The Immunological Basis for Immunization Series

Module 17: Rabies
The Immunological Basis for Immunization Series

Module 17: Rabies
The Department of Immunization, Vaccines and Biologicals thanks the donors whose unspecified financial support has made the production of this document possible.

This module was produced for Immunization, Vaccines and Biologicals, WHO, by:

Dr Deborah J Briggs, Executive Director
Global Alliance for Rabies Control
Manhattan Kansas, USA.

The Department of Immunization, Vaccines and Biologicals, WHO, also gratefully acknowledges the kind assistance and contribution of the Department of Control of Neglected Tropical Diseases in the preparation and review of this module.

Printed in February 2011

Copies of this publication as well as additional materials on immunization, vaccines and biological may be requested from:

World Health Organization
Department of Immunization, Vaccines and Biologicals
CH-1211 Geneva 27, Switzerland

Fax: +41 22 791 4227 • Email: vaccines@who.int

© World Health Organization 2010

All rights reserved. Publications of the World Health Organization can be obtained from WHO Press, World Health Organization, 20 Avenue Appia, 1211 Geneva 27, Switzerland (tel: +41 22 791 3264; fax: +41 22 791 4857; email: bookorders@who.int). Requests for permission to reproduce or translate WHO publications – whether for sale or for noncommercial distribution – should be addressed to WHO Press, at the above address (fax: +41 22 791 4806; email: permissions@who.int).

The designations employed and the presentation of the material in this publication do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted lines on maps represent approximate border lines for which there may not yet be full agreement.

The mention of specific companies or of certain manufacturers’ products does not imply that they are endorsed or recommended by the World Health Organization in preference to others of a similar nature that are not mentioned. Errors and omissions excepted, the names of proprietary products are distinguished by initial capital letters.

All reasonable precautions have been taken by the World Health Organization to verify the information contained in this publication. However, the published material is being distributed without warranty of any kind, either expressed or implied. The responsibility for the interpretation and use of the material lies with the reader. In no event shall the World Health Organization be liable for damages arising from its use.

The named authors alone are responsible for the views expressed in this publication.

Printed by the WHO Document Production Services, Geneva, Switzerland
## Contents

_Abbreviations and acronyms_ ............................................................................................. v
_Preface_ .............................................................................................................................. vii

1. **Rabies virus and disease** ......................................................................................... 1
   1.1 *Structure of the virus* ................................................................................... 1
   1.2 *Classification* ............................................................................................. 1
   1.3 *Pathology* ...................................................................................................... 2
   1.4 *Epidemiology* ............................................................................................... 2

2. **Immunity to rabies** ................................................................................................. 3
   2.1 *Preventing clinical disease* .......................................................................... 3
   2.2 *Rabies vaccines* ........................................................................................... 4
   2.3 *Response to immunization* .......................................................................... 4
   2.4 *Role of passive immunity* ............................................................................ 5
   2.5 *Routes of active immunization* .................................................................. 6
   2.6 *Immune response in different populations* .............................................. 6

3. **Duration of immunity after immunization** ............................................................... 8
   3.1 *Development of immunity* ......................................................................... 8
   3.2 *Duration of rabies virus-neutralizing antibody* ........................................... 8
   3.3 *Anamnestic response* ................................................................................... 10
   3.4 *Timeliness of routine booster vaccination* ............................................... 11

4. **Techniques to measure the immune response** ....................................................... 12
   4.1 *Choosing the test to fit the purpose* ......................................................... 12
   4.2 *Virus neutralization assays* ........................................................................ 12
   4.3 *Binding assays* ............................................................................................ 13
   4.4 *Measuring cell-mediated immunity* ............................................................ 13

5. **Innocuity and efficacy of rabies biologicals** .......................................................... 14

6. **Future prospects** .................................................................................................. 15

_References_ ......................................................................................................................... 16
Abbreviations and acronyms

