RESTRICTED
EFMC (59) STC PV.2

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

Research Members of the Standing Technical Committee

MINUTES

of the Meeting held on the Island of Lindholm (Denmark) on 7-9 July 1959

A meeting of the Research Group of the Standing Technical Committee of the Commission was held at the Virus Research Institute, Lindholm, Denmark on 7 - 9 July 1959. The following members of the group were present:- Dr. H. Frenkel (Netherlands), Dr. I.A. Galloway (United Kingdom), Dr. E. Michelsen (Denmark) and Professor B. Ubertini (Italy). Sir Thomas Dalling and Dr. E. Fogedby from the Commission's Secretariat were also present with, in addition, Dr. L. Nardelli (Italy) and Dr. J.G. van Bekkum (Netherlands), by special invitation. In accordance with the decision taken in Paris on 9 May 1959, the President and Secretary of the OIE Standing Commission for the Study of Problems concerning the Immunization against Foot-and-Mouth Disease, Dr. R. Willems (Belgium) and Dr. G. Moosbrugger (Switzerland), also attended.

The Chairman of the meeting was Dr. E. Michelsen: this was in accordance with the decision of the Executive Committee concerning laboratory meetings that the Director of the Institute at which the meeting was held should be the Chairman.

The subjects for discussion were: 1. Cultivation of foot-and-mouth disease virus in kidney tissue cells; 2. Vaccination of pigs; 3. Reanimalization of culture virus in the production of vaccine; 4. Inactivation and concentration of virus; 5. Special equipment for the production of the newer types of vaccine; 6. Value of disinfectants at present in use; 7. Prevention of the introduction of exotic strains of the virus into European countries; 8. Suggestions for items for the agenda of the next meeting of the OIE Foot-and-Mouth Disease Commission.

Cultivation of foot-and-mouth disease virus in kidney tissue cells

Dr. Michelsen introduced the subject under three headings:-

(a) production of virus on a large scale; (b) titration of virus in kidney tissue cell cultures; (c) control of the innocuity of vaccines by this method.

Dr. Ubertini explained that a pilot plant had been set up in his institute in Brescia. At present some three hundred litres of virus cultivated in calf kidney tissue cells are being produced each week: it is possible to extend the amount to 450 - 500 litres and it is hoped that, by improved techniques under study, still larger scale production may be attained in the pilot plant. Some 30 kidneys are required to produce the 300 litres of virus and the total time between the receipt of the kidneys and the completion of the cultivation

of a batch of virus is six days. Virus is always cultivated in primary tissue cell cultures. From the virus so cultivated monovalent vaccine containing 34 percent virus has been prepared: the virus was first inactivated with formalin and then adsorbed on aluminium hydroxide. The amount of formalin used was 0.25 percent instead of the usual 0.1 percent. Vaccines have been prepared from virus type O_2, A_7 and C cultivated in kidney tissue cells: their immunizing value, tested on guinea-pigs challenged some 3 - 4 weeks after injection, is comparable to that of vaccines prepared from virus cultivated by the Frenkel technique.

Virus produced on a large scale by cultivation in kidney tissue cells has been titrated both in baby mice and in cultures of kidney tissue cells: in mice, a titre of 10^{-6} to $10^{-6.5}$ was obtained and this was somewhat higher than that obtained by titration in kidney cell cultures.

Dr. Willems gave an account of the work in progress in his institute, where the tissue used is foetal calf kidney. The collection of this tissue obviates difficulties with contaminations experienced with calf kidneys: it has also been found that the use of the foetal kidneys produces better cell growth. The virus is always cultivated on primary tissue cell cultures but for titration of the virus, subcultures (2-3 passages) of the tissue cells are used. Virus cultivated in such tissue cell cultures had a titre of 10^{-6} to 10^{-7}. It has been observed that a virus so cultivated may often have a high titre when only comparatively low complement deviation is found. The virus titrated in cattle has shown a tenfold lower titre than when titrated in tissue cultures. Using this virus, monovalent and trivalent vaccines, containing 10 - 20 percent of virus have been produced, 0.075 percent formalin being used.

