This report contains the collective views of an international group of experts and does not necessarily represent the decisions or the stated policy of the World Health Organization.

WORLD HEALTH ORGANIZATION
TECHNICAL REPORT SERIES

No. 82

EXPERT COMMITTEE ON RABIES

Second Report

WORLD HEALTH ORGANIZATION
PALAIS DES NATIONS
GENEVA

APRIL 1954

EXPERT COMMITTEE ON RABIES

Second Session

Rome, 14-19 September 1953

Members :

Dr. M. Baltazard, Directeur de l'Institut Pasteur de l'Iran, Teheran, Iran

Dr. K. Habel, Chief, Laboratory of Infectious Diseases, National Microbiological Institute, National Institutes of Health (Public Health Service), Bethesda, Md., USA (*Chairman*)

Dr. H. N. Johnson, Virus Research Centre, Poona, India

Dr. A. Komarov, Director, Government Virus Diseases Laboratory, Haifa, Israel

Dr. H. Koprowski, Assistant Director, Viral and Rickettsial Research, Lederle Laboratories Division, American Cyanamid Company, Pearl River, N.Y., USA (*Rapporteur*)

Dr. P. Lépine, Chef du Service des Virus, Institut Pasteur, Paris, France

Dr. F. Pérez Gallardo, Chief, Virus Laboratory, National School of Health, Madrid, Spain

Dr. N. Veeraraghavan, Director, Pasteur Institute of Southern India, Coonoor, India (*Vice-Chairman*)

Secretariat :

Dr. M. M. Kaplan, Chief Veterinary Public Health Officer, Division of Communicable Disease Services, WHO (*Secretary*)

Dr. E. S. Tierkel, Rabies Consultant, Division of Communicable Disease Services, WHO

Observer :

Sir Thomas Dalling, Chief Veterinary Consultant, Animal Production Branch, Agriculture Division, FAO

The report on the second session of this committee was originally issued in mimeographed form as document WHO/Rabies/45, 30 October 1953.

PRINTED IN SWITZERLAND

EXPERT COMMITTEE ON RABIES

Second Report *

The Third World Health Assembly noted the report of the Expert Committee on Rabies on its first session (held in 1950) [1] and, inter alia, requested the Director-General:

> "(1) to arrange, as approved by the Executive Board at its fifth session,[2] the undertaking of a field trial using hyper-immune serum-vaccine as a preventive measure for rabies in man, and a field demonstration on the control of rabies in dogs using a new avianized vaccine;
>
> "(2) to co-ordinate the exchange of virus strains for the production and testing of rabies vaccines;
>
> "(3) to arrange regional meetings of appropriate authorities from neighbouring and nearby countries where rabies is a problem so that concerted attacks on this disease will be possible;
>
> "(4) to encourage, wherever possible, research on problems in rabies requiring clarification, as indicated in section 16 of the report of the Expert Committee on Rabies, and
>
> "3. DECIDES to defer a decision on convening an international conference on rabies pending examination of the results of the afore-mentioned field trials at the second session of the Expert Committee on Rabies."[3]

The second session of the committee was convened in Rome, from 14 to 19 September 1953, to consider the results of the work undertaken in implementation of the above resolution, and to formulate technical recommendations in the light of advances in the field of rabies made in the past few years. For the second session Dr. K. Habel was elected Chairman, Dr. N. Veeraraghavan, Vice-Chairman, and Dr. H. Koprowski, Rapporteur.

---

* The Executive Board, at its thirteenth session, adopted the following resolution:
The Executive Board
1. NOTES the second report of the Expert Committee on Rabies;
2. THANKS the members of the committee for their work; and
3. AUTHORIZES publication of the report.
(Resolution EB13.R7, *Off. Rec. Wld Hlth Org.* **52**, 3)

[1] *Wld Hlth Org. techn. Rep. Ser.* 1950, **28**

[2] *Off. Rec. Wld Hlth Org.* **25**, 8, section 2.3.5

[3] Resolution WHA3.20, *Off. Rec. Wld Hlth Org.* **28**, 22

During the second session, the committee followed closely the report on the first session and references to it have been made throughout the report now submitted. The reader would therefore find it convenient to refer to both reports in parallel.

1. Introduction

1.1 *Scientific aspects*

During the past three years great strides have been made in our understanding of some aspects of rabies which were not clear at the time of the first session of the committee. This applies particularly to antirabies hyperimmune serum, chicken-embryo-adapted virus vaccine,[4] recently reported profound modifications of the rabies virus in certain chicken-embryo-adapted strains, and some laboratory procedures including potency tests of vaccines.

In formulating this report, the committee has examined experimental data accumulated during the past few years, and special reports submitted to the committee of work accomplished during this period with the assistance and co-ordination of WHO.[5] It should be noted that all recommendations made in this report dealing with the duration and degree of immunity, which are based largely on experimental results, are subject to variables encountered with the use of all biological products, such as care in handling of the material, differing responses of individuals to antigenic stimuli, and overwhelming exposure to infection. Thus, occasional " breaks " may well be encountered in the field which cannot be anticipated with any degree of accuracy compared with results obtained from carefully controlled laboratory studies.

1.2 *Monograph on laboratory techniques*

Many references are made to laboratory procedures throughout the report. In most instances details of these procedures are obtainable in a monograph entitled *Laboratory techniques in rabies*, shortly to be published by WHO.[6] The committee has reviewed the contents of this publication and strongly recommends it for laboratory workers dealing with rabies throughout the world. The committee believes that not only will the monograph

---

[4] In this report, the term " chicken-embryo vaccine " refers to the vaccine prepared with the Flury strain of rabies virus, unless otherwise stated.

[5] The committee has also considered communications from several distinguished workers, e.g., Dr. C. Fermi and Dr. P. Remlinger, in the field of rabies, and these have proved most useful in preparing the present report.

[6] World Health Organization (1954) *Laboratory techniques in rabies*, Geneva (*World Health Organization : Monograph Series*, No. 23)

be a valuable source of instructions for technical procedures, but also that the adoption of the main principles of the described methods will bring about a desirable uniformity of techniques used in various laboratories which will, in turn, permit of a more valid comparison of results obtained. This is especially needed with respect to the preparation and potency testing of vaccines and serum.

1.3 *Regional meetings*

The committee considers that the organization by WHO of such meetings as the one held in Coonoor, India, in 1952, and attended by 55 rabies workers from 23 countries, provides a unique and highly effective method for demonstrating and teaching rabies techniques, so that they can be adopted by a maximum number of countries within a relatively short period. The committee recommends that this type of meeting be utilized by WHO, wherever possible, to serve other regions where rabies is a major problem. Apart from the knowledge gained of new laboratory techniques, neighbouring and nearby countries can exchange information and experience with respect to local control problems, particularly in connexion with wildlife and dog control along common frontiers.

