EUROPEAN COMMISSION FOR THE CONTROL OF FOOT-AND-MOUTH DISEASE

STANDING TECHNICAL COMMITTEE

AN/61

RESEARCH MEMBERS

Brussels, 9-10 May 1961

A meeting of the Research Members of the Standing Technical Committee of the European Commission for the Control of the Foot-and-Mouth Disease was held at the National Institute for Veterinary Research, Brussels, on 9-10 May 1961 and was continued at the Bureau of the Office International des Epizooties, Paris on 18 May 1961.

The Research Members present were: Dr. R. Willems (Belgium), Dr. Ubertini (Italy), Dr. I. Galloway (Great Britain), Dr. Michelsen (Denmark). Others present were: Dr. G. Mossbrugger (Secretary-General OIE Commission on Foot-and-Mouth Disease), Dr. Nardelli (Italy), Dr. van Bekkum (The Netherlands), Dr. Leunen (Belgium) and Dr. M. Shahan (Plum Island, U.S.A.).

Sir Thomas Dalling and Dr. E. Fogedby, Consultant and Secretary, respectively, European Commission for the Control of Foot-and-Mouth Disease also attended.

The Agenda for the meeting, which was adopted, was:

1. Adoption of the Agenda
2. Production of virus and vaccine with demonstrations
3. Vaccines prepared from virus cultivated on kidney cell monolayers
4. Vaccination of pigs
5. Immunising value of vaccines
6. Escape of virus from laboratories
7. Establishment of a new foot-and-mouth disease institute in Yugoslavia
8. Any other business.

The Secretary opened the meeting and, in accordance with usual procedure, Dr. Willems, Director of the Institute, was appointed Chairman of the Meeting.

Production of Virus and Vaccine with Demonstrations

A considerable amount of time was devoted to demonstrations and explanations on the production of virus and vaccine as carried out at the Institute.

The production of aluminium hydroxide was demonstrated in detail: a full description of the method is to be published in the near future. Aluminium hydroxide, as prepared at this Institute, conserves the immunising value of the vaccine and so prolongs its expiry date for a longer period than is usually given for other foot-and-mouth disease vaccines.

All the stages in the production of virus and vaccine were demonstrated and detailed explanations were given. The vaccine is trivalent - O, A, C - and is prepared from both natural virus and virus cultivated by a modification of the Frenkel method (reanimalisation). One of the virus types is used in the form of natural virus while the other two are culture virus.

The procedure of reanimalisation in the National Institute, Brussels, is:

The natural virus is adapted to culture by the Frenkel method, by 5 to 7 passages. It is, then, injected, intradermally, in the tongues of 20-30 susceptible Irish cattle and the tongue epithelium and vesicular fluid are collected and pooled. This material is conserved in the deep-freeze and is the future seed for virus cultivation by the Frenkel method. For each batch of virus to be used for vaccine preparation, the cultures are seeded from the above-mentioned pool and, in addition, a small amount of virus from the preceding batch is added. The resulting cultures are, then, treated in the usual way for vaccine preparation.

A visit was made to the large, new, modern establishment at Machelen which is to be brought into operation at an early date for the production of natural virus. Up to 240 cattle can be dealt with each week. There is also provision for the testing of vaccine for innocuity and potency in cattle. The many arrangements for all this work in cattle were demonstrated and explained.

Vaccines Prepared from Virus Cultivated on Kidney Cell Monolayers

It was recalled that an experiment was arranged, jointly, by the Veterinary Institute at Brescia, Italy and the Veterinary Institute, Lindholm, Denmark, to test the immunising value of vaccine prepared from virus cultivated on kidney cell monolayers; this is referred to as the Italian/Danish experiment. An account was given of the results.

The vaccine, monovalent type A, was prepared in Brescia and, as reported at the meeting of the group in Brescia in 1960, some experiments in guinea pigs and a few cattle in Italy, gave indications that it had a good immunising value.

The vaccine was shown to be innocuous by tests in cattle and in mice. In February 1960, 36 susceptible cattle were each given 40 ml of the vaccine, subcutaneously. (The vaccine contained 40 percent of virus, that is, each dose of vaccine contained 16 ml of virus). Samples of blood were taken from the vaccinated animals at intervals for serum-neutralisation tests. At 3 months after vaccination, 12 of the vaccinated animals were subjected to severe challenge under carefully controlled conditions: they showed a very high degree of immunity.

