REPORT OF A WHO CONSULTATION ON TRANSFER OF TECHNOLOGY FOR PRODUCTION OF RABIES VACCINE *

Geneva, 23-26 October 1984

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INTRODUCTION

A group of experts on rabies vaccines met in Geneva from 23 to 26 October 1984 to discuss international collaboration in the transfer of technology for rabies vaccine production to those countries with a need to extend rabies control. This report summarizes the recommendations of the group concerning the appropriate technologies and the strategies for their transfer.

Dr F. Assaad, Director, Division of Communicable Diseases, opened the meeting on behalf of the Director-General of the World Health Organization and welcomed the participants (Annex 1 - List of Participants).

He reminded the meeting that in 1985 the centenary of the first use of rabies vaccine by Louis Pasteur would be celebrated. Despite 100 years of experience with rabies vaccines much remained to be done in the prevention and control of human and animal rabies. The early nervous tissue-derived rabies vaccine of Pasteur had now been superseded in purity, potency and safety by products prepared in cell cultures. Every effort should be made to replace vaccines derived from nervous tissue of adult animals by such improved products. WHO should meet the important challenge of prevention and control of rabies in the world by fostering and coordinating international efforts to increase the availability and use of high quality cell-culture rabies vaccines for man and animals. An important aspect of these activities would be the transfer of technologies for production in cell-cultures of safe and effective rabies vaccine to developing countries. Extending the production of vaccine with both existing and new manufacturing units also requires that careful attention be given to maintaining high standards of safety and efficacy of the vaccines by application of appropriate standardization and control procedures.

In a 1982 survey there were at least 74 producers of rabies vaccines in the world, many of whom produce vaccines on a small scale. In most cases the vaccine were prepared from adult animal nervous tissue, despite the fact that techniques are available for the preparation of vaccines of improved potency, safety and purity in cell-cultures.

Most vaccines are required in areas where rabies is endemic in dogs. Canine rabies is still endemic in 87 countries (or territories) and these reservoirs account for over 99% of all human rabies cases in the world and for over 90% of post-exposure treatments. For example, in Latin America some 300 cases of human rabies are reported per year; in Asia over 30,000 human cases and in Africa over 5,000 cases are recorded per annum. In Europe, where the sylvatic form of the disease dominates, only about 100 human deaths are observed yearly.

In recognition of the threat of canine rabies, the World Health Organization, with the assistance of experts from several countries, has issued a document entitled "Guidelines for Dog Rabies Control" (V&83.43).

It is estimated that globally about 5.6 million people need rabies treatment after exposure each year, but only 3.5 million people are actually vaccinated. Many of those who receive vaccine derived from nervous tissue of adult animals develop severe neuropathological complications.

For a successful dog immunization programme, at least 280 million dogs in canine rabies infected areas should be vaccinated. At present less than 10% of these dogs receive vaccine.
The Latin American countries decided to embark on a regional programme for controlling urban rabies and have established strategies and a plan of action for reaching this goal before the end of the present decade. It was estimated that some 30,000,000 doses of vaccine would be required annually by 1986 to give 80% vaccination coverage in dogs in this region.

**Vaccines for use in man**

A variety of cell-culture systems are available for the preparation of inactivated rabies vaccines intended for use in man. The most important of these are listed below. All are inactivated by treatment with beta-propiolactone:

1. Three manufacturing organizations use human diploid cells (HDC) for production of vaccines for use in humans. There is ten years' experience on the use of these vaccines and they are generally safe and effective. However, the yield of virus is low and considerable concentration (X20) of virus harvests is necessary to provide a vaccine of suitable potency. Consequently, difficulties have been encountered in producing products of uniformly acceptable potency. The cost per dose for HDC-derived vaccine is high.

2. An alternative cell substrate is Vero, a continuous, aneuploid cell line derived from vervet monkey kidney. Higher yields of antigen are produced by Vero cells than by HDC. In addition, the cells can be grown in microcarrier cultures to high population density. Their cultivation in large volume fermenters makes large scale commercial vaccine production feasible, and such techniques can reduce the cost of vaccine production. If vaccines for human use are prepared in Vero cells it will be necessary to ensure that DNA of cellular origin is reduced to acceptably low levels by a suitable purification procedure.

3. Primary chick embryo fibroblast (CEF) cell-cultures have been used to prepare vaccines for use in man. In one laboratory high yields of rabies antigen have been produced on this substrate with the Flury (LEP) strain. The vaccine has been clinically tested with satisfactory results.

4. Certain other cell substrates are currently used in various countries for rabies vaccine production. These include Japanese quail embryo fibroblasts, primary hamster kidney cells, dog kidney cells and foetal bovine kidney cells.

There are also other types of vaccine which, although not of tissue culture origin, are an improvement over the currently used vaccines derived from adult animal nervous tissue.

1. A highly purified vaccine prepared in embryonated duck eggs has been developed by one manufacturer. The vaccine contains intact virions isolated from the virus harvests.