1.4 *International rabies conference* (see resolution on page 3 of this report)

The committee considers that, in view of the detailed attention given to rabies at various recently held international congresses such as the Sixth International Congress for Microbiology and the Fifteenth International Veterinary Congress, in addition to the periodic meetings of the Expert Committee on Rabies and the regular dissemination of information by WHO and other international organizations, an international conference devoted solely to the problem of rabies is unwarranted, at least for the next several years.

2. Results of WHO-Sponsored Field Trial and Demonstration of Rabies Control Using Chicken-Embryo Vaccine in Dogs

In the first report of the committee, a recommendation was made that WHO sponsor a field trial and demonstration of rabies control in animals using chicken-embryo vaccine for the vaccination of dogs. After approval of this recommendation by the Executive Board and the Third World Health Assembly, the work was undertaken in Israel in October 1950. In 1952, the opportunity to extend this work was presented by a request from Malaya for technical assistance from WHO to control an epizootic of rabies. A summary of the results obtained in both Israel and Malaya

is given in Annex 1 (see page 20). In both countries rabies was controlled very successfully, and mass vaccination of dogs with chicken-embryo vaccine, which was subjected to a severe trial under difficult field conditions, was undoubtedly the major factor in the successes obtained.

3. New Developments in Antirabies Vaccines

3.1 *Nervous-tissue vaccines*

The committee notes that new types of inactivated virus vaccines have been and will continue to be developed in various laboratories. The committee feels that as long as these new vaccines meet the requirements of a standard potency and safety test, and can be shown to be innocuous in human beings or the species of animal for which they are intended, there are no contra-indications for their use in those countries desiring to do so. Accumulated experience of their efficacy in the field will, of course, be the final criterion of their usefulness.

At the time of writing this report the committee is not aware of any proven method applicable to large-scale production for the successful removal of the paralysis-producing factor from nervous-tissue vaccines (see section 9.4, page 19).

3.2 *Chicken-embryo vaccines*

The committee recognizes that vaccine prepared from chicken-embryo-adapted Flury strain, at the level of 40th-50th egg passage, is of high immunogenic potency, as indicated by results of experiments and substantiated by the results of mass immunization of canine populations (see section 2). Experimentally, the same vaccine was found to protect cats against challenge inoculation with street virus, and it can be used for immunization of this species.

In the course of continued chicken-embryo passages the Flury strain, at the level of the 180th egg passage, became non-virulent for adult mice, dogs, and rabbits injected intracerebrally. This high egg-passage strain was found in preliminary experiments to be antigenic for dogs and cattle, and entirely devoid of pathogenic properties for the latter species when given intramuscularly.

At the same time another chicken-embryo-modified strain of rabies virus, the Kelev strain, was developed which was devoid of pathogenic properties for intracerebrally injected rabbits, hamsters, and guinea-pigs. This strain was also found, in preliminary experiments, to be antigenic for dogs and cattle.

In view of these new developments, the committee feels that vaccines prepared from chicken-embryo-modified strains may find even wider application than at present in the field of prophylaxis.

At the present time, chicken-embryo vaccines are not recommended for human treatment.

The committee stresses the fact that, on the basis of experimental results, only certain strains of rabies virus cultivated in the chicken embryo can be considered as immunogenic and safe for vaccination purposes (see also section 7, page 11).

The committee feels that it should discourage any attempts to prepare a chicken-embryo vaccine from virus strains which have not been tested for their innocuousness and broad antigenicity in a large number of animals of different species, particularly in view of the availability for such purposes of well-studied modified strains.[7]

3.3 *Vaccine potency tests*

3.3.1 *Nervous-tissue vaccines.* Following the recommendations made in the first report, this committee welcomes the arrangements made by WHO for the exchange of virus strains [7] for the production and potency testing of rabies vaccines, and for the checking of potencies of vaccines produced in those countries desiring an evaluation. This service made available by WHO should be used by countries producing their own vaccine where they are unable to carry out adequate potency tests, or wish to have their own tests checked. It cannot be too strongly emphasized that reliance should not be placed on routine production of vaccines even if apparently identical methods are used, as it has been repeatedly observed that successive batches of vaccine can vary in potency. Hence, it is important to test each batch of vaccine wherever possible.

There are several standardized types of potency tests available for checking the antigenicity of rabies vaccines. Details of the techniques of these tests, and consideration of the indications for their use, are set forth in the monograph on laboratory techniques. Although the committee, in agreement with recommendations made in the first report, stresses the desirability of continuing *quantitative* evaluation of potency of all batches of vaccine produced in any laboratory, it recognizes the difficulties some laboratories have in performing these more costly quantitative tests. However, the importance of carrying out *some* test on *every* batch of vaccine leads the committee to recommend strongly that, where

---

[7] These strains are available to national laboratories on request to the Secretary, Expert Panel on Rabies, World Health Organization, Palais des Nations, Geneva, Switzerland.

laboratories are unable to carry out the quantitative tests, a more qualitative test, described in the monograph as the "modified Habel test for potency", should be employed for routine testing. A more complete quantitative test should be done as a check every six months, or at least once a year, and here the arrangements offered by WHO may be used if necessary.

3.3.2 *Chicken-embryo vaccines.* The committee recommends that every batch of chicken-embryo vaccine (Flury and Kelev strains) should be submitted to a guinea-pig potency test developed especially for such types of vaccine. This is the only test available, at present, for proper evaluation of immunogenic potency of chicken-embryo-adapted vaccines, and the technique outlined in the monograph should be followed closely. Work is under way at present towards a simplification of potency tests for these vaccines.

4. Antirabies Hyperimmune Serum

In the first report it was recommended that a field trial should be undertaken in Iran to evaluate the use of antirabies serum followed by vaccine in the post-exposure treatment of human beings bitten by rabid wolves.[8] However, during the ensuing three years the number of persons exposed to bites of rabid wolves in Iran was very small and no large series of simultaneous exposures was seen, although numerous cases of bites by non-rabid wolves were recorded during this time. Serum was therefore used rarely, and only in numerically small series which have not yet reached statistical significance. Because of growing experimental evidence in favour of antirabies hyperimmune serum prophylaxis, however, the committee recommends that serum followed by vaccine should be used in all human cases of severe exposure. In addition, it also recommends that, in such cases, the antirabies serum treatment should be given within the shortest possible time after exposure, since under laboratory conditions the best results are obtained only when the serum is administered within 72 hours. Since there are no contra-indications other than those arising from other types of serum therapy, antirabies serum, in addition to vaccine, can also be administered in instances other than those involving severe exposure, according to circumstances accompanying each particular case.