Some of the cattle in the group which had not been subjected to challenge were retained and were used in a similar challenge, six months after the original vaccination. In the interval, serum-neutralisation tests were carried out on samples of their serum. From the result of the challenge it appeared that the immunity from the vaccination had almost completely disappeared.

There still remained 8 vaccinated cattle which had not been subjected to challenge. They were revaccinated at 6½ months after the original vaccination and the serum titre of 4 of them was followed during the next 6 months.

It was found that the revaccination caused a rapid rise in the titre to a level higher than that after the first vaccination and this was maintained throughout the period of observation.

The four revaccinated animals were challenged by contact, six months after the revaccination, and were found to be completely protected.

It is now intended to extend the Italian/Danish experiments to the testing of a trivalent vaccine prepared from virus cultivated on kidney cell monolayers.

An account was given of the use of this type of vaccine in the field in Italy during the recent epizootic of foot-and-mouth disease caused by virus type C. Four batches of vaccine were prepared comprising, in all, some 20,000 doses. Details of its use was given in 4 herds: in each of the herds there were several separate stables and it was quite clear that the effect of the vaccination had been stri/king in preventing spread among them.

A description was given of the work on this type of vaccine carried out at the National Institute for Veterinary Research, Brussels. Vaccines were tested in Brussels for innocuity, but intradermolingual inoculation showed that sometimes they contained some active virus. The vaccines tested were prepared by the addition of formalin alone and also by the addition of formalin after adsorption on aluminium hydroxide. The virus had not been filtered. None of the vaccines containing 5,10 or 20 percent virus were satisfactory immunising agents: it was necessary for a satisfactory vaccine to contain 50 percent virus.

Some preliminary work had also been carried out at the Institute, Brussels, on the preparation of this type of vaccine.

It was reported that in Greece, some 50 litres of this type of vaccine is now being produced each week; each dose contains 3.6 ml of the culture. The vaccine has proved useful in preventing spread of invasions of the three types O, A and C, although it is probably doubtful if it has as high an immunising value as vaccine produced from the natural virus.

In the Netherlands some work has been carried out with kidney cell cultures. Foetal bovine kidneys cells have been used in suspension rather than in monolayers - a modification of the technique used in the Plum Island Laboratory.

The meeting agreed that the practical results of monovalent vaccine prepared from virus grown on kidney cell cultures were definitely encouraging and that further work on the method should be pursued in all vaccine-producing laboratories. While the present evidence might suggest that vaccine prepared from natural virus and from virus cultivated by the Frenkel method were somewhat better immunising agents, they had some disadvantages, related mainly to expense and the difficulty in some countries to obtain the necessary supply of cattle tongues. The danger of unfiltered vaccine containing active virus has to be stressed, even although it may not always be easy to demonstrate its presence. Even although incubation is carried out for a week in the course of preparation of the vaccine, active virus may, sometimes, be present. It would appear that a considerable amount of virus produced in kidney cell cultures is necessary in vaccine to stimulate a satisfactory immunity.

Vaccination of Pigs

In the discussion on foot-and-mouth disease in pigs, stress was laid on the importance of the disease in these animals as spreaders of the virus and the urgent need to study the preparation and use of vaccines for their i immunisation.

An account was given of the recent experimental work at Lindholm (Denmark). An experiment was carried out to test the immunising value of vaccines, injected into pigs by the intradermal and the subcutaneous routes. Two types of vaccine, both prepared from virus type O, of bovine origin, were used: in one, a special adjuvant - Twean, Span substance (oil) and liquid paraffin was used and the other vaccine was the usual aluminium hydroxide adsorbed vaccine. Each vaccine contained 2.8 percent of virus.

In all, 60 pigs were vaccinated: 30 pigs were given intradermal injections of 1 ml of the vaccines (each vaccine to 15 pigs) and 30 pigs received 25 ml of the vaccines subcutaneously (each vaccine to 15 pigs).

After a month, each of the 4 groups of 15 pigs was subjected to challenge by contact for 14 days with two donor and four control pigs. All the donors and the controls reacted satisfactorily.