Dr. Galloway reported that since 1955 when Sellers showed that the virus of foot-and-mouth disease could be cultivated in pig and calf cell monolayers and that plaques could be produced, studies had been in progress at his Institute with tissue cultures, monolayers and suspended cells, of pig, calf, ox and lamb kidneys. Monolayer cultures of pig kidney tissue were being used extensively and increasingly for assay of virus, titration by the plaque method and for serum neutralisation tests and for other purposes including the study of attenuated strains in kidney cultures. Work was in progress also to investigate the suitability of kidney tissue culture virus for large scale vaccine production. One point which required more investigations was the effect of long passage of virus on the characteristics of different strains of virus. Some evidence had been obtained which indicated that long passage in pig kidney tissue cultures may modify a cattle strain of virus in respect of its invasiveness both for cattle (decreased) and for pigs (increased).

With regard to (C) the possibility of control of the innocuity of inactivated vaccines by tests in kidney tissue cultures, the results of comparative tests in kidney tissue cultures, unweaned mice (intraperitoneal inoculation) and cattle (I.D. tongue inoculation) indicate that the most sensitive test for the detection of virus infectivity in a preparation which contains virus that has received physical or chemical treatment with a view to rendering it non-infective is I.D. cattle tongue inoculation (20 sites in not less than 6 susceptible cattle). With regard to the

possibility of using kidney cultures for testing of vaccines of the Schmidt Waldmann or Frenkel type, there are other factors to consider such as the presence of formalin which can to some degree impair the capacity of the virus for cell infection and the presence of aluminium hydroxide and its effect on cell sheets etc.

Following discussion, the Meeting concluded that large-scale cultivation of virus in kidney tissue cultures for vaccine production is distinctly promising and that the time is now opportune to confirm the antigenicity of such vaccine on cattle under well-controlled laboratory conditions.

Vaccination of pigs

Dr. Fogedby stated that only a limited number of experiments had been carried out on the vaccination of pigs. He referred to two papers on the subject published in Germany. One was by Möhlmann (1950) who reported the successful vaccination of pigs with vaccines prepared by the Schmidt-Waldmann method, using cattle virus adapted to swine and recovered from them. In the same paper successful results are recorded following the vaccination of swine with vaccine prepared with virus from the tongues of cattle, inoculated with pig-adapted virus. The other paper was by Geiger and Otto (1958) in which it was reported that the Möhlmann results could not be confirmed. The author recommended, however, the use of large doses of current cattle vaccine to ameliorate the disease in pigs. Dr. Ubertini drew attention to a recent paper by Traub in which unsuccessful immunization results in pigs with kidney tissue virus vaccine were reported.

Dr. Fogedby referred to the experiments on the vaccination of pigs carried out at the Lindholm Institute over several years. In 1937 experiments were made in collaboration with the Dutch Institute. The vaccine used was prepared from virus cultivated by the Frenkel method on embryonic calf skin and adsorbed on aluminium hydroxide. No further inactivation was carried out. The results were unsatisfactory. In 1939 and 1942 some experiments were made, using the current Danish cattle vaccine prepared according to the Schmidt-Waldmann method. Although, in general, the results were unsatisfactory, some slight protection was noted in pigs which had received large doses of vaccine. Following the 1951-52 epizootic when many outbreaks occurred in pigs, further consideration was given to their immunization. Möhlmann's work was repeated and it was found that vaccine prepared from swine-adapted virus recovered from infected pigs successfully produced immunity in pigs while vaccine made from virus recovered from the tongues of cattle inoculated with swine-adapted virus showed no immunizing value. Reference was also made to a study on the cultivation of pig-adapted virus on the epitholium of the tongues of pigs. It was foud that such cultivation was easily accomplished, that the titre of the cultured virus was, generally speaking, low and that vaccine prepared from it showed no immunizing properties. It was pointed out that a difficulty in these experiments had been the failure of the control pigs to react clinically to exposure to the virus by contact.

Dr. Michelsen reported that in recent experiments at the Lindholm Institute, pigs vaccinated with normal and concentrated cattle vaccine had failed to show immunity. He stated that the current program at Lindholm includes a study of the use of adjuvants other than aluminium hydroxide in the preparation of vaccine and that the experimental work will concern large numbers of pigs.