Hyperimmune serum production in horses is described in the monograph on laboratory techniques. It should be noted here that limited experience has shown that antirabies serum produced in sheep may cause an unusually high incidence of serum sickness in man, whereas this has not been true of equine serum, particularly when it has been partially purified. With all types of sera it is necessary to test for immediate sensitivity in the patient before

[8] *Wld Hlth Org. techn. Rep. Ser.* 1950, **28**, 8

serum treatment. If the patient is sensitive and the administration of serum is imperative, the usual measures to desensitize should be undertaken.

The committee encourages the continuation of field trials in Iran and other parts of the world to evaluate antirabies serum prophylaxis in man, and recommends strongly that products used in such trials be of known standardized potency. The committee is of the opinion that it is desirable for certain research centres to prepare a mutually comparable standard antirabies serum, which would be made available to laboratories throughout the world through WHO, for comparison with their own product (see Annex 2, page 25). The committee recommends that provisional international standardization (see Annex 2) should be applied to antirabies serum, subject to revision in the light of research data obtained in the future.

5. Results of WHO Co-ordinated Serum-Virus Neutralization Tests on Non-Exposed Individuals Receiving Antirabies Hyperimmune Serum and Varying Doses of Vaccines [9]

In an attempt to evaluate the effectiveness of hyperimmune serum either alone or combined with vaccine when administered to humans, the presence of serum antibody at varying periods of time after the start of immunization was tested in groups of non-exposed normal individuals.

5.1 *Results to date (trials are continuing)*

1. After a single dose of hyperimmune serum given intramuscularly (0.5 ml per kg of body-weight), antibody was demonstrable at the 1st day and present up to the 14th day.

2. A single dose of potent phenolized vaccine (3.5 ml of 20% nervous tissue suspension) alone caused only minimal evidence of antibody after 14, 21, and 28 days.

---

[9] The following laboratories participated in this work:

France: Institut Pasteur, Paris (Dr. P. Lépine, Chef du Service des Virus)

Iran: Institut Pasteur de l'Iran, Teheran (Dr. M. Baltazard, Directeur)

Israel: Government Virus Diseases Laboratory, Haifa (Dr. A. Komarov, Director)

Spain: National School of Health, Madrid (Dr. F. Pérez Gallardo, Chief, Virus Laboratory)

USA: Communicable Disease Center, Montgomery, Ala. (Dr. M. Schaeffer, Director, Virus and Rickettsia Section)

Lederle Laboratories Division, American Cyanamid Company, Pearl River, N.Y. (Dr. H. Koprowski, Assistant Director, Viral and Rickettsial Research)

National Microbiological Institute, National Institutes of Health (Public Health Service), Bethesda, Md. (Dr. K. Habel, Chief, Laboratory of Infectious Diseases)

3. Seven daily doses (0.5 ml) of phenolized vaccine produced a good antibody response, starting at 14 days and continuing at 21 and 28 days, as did also 14 daily doses of the same vaccine.

4. A single dose of chicken-embryo vaccine (3 ml of 70% tissue suspension) gave no evidence of antibody response up to 28 days.

5. In no instance did the administration of either chicken-embryo or phenolized vaccine after a dose of hyperimmune serum affect the demonstration of the passive antibody due to the hyperimmune serum.

6. Likewise, the active antibody response to a course of phenolized vaccine occurring from the 14th day onwards was not affected by the early presence of passive antibody resulting from a dose of hyperimmune serum.

7. Only in the group receiving serum followed 24 hours later by a course of 14 daily doses of phenolized vaccine was there a demonstration of the continuous presence of antibody over the entire period of the 28-day test.

5.2 *Conclusion (provisional)*

Although the presence of serum antibody in humans during or after any antirabies treatment is only indirect evidence of immunity to rabies, it is still the only available experimental evaluation that can be carried out in man.

For maintenance of continuous antibody over a period, the combined use of a dose of hyperimmune serum and 14 subsequent daily doses of phenolized vaccine would appear to be the best procedure in the light of these experiments.

6. Post-Exposure Treatment of Man

6.1 *Local treatment of wounds*

The committee recommends the immediate treatment of all bite wounds inflicted by animals, especially those suspected of being rabid, by thorough cleansing with soap or detergent solution. Such treatment does not preclude the subsequent use of strong mineral acids, such as nitric acid, which may be introduced into the depths of puncture wounds which cannot be cleansed efficiently with soap or detergent solution. The application of ordinary antiseptics and the local or parenteral use of antibiotics has no prophylactic value against the rabies virus, but may be used following the local treatment recommended above to combat bacterial infections. Experimental evidence suggests that the immediate infiltration of the tissue beneath the wound

with antirabies serum may be used as a local prophylactic measure. Sufficient evidence of the effectiveness of hyperimmune serum and other substances used in local treatment is not as yet available, however, and further experimental trials are in progress under WHO auspices.

6.2 *Indications for specific treatment*

In reviewing the indications for treatment given in the first report the committee feels that some modifications are required (see table I, page 12).

The following paragraphs, quoted from the first report, state well the position concerning the treatment of cases of re-exposure.

" Fairly often a situation arises in which a person previously exposed to infection and treated with vaccine is re-exposed to infection with rabies. The question as to whether treatment should be re-instituted and, if so, on what basis, must be answered. It is recommended that, if this situation arises within three months of the first course of vaccine, no further treatment is necessary unless the second exposure is of a severe type. If the interval is between three and six months, two reinforcing doses of vaccine, one week apart, are indicated, whereas if more than a six-months' interval has elapsed the treatment should be on the same basis as if it were an original exposure.

" Occasionally, marked allergy to rabies vaccine manifested by angioneurotic oedema, fever, adenopathy, shock, etc., is encountered. This may be during the course of immunization or, more often, following the administration of the first dose to a person who has previously received rabies vaccine. It is suggested that this difficulty may be circumvented by a change to vaccine made from the brain tissue of another species of animal (i.e., from rabbit-brain vaccine to sheep-brain vaccine)." [10]

7. Rabies Control in Animals

7.1 *Control procedures*

An excellent summary of certain basic principles in rabies control was given in the first report:

" Experience has shown that the efficient organization of a rabies-control programme in an infected area is best accomplished by means of a central authority headed by a public-health officer, preferably a veterinarian, who has full executive power and who devotes his full time to this work. A system of weekly reports of rabies cases should be instituted to enable the officer to keep abreast of the problem. He should enlist the support of all local groups directly or indirectly concerned with rabies, such as public-health authorities, veterinary and medical practitioners, livestock organizations, animal protection societies, etc. These groups can provide material assistance to the rabies-control officer by publicizing the programme and otherwise informing the general public whose co-operation must be obtained before specific measures can be successfully applied. If possible, an antirabies campaign should be co-ordinated on a national basis, or at least in adjacent infected areas.