2. Suckling mouse brain-derived vaccines are extensively used in all countries of Latin America with some 5,000,000 doses being produced annually.
Vaccines for veterinary use

Live, attenuated and inactivated vaccines have been used for immunization in animals. However, because of concern regarding the genetic stability and safety of live vaccines, only inactivated products should be considered as candidates for technology transfer.

A variety of cell substrates are used to prepare vaccines for veterinary use. These include primary cell-cultures and cell-lines from a number of species. Suckling mouse brain-derived vaccine has been extensively used in dogs in the Americas where 20 million doses are produced yearly. Its routine use over 10 years has been reported to markedly reduce incidence of canine rabies in some countries.

Transfer of technology

A. Principles of technology transfer

1. Transfer of the ability to manufacture improved rabies vaccines for animals or humans is dependent on two important factors:

   (a) A long-term commitment of the government of the recipient country to a programme on the prevention and control of rabies; and

   (b) An assurance that appropriate resources are, or will be, available to support animal and human rabies vaccine production at a level necessary to meet national needs. It should again be emphasized that managers, technicians, technical assistants and equipment service engineers are essential to the success of a vaccine production facility. Great care needs to be given to the selection of well trained personnel who are expected to have a long-term association with the project.

2. Programme objectives

For those countries which provide adequate assurances regarding commitment to rabies control, WHO recommends a three phase programme of technology development involving:

   (a) Production of relevant primary or continuous cell-cultures at a scale adequate for vaccine manufacture.

   (b) The use of seed virus and tissue cultures, according to WHO recommendations, to manufacture rabies vaccine for use in animals on a scale necessary to meet national needs.

   (c) The use of seed virus and tissue cultures, according to WHO recommendations, to manufacture rabies vaccine for use in humans on a scale adequate for national needs.

The implementation of any of these objectives should begin at a scale appropriate to national technological and economic resources but may be assisted by external experts, for example consultants working through the auspices of WHO. Countries which now produce potent vaccines in suckling mice may elect to develop tissue culture facilities for the preparation of human vaccines. Another way vaccine technology may be transferred involves importation of final container product with quality control, labelling and packaging being done in the recipient country. As a second step, bulk or
concentrates might be imported and quality control tests, filling, labelling and packaging done by the importing nation. Successful management of these processes would form the technical basis for transfer of complete manufacturing capability.

Requirements for biological safety and the welfare of laboratory animals must be borne in mind in technology transfer.

3. Technical options

Large scale production of tissue culture-derived vaccines may be achieved using monolayer cultures either in stationary or roller bottles, or suspension or microcarrier cultures in fermentation vessels. The former method is labour intensive, but requires only modest investment in equipment. The latter method is capital-intensive requiring local availability of an appropriately advanced technical infrastructure.

The expertise required to develop stationary or roller bottle technology can be found in a variety of research laboratories and some WHO collaborating laboratories or commercial vaccine manufacturers. Assistance in the development of automated fermentation technology is available from commercial manufacturers, state vaccine manufacturers and some WHO collaborating centres.

In some countries where it can be foreseen that the demand for human rabies vaccine may decrease in a short time, the use of vaccine produced in suckling mouse brain, because of the ease in which it can be transferred for small scale production, may be considered.

Based on the advice of its expert consultants, WHO activities in this programme are likely to be directed mainly towards the development of vaccine production employing conventional monolayer cell-culture techniques.

4. WHO assistance programme

There are various ways in which such a programme will provide assistance:

(a) in promoting internal cooperation and technical cooperation within a country for animal and/or human rabies vaccine production;

(b) in promoting inter-country technical co-operation;

(c) in identifying training laboratories suitable to meet programme requirements, e.g. those competent in large-scale tissue culture production, animal or human rabies vaccine production and which support suitable training programmes for appropriate individuals;

(d) in developing a roster of experienced consultants who will work with the recipient countries to assist in the design of production facilities; in the identification and installation of appropriate equipment; in training for equipment maintenance and repair; in management of small or large scale tissue culture and vaccine production; in preparation of glassware, media and other essential reagents; in training of tissue culture vaccine production and in potency and safety testing in accordance with the recommendations of the WHO Expert Committee on Biological Standardization\(^{(1)}\). Such technical and management support should be provided for the duration of the project and when possible by the same individual or group of individuals;

\(^{(1)}\) WHO Technical Report Series No. 658, 1981 (Thirty-first report of the WHO Expert Committee on Biological Standardization)
(e) in identifying technical experts on an ad hoc basis to meet the unusual requirements of countries which have established their own vaccine development programmes, but are encountering technical or other difficulties.

B. Economic Aspects of Technology Transfer of Rabies Vaccine Products

Basic assumptions have to be made that will influence the type of technology to be transferred. Among factors to be considered are:

1. the size of the demand for rabies vaccine. There is a level below which transfer is uneconomic;
2. the expected duration of the demand. It should be noted that epidemiological changes in rabies may occur after the extended use of vaccines;
3. changes in present international prices for vaccines that may occur due to market changes or improvements in production methods; and
4. whether the facilities installed for production and quality control of rabies vaccines might also be available for production of other vaccines. This would have obvious economic and public health advantages.