Dr. Galloway remarked on the difficulties in experimentation in immunizing pigs. It was natural to expect that pig-adapted strains of virus would give better immunizing results than would cattle strains. He stated that pig-adapted strains of the virus have been collected from the field. He emphasized the non-clinical reactions of pigs exposed to the infection by contact and cited an example of only 13 of 30 exposed pigs showing clinical reactions. The 17 pigs which failed to show clinical evidence of the infection were found to be immune when challenged at a later date. It is useful in such exposed animals to follow antibody levels. Dr. Galloway held the view that better results might be obtained from the use of attenuated rather than inactivated virus and stated that pigs are included in the work on attenuated strains at the Pirbright Institute.

Dr. Willems stated that vaccination of pigs, including piglets and heavy pigs is sometimes practised in Belgium. He referred to an epizootic among pigs in East Flanders, caused by virus, type C, which continued to spread. In all, some 20,000 pigs were vaccinated with ordinary cattle monovalent vaccine, the dose being 15 ml. He stated that this widespread vaccination had arrested the spread of the infection.

Dr. Moosbrugger remarked on the differences between the results of vaccinating pigs in laboratory experiments and field use of vaccine. He referred to an outbreak among pigs which had been arrested by vaccination. Some three months later some unvaccinated pigs were added to the herd. They developed foot-and-mouth disease while the original pigs in the herd, which had been vaccinated during the outbreak, remained healthy.

Dr. Ubertini's experience was not in keeping with the attachment of any value of vaccination in prevention of the disease in pigs.

During the general discussion it was evident that the meeting attached considerable importance to the production of efficient vaccine for the immunization of pigs. Note was taken of the work in the program of the Pirbright Institute on live attenuated strains for vaccination and of experiments on the use of adjuvants other than aluminium hydroxide at the Lindholm Institute where the cultivation of pig-adapted strains of the virus will also be given attention.

Re-animalization of culture virus in the production of vaccine

The Chairman remarked on the interest of the subject because of the difficulties experienced in some vaccine-producing laboratories in the regular production of virus of high immunizing value by the classical Frenkel technique.

Dr. Ubertini said that, following about 100 passages of the virus in culture by the Frenkel technique, he had experienced difficulties in maintaining the antigenicity of the virus. At this stage he had found that re-animalization restored the antigenicity.

Dr. Willems explained that the following procedure is carried out at the Institute at Uccle:-

When a strain has been adapted to culture by some successive passages it is inoculated into some 20 bovines. Following this re-animalization by having been passed through cattle, the virus is pooled and kept under deep freeze conditions. This pooled virus is subsequently used for seeding the large cultures for the production of virus by the Frenkel method for vaccine preparation. Thus there are no further culture passages of the virus before large scale production is carried out.

Dr. van Bekkum said that in the Institute in Amsterdam, virus strains of types O, A and C had all been successfully cultivated through several hundred passages without any apparent loss of antigenicity. At the present time virus from recently infected cattle in the field was being used for virus production. He said that a difference of one log in the virus titre has been found to occur from time to time and that the original titre can be restored by one cattle passage.

Dr. Frenkel spoke of lowered titres which occasionally occurred in virus cultivated by this technique. He had found that the original titre was re-established by continued cultivation. In order to prevent loss of time, however, the normal procedure when too low a titre of the virus was found in culture, was to begin a new culture using the original virus which had been preserved. These preserved samples of virus are always readily adapted to culture.

Dr. Michelsen said that although re-animalization of virus had not been carried out to any large extent at the Lindholm Institute, the strong indication, from the observations already made, are that better antigenic results follow its adoption. He presented a table which showed that of 20 batches of vaccine prepared with culture virus of high titres only nine gave a satisfactory immunity in cattle. He stated that all strains of the virus do not become adapted equally well to culture by the Frenkel technique and that, so far as work done up to the present time is concerned, the indications are that strains of virus become adapted more rapidly to cultivation on renal tissue cultures.