---

[10] *Wld Hlth Org. techn. Rep. Ser.* 1950, **28**, 10

TABLE I. INDICATIONS FOR SPECIFIC POST-EXPOSURE TREATMENT

Nature of exposure	Condition of biting animal		Recommended treatment
	At time of exposure	During observation period of 10 days	
I. No lesions; indirect contact only	rabid	—	none *
II. Licks: (1) unabraded skin	rabid	—	none *
(2) abraded skin and abraded or unabraded mucosa	(a) healthy	healthy	none
	(b) healthy	clinical signs of rabies or proven rabid	start vaccine at first signs of rabies in animal
	(c) signs suggestive of rabies	healthy	start vaccine immediately; stop treatment if animal is normal on 5th day after exposure **
	(d) rabid, escaped, killed, or unknown	—	start vaccine immediately
III. Bites: (1) simple exposure	(a) healthy	healthy	none
	(b) healthy	clinical signs of rabies or proven rabid	start vaccine at first signs of rabies in animal
	(c) signs suggestive of rabies	healthy	start vaccine immediately; stop treatment if animal is normal on 5th day after exposure **
	(d) rabid, escaped, killed, or unknown; or any bite by wolf, jackal, fox, or other wild animal	—	start vaccine immediately
(2) severe exposure: (multiple; or face, head, or neck bites)	(a) healthy	healthy	hyperimmune serum immediately; no vaccine as long as animal remains normal
	(b) healthy	clinical signs of rabies or proven rabid	hyperimmune serum immediately; start vaccine at first sign of rabies
	(c) signs suggestive of rabies	healthy	hyperimmune serum immediately, followed by vaccine; vaccine may be stopped if animal is normal on 5th day after exposure
	(d) rabid, escaped, killed, or unknown. Any bite by wild animal	—	hyperimmune serum immediately, followed by vaccine

* Start vaccine immediately in young children and in patients where a reliable history cannot be obtained.

** An alternative treatment would be to give hyperimmune serum and not start vaccine as long as the animal remained normal.

Note: To be effective hyperimmune serum must be given within 72 hours of exposure. **Dose:** 0.5 ml per kg of body-weight (for serum potency, see Annex 2, page 25).

These indications apply equally well whether or not the biting animal has been previously vaccinated.

"The committee recommends that the following specific measures be applied in affected regions:

(1) Registration, licensing, and taxation of dogs
(2) Elimination of stray animals
(3) Restraint of dogs while the control campaign is under way
(4) Mass vaccination of dogs free of charge
(5) Provision of adequate facilities for diagnosis
(6) Reduction in number of wildlife species where these are a reservoir of the disease
(7) A continual and energetic publicity campaign." [11]

The three basic principles of an operational programme are elimination of stray dogs, canine vaccination, and control of wildlife vector populations.

7.1.1 *Elimination of stray dogs*

It has been found that registration or licensing of dogs is an important adjunct to a successful control programme. If properly enforced, this measure rids the area of ownerless stray dogs and assures a reasonable dog census. An efficiently conducted programme requires the operation of a local pound or humane shelter where stray animals may be kept for a few days, and if unclaimed at the end of that period they should be humanely destroyed. Collection of strays should be carried out by teams of dog wardens and assistants in properly equipped trucks.

7.1.2 *Canine vaccination*

The committee recognizes that the chicken-embryo vaccine (Flury strain) produces excellent immunity in dogs for at least three years following a single intramuscular inoculation (posterior thigh muscles), and recommends the use of this vaccine in mass immunization programmes.

All dogs three months of age and older can be effectively immunized with this product. Although this vaccine appears to be harmless in dogs younger than three months, there is no information yet available concerning its immunizing value in this lower age-range.

The committee also recognizes the efficacy of a single dose of nervous-tissue vaccine, and therefore recommends the use of this type of vaccine for mass vaccination of dogs in areas where chicken-embryo vaccine is not available or is impracticable. Potency tested nervous-tissue vaccine confers good immunity for one year, and three years after vaccination by the intramuscular route there is still significant protection. (See section 9.4, page 19, on paralytic factor.)

Dogs which have been vaccinated with either vaccine when under six months of age should be revaccinated within the first year of life.

---

[11] *Wld Hlth Org. techn. Rep. Ser.* 1950, **28**, 13

Since approximately one month is required for canine vaccines of either type to elicit a maximum level of immunity, restrictive measures (leashing, confinement) for dogs which are involved during an epizootic may be lifted 30 days following vaccination.

With regard to methods which will rid an area of enzootic or epizootic canine rabies, the committee feels that no significant degree of success can be expected unless there is a well-organized, intensified programme of mass immunization. This necessitates the establishment and operation of temporary clinic sites strategically located throughout the problem area, as well as a substantial zone surrounding the geographical focus of infection. The programme should be directed towards the swift reduction of susceptible animals, and this can be achieved by the immunization of at least 70% of the entire dog population of the area in the shortest possible period.

In rabies-free areas faced by the constant danger of introduction of the disease, continued programmes of vaccination should be adopted which would provide for revaccination of dogs every three years with chicken-embryo vaccine or for annual revaccination with nervous-tissue vaccines.

Canine immunization should be made an integral part of all long-range rabies-control programmes and, as a sound public-health procedure, dog owners should be encouraged to have their pets vaccinated by the time they are six months of age.

The committee again stresses that all vaccines used for immunization should have previously passed an adequate potency test.

7.1.3 *Control of wildlife vectors*

In areas where rabies is present in wild animals such as foxes, wolves, mongooses, jackals, skunks, and coyotes, either alone or with canine rabies, well-organized campaigns for the reduction of excessive numbers of wild-vector populations should be co-ordinated with an overall control programme. It has been observed that outbreaks of rabies in wildlife occur most often in areas where the vector species has reached unusually high population densities, and the objective should be to reduce the number of susceptible animals to a level which would no longer support an epizootic and thereby prevent the danger of its spread to man and his domestic animals. These programmes should be directed by experienced or professionally trained predator-control specialists who may utilize trapping, shooting, gassing of dens, and/or poisoning techniques best suited to the problem area.

Vampire-bat rabies continues to be the source of a large rabies problem in Mexico and in Central and South America. Some progress has been noted in controlling the disease in these areas by eradication schemes which employ dynamiting, gassing, and shooting of vampire-bats in their diurnal resting-places.

