W.H.O Expert committee Report on Rabies

WHO Report on Rabies will be published fully in JIVA, and here is the first part-(Editor)

The WHO Expert Committee on Rabies met in Geneva from 24 to 30 September 1991. Opening the meeting on behalf of the Director-General. Dr. R. Henderson, Assistant Director-General, pointed out that Rabies continues to be a major health hazard in many countries in Africa, South America and Asia, and an economic burden for both developed and developing countries, in spite of recent advances in diagnosis, human post-exposure treatment, the production of vaccines for human and veterinary use and the control of rabies in dogs and wild animals.

1.1 Recent advances

Since the last meeting of the WHO Expert committee on Rabies in September 1983. Many advances have been made in basic and applied research on the disease. In particular, the Committee noted the application of molecular biology techniques, not only in the laboratory but also in field control operations, as in the oral immunization of foxes in Canada and Europe and in trials for the oral immunization of racoons in the USA. The Committee also reviewed the new strategies for controlling canine rabies that had been developed by the WHO regional programme for the elimination of urban rabies in Latin America and the interregional project for human and canine rabies control in developing countries, which was supported by the Arab Gulf Programme for United Nations Development Organizations (AGFUND) and WHO. The Committee also noted that several consultations and regional conferences on these subjects had been held and a number of training seminars had been organized. These advances were taken into account by the Committee in formulating its recommendations. The Committee urged rabies control authorities and research groups to take note of these recommendations and to revise their policies and procedures accordingly.

1.2 Canine rabies

In view of the need for a special initiative for canine rabies control, the Committee expressed its support for the conclusions of the WHO Cosultation on the feasibility of global control and elimination of urban rabies, and urged rabies control authorities and funding agencies to take the necessary measures to eliminate rabies.

1.3 Laboratory techniques in rabies

The draft of the fourth edition of the WHO monograph Laboratory techniques in rabies was reviewed by the Committee. The Committee commended this book as an excellent source of information on the laboratory aspects of rabies. The revised edition, which is referred to frequently throughout the report, will contain many new chapters dealing with recent procedures, and include updated descriptions of techniques described in the previous edition.

2. Advances in rabies research

2.1 Monoclonal antibodies and the classification of rabies and rabies-related viruses

Since the early 1980s, monoclonal antibodies have been used extensively in identifying rabies virus strains and in diagnosing human and animal rabies.

Monoclonal and polyclonal antibody studies of rabies isolates from many animal species worldwide have led to the following classification of the rabies group of Rhabdovirae, genus Lyssavirus:

Serotype1.: prototype strain Challenge Virus Standard (CVS); includes the majority of field viruses isolated from terrestrial mammals as well as isolates from insectivorous bats in North America and haematophagous bats in Latin America; also includes fixed virus laboratory strains.

Serotype 2: prototype strain Lagos bat, first isolated from pooled brains of bats in Nigeria (Lagos-bat.1), then from a bat in the Central African Republic (Lagos bat2) and from a bat in Guinea and a cat in Zimbabwe (Lagos-bat 3).

Serotype 3: Prototype strain Mokola, first isolated from shrews in Nigeria and then from a human (Mokola 1); further isolates have been obtained from shrews in Cameroon (Mokola2) and in the Central African Republic (Mokola3) and from dogs in Zimbabwe (Mokola 5).

Serotype 4: prototype strain Duvenhage, first isolated from a human in South Africa (Duvenhage 1), and then from bats in South Africa (Duvenhage 2) and Zimbabwe (Duvenhage 3).

A number of viruses still remain to be typed. These include the recently identified European Bat Lyssaviruses (EBL) isolated from Eptesicus serotinus bats (EBL 1) and Myotis bats (EBL 2), as well as isolates from human exposed to bats in Finland and Ukraine .

2.2 Molecular biology of the rabies virus

Over the past decade, considerable progress has been made in understanding the structure of lyssaviruses.