From the general discussion it was concluded that loss of antigenicity of virus in cultivation by the Frenkel method can be recovered by re-animalization.

Inactivation and concentration of virus

The Chairman explained that this subject had already been discussed at the meeting of the group in Pirbright in June 1958 and that the Commission desired some further information which was now available from Brescia.

Dr. Ubertini circulated reports of his work. Five groups of experiments were carried out, the results of which may be summarized as follows:-

(a) Immunizing value of foot-and-mouth disease vaccines prepared with virus, formolised before and after adsorption with aluminium hydroxide.

Virus cultivated on kidney cells was used in one set of experiments and virus cultivated by the Frenkel method in another: their immunizing value was tested in guinea-pigs. From statistical analyses of the results it was concluded that the differences between I.D. 50 protective doses of the vaccines prepared from virus formolised before and after adsorption with aluminium hydroxide were not significant. Although this result had to be accepted from the statistical evidence, it was stated that, judging largely by the amount of generalization in the guinea-pigs, the impression was left that the vaccines prepared with virus formolised after adsorption by aluminium hydroxide appeared to give better protection.

(b) Immunizing value of foot-and-mouth disease vaccines prepared with virus formolised before and after adsorption with aluminium hydroxide, various amounts of formalin being used.

The virus was type C and was cultivated in calf kidney cells, fourth passage.

The amounts of formalin were nil; 0.5%; 0.75%; 1.0%; 1.5%; 2.0%.

The tests were carried out on guinea-pigs. The following conclusions were made:- The addition of increasing amounts of formalin causes a gradual and parallel lowering of P_H but by the addition of glycocol buffer the P_H can be maintained at an optimal level. The addition of formalin to extents of 0.5% and 0.75% does not inactivate, with certainty, virus cultivated on kidney cells when tested in mice 48 hours after the addition of the formalin. Statistical analyses show that the addition of increasing amounts of formalin from 0.5% to 2.0% does not interfere with the protective value of the vaccines, whether the formalin is added before or after adsorption with aluminium hydroxide. On the other hand, again in this series of experiments, the impression was gained that all the vaccines prepared with virus formolised after adsorption gave somewhat better protection. Vaccines prepared with virus to which no formalin was added and which had an infective titre for mice of $10^{-3.83}$ and of $10^{-1.34}$ for guinea-pigs by intradermal plantar injection, did not give any better protective results in guinea-pigs following subcutaneous injection than did formolised vaccines. These observations would indicate that formalin does not destroy or alter to any appreciable extent, the antigenic condition and immunizing value of foot-and-mouth disease virus. It has to be pointed out, however, that all the vaccines used in these experiments were injected into guinea-pigs three weeks after preparation and storage at +5°C. Different results might be obtained with

vaccines stored for longer periods during which excess of formalin might influence the antigenic position and, hence, the immunizing value.

(c) Concentration of foot-and-mouth disease virus cultivated on kidney cells and by the Frenkel method with ammonium sulphate and by vacuum.

The results show that, virus cultivated by either of these methods can be concentrated to one tenth of its volume by vacuum at low temperature, without interfering with its infective ability. In the concentration, however, the influence of the P_H of the virus has to be considered. It was found that in one of the experiments in which the P_H rose from 8.1 to 8.76, the infective titre was considerably reduced. When the original P_H was adjusted to an optimal degree before concentration was carried out, however, the infective titre was maintained. It was also found that concentration under vacuum at low temperature did not effect the complement deviation state of the virus.

(d) Immunizing value of foot-and-mouth disease vaccines prepared with virus cultivated on calf kidney cells and concentrated under vacuum, where used in reduced doses in the concentrated form and in amounts diluted to the original volume.

The experiments included vaccines prepared from viruses cultivated by the above method, to which formalin was added both before and after concentration. Statistical examination of the results showed that in the doses used the differences of the protective value of the vaccines was not significant. It is concluded, therefore, that the actual dose of a vaccine can be reduced so long as the required amount of antigen is retained.

(e) Conservation of the immunizing value of foot-and-mouth disease vaccines prepared with concentrated anavirus and stored at +2°C and +5°C.