The results showed that the route of administration of the vaccine (intradermal or subcutaneous) yielded very similar results, although the dose injected subcutaneously was 25 times larger than that injected intradermally, and that the vaccine with the special adjuvant gave the better immunity.

Although these results were apparently very definite, it was pointed out that the same virus may behave differently in pigs on different occasions and that it was very necessary to have a full appreciation of the characteristics and behaviour of a virus to be used in pig experiments.

It was reported that many pigs had been vaccinated in Italy with bovine vaccines types A and C, the dose being that used for calves: no good results were found.

In the Netherlands, data on the serology of pigs vaccinated with bovine vaccine is being collected. Pigs have been vaccinated with 40 percent of the dose given to cattle. It is found that antibodies can be demonstrated in only a limited number of the animals and that, although after revaccination, there is a greater response, the antibody titres are not high and do not reach the level of those in cattle.

This limited antibody response was confirmed by observations made in Denmark.

As evidence that vaccine containing virus of bovine origin was of value in preventing the spread of foot-and-mouth disease in pigs, the experience in Flanders was quoted: a large number of pigs was injected with a monovalent type C vaccine and the epizootic in the pigs was arrested. Although the immunity was of short duration, the period of protection was sufficiently long for the infection to be suppressed in the country.

It was agreed that, economically, the protection of pigs against foot-and-mouth disease is of real importance, especially in countries which practise vaccination, and that every effort should be made to continue or institute research on the production of a satisfactory vaccine for use in pigs. The question of strains of the virus - cattle and pig - for use in vaccine preparation should be studied. Different types of adjuvants should receive attention. The method of challenge to demonstrate the value of vaccines in pigs should be fully considered. It was felt by some that the amount of work on vaccines for cattle left little time for including much investigation on vaccines for pigs in their programs. The general view, however, was that the importance of immunising pigs was so acute, that every effort should be made to concentrate on the problem. It was suggested that it might be possible for the research work at Lindholm to be continued and further developed, that Lindholm could perhaps be regarded as the centre for research work on foot-and-mouth vaccines for pigs and that other institutes might collaborate, for example, by assisting in the testing of vaccines for potency, when the appropriate stage had been reached in the work at Lindholm. Note was also made of the research work on the subject at the Plum Island Institute, especially on the method of exposure of vaccinated pigs and strains of virus for use in the preparation of vaccine for pigs. The hope was expressed that this work would be continued and that any results would be made available to the group in due course.

Immunising Value of Vaccines

It was explained that this subject was brought up last year at the meeting of the group in Brescia, the view being held by some members that vaccines now being produced are less effective in the degree of immunity they induce and that the immunity is of shorter duration than that found with vaccines prepared when vaccination against foot-and-mouth disease was first introduced. The various reasons suggested at the meeting in Brescia were reiterated.

The discussion on this subject now revolved around the strain of the virus used for vaccine preparation. Two important findings were reported. In Italy, there was distinct evidence that something had gone wrong with the immunity from vaccine types A and O in the field last year. There were breaks in the immunity.

No difference could be found, however, from the results of the complement fixation test, i.e. between the virus of the vaccine and the virus which caused the break in the immunity. The strains of virus type A used for vaccine production had been isolated in 1951 and had been in constant use for vaccine preparation since that time. Recently, the type of O virus used in vaccine production has been changed in the Brescia Institute and, although the results of complement fixation tests fail to show any difference between this and the former strain of virus used for vaccine production, the new vaccine appears to be giving better immunity in the field. Also in Italy, vaccine prepared with the current C type virus, used in about 3½ million cattle has given excellent immunity in controlling the present epizootic.

In Belgium, a most interesting observation has been made in connection with a change in virus type A_5. Because of field evidence of the somewhat poor immunity from the A_5 type vaccine, a comparison was made between the virus used for vaccine production and the virus isolated from outbreaks. The A_5 virus used in the vaccine production had been passaged some 50 times in culture by the Frenkel method. All the usual tests were carried out with the laboratory and the field strains of the virus marked differences between them were found in complement fixation tests, agar gel, precipitin tests and serum-neutralization tests. The immunising value of vaccines prepared from the laboratory and field strains was tested on 36 susceptible Irish cattle and the results showed that each vaccine was markedly better when challenged with its homologous strain.