The virions or virus particles have a bullet-shaped structure with an average length of 180 nm and a diameter of 75nm. Each particle contains a helical nucleocapsid surrounded by a lipid bilayer. The outer surface is coverd with spike-like projections, 10nm in legth, anchored in a lipid bilayer. Five proteins have been identified following disruption of rabies virus with sodium dodecyl sulfate. The ribonucleoprotein contains the genomic RNA associated with three internal proteins, the trascriptase (L) (M_1 = 190000) the nucleoprotein (N) (M_1 = 55000) and a phosphoprotein (NS) (M_1 = 38000). These proteins together with the RNA form an active RNA complex, which controls both transcription and replication. The other structural proteins are the matrix protein (M) (M_1 = 26000), which is located on the inner side of the virus envelope, and the glycoprotein (G) (M_1=67000), which forms the surface projections. The complete amino acid sequences of these five proteins have been deduced from the primary nucleotide sequences of cloned rabies genome and individual mRNA trascripts.

Both rabies virus and rabies-related virus have the same genomic structure. The virion contains an unsegmented and non-polyadenylated negative-stranded RNA genome. Although complete nucleotide sequences of all the five rabies genes have been determined for several fixed rabies strains, this information is not available for field virus strains.

Of the five proteins, the G and N proteins are the most extensively characterized.. The G protein is the only viral antigen that induces virus-neutralizing antibodies; it is also a target for virus-immune T helper cells and cytotoxic T cells. Rabies virus-neutralizing antibodies directed against the G protein ap-

pear to be an important component in the immune response to rabies. Antigenic determinants in the G protein recognized by rabies virus-specific B and T cells have been identified, and synthetic peptides incorporating these antigenic determinants offer an approach to the development of vaccines against rabies.

Of the three proteins that form the helical nucleocapsid in conjuction with the RNA, the N Protein represents the major internal protein of the virus. Analysis of the primary nucleotide sequence of the N protein gene of several rabies virus strains has demonstrated an exceptionally high level of conservation, reflected by a high degree of antigenic homology between rabies and rabies-related virus strains in the ribonucleprotein. The N protein has been shown to be a major antigen, capable of inducing T helper cells that cross react between different rabies and rabies-related viruses. The finding that ribonucleoprotein can confer protective immunity may have clinical significance for immunization against heterologous virus strains.

Since the G and N proteins are the major antigens capable of inducing immunity against lethal rabies infection, both should logically be included in the development of genetically engineered vaccines. Both proteins have been expressed in a variety of prokaryotic and eukaryotic expression systems (see section 2.4). The capacity to produce large quantities of genetically engineered rabies virus proteins will provide research teams with new tools for diagnosis and vaccine development.

2.3 Molecular epidemiology

Recent progress in research on the molecular genetics of lyssaviruses suggests that powerful tools for the identification of rabies viruses may be available in the future. Cloning and sequencing techniques together with other techniques such as the polymerase chain reaction (PCR) have been successfully applied to the rabies virus and may lead to the characterization of certain regions of the viral genome.

The PCR technique, however, is not yet suffficiently developed for the routine diagnosis of rabies and should only be carried out by molecular biology laboratories with the necessary facilities and expertise.

2.4 Research in vaccine development

Since the last meeting of the Committee in 1983, significant progress has been made in the preparation and delivery of vaccines to animals and humans, including a major shift to the use of cell culture for vaccine preparations.

2.4.1 Candidate vaccines

Advances in cloning and gene expression have resulted in the production of many unique recombinant rabies vaccines, which have been tested for possible use in animals and humans. These recombinant vaccines include the following groups.

Orthopox Viruses

A rabies G-protein orthopoxvirus recombinant has been characterized extensively and used for oral immunization of foxes and racoons in several countries (see sections 4.3.2 and 4.4.2). This vaccice is not considered suitable for human immunization because of safety considerations in the use of live vaccinea virus. Another vector, racoon poxvirus, has been used for preparing a recombinant vaccine containing the G and N proteins; this vaccine is an effective oral immunogen in racoons, foxes and dogs.

The N protein has been shown to be a major antigen, capable of inducing T helper cells that cross react between different rabies and rabies-related viruses.