In this series of experiments vaccines prepared with formolised concentrated virus were used in reduced doses and in a dilution representing the original volume. The results of the experiments showed that storage of concentrated virus in the ice box (+2°C) for $5\frac{1}{2}$ months did not interfere with its immunizing value.

Special equipment for the production of newer types of vaccine

Dr. Fogedby explained that this subject had been referred to this group by the Commission's Executive Committee. Technical assistance by FAO in the production of foot-and-mouth disease vaccine had, up to the present time, included the provision of some equipment for carrying out the Frenkel method. In the light of the work now in progress in several parts of the world on vaccines prepared from virus cultivated in kidney tissue cultures and on live attenuated strains, the group's opinion was sought on the advisability or otherwise of making changes in the type of equipment which FAO might supply.

The Meeting gave full consideration to the subject and came to the conclusion that it was not yet possible to predict if and when the types of vaccine now in use would be replaced by those now under study. The Meeting felt, therefore, that it was premature to make any suggestions concerning any change in the type of equipment now in use.

Value of disinfectants at present in use

Dr. Fogedby explained that this subject was mentioned at the last Session of the Commission and was referred to this group.

The Meeting examined the question and came to the conclusion that the disinfectants now commonly used in different countries and which have been in use for many years have given satisfactory results.

The Meeting agreed that special attention should be given to the disinfection of railway waggons and trucks.

Prevention of the introduction of exotic strains of virus into European countries

Following a short discussion, the Meeting felt that this subject would be dealt with more satisfactorily at a meeting of the whole of the Commission's Standing Technical Committee.

Suggestions for items for the agenda of the next meeting of the OIE Standing Commission for the Study of Problems concerning the Immunization against Foot-and-Mouth Disease

It was explained that in the proposed agreement between the European Commission and the OIE formulated in Paris on 9 May 1959, there should be suggestions made at this meeting of the research workers of the Commission's Standing Technical Committee for items of the agenda for the next meeting of the OIE Standing Commission for the Study of Problems concerning the Immunization against Foot-and-Mouth Disease.

The meeting was informed that the meeting of the OIE Standing Commission would take place during the spring of 1960.

The following subjects were suggested:-

Testing foot-and-mouth disease vaccine for efficacy

Present knowledge concerning live attenuated virus strains as immunizing agents

Immunizing value of virus cultivated in kidney tissue cell culture

Vaccination of pigs

Concentrated vaccines

Disinfection of wagons and lorries

Control of foot-and-mouth disease caused by exotic strains of virus which may be introduced into a region

Epizootology of foot-and-mouth disease including virus carriers

Non-specific immunity (para-specific immunity) in foot-and-mouth disease

Production of foot-and-mouth disease anti-serum.

The final selection of the subjects for the agenda of the meeting will, course, be made by the OIE.

t Meeting

It was agreed that the next meeting of the group should be held at Istituto Zooprofilattico Sperimentale in Brescia.

---

eral Conclusions

The meeting agreed that the following conclusions could be made as a lt of the discussions:-

The results of the cultivation in kidney tissue cells of the virus for vaccine production are very promising. There are still some details to be considered. Work on the subject should be encouraged to the fullest extent in all laboratories in which the necessary facilities exist or may be provided.

Up to the present time, only a limited amount of experimental work has been carried out on the immunization of pigs. The approach to the problem should be on lines somewhat different from those concerning immunization of cattle. Wherever possible renewed efforts should be made to include experiments on the subject in the program of work of research centres.

According to some workers the virus adapted to growth in culture may show a loss in antigenicity. In this case re-animalization has successfully been used in some laboratories.

Much valuable information has been obtained from experiments in guinea-pigs on the possibility of inactivating virus before the addition of aluminium hydroxide towards the development of vaccine of satisfactory potency. The work should now be extended to cattle.

Similarly, valuable information has been obtained in methods of concentrating virus, with the advantage of small volumes of vaccine for storage, transport and inoculation.

Until more information on the value of the newer types of vaccine becomes available, equipment for vaccine production at present in use should not be replaced.

The disinfectants at present in use are satisfactory and there seems to be no reason to suggest any changes. An essential part of disinfection is thorough cleansing.