All this evidence shows that the strain of virus used for vaccine preparation is of the utmost importance and that it is essential to examine the strain from time to time to ensure that it is still producing a satisfactory vaccine to protect against field strains causing outbreaks. It is also evident that in testing strains for antigenic properties, all the various methods should be used and reliance should not be placed on one single method. These should include complement fixation, agar gel precipitin, serum-neutralization and cross-immunisation tests in cattle. It was recognised, of course, that all strains are not necessarily immunogenic.

Escapes of Virus from Laboratories

It was explained that this subject was causing some concern to the Commission, because of the evidence that was accumulating of the occurrences of the escape of virus from Laboratories. It was pointed out that the prevention of the escape of virus was the responsibility of the laboratories and was of real importance and would present a serious problem if exotic types of the virus should escape.

While there is definite evidence that escapes have taken place, it was felt by the meeting that it must be pointed out that the possibility must not be overlooked of blame being attached to laboratories for outbreaks in the vicinity of a laboratory from entirely different origin.

A full description of the escape of virus SAT II from the Pirbright Institute as well as the additional precautions now in force further to minimize the risk of any escape is given below:

On 18th January, 1960, foot-and-mouth disease was detected in cattle on a farm (13 cattle and 9 pigs) approximately one mile from the Institute. The cattle and pigs on this farm were valued and destroyed in the afternoon and evening of 18th January and disinfection of the premises was completed by 1st February. Contiguous stock (17 cows) on a neighbouring farm were destroyed also, as dangerous contacts had taken place. The son of the owner of the infected farm had visited this farm daily to collect milk. None of the animals on the second farm showed signs of infection at the time of slaughter.

Enquiries elicited the information that 3 pigs from the infected farm had been sent to a slaughter house on the day before the outbreak was detected, i.e. 17th January. This slaughter house is at Brighton, about 25 miles away. These pigs were slaughtered on 18th January. The lorry involved in transporting the pigs was traced and all necessary action taken for disinfection. One farm visited by the lorry after collecting the pigs from the infected farm was placed under restrictions. No disease was detected on this farm and there were no other outbreaks of disease in the district around the infected farm. The intervening space between the Research Institute and the infected farm is an estate on which there are 3 farms with about 141 cattle, 120 sheep and 218 pigs. No further outbreaks of disease occurred in the district or elsewhere.

It was demonstrated that the type of virus involved was type SAT 2 and it was confirmed that it was a sub-type similar to that which was being used in current experiment in one of the large animal isolation units in the Institute. In spite of exhaustive enquiries, no positive information was obtained which would establish irrefutably the origin of the outbreak or the link between it and the Institute. That there was no such clear explanation of the apparent escape of virus from the Institute was very disappointing and disquieting. If it had been determined that there was a breakdown of the very stringent disease security precautions, it would have been possible to take definite action to avoid the risk of a recurrence. (During 1959 the work in the five cattle units proceeded at a greater rate than had hitherto been possible. All units were in operation and all seven types of virus were worked with during the year in very considerable numbers of animals. No untoward circumstances occurred.)

It was concluded that airborne virus must have escaped through the ventilators of this isolation unit and possibly contaminated the ground in the vicinity and that thereafter some mechanical transference of the virus took place, possibly by foxes or other fauna or birds. It was estimated that up to 1,000 wild pigeons were constantly flying to and from a field of kale on the farm which became infected a short time before 18th January. Pigeons were also active on the kale and cabbage plots around the large animal isolation units at that time. It is known that foxes are prevalent in the district. A fox hole was seen in the kale field on the farm and on various occasions foxes have been seen within the perimeter of the fence of the Institute.

It was clear that it would be necessary to install a system of air filtration to treat the outgoing air from the large animal isolation units. At the same time, the risks of dissemination of virus from the large-scale vaccine production unit were considered.

Steps were also taken to reduce contact by persons and wild fauna with the areas in the immediate vicinity of the sources of infection. These measures included the erection of an inner perimeter fox-proof fence around cattle isolation units, the prohibition of growing crops in the perimeter fence. A number of other detailed disease security precautions were reinforced, including those applicable to the incineration of carcasses of experimental animals and manure, which are a possible source of virus aerosols also.