Some factors in the epizootiology of wild-animal rabies are considered in Annex 3 (see page 26).

7.2 *Pre-exposure immunization of animals other than dogs*

Experiments have shown that cats can be effectively immunized with chicken-embryo vaccine. Cat owners may therefore have their animals vaccinated for their own protection, but there is no evidence at present that rabies persists among pet cats in urban areas when dog rabies has been eliminated.

In certain livestock producing areas where rabies is enzootic in wild-animal vectors, such as vampire-bats, foxes, jackals, etc., pre-exposure immunization may be carried out in cattle, where economically feasible.

The chicken-embryo vaccine (Flury strain) used at present for the immunization of dogs is not recommended for the vaccination of cattle. The experimental work mentioned in section 3.2 (see page 6) describes further modification of rabies virus strains cultivated in chicken embryos. The two modified strains referred to have not produced any signs of illness in inoculated cattle. One of the strains (Flury) has been used for prophylactic immunization of cattle in areas where vampire-bat rabies is the major source of cattle infection. No cases of rabies have been observed in the vaccinated animals in these areas. Results of experiments and these field studies indicate that this further modified chicken-embryo-adapted virus may be used for the vaccination of cattle.

The committee recognizes the possible usefulness of nervous-tissue vaccines for pre-exposure vaccination of animals other than dogs, but conclusive experimental data are not available as to their immunizing value.

7.3 *Handling of animals bitten by rabid animals*

The committee recommends that dogs and cats bitten by a known rabid animal should be immediately destroyed. If the owner is unwilling to destroy the exposed animal, the following alternatives are recommended:

(1) Strict isolation of the animal in a kennel for six months.

(2) If no previous vaccination has been given within a period of three years with chicken-embryo vaccine, or within one year with nervous-tissue vaccine, administer post-exposure treatment [12] and confine in a kennel for three months.

---

[12] Post-exposure treatment may consist of administration of antirabies hyperimmune serum (0.5 ml per kg of body-weight) not later than 72 hours after exposure, followed by a single dose of chicken-embryo vaccine within the next seven days or a course of nervous-tissue vaccine.

(3) If the animal has been previously vaccinated within one year with nervous-tissue vaccine, or within three years with chicken-embryo vaccine, revaccinate and restrain (leashing, confinement) for 30 days.

Although no experimental evidence is available, the post-exposure treatment described in footnote 12 may be applicable to animals other than dogs and cats.

7.4 *International transfer of dogs and cats*

In the first report consideration was given to preventive measures indicated in the international movement of dogs and cats. In view, however, of the advances in our knowledge of the vaccines discussed in the present report, the committee feels that certain elaborations can now be made on the recommendations quoted above.

The committee fully supports the following statement contained in the first report.

> " The committee recognizes that countries now free of rabies should continue either to prohibit the importation of dogs and cats, or subject them to a prolonged period of quarantine, preferably six months, at the port of entry. In the case of countries with extensive land borders, and where rabies is already present in domestic or wild animals, it is recognized that such strict quarantine measures are impracticable." [13]

For this latter group of countries the present committee recommends that :

(*a*) Animals (dogs and cats [14]) originating in countries where rabies infection is known to exist should be vaccinated more than 1 month but within 12 months before departure with nervous-tissue vaccine, or within 36 months before departure with chicken-embryo vaccine, both vaccines having previously passed satisfactory potency tests. Animals should be revaccinated with either type of vaccine as soon as possible after arrival. Certificates signed by the appropriate veterinary authorities in the country of origin should accompany each animal. These certificates should clearly identify the animal, and should contain a record of the date of vaccination, the vaccine used, lot number of the vaccine, and name of the production laboratory. Where any doubt exists with respect to the potency of the vaccine used in the animals' country of origin, the animals should be considered as unvaccinated.

(*b*) Unvaccinated animals originating in rabies-infected countries should be vaccinated upon arrival and quarantined for 30-45 days, or where quarantine measures are impossible to apply the animal should

---

[13] *Wld Hlth Org. techn. Rep. Ser.* 1950, **28**, 12

[14] The committee has no evidence with respect to the duration of immunity conferred by vaccine in cats.

be kept under surveillance for a similar period and not allowed to run at large.

(*c*) Unvaccinated animals originating in countries free of rabies, and not exposed en route, should be vaccinated upon arrival and kept under restraint by the owner for one month.

These recommendations are made in consideration of the varied conditions encountered throughout the world and should not be construed as discouraging more-stringent measures, such as maximum quarantine or restraint periods upon entry. This is especially true where receiving countries are free of rabies, and do not carry out vaccination procedures.

8. Diagnosis

The attack against an infectious disease like rabies must necessarily begin with adequate facilities for detecting and measuring the problem as quickly and accurately as possible. The committee wishes to call attention to the necessity of carrying out laboratory diagnostic procedures which embrace the highest degree of accuracy, speed, and economy. Techniques for demonstration of Negri bodies in original brain specimens and virus isolation by animal inoculation are available in the monograph on laboratory techniques in rabies. The importance of the animal inoculation tests for the isolation of virus from suspected brain tissue in Negri-negative specimens cannot be over-emphasized. Exhaustive surveys of large numbers of routine specimens submitted for diagnosis have shown that 10%-15% of those cases proved positive by mouse inoculation had been missed by direct microscopic examination for Negri bodies. The use of antibiotics for suppressing contaminating bacteria without destroying the virus present in decomposed tissue specimens has given the mouse inoculation test wider applicability in recent years than was formerly possible. The same antibiotics make it possible to confirm ante-mortem diagnosis of human rabies cases by isolation of virus from saliva.

The committee recommends two additional procedures, both of which are described in the monograph. The first is the examination of the submaxillary salivary glands of biting animals for the presence of virus, which is of obvious value in providing definitive evidence of whether or not a bite has entailed a risk. The diagnosis of rabies in an animal by either demonstration of Negri bodies or virus isolation from the brain does not necessarily indicate infective saliva. Isolation of virus from the salivary glands is carried out in the same manner as the mouse inoculation test for virus in brain-tissue specimens. The second procedure is the serum-virus neutralization test for identification of the isolated virus. This test is of great value

as a specific confirmatory procedure in those cases which may be complicated by the possible presence of other infectious encephalitic agents.

9. Suggestions for Future Research

9.1 *Studies on veterinary vaccines*

9.1.1 *Chicken-embryo vaccines.* In view of the fact that certain modified chicken-embryo viruses (Kelev and Flury) have been found to be antigenic and devoid of pathogenic properties for cattle (see section 3.2), it is advisable to encourage further research on the use of such vaccines in this species. The committee feels that work should be initiated with the aim of studying the immunogenic response of sheep, goats, mules, and horses to these vaccines.