ABL V  Australian bat lyssavirus
ARAV  Aravan virus
CCV  cell culture rabies vaccine
CNS  central nervous system
dRIT  direct rapid immunohistochemical test
DTP-IPV combined diphtheria, tetanus, whole cell pertussis and inactivated poliomyelitis vaccine
DUVV  Duvenhage virus
EBLV-1  European bat lyssavirus type 1
EBLV-2  European bat lyssavirus type 2
ELISA  enzyme-linked immunosorbent assay
ERIG  equine rabies immune globulin
FAT  fluorescent antibody test
FAVN  fluorescent antibody virus neutralization
GBS  Guillain-Barré syndrome
HAART  highly active antiretroviral therapy
HDCV  human diploid cell vaccine
HIV  human immunodeficiency virus
HRIG  human rabies immune globulin
ICTC  International Committee on Taxonomy of Viruses
ID  intradermal
Ig  immunoglobulin
IM  intramuscular
IRKV  Irkut virus
JEV  Japanese encephalitis vaccine
KHUV: Khujand virus
LBV: Lagos bat virus
MHCII: major histocompatibility complex class II
MNT: mouse neutralization test
MOKV: Mokola virus
NTV: nerve tissue vaccines
PCECV: purified chick embryo cell vaccine
PCR: polymerase chain reaction
PDEV: purified duck embryo cell vaccine
PEP: post-exposure prophylaxis
PrEP: pre-exposure vaccination
PVRV: purified Vero cell rabies vaccine
RABV: rabies virus
RFFIT: rapid fluorescent focus inhibition test
RIDT: rapid immunodiagnostic test
RIG: rabies immune globulin
RNA: ribonucleic acid
RTCIT: rabies tissue culture infection test
RT-PCR: reverse transcription-polymerase chain reaction
RVNA: rabies virus-neutralizing antibody
SHIBV: Shimoni bat virus
WCBV: West Caucasian bat virus
WHO: World Health Organization
Preface

This module is part of the series *The Immunological Basis for Immunization*, which was initially developed in 1993 as a set of eight modules focusing on the vaccines included in the Expanded Programme on Immunization (EPI). In addition to a general immunology module, each of the seven other modules covered one of the vaccines recommended as part of the EPI programme — diphtheria, measles, pertussis, polio, tetanus, tuberculosis and yellow fever. The modules have become some of the most widely used documents in the field of immunization.

With the development of the Global Immunization Vision and Strategy (GIVS) (2005–2015) (http://www.who.int/vaccines-documents/DocsPDF05/GIVS_Final_EN.pdf) and the expansion of immunization programmes in general, as well as the large accumulation of new knowledge since 1993, the decision was taken to update and extend this series.

The main purpose of the modules — which are published as separate disease/vaccine-specific modules — is to give immunization managers and vaccination professionals a brief and easily-understood overview of the scientific basis of vaccination, and also of the immunological basis for the World Health Organization (WHO) recommendations on vaccine use that, since 1998, have been published in the Vaccine Position Papers (http://www.who.int/immunization/documents/positionpapers_intro/en/index.html).

WHO would like to thank all the people who were involved in the development of the initial *Immunological Basis for Immunization* series, as well as those involved in its updating, and the development of new modules.

---

1 This programme was established in 1974 with the main aim of providing immunization for children in developing countries.
1. Rabies virus and disease

1.1 Structure of the virus

Rabies virus is an enveloped, rod-shaped virus containing a single-strand negative-sense non-segmental ribonucleic acid (RNA) genome. It has a simple genome organization that encodes five structural proteins: RNA polymerase (L); nucleocapsid protein (N); phosphoprotein (P); matrix protein (M), and a surface glycoprotein (G). It is the G that induces the production of rabies virus-neutralizing antibodies (RVNA) that are the major immune effectors in protecting against infection with rabies virus (1,2). The ribonucleoprotein complex consisting of the N, P, L, and negative-strand genomic RNA has been reported to potentially play a role in the establishment of immunologic memory and long-lasting immunity (3).