Laboratory investigations confirmed that, of the several materials currently used for air filtration, a glass fibre paper was the most efficient for the virus of foot-and-mouth disease in that it had the lowest penetration: 0.001 percent. A bank of glass fibre filters with an appropriate ventilation system and ducting has now been installed in the large animal isolation units and the large-scale vaccine production unit. Gradually the same filtration system is being installed in all the other large animal isolation units on a forced extract basis.

Extensive modifications were made in the vaccine production unit also to endeavour to eliminate the possible production of aerosols. To do this, various kinds of sterilising equipment were attached to the vessels in which the virus is grown and to the various pieces of apparatus involved in the preparation of vaccine and many of these were also encased in cabinets.

It is hoped that the completion of air filtration installations and the various other minor modifications of the disease security precautions will prevent similar accidental infections in the future but it must be realised that in working with such a highly infective agent as the virus of foot-and-mouth disease even the most rigorous precautions may break down. The history of the Pirbright Institute has shown a gradual increase in effectiveness of the disease security precautions necessitated by the strain put on them by the ever-increasing tempo of research. It is hoped that the additional measures now being taken will make the odds against any accidental infection much higher than they were before.

(Mention may be made of the fact that, in addition to all the disease security regulations, all members of the staff are forbidden to come in contact with susceptible animals away from the Institute. They must not visit farms, cattle markets, cattle shows, etc. They must not live in the same house as people tending such stocks, or keep any themselves.

The view of the Institute on this type of precaution has always been that, if all the disease security regulations are maintained, there should be no risk of the staff carrying the virus away from the Institute but, in the event of a coincidental outbreak associated with a visit or the movement of such persons, the suspicion would inevitably be directed at the Institute. To avoid this arising, it has been deemed advisable to forbid staff the contacts indicated. The same rule applies to visitors to the Institute.)

Circumstantial evidence indicates that dissemination of foot-and-mouth disease virus also occasionally occurred from the Amsterdam laboratory. However, additional security measures were taken and these appeared to have been effective.

The meeting discussed the various ways in which escapes of virus might occur and the precautions which must be observed for their prevention. There is little doubt that the arrangements now in force at the different laboratories go a long way to minimize the risks of virus leaving the laboratory.

It was reported, for example that there is no evidence of escape of virus from the Lindholm Institute although, on occasion, several hundred animals are under experiment at the same time and it is necessary to employ extra and inexperienced staff to meet the heavy work at these times. Escapes have occurred within the Institute when many infected animals were being slaughtered.

The importance of implicit observation of regulations by personnel of laboratories and by any visitors to laboratories concerning bathing, changing clothing and keeping away from susceptible animals for a period after leaving a laboratory was stressed. There may be a tendency, at times, for personnel to adopt a certain measure of carelessness in the strict observation of the somewhat tedious precautions which must be taken all the time to ensure safety.

An example of the occurrence of an outbreak of foot-and-mouth disease which could be traced to a visitor who failed to observe instructions was given from Switzerland. Students from the Universities of Zürich and Berne visit the Basle Institute each year. They are required to undergo the various precautionary measures concerning changing clothing, bathing etc. and they have to undertake that they would not come into contact with any cattle for 48 hours after leaving the Institute. On one occasion, a student failed to observe this latter instruction and milked a cow soon after leaving the Institute: in two days the cow developed foot-and-mouth disease.

The meeting was informed of the precautions taken to prevent personnel from disseminating virus from the Plum Island Institute: included is that personnel may not contact livestock for a week after leaving the laboratory.

Mention was made of the possible spread of virus by carcasses of cattle which had been used in experiments and for vaccine testing in many countries, the carcasses, after hanging for 48 hours at room temperature and with or without treatment with lactic acid, are distributed to the trade. From the discussion, it appears that some spread has taken place by this procedure.

It was agreed that the meeting should advise the Commission that the precautions to prevent possible spread of foot-and-mouth disease virus from laboratories should include the following:

(1) Personnel

Strict supervision of the rules and regulations on all matters concerning bathing, clothing changing, disinfection, handling of animals outside the laboratory, i.e. periodic warnings to the personnel.