The committee suggests that studies should be undertaken on the immunogenic response and duration of immunity in puppies vaccinated with these vaccines.

The committee urges that further research work should be carried out with the purpose of developing a more simple procedure for testing the potency of chicken-embryo vaccines (see section 3.3).

9.1.2 *Nervous-tissue vaccines.* Studies on immunity similar to and parallel with those outlined for chicken-embryo vaccines are recommended. In view of one series of experiments which suggest possible differences in effectiveness of nervous-tissue vaccines given subcutaneously as compared to the intramuscular route in dogs, investigations along these lines are recommended.

9.2 *Studies on the value of antirabies hyperimmune serum in human treatment*

The committee suggests that controlled studies should be undertaken to compare the value of hyperimmune serum followed by vaccine with that of vaccine alone in humans exposed to severe risk of infection from the bites of rabid jackals and wolves. This would enable further evaluation to be made of antirabies hyperimmune serum in human treatment.

9.3 *Antibody studies in humans*

After considering the results already obtained in the serum-virus neutralization tests of non-exposed individuals receiving varying doses of vaccines with and without hyperimmune serum, the committee feels that these important studies should be continued.

9.4 *Paralysis-producing factor in vaccines*

Although efforts to date have been unsuccessful with respect to large-scale production of potent nervous-tissue vaccines free of the paralysis-producing factor, further studies in this field would be advisable. Elucidation of this factor, pathogenesis of the phenomenon, and therapeutic studies in paralysis caused by nervous tissue in rabies vaccines would be valuable.

9.5 *Local treatment of wounds*

As stated in section 6.1, WHO has supported experiments in local treatment of wounds. The committee feels that a thorough study of this particular field should be encouraged so that definite information on this important aspect of post-exposure treatment could be obtained.

9.6 *Chemotherapy*

It is suggested that, in any research programme on chemotherapy of virus diseases, consideration should be given to the use of peripherally inoculated rabies virus in experimental animals, since experimental and natural infections with rabies virus lend themselves very well to chemotherapeutic studies.

9.7 *Ecological studies on wildlife rabies*

The success which has been achieved in the control of dog rabies in many areas of the world has focused attention on the importance of wildlife rabies reservoirs as factors in the perpetuation and spread of the disease. Investigations on ecological studies as they relate to the epizootiology of the sylvatic or campestral form of the disease have begun in red and grey foxes and small mammalian prey (see Annex 3, page 26). The committee recommends that similar studies should be undertaken in different parts of the world where other species serve as principal transmitters. Special attention should be given to investigation of the epizootiology of rabies in insectivorous and fructivorous bats in areas where vampire-bats are not found. It is also suggested that searches should be made for the possibility of asymptomatic carriers in wild species, with special attention being given to small mammals, by testing saliva and salivary gland specimens as well as brain tissue for isolation of the virus.

Annex 1

RABIES CONTROL USING CHICKEN-EMBRYO VACCINE IN DOGS: SUMMARY OF RESULTS OF WHO-SPONSORED FIELD TRIALS IN ISRAEL AND MALAYA *

1. Israel

At the first session of the Expert Committee on Rabies in 1950 it was recommended that WHO should sponsor a demonstration programme of rabies control in dogs in some area where canine rabies was enzootic. Mass immunization of dogs with chicken-embryo vaccine was recommended as an adjunct to the usual sanitary measures of control. Careful laboratory studies and a limited use in the field had demonstrated the vaccine to be safe and of high immunizing value. The Third World Health Assembly approved the recommendation of the committee.

In order for the field demonstration to be carried out adequately, and to obtain some assessment of the value of the vaccine, it was necessary to select an area of limited size where rabies was highly enzootic. Other requirements were well-organized veterinary and public-health services with suitable facilities, and a willingness to carry out the campaign along established technical lines.

After considering several possibilities, the State of Israel was selected as a suitable locality. From 1932 to 1950 (the year the demonstration started), the annual number of cases of rabies in animals varied between 50 and 333 —a high incidence in view of the size of the area (see table II). The Israeli veterinary and public-health services were well organized and adequately staffed. This ensured good reporting and execution of the control programmes with the necessary careful checking and follow-up of all suspected and proven cases of rabies in animals. The campaign was conducted by the Government veterinary services with WHO providing technical guidance.

Rabies had become a serious disease problem in all parts of Israel in 1949, when 194 animal cases, including laboratory confirmed cases in 80 dogs, 20 jackals, and 38 other animals, were reported (see table III). From October 1950 to June 1953, a total of approximately 30,000 dogs

---

* The complete details of these field trials will be published in the *Bulletin of the World Health Organization*.

TABLE II. RABIES INCIDENCE AND CONTROL ACTIVITIES IN ISRAEL, 1932-53 *

Year	Number of rabid animals reported	Control activities	
		Number of animals destroyed	Number of animals vaccinated
1932	172	21,466	Negligible
1933	112	29,433	"
1934	129	10,640	"
1935	105	21,352	"
1936	87	16,426	"
1937	165	19,930	"
1938	85	25,000	"
1939	145	13,000	"
1940	333	30,824	"
1941	330	54,052	"
1942	71	62,750	"
1943	73	38,603	"
1944	67	37,357	"
1945	50	38,343	"
1946	74	38,780	"
1947	84	46,328	"
1948	99	15,181	"
1949	194	17,098	"
1950	68	4,072	1,620
1951	10	5,118	14,147
1952	11	15,003	9,253
1953*	3		5,000

* First six months

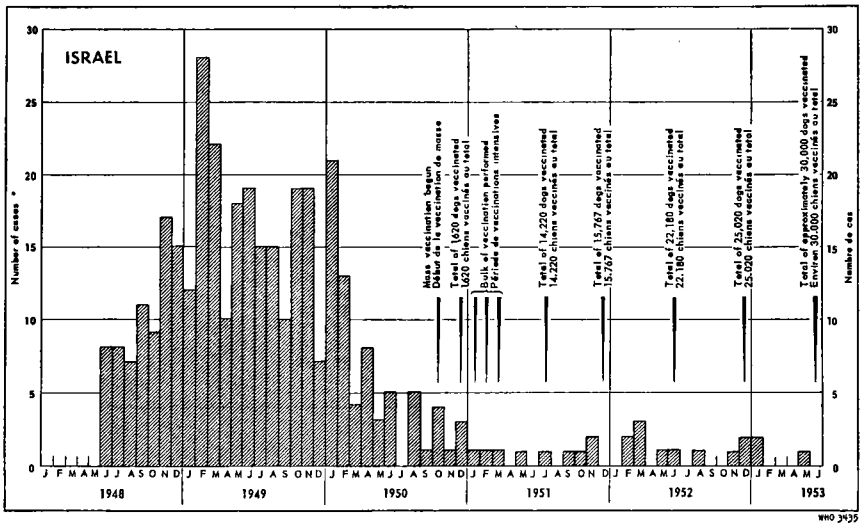
were vaccinated with chicken-embryo vaccine—28,000 with the Flury strain and 2,000 with the Kelev strain. From January 1951 (the time when the vaccination programme began to reach large numbers of dogs) to June 1953, a total of 24 cases of animal rabies—20 dogs, 2 bovines, 1 jackal, and 1 mule—was reported (see fig. 1). Of the dogs, only two had been vaccinated. One of these developed the disease two weeks after vaccination and therefore had undoubtedly been incubating the disease at the time of vaccination. The other dog was bitten 51 days after vaccination, and developed rabies after an incubation period of 53 days.