Other poxviruses may be safer for human vaccination; these include avipoxes

such as canary pox, and vaccinia attenuated through the removal of 18 genes accounting for the virulence of the virus. In one study, human volunteers injected with vaccine prepared from canary poxvirus and rabies G protein produced a level of virus-neutralizing anitibodies equal to that observed in subjects injected with a standard tissue-culture vaccine. Moreover, orthopox vectoring may permit the incorporation of several antigens (e.g. measles, mumps, rubella, rabies, pertussis) into a single vaccine, which would reduce the medical facilities required for human prophylactic immunization.

Baculoviruses

Both rabies G-protein and N-protein baculovirus recombinants are now available and could be considered for the production of rabies vaccine for immunization of animals. Although the purification of N protein from the baculovirus recombinant may be too complicated and too costly for the production of a reasonably priced vaccine, it may be possible to use N protein for human vaccination if sufficient amounts are generated by mammalian cells transfected with the recombinant.

Adenoviruses

Human and animal adenoviruses are also sufficiently large to accommodate foreign genes. When inserted into the adenovirus genome., the complementary DNA (cDNA) of rabies glycoprotein gene is expressed on the surface of infected cells, but not on the virion surface. Such vaccines would be especially useful to protect animal species that have been difficult to immunize with existing oral vaccines, such as dogs and skunks. A recombinant vaccine prepared by inserting the cDNA of rabies glycoprotein gene into human adenovirus 5 has been found to be immunogenic in a variety of animals, including skunks, racoons, foxes and dogs. Since dogs tend not to chew baits much before swallowing them, the vectored vaccine should, ideally, be resistant to the low pH conditions in the stomach and infect the intestinal tissues.

No recombinants have yet been prepared using canine adenoviruses as vectors, but preliminary studies indicate that these viruses may be suitable candidate vaccines for various species (e.g., skunks, foxes, racoons, mongooses and dogs) and studies are continuing in several laboratories worldwide.

Other recombinant vaccines

BCG (bacille Calmette-Guerin) and attenuated salmonellae are also potential candidate vectors for preparing rabies vaccines. Extrachromosomal and integrative expression vectors carrying the regulatory sequences for the major BCG heal-shock proteins have been developed which could allow expression of rabies virus G or N protein.

Other mechanisms by which antigens may be delivered to the immune system are summarized in section 13.

2.4.2 Safety aspects

Further development of recombinant rabies vaccines will increase the number of prototypes constructed in different vectors and proposed by different authors or manufacturers. Strict observation of international safety norms will be required before field testing.

A number of rabies vaccines have induced virus-neutralizing antibodies when fed to dogs. These include both attenuated vaccines, such as SAD and SAG1 (a derivative of SAD virus), and recombinant vaccines, such as those prepared from vaccinia and racoon poxvirus. To date none of these vaccines have been sufficiently developed for field testing in dogs, although considerable time and effort have gone into testing their safety and efficacy. standard procedures for testing the safety and efficacy of candidate vaccines have been developed. Since children or even adults may come

into contact with oral rabies vaccines intended for canine use any vaccine to be field tested must first be carelully examined for safety in selected non-target species to address the question of possible hazard to humans.

2.5 New substances for post-exposure treatment and new post-exposure vaccination regimens

2.5.1 Monoclonal antibodies

Research is continuing on the possible use of monoclonal antibodies for post-exposure treatment of humans and animals. In a recent study, monoclonal antibodies were shown to protect Syrian hamsters against rabies when given intramuscularly 24 hours or more after intramuscular challenge with a field strain. Although these antibodies were of murine origin, murine antibodies have been used extensively in the treatment of cancer patients over the past decade, without any significant side-effects being reported. However, recombinant DNA techniques are now available to prepare chimeric (murine-human) antibodies and also to "humanize" monoclonal antibodies of murine origin. Moreover, following cloning and sequencing of these latter anibodies, it is possible to have them expressed in vectors such as baculovirus.

2.5.2 Interferon and interferon-inducers

Both exogenous interferon preparations and "interferon-inducers" have been shown to be highly effective in reducing mortality in laboratory mice and subhuman primates challenged intramuscularly with a field virus strain. Neither interferon nor interferon-inducers appear to suppress the virus-neutralizing anitibody response induced by vaccination. In addition, it has been shown that recombinant α-interferon administered with vaccine can be as effective as exogenous interferon and vaccine in reducing rabies mortality in subhuman primates. Exogenous interferon has already been shown to be effective in a patient given a corneal transplant from a person with rabies; further studies are in progress.