(2) Disposal of Meat

Consideration should be given to the burning or burial of carcasses of animals used in experiments or for testing vaccines and to the salvage of carcasses of slaughtered animals in outbreaks.

(3) Careful Examination

of ventilators and filters for air leaving the laboratories to ensure that reliable filtration is being done: consideration of spread of virus from and within the laboratory by infected aerosols.

(4) Consideration of Vaccination

of all animals in an area surrounding the laboratory: some members felt that such vaccination should not be practised as susceptible animals in the area act as "sentinels" and so escapes of virus may be rapidly detected.

(5) Slaughter of All Animals

in outbreaks in an area surrouding the laboratory, irrespective of the control method employed in the country, and satisfactory disposal of the carcasses.

(6) Careful Observations

of all livestock in the area around the laboratory to detect any outbreak at once.

It was pointed out thqt while every precaution must be taken to prevent the escape of virus from laboratories, even under the best conditions, it is impossible to guarantee that no escape will ever occur. The Minister of Agriculture, Fisheries and Food, Great Britain, when asked about the precautions taken at the Pirbright Institute to prevent escape of the virus included such a remark in his reply to the question.

It is also pertinent to note that, in discussions with the United States Government on foot-and-mouth disease, it is recorded that there is more risk in the introduction of the virus of foot-and-mouth disease by international trade than by its escape from a laboratory.

Establishment of a New Foot-and-Mouth Disease Institute in Yugoslavia

It was explained that the Commission felt that it should give all possible advice to the Government of Yugoslavia concerning the establishment of the new foot-and-mouth disease institute. The meeting was, therefore, asked to consider the advice which the Commission might give.

The Secretary (Dr. E. Fogedby) and Dr. R. Willems (Belgium), had, separately, visited Yugoslavia recently and gave their views.

The Government of Yugoslavia had decided to place the new institute near Sarajevo at some little distance from the town. While it was felt that the establishment of the Institute in a large town would be advisable for several reasons, its location must now be accepted. Plans have already been prepared for the Institute and some criticism of them have been made by Dr. Willems.

He informed the meeting that the Institute was planned for work not only on foot-and-mouth disease, including the production of virus and vaccine, but also on other viruses which are causes of diseases of animals. It was necessary therefore, that ample accommodation be provided so that work on the various viruses would be carried out under conditions whereby there would be no chances of cross-infection. This includes not only laboratories and laboratory space but also housing for laboratory and large animals with separate food stores and attendants.

The ultimate overall size of the Institute must be taken into consideration so that the various units could be made available.

It appears that full consideration has not yet been given to the types of foot-and-mouth disease vaccine to be produced. It would seem that there will be difficulties in obtaining sufficient supplies of cattle tongues, which are of small size in the area, for large-scale cultivation of the virus by the Frenkel method. It would be advisable, therefore, that arrangements be made in the Institute whereby vaccine may be prepared by different methods, including the use of virus cultivated on kidney cell monolayers.

The meeting felt that the Commission cannot give clear-cut advice to the Yugoslav Government on all details but could point out the points referred to above.

Next Meeting and Program

The meeting felt that the next meeting of the group, in 1962, might be held at the Foot-and-Mouth Disease Institute, Basle, Switzerland. Dr. Mossbrugger was prepared to make the necessary arrangements, subject to the approval of his Government. It was intimated that the OIE Permanent Commission on Foot-and-Mouth Disease would probably hold a meeting in Lyons, France, in 1962, on the occasion of the bicentenary of the Lyons Veterinary School. The date of the meeting of the group was not fixed but it was felt that sometime during late June would be convenient.

Some discussion took place on the form which future meetings of the group should take. The view was expressed that the meetings might be divided into three parts:

(a) demonstrations given by the Institute at which the meeting is held;

(b) discussion on subjects referred to the group by the Commission;

(c) discussion on special subjects brought forward by members of the group.

In connection with this last point, members should be asked to inform the Secretariat of the subject which they wish to discuss. Such subject will, probably, refer to recent research problems in the various laboratories. It was also suggested that it would be of considerable advantage if it could be arranged that the actual worker on the subject to be brought forward were invited to attend the meeting.

For the meeting in 1962, it was agreed that the general subject for discussion should be the relationship between immunity and antibodies as demonstrated by the different techniques.