1.2 Classification

Rabies virus belongs to the genus Lyssavirus in the family Rhabdoviridae (1). According to the International Committee on Taxonomy of Viruses (ICTV), as of 2009, 11 species were classified under the Lyssavirus genus (http://www.ictvonline.org). The 11 species include: rabies virus (RABV), Lagos bat virus (LBV), Mokola virus (MOKV), Duvenhage virus (DUVV), European bat lyssavirus type 1 (EBLV-1), European bat lyssavirus type 2 (EBLV-2) and Australian bat lyssavirus (ABLV) (4,5), Aravan virus (ARAV), Khujand virus (KHUV), Irkut virus (IRKV) and West Caucasian bat virus (WCBV) (6–10). In addition, a new virus, Shimoni bat virus (SHIBV) was reported in 2010, and needs further classification (11).

Besides rabies virus, viruses belonging to all other known lyssavirus genotypes have been demonstrated (i.e. ABLV, DUVV, EBLV, MOKV), or can all be expected (ARAV, KHUV, IRKV, WCBV and SHIBV) to cause a rabies-like lethal encephalitis in humans.

Reports available on genetic classification of seven lyssavirus species/genotypes (i.e. RABV, LBV, MOKV, DUVV, EBLV-1, EBLV-2 and ABLV) suggest that they are divided into two phylogroups according to differences in genetic makeup, serologic cross-reactivity and animal pathogenesis. Phylogroup I includes RABV, DUVV, EBLV-1, EBLV-2 and ABLV, while phylogroup II includes LBV and MOKV (6). The level of immunity against phylogroup II viruses induced by administering the currently licensed rabies vaccines is dependent on how far the genetic makeup of these viruses converges or diverges from that of RABV (7). The level of protection provided against newly identified lyssaviruses by the currently available rabies vaccines or immunoglobulins has, as yet, not been completely evaluated, and they may provide reduced or no protection.
1.3 Pathology

Human rabies as a disease has the highest case-fatality rate ever reported (12). Rabies virus infection can cause an acute progressive encephalopathy in a wide variety of mammals, including humans, in which the disease almost invariably results in the death of the host (12,13). After an exposure occurs, generally through infiltration of virus-contaminated saliva from a rabid animal into a bite wound, or contact with mucous membrane, the highly neurotropic rabies virus replicates in muscle tissue and enters peripheral nerves, spreads by way of the peripheral nervous system to the spinal cord and ascends to the brain. After dissemination within the central nervous system (CNS), the virus spreads centrifugally from the CNS back along the nerves to various organs, including the salivary glands, where it is emitted into the saliva and passed on to the next victim, again usually through a bite wound or contamination of virus-carrying saliva on to a mucous membrane (14).

1.4 Epidemiology

Rabies is an underreported disease that is present on every continent, except Antarctica, with more than 55 000 human deaths occurring annually (15,16). Most human deaths occur in Africa and Asia (15–18). Although all mammals are, to varying degrees, susceptible to rabies, the primary reservoirs of the disease belong to the Orders Carnivora and Chiroptera (i.e. dogs, foxes, jackals, coyotes, raccoon dogs, skunks, raccoons, mongoose and bats) (13,19). Globally, over 98% of all human rabies deaths occur following exposures to infected dogs. Millions of exposures to dogs occur annually, with tens of thousands of human deaths resulting from cases of untreated exposures (12,13,15). Human rabies, especially paralytic rabies (which may represent as much as 30% of total clinical rabies presentations), is often misdiagnosed as other encephalitic diseases, such as malaria or Guillain-Barré syndrome (GBS), thus masking the true global burden of the disease (20,21,22).
2. Immunity to rabies