2.5.3 New post-exposure vaccination regimens

A variety of new vaccination schedules have been evaluated in humans in an effort to reduce the number of vaccinations and amount of vaccine required after exposure to rabies. One of these schedules, the 3-1 schedule, is an abbreviated multisite regimen consisting of three vaccine doses applied in the deltoid muscle of the right and left arm at day 0, and one dose applied at day 7. Application of the 3-1 regimen to human volunteers produced an early and elevated cellular (starting 6 hours after vaccination) and humoral (starting at day 5) immune response. When this regimen was combined with administration of anit-rabies human immunoglobulin, however, the initial anibody response was suppressed, although subsequent titres reached expected levels after 2 weeks.

3. Diagnosis

3.1 Clinical diagnosis

Rabies in animals and humans is still diagnosed on the basis of clinical signs and symptoms in many areas of the world.

Clinical diagnosis of rabies in animals is, however, sometimes difficult and rabid dogs may be judged to be infected, which could result in danger in humans: equally, persons bitten by animals with other diseases or conditions (such as distemper) could be vaccinated against rabies unnecessarily. Clinical diagnosis of rabies in humans can also be difficult, since patients may present with a paralytic or Guillain-Barre-like syndrome. Signs of brain involvememnt are spasms in response to tactile, auditory, visual or olfactory stimuli(e.g. aerophobia, hydrophobia), alternating with periods of lucidity, agitation, confusion, and signs of autonomic dysfunction. These spasms occur at some time in almost all rabid patients in whom excitation is promi-

nent, but spontaneous inspiratory spasms usually occur continuously until death; their presence often facilitate clinical diagnosis. Excitation is less evident in paralytic rabies, and phobic spasms appear in only 50% of these patients . During the early stages of paralytic rabies, notable signs include myoedema at percussion sites, usually in the region of the chest, deltoid musle and thigh and piloerection.

Side - effects following the inoculation of adult or suckling - mouse brain rabies vaccines are occasionally misdiagnosed as rabies and a test for antibody to myelin basic protein may be useful in identifying such patients. Great care needs to be exercised before a diagnosis of rabies is made on clinical grounds.

Since imported cases of human and animal rabies have been noted in rabies -free countries (or rabies- free areas of infected countries) the Committee emphasize that rabies must be included in the differential diagnosis of all persons who present with signs of neurological involvement.

3.2 Laboratory diagnosis

3.2.1 Postmortem diagnosis of rabies in animals and humans

Antigen detection

The fluorescent antibody (FA) technique is a rapid and sensitive method for diagnosing rabies infection in animals and humans. The test is based upon microscopic examination, under ultraviolet light, of impressions, smears or frozen section of tissue after treatment with anti - rabies serum or globulin conjugated with fluorescein isothiocyanate.

Bilateral impressions (or smears) of tissue samples from the hippocampus (Ammon's horns) and brain stem are recommended for increased sensitivity of the test; some laboratories also stain samples of cerebellar tissue.

An enzyme-linked immunosorbent assay (ELISA) called rapid rabies enzyme immunodiagnosis (RREID) was developed for the diagnosis of rabies, based upon the detection of rabies virus nucleocapsid antigen in brain tissue. Since the antigen can be visualized with the naked eye, the test can be carried out (with the aid of a special kit) under field conditions.

RREID is a rapid technique which can be especially useful for epidemiological surveys. The test may be used to examine partially decomposed tissue specimens for evidence of rabies infection, but it cannot be used with specimens that have been fixed in formalin. It should be noted, in addition, that the FA test may yield positive results when the RREID is negative.

Virus isolation in vitro

Virus isolation may be necessary for confirming the results of antigen detection tests and for further characterizing the isolate.