TABLE III. LABORATORY CONFIRMED CASES OF RABIES IN ANIMALS IN ISRAEL: MARCH 1948 — MAY 1953

Animal	1948	1949	1950	1951	1952	1953
Dog	41	80	28	7	7	3
Cat	9	4	2	—	—	—
Jackal	3	20	3	—	—	—
Ruminants	15	27	11	1	2	—
Equines	2	7	—	—	1	—
Total.	70	138	44	8	10	3

In spite of the continued prevalence of rabies in adjacent countries, only three cases of animal rabies were observed during the first six months of 1953 and these were in border areas.[1]

FIG. 1. MONTHLY INCIDENCE OF RABIES IN ANIMALS BEFORE AND AFTER MASS VACCINATION OF DOGS IN ISRAEL: MAY 1948 — JUNE 1953



It is significant that although ancillary measures, such as registration of dogs, good reporting, adequate diagnostic facilities, elimination of stray animals, and destruction of wildlife, were all applied during the years preceding the campaign, it was not until mass vaccination of dogs was introduced that the disease was brought under control.

2. Malaya

According to available records, rabies had been present in Malaya at a substantial enzootic level in the northern half of the country since 1924. In 1946 the disease began to spread, and by 1952 it had reached the southern part of the State of Selangor with an outbreak in the Federal capital of Kuala Lumpur.

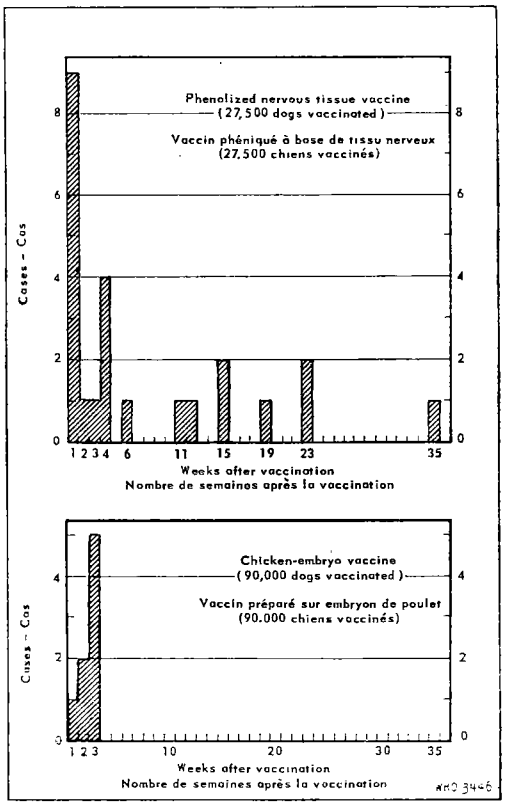
Previous attempts at rabies-control measures had included continual stray-dog destruction and sporadic compulsory vaccination campaigns held

[1] Since this report was written two further cases of rabies in animals have been reported—one in a jackal (August) and one in an unvaccinated dog (October). Both cases occurred in the northeastern border area.

from 1932 to 1937 (20,000 dogs vaccinated), and again in 1946 and 1947 (16,000 dogs vaccinated) using locally produced phenolized buffalo-brain vaccine.

The high enzootic level of the disease reached epizootic proportions in the middle of 1952. Compulsory vaccination of 18,000 dogs with chicken-embryo vaccine (Flury strain) was immediately carried out in the Kuala Lumpur area, and 12,000 dogs were vaccinated with phenolized nervous-tissue vaccine (buffalo origin) in the most seriously infected areas of Perak.

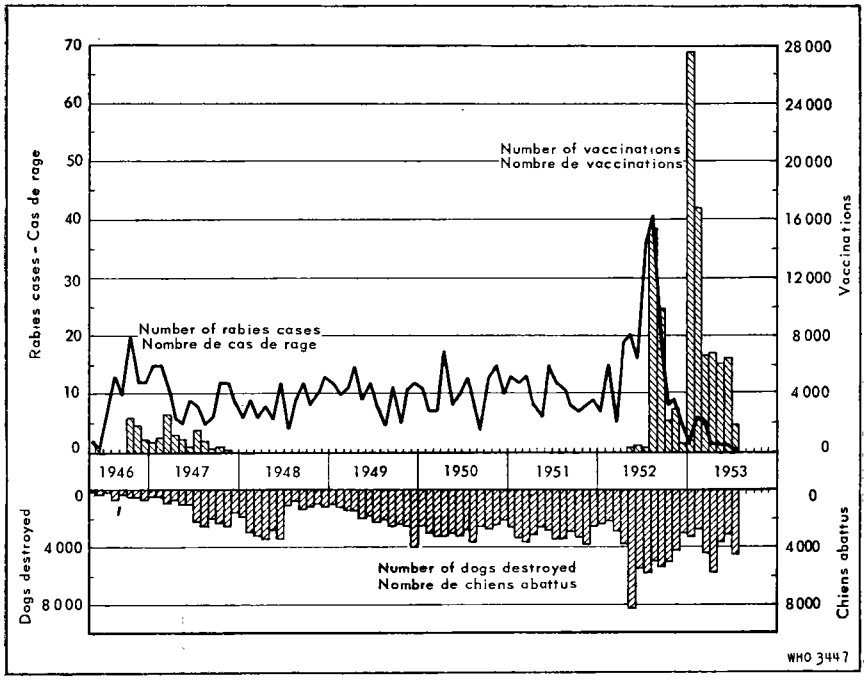
FIG. 2. RABIES IN VACCINATED DOGS IN THE FEDERATION OF MALAYA, 1946-53



In January 1953, a Federally-sponsored intensified mass canine-immunization campaign, using chicken-embryo vaccine alone, began simultaneously in all infected States, and continued through July of that year. (At present, vaccination of young dogs as they reach four months of age is practised.) The regulations requiring vaccination were augmented by well-organized

educational campaigns using every available publicity medium. Up to September 1953, approximately 73,000 dogs had been vaccinated, giving a total of about 103,000 since the inception of the programme in August 1952. Stray-dog elimination was co-ordinated with vaccination throughout the intensified programme. In 1952, 53,000 dogs were destroyed, and from January to September 1953 an additional 36,000 were eliminated.