2.1. Preventing clinical disease

Rabies is almost unique compared to other infections in that the development of clinical disease following exposure to the virus is preventable, even in patients that have not been previously vaccinated, through timely administration of post-exposure prophylaxis (PEP). PEP, as recommended by WHO, comprises three components: (a) wound treatment with cleansing, flushing and disinfection; (b) vaccine administration over 28 to 90 days, depending on regimens and route of administration; (c) administration of rabies immune globulin (RIG) with, or within the week which follows administration of the first dose of vaccine in all category III (severe\(^1\)) exposures (13,23). The outcome of an exposure to rabies depends on many factors, including: the site and severity of the exposure; the dose and variant (genotype or biotype) of virus inoculated into the wound(s), and the timeliness of administering and adherence to WHO recommendations for PEP (12,13,23). Both the innate (basic immune system inducing ‘non-specific’ resistance to disease) and adaptive (highly specialized, systemic cells and processes) immune responses of a patient, are involved in securing protection against developing rabies (24,25).

Besides wound treatment, whose action is mechanical and chemical, the primary immunological objective of PEP is to neutralize and destroy rabies virus that was inoculated into a victim’s body at the time of exposure. This needs to be achieved as quickly as possible by increasing the amount of rabies virus neutralizing antibody (RVNA) available to complete the task. Thus, it is critical for a protective immune response to ensure that RVNA directed against the G of the virus is produced as soon as possible (3,24). The level of RVNA is almost always high enough to be detected between 7 to 14 days after primary vaccination (adaptive or active immunity) (41,42,43). However, because human rabies is invariably fatal, the administration of rabies immune globulin (RIG) (passive immunity) early in the vaccination regimen aims at providing additional protection, especially for patients with severe and/or multiple wounds (13,23,26,27,28).

---

\(^1\) Severe exposure is defined by WHO as single or multiple transdermal bites or scratches and licks on broken skin, and contamination of mucous membrane with saliva.
2.2 Rabies vaccines

Since their development over three decades ago, cell culture- and embryonated egg-based rabies vaccines (CCVs) have proved to be highly effective in preventing human rabies, both when administered as pre-exposure vaccination (PrEP) and when used in association with RIG for PEP (29). The production of CCVs represented a significant advance, particularly over the first crude nerve tissue vaccines (NTV) for rabies manufactured over a century ago using the brain material of infected animals (29). It should be noted that all NTVs are reactogenic and the WHO has recommended that they be replaced with CCVs (13). Several different cell substrates have been used for the production of rabies vaccines, including Syrian baby hamster kidney cells, human diploid cells, primary cell lines produced from embryonated chicken and duck eggs, and continuous cell lines produced from Vero cells (29). Rabies vaccines produced on Vero cells and primary cell lines originating from embryonated eggs have expanded the safe use and availability of CCVs throughout the world. CCVs have also allowed a broader use of vaccines for PrEP, so protecting those at constant risk of exposure (13, 23). Over the past two decades numerous data have been published proving the efficacy and safety of CCVs (7, 23, 30–33). The cost of administering a 5-dose intramuscular (IM) PEP regimen using CCVs is often beyond the financial capability of many persons living in developing countries. Therefore, where budgetary limitations may deter the use of CCVs for PEP, the WHO has recommended the administration of intradermal (ID) PEP using CCVs that meet specific potency and immunological criteria (13). Ongoing research specifically aimed at developing new, low cost and effective rabies vaccines, could eventually reduce the global cost of preventing rabies (26).

2.3 Response to immunization

Early experiments designed to identify the components of the immune system responsible for protection against rabies virus infection have proved that inactivated rabies vaccines can induce the production of cytotoxic T-cells (34). Additional experimental evidence investigating the role of cell-mediated immunity in mice confirmed the fact that cytotoxic T-cells alone do not protect against rabies, as the depletion of CD8+ T-cells had no effect on the resistance to disease nor on the survival rate of vaccinated animals (3, 24, 35). Research indicates that inactivated rabies vaccines stimulate B-cells as well as CD4+ cells using major histocompatibility complex class II (MHCII) mechanisms and confer protection through the induction of an immune response including the activation of lymphocytes, CD4+ antibody-secreting plasmocytes and neutralizing antibodies that migrate into the nervous system parenchyma (3, 24, 35). The activation of CD4+ T-lymphocytes ultimately results in the production of RVNAs that target and destroy rabies virus, thus playing a major role in protecting victims exposed to rabies against developing the disease (24, 35, 36).

