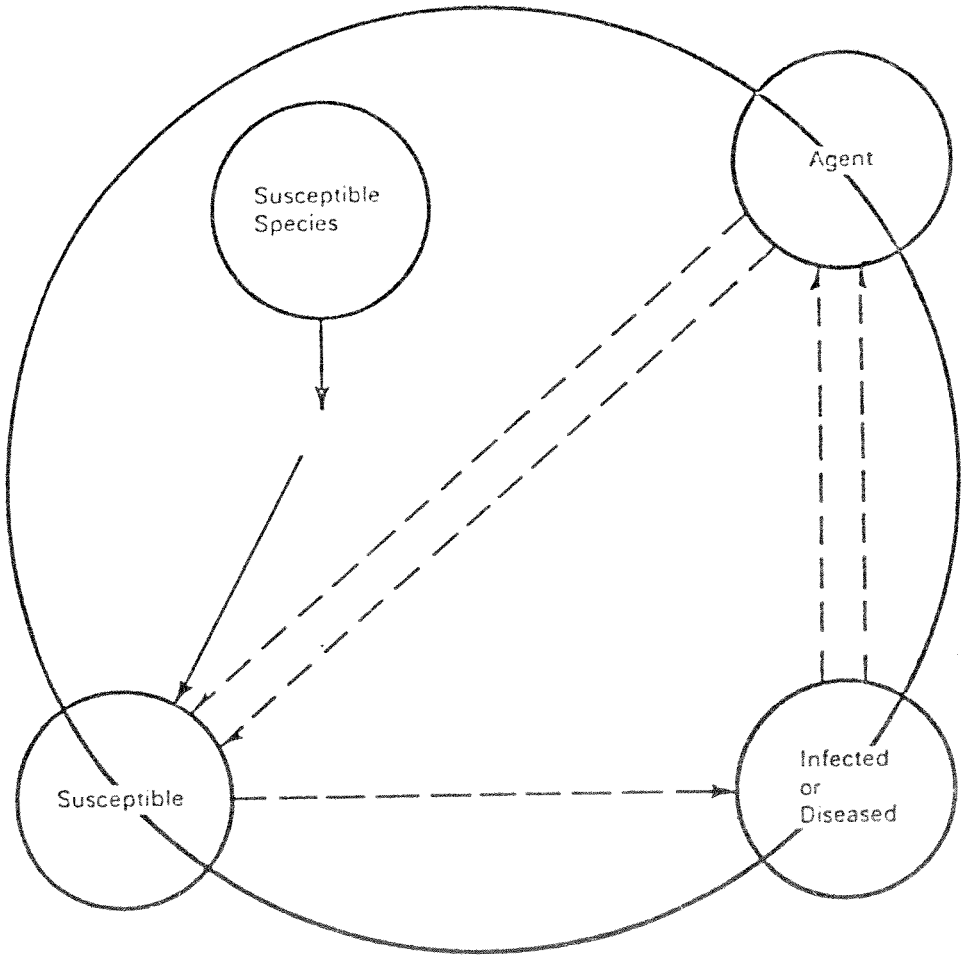


Figure 3: Paraendemic Ecosystem: the ecosystem lacks the elements necessary to the maintenance of the disease. Its sporadic occurrence is due to a combination of interactions with external elements.

Appendix 20

# LARGE SCALE APPLICATION OF OIL ADJUVANT FOOT-AND-MOUTH DISEASE VACCINE

Hans G. Bahnemann*

The immunogenicity of oil adjuvanted foot-and-mouth disease (FMD) vaccines as well as the duration of immunity induced by these vaccines in young and adult cattle at primovaccination and at revaccination was studied by the Pan American Foot-and-Mouth Disease Center (PAFMDC) since 1968 in extensive laboratory experiments and field trials in Brazil (1, 2, 3, 5, 8). The serum antibody titers are higher for the oil adjuvanted vaccine in comparison to the aluminum hydroxide vaccine for a longer period of time already in primovaccinated animals. This effect is much more pronounced upon revaccination. The recommendation of the PAFMDC for application of the oil adjuvanted vaccine is therefore that young animals under two years of age should be revaccinated at six month intervals while for animals more than two years old and previously immunized one annual revaccination is sufficient.

## Field Application

All laboratory and field studies at the PAFMDC of the immunogenicity of oil adjuvanted FMD vaccine indicated that this type of vaccine induced high levels of antibody to FMD virus and immunity to challenge for longer periods of time than the conventional aluminum hydroxide FMD vaccine. Two countries in South America, Argentina and Brazil, tested the oil adjuvant vaccine prepared at the PAFMDC in large scale field application programs in comparison to the standard aluminum hydroxide vaccine. In Argentina about 68,000 cattle were vaccinated from 1977 to 1981 in Hipolito Yrigoyen, Province of Buenos Aires, with oil adjuvanted FMD vaccine and the incidence of FMD in these animals was compared with that in a cattle population in the same area vaccinated with commercial aluminum hydroxide vaccine. C.H. Caggiano and collaborators reported on this experiment in 1982 (4) and the results are summarized in Table 1. Recently J.F.P. Dora and collaborators in Brazil published a report (6) comparing the incidence of FMD during an epidemic in Bage, R.S., in 1980 in two cattle populations vaccinated with commercial aluminum hydroxide and oil adjuvanted FMD vaccine respectively. The results of their publication are summarized in Table 2.