Murine neuroblastoma (NA C 1300) cells are more susceptible to rabies field virus infection than any other cell lines tested. Virus isolation in cell culture (with neuroblastoma cells) is at least as efficient as mouse inoculation for demonstrating small amounts of rabies virus. It also reduces the time required for diagnosis from 10-15 days to 2 days, eliminates the need for experimental animals, and is considerably less expensive to perform. This technique is not feasible in every laboratory, however, and intracerebral mouse inoculation is still a useful test in the laboratory diagnosis of rabies. Suckling mice (less than 3 days old) are more susceptible to rabies than weanling or adult mice and should be used whenever possible. The observation period may be shortened by FA examination of brains of inoculated mice killed 3 to 4 days (or more) after inoculation.

Virus identification using monoclonal antibodies: epidemiological considerations.

To date, several hundred lyssavirus isolates from humans, domestic animals, and wild animals in Africa, the Americas, Asia and Western Europe have been compared using monoclonal antibodies. These studies demonstrate that rabies virus can be distinguished from other lyssaviruses and that rabies isolates from given geographical area or species have unique reactivity patterns both in the nucleocapsid and glycoprotein components of the virion. In relatively simple ecosystems, a few principal carnivore hosts (e.g. wild canids) serve as primary rabies reservoirs. In Canada and the USA, field rabies viruses are maintained in "compartments" in specific geographical regions by species such as foxes, skunks, racoons and bats; transfer of the disease to other species is relatively unimportant for maintenance of infection. Striking differences are apparent between viruses isolated from bats and those isolated from terrestrial carnivores, which confirm previous epidemiological findings.

Detection by molecular techniques

The use of molecular probes and the polymerase chain reaction is not currently recommended for the routine diagnosis of rabies.

3.2.2 Intra vitam diagnosis of rabies in humans

The choice of techniques for intra vitam diagnosis varies greatly according to the stage of the disease; antigen detection is generally sensitive during the first few days, while virus-neutralizing antibodies in cerebrospinal antibodies in cerebrospinal fluid and serum usually tend to appear after 7-10 days of illness.

Viral antigen may be detected by FA in corneal impressions or skin biopsies from patients with rabies; however, FA-positive specimens are more common during the final stages of the disease. Skin biopsies are usually taken from the nuchal area of the neck, with hair follicles containing peripheral nerves. Corneal impressions (never scrapings) are taken from patients with encephalitis by lightly touching the central part of the cornea with a microscope slide.

The quality of the samples - both corneal impressions and skin biopsies - is paramount; they should be refrigerated immediately after collection and until the test is carried out.

Nevertheless, the sensitivity of the FA technique for intra vitam diagnosis is limited:

Rabies antigen has been demonstrated in corneal impressions taken from patients and naturally and experimentally infected animals. However, while a positive result is indicative of rabies, a negative result does not rule out the possibility of infection.

Although rabies antigen may be detected in skin biopsies at the onset of clinical signs, the proportion of positive results tends to increase as the disease progresses. With nuchal skin biopsies, only some patients show positive results, especially during the early phase of clinical illness. Nevertheless, the overall sensitivity of FA is higher with skin biopsies than with corneal impressions.

Rabies virus may be isolated in cell culture from certain body tissues and fluids, especially saliva and cerebrospinal fluid.

Saliva samples should be maintained frozen after collection; the contents of the swab should be expressed in the collection medium, the swab removed and the specimen sent frozen for further examination. Biopsy material and cerebrospinal fluid should be frozen after removal. Under no circumstances should preservatives be added to the collection medium.

Antibody titration

Neutralizing antibodies in the serum or cerebrospinal fluid of non-vaccinated patients may be measured either by the mouse serum neutralization test

(MNT) or by the rapid fluorescent focus inhibition test (RFFIT). The Committee recommended that where possible, the MNT be replaced by the RFFIT, since the latter test is more rapid and at least as sensitive as the MNT.

An enzyme-linked immunosorbent assay (ELISA) using purified rabies glycoprotein has been used to determine virus-neutralizing antibody levels in the serum of several species, including humans. The test can be carried out (with the aid of a special kit) in the field and provides results within a few hours. It also appears to be quite reproducible. Nevertheless, the sensitivity of the test is limited; the measurement may include a variety of antibodies in addition to virus-neutralizing antibodies.