The immune characteristics of both the humoral and cellular immune responses after rabies vaccination was recently studied in 17 healthy patients and in five patients suffering from a combined B- and T-cellular immunodeficiency (37). In all healthy patients the enzyme-linked immunosorbent assay (ELISA) test results indicated that at one week after primary vaccination there was a significant rise in the level of immunoglobulin (Ig) M, and at two weeks after primary vaccination there was a significant rise in the level of IgG (IgG1 and IgG3) and IgA. In the same study, after a booster vaccination was administered, the level of IgG increased significantly faster (measured one week after the booster dose) than it did after the primary series of doses was administered.
Overall, IgG1 is the major IgG subclass present after primary and booster rabies vaccination. The five patients with a combined immunodeficiency, vaccinated using the same protocol, showed a number of abnormalities in their humoral and cellular immune responses.

Following inoculation of virulent rabies virus into animal models, the virus may either replicate at the site of inoculation (usually in muscle tissue) or enter directly into the peripheral nerves innervating the wound site without replication (38). Once rabies virus enters the neurons neutralization could potentially be possible, although, according to earlier research, it seems to be less likely (39). However, the pathogenesis of rabies is, as yet, not completely defined and, because the administration of PEP has been effective several days to months after an exposure has occurred, it is possible that RVNA can occasionally clear rabies virus from the CNS.

Following administration of rabies vaccines, antibodies produced against other viral proteins besides the G (specifically the N) have been detected in the sera of human subjects. Published reports indicate that antibodies directed against the N do not neutralize rabies virus, and therefore these specific ‘anti-N’ antibodies are unlikely to play a major role in the development of protective humoral immunity. At present, the role of non-neutralizing viral antibodies in providing immunity against disease is not fully understood (25,40). There is no specific level of RVNA that is recognized as being ‘protective’ against rabies in humans, although WHO recommends an antibody level of 0.5 IU/mL as being proof of an adequate immune response after vaccination (13,23).

2.4 Role of passive immunity

Due to the critical role that RVNA plays in destroying by neutralizing rabies virus, the level of protection against this disease can be enhanced through the immediate administration of RIG into wounds inflicted by a rabid animal. RIG administration delivers RVNA specifically targeted against rabies virus to the anatomical region where it was injected during the trauma of the exposure. Convincing clinical evidence proving that the administration of anti-rabies antiserum (in conjunction with vaccine) into patients that were severely exposed to rabid animals reduced the risk of rabies, was collected during a field study in Iran in 1954 (44). In this early study, different doses of anti-rabies serum and/or vaccine were administered to 29 patients that had received severe bite wounds from one rabid wolf. Of the 29 bite victims, 17 that had incurred severe head wounds were treated as follows: five patients received two doses of anti-rabies serum plus vaccine (all five patients survived); seven patients received one dose of anti-rabies serum plus vaccine (one patient subsequently died of rabies), and five patients received only vaccine (three patients subsequently died of rabies). One six-year old patient that had received exceptionally deep head wounds including a crushed skull received six doses of serum over a six-day period, plus vaccine, and survived. The other patients involved in the exposure were bitten in the trunk and legs and were administered either vaccine alone, or vaccine and serum. All of these patients survived.
RIGs should be infiltrated into and around the wound sites of patients bitten by rabid animals in order to neutralize rabies virus that may have been deposited in tissues during an exposure (13). Human rabies immune globulin (HRIG) produced in human subjects is administered at a dose of 20 IU/Kg of body weight, and equine rabies immune globulin (ERIG) produced in horses is administered at a dose of 40 IU/Kg of body weight. Unfortunately, due to the expense and lack of availability of RIGs, not all patients that should receive passive immunity as part of PEP actually have access to this life-saving product (13,15,45). Although the administration of vaccine alone will save most patients, some patients will need to receive passive immunity immediately in order to survive (46). Patients with bites into highly innervated regions such as the head or hands, and those that have deep or multiple wounds, are the most vulnerable and most in need of RIG (46,47). Specific recommendations for administration of RIG as part of PEP is detailed elsewhere (http://www.who.int/rabies/human/en/index.html) (13,23).