Although in the Argentinian experiment the number of animals vaccinated with oil adjuvant vaccine is considerably smaller than in the control group, it can clearly be seen that the disease incidence is greatly reduced in the oil adjuvant group. The morbidity rate in the aluminum hydroxide vaccine group is 55 per 1,000 animals while it is only seven for the oil adjuvant group.

The Brazilian experiment has comparable cattle populations of over 200,000 animals for each vaccine type. The morbidity rates for the aluminum hydroxide vaccine and for the oil adjuvanted vaccine were 45 and six respectively, which is surprisingly similar to those in the Argentinian experiment (Table 3). Also very interesting is a comparison of the animals vaccinated once or twice and more times with the oil

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adjuvanted vaccine. The primovaccinated animals had a morbidity rate that was lower by two thirds in comparison to the aluminum hydroxide vaccine. But the animals vaccinated two or more times with this type of vaccine had a morbidity rate more than twenty times lower than the animals vaccinated with aluminum hydroxide vaccine.

These figures show that the incidence of FMD is greatly reduced in animal populations vaccinated with an oil adjuvant vaccine. One can only agree with the conclusion of Dora and his collaborators, that if all the animals in the particular area had been vaccinated with an oil adjuvant vaccine the incidence of the disease would have been insignificant. This opinion is supported by the observation that the cattle population in Bage, which continues to be vaccinated with oil adjuvant vaccine, has not had a single case of FMD in the five years following the described epidemic in 1980, although there are still outbreaks of FMD in Rio Grande do Sul (7). However the incidence of FMD in Rio Grande do Sul has been reduced sharply as almost half the bovine population in this State, 5.5 million animals out of a total of about 12 million cattle, is now systematically vaccinated with oil adjuvant vaccine.

## Present and Future FMD Oil Adjuvant Vaccine Production

The PAFMDC prepares at present oil adjuvanted inactivated FMD vaccines for Government field projects in Argentina, Bolivia, Brazil, Ecuador, Paraguay, Peru, Uruguay and Venezuela and keeps a stock of 50,000 vaccine doses for emergency application at outbreaks in disease-free areas.

The excellent experience with this vaccine in FMD epidemics in southern Brazil and the great interest of farmers in the application of this vaccine lead government authorities in Brazil to establish oil adjuvant vaccine production laboratories in Campinas (Federal Government) and Porto Alegre (State Government, Rio Grande do Sul), which have been in operation since 1984.

Private vaccine production laboratories in Brazil are interested in this vaccine and have already produced experimental batches. Through the experience of government field projects, supported by the PAFMDC, several countries in South America are also interested in the production of this type of FMD vaccine in the near future. It is therefore very likely that the production volume of oil adjuvanted FMD vaccine in South America will soon increase considerably. A systematic and massive application of this type of vaccine mainly in primary endemic areas should have a profound effect on the epidemiological situation of FMD on this continent.

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Table 1

Field Application of Oil Adjuvanted Foot-and-Mouth Disease Vaccine

Hipolito Yrigoyen, Buenos Aires Province, Argentina, 1977-1981

| Cattle | Alum. Hydr. Vaccine | Oil Adjuv. Vaccine |
|---|---|---|
| Herds vaccinated | 1719 | 494 |
| Anim. vaccinated | 620462 | 67715 |
| Herds affected | 365 | 23 |
| Animals exposed | 245516 | 2874 |
| Anim. diseased | 34373 | 441 |
| Morbidity / 1000 | 55 | 7 |

[Data from Caggiano et al. (4)]

Table 2

Field Application of Oil Adjuvanted Foot-and-Mouth Disease Vaccine

Bage, Rio Grande do Sul, Brasil, 1980

| Cattle | Alum.Hydr. Vaccine | Oil Adjuv. Vaccine | | |
|---|---|---|---|---|
| | | total | primovacc | revacc |
| Herds vaccinated | 1813 | 620 | 316 | 304 |
| Anim. vaccinated | 220532 | 240616 | 87831 | 152785 |
| Herds affected | 73 | 20 | 9 | 11 |
| Animals exposed | 30780 | 17164 | 5647 | 11517 |
| Anim. diseased | 9958 | 1546 | 1218 | 328 |
| Morbidity / 1000 | 45 | 6 | 14 | 2 |

[Data from Dora et al. (6)]

Table 3

Morbidity Rate (per 1000) of Foot-and-Mouth Disease in Two Large Scale Field Application Experiments with Aluminum Hydroxide and Oil Adjuvant Vaccines

| Country | Alum. Hydr. Vaccine | Oil Adjuv. Vaccine |
|---|---|---|
| Argentina 1977/81 | 55 | 7 |
| Brasil 1980 | 45 | 6 |

Appendix 21

EUROPEAN COMMISSION FOR THE CONTROL OF FOOT-AND-MOUTH DISEASE
Session of the Research Group of the Standing Technical Committee
Centro Panamericano de Fiebre Aftosa, Rio de Janeiro
15 to 18 October 1985

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REPORT OF THE SESSION OF THE RESEARCH GROUP OF THE STANDING TECHNICAL COMMITTEE OF THE EUROPEAN COMMISSION FOR THE CONTROL OF FOOT-AND-MOUTH DISEASE
Rio de Janeiro, Brazil, 15-18 October 1985

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