FIG. 3. INCIDENCE OF CANINE RABIES IN RELATION TO DOGS VACCINATED AND TO DOGS DESTROYED IN MALAYA DURING THE PERIOD MAY 1946—JULY 1953



The results of the campaign are reflected in the incidence of the disease which had risen to an all-time high of 41 laboratory confirmed cases during the month of August 1952 alone, when the mass immunization programme began, and which then declined each succeeding month until the country was entirely free of rabies by the end of June 1953 (see fig. 2 and 3). No case of rabies in man or animals had been reported in Malaya from the middle of June 1953 up to the end of December 1953, when the latest report was received.

Annex 2

SUGGESTED MINIMUM REQUIREMENTS OF POTENCY OF ANTIRABIES HYPERIMMUNE SERUM

1. Potency Test *

This test is essentially the same as the serum-virus neutralization test in mice described in the monograph on laboratory techniques in rabies. The details of the potency test follow:

1.1 *The test animal*

Normal mice of either sex weighing 10-14 g each are used. In any one test, mice of only one sex are used.

1.2 *The test virus*

Any standard strain of rabies virus of known potency may be used.

1.3 *Reference antirabies serum*

A reference serum distributed by WHO will be available for use in the potency test for comparative purposes when standardizing all antirabies sera. This serum will be supplied in the dry form, and instructions for redissolving will be sent with each shipment. Its potency is adjusted so that when mixed in final concentration with not less than 31.6 LD_{50} and not more than 316 LD_{50} of one part of virus suspension, the minimum protective titre (50% end-point) will be not less than 1 : 300 dilution.

1.4 *Procedure*

Serial twofold dilutions of both the serum under test and the reference serum are prepared in 2% normal serum in distilled water or physiological salt solution. Six serial twofold dilutions starting at 1 : 50, and continuing through 1 : 1,600, are usually sufficient for the reference serum, and 1 : 125 through 1 : 4,000 for the serum under test. These dilutions will reveal a 2.5 potency factor required of the serum under test. Equal quantities of

* Reproduced with slight modifications from the monograph entitled *Laboratory techniques in rabies*.

a suspension of test virus are added to the dilutions of serum. The mixtures are incubated in a 37°C water-bath for one hour, and 0.03-ml quantities are injected intracerebrally into the mice. At least 10 mice are injected for each mixture. The amount of virus used is such that each mouse receives not less than 31.6 LD_{50} and not more than 316 LD_{50}. The mice are observed for two weeks. For a serum under test, its minimum acceptable potency should be 2.5 times that of the reference serum.[1]

NOTE. The neutralization test must be performed with serum before the addition of any chemical preservatives.

2. Sterility Test

The contents of the final container should be sterile, as indicated by culturing the entire recommended dose, except that the amount cultured need not exceed 5.0 ml. The culture should be made in one or more tubes of fluid thioglycollate medium or other standard media with sufficient dilution for the preservative no longer to exert a bacteriostatic effect. Incubation is at 32°C, with observation for at least seven days.

Annex 3

EPIZOOTIOLOGY OF WILD-ANIMAL RABIES

Since the publication of the first report, rabies has been discovered in mongooses in Puerto Rico. This is the first major outbreak of rabies in the Western hemisphere attributed to the Indian mongoose (*Herpestes javanicus*). More recently, rabies has been discovered in insectivorous bats in the States of Florida and Pennsylvania (USA), where vampire-bats are unknown. Thus far, the infection has been diagnosed in the yellow bat (*Dasypterus floridanus*) and the Seminole bat (*Lasiurus seminola*). Investigations are progressing to determine the significance of these findings in the epizootiology of the disease in these areas and its possible importance to the entire rabies problem.

The first report pointed out the large gaps in our knowledge of the epizootiology of wildlife rabies and suggested ecological studies of the various principal sylvatic vectors in association with characteristics of the

[1] Laboratories encountering difficulties in the performance of the test can submit samples of their hyperimmune serum to WHO for testing in specialized laboratories.

disease in these species. Such studies are now under way in certain areas of the world. The projects include ecology and basic biological behaviour of the species under study, determination of the extent of the movement of rabid wild animals, determination of factors which influence relative susceptibility or resistance of these animals to rabies, methods for developing accurate census techniques, estimates of the threshold of population density in a particular species which is required to support the disease, development of measures to regulate this threshold, and an evaluation of the methods and technical implements required in any control activity.

WORLD HEALTH ORGANIZATION
TECHNICAL REPORT SERIES

	Number	Price s. d.	Price $	Price Sw. fr.
Bilharziasis, Expert Committee on				
First report	**65**	2/3	0.30	1.20
Bilharziasis in Africa, Joint OIHP/WHO Study-Group on				
Report on the first session	**17**	9d.	0.10	0.40
Brucellosis, Joint FAO/WHO Expert Committee on				
Report on the first session	**37**	2/–	0.25	1.—
Second report	**67**	2/–	0.25	1.—
Cholera, Expert Committee on				
First report	**52**	1/3	0.15	0.60
Cholera, Joint OIHP/WHO Study-Group on				
Report on the third session	**18**	1/3	0.15	0.60
Hepatitis, Expert Committee on				
First report	**62**	1/6	0.20	0.80
Influenza, Expert Committee on				
First report	**64**	1/6	0.20	0.80
Leprosy, Expert Committee on				
First report	**71**	1/6	0.20	0.80
Malaria, Expert Committee on				
Report on the third session	**8**	2/3	0.30	1.20
Report on the fourth session	**39**	1/6	0.20	0.80
Fifth report	**80**	*In preparation*		
Malaria Conference in Equatorial Africa				
Report	**38**	3/6	0.45	1.80
Plague, Expert Committee on				
Report on the first session	**11**	1/6	0.20	0.80
Second report	**74**	9d.	0.10	0.40
Poliomyelitis, Expert Committee on				
First report	**81**	3/6	0.50	2.—
Rabies, Expert Committee on				
Report on the first session	**28**	1/6	0.20	0.80
Second report	**82**	1/9	0.25	1.—
Rheumatic Diseases, Expert Committee on				
First report	**78**	1/9	0.25	1.—