2.5 Routes of active immunization

The first CCVs, initially administered IM, were seen as the solution to replace early reactogenic nerve tissue vaccines (NTVs) that normally induced a low or moderate immune response (48). However, the high cost of CCVs relative to the cost of NTVs, and the large number of patients that required PEP in canine rabies endemic countries, initially curtailed the widespread use of CCVs. In an effort to alleviate the situation by reducing the cost of CCVs without lowering the efficiency of the vaccine, clinical trials were conducted to investigate the efficacy of ID regimens using a fraction (60% to 80%) of the IM vaccine dose for PEP (49,50,51).

Over the past two decades, results published from several clinical trials have confirmed the immunogenicity and efficacy of the ID route for rabies PEP which is currently being used effectively in many Asian countries, including India, the Philippines, Sri Lanka and Thailand (51,52,53). The ability of the ID route to induce an immunological response results from the fact that the skin is an effective immune organ and vaccine efficacy is enhanced when antigens are presented into the dermal layer (54,55,56). Furthermore, the administration of antigens into the skin layer facilitates their exposure to the numerous antigen-presenting cells, such as macrophages and dendritic cells, that are present in higher numbers in skin than in muscle.

2.6 Immune response in different populations

CCVs are among the most immunogenic vaccines in the world, as is evidenced by the very few reported human rabies deaths in patients that received prompt PEP according to the WHO recommendations (25,28,57). Rabies vaccines are highly immunogenic in almost every population, with perhaps the exception of patients having very low CD4+ cells (58–61). In one study which investigated the immune response of CCVs in selected populations of human immunodeficiency virus (HIV) infected adults, only 57% of symptomatic HIV-infected patients with CD4+ counts below 400 developed a measurable RVNA response above 0.5 IU/mL after receiving a 5-dose regimen of PEP intramuscularly on days 0, 3, 7, 14 and 30 (58). In another study, 10 HIV-infected adults with CD4+ counts between 25 and 472 were given a multi-site PEP regimen whereby four doses of CCV were administered ID on days 0, 3 and 7, and two doses of CCV were administered ID on days 28 and 90 (“4-4-4-0-2-2”) (60).
A lower than expected immune response was reported in all 10 patients; two of the patients did not develop RVNA titres above 0.5 IU/ml by day 14, and one of those patients did not develop RVNA above 0.5 IU/ml by day 30. In another study, the immune response to a three-dose IM PrEP regimen was examined in 13 HIV-infected children with CD4+ counts that were below normal, and compared to the immune response in nine uninfected children (62). In this study, children that had fewer than 15% of the normal CD4+ cells had significantly lower RVNA titres when compared with the control group and four of the 13 HIV-infected children failed to develop any measurable RVNA. In a more recent study that examined the immune response after vaccination with CCVs in HIV-infected patients receiving highly-active antiretroviral therapy (HAART), slightly lower IgG and IgM titres were reported in older patients infected with HIV (63). However, this study also reported that 63% of patients receiving HAART still had measurable antibody titres five years after primary vaccination.

In another study evaluating more effective vaccination protocols in immunosuppressed patients, two groups of HIV-positive subjects, one with CD4+ counts below 200 and the other with CD4+ counts above 200, received