For countries whose requirement is less than 100,000 doses of vaccine per year it may not be worthwhile to transfer technology. An alternative to this would be the transfer of technology to groups of countries.

For quantities between 100,000 and 1 million doses, conventional methods of vaccine production, including the use of monolayer cell cultures or purified duck egg vaccine should be considered.

For quantities of 1 million or more vaccine doses per year, fermenter-style production might be considered.

The initial cost for high technology transfer (fermenter-style production) is high as is the maintenance of sophisticated, essential ancillary equipment and supplies. Installation of this type of advanced technology pre-supposes a continuing demand for vaccine over several years and should only be attempted in countries where the appropriate level of scientific and technical infrastructure exists.

Along with any technology transfer a well planned rabies control programme, including strategy for use of vaccines in animals and man, should be developed.

C. Selection of Rabies Vaccine Production Technologies for Transfer

Vaccines to be transferred to developing countries should meet the requirements for safety, antigenicity and protective capacity proposed by WHO in 1981 [1]. Veterinary vaccines should be able to produce long-lasting immunity.

For human use, the "second generation" tissue culture vaccines are preferred for transfer because of their potency and reduced cost. Such vaccines are currently prepared either on primary cell substrates (e.g. foetal bovine kidney, primary chick embryo fibroblast, Syrian hamster kidney cells, etc.).

1 WHO Technical Report Series No.658, 1981 (Thirty-first report of the WHO Expert Committee on Biological Standardization)
primary dog kidney cells and others) or on non-tumorigenic continuous cell lines (e.g. Vero cells or others). Although not of tissue culture origin, newly developed vaccine such as purified duck embryo vaccine must also be considered for transfer of technology. In order to discontinue as soon as possible the use of dangerous "adult brain" vaccines, the replacement of those products by vaccines derived from brain of suckling animals, especially mice, may be a temporary improvement.

Only inactivated veterinary vaccines should be considered for technology transfer. Because of price, preference should be given to tissue culture vaccines. As with the human vaccines, the adult brain vaccines for use in animals, should be replaced as soon as possible.

It should be left to the decision of the individual country, in collaboration with WHO and industry, to select the most suitable technological approach for production of rabies vaccine. The country should consider its existing rabies control programme when making a selection.

D. Standardization and Control of Rabies Vaccines

The transfer of technology for the production of rabies vaccines for use in animals and man must be accompanied by the development of technical expertise in vaccine quality control. Developing countries should have the option of receiving the technology for rabies vaccine production in a sequential fashion. The expertise for the control testing required at each production step must be acquired before production begins.

All vaccines should be tested in accordance with the control procedures stated in the requirements for rabies vaccine for human and veterinary use (1981) and shall have adequate stability by appropriate control tests. As stated in the WHO General requirements for Biological Standards. Manufacturing Establishments and Control Laboratories (1966)\(^1\), the control laboratory shall be independent of the production laboratory and shall be responsible to the national control authority.

In view of the recent advances in rabies vaccine technology, it was recommended by the Group that WHO should update the requirements for rabies vaccines, especially in terms of vaccine substrates and potency testing. The use of in vitro assays, e.g. single radial diffusion (SRD) tests, for virus glycoprotein content should be encouraged as a method of establishing consistency of production. In addition, further collaborative studies should be carried out, coordinated by WHO, to determine the correlation between the amount of antigen measured by in vitro tests and immunogenicity in man or animals.

Rabies vaccine control testing requires adequate laboratory and animal facilities. It is recognized that the cost of quality control procedures make a significant contribution to the overall production cost and need to be taken into account in economic planning. Despite its high cost, quality control should not be compromised.

RECOMMENDATIONS

The Group expressed the conviction that technology transfer should be mediated through a framework, to be established and maintained by WHO, involving national institutions, expert panels, WHO Collaborating Centres, production and control laboratories and other relevant institutions. It is hoped that such a framework will soon be formulated and made known to member states and production laboratories.

Based on this statement the following recommendations were made:

1. Only inactivated vaccines prepared in cell cultures are considered as candidates for technology transfer.

2. Adult brain tissue-derived vaccine should be replaced as soon as possible by cell culture-derived vaccines.

3. Priority should be given to the development of vaccine production in areas of particularly high prevalence of canine rabies and where no or unacceptable brain-tissue derived vaccines are available.

4. The strategy for and priorities of WHO efforts for the transfer of rabies vaccine production techniques should be coordinated by a specially constituted Group of Experts on Rabies Vaccines. This should identify individual national projects to be given priority and periodically review progress.

5. The Organization, in cooperation with the relevant WHO Collaborating Centres, together with the Group of Experts, should provide a framework for technical cooperation with the vaccine manufacturing industry, to assist the transfer of technology for the preparation of cell culture-derived rabies vaccine.

6. The development of cell culture rabies vaccines should be in accordance with a well considered, long-term strategy for rabies control. Initial success in reducing dog rabies will reduce requirements for vaccines for use in man. In countries where wild-life rabies is prevalent there will be a continuing need for vaccines and this should be reflected in the technology transfer strategy.

7. The level of technology transfer to recipient countries should take into account local factors, including the technical development of that country and the prevalence of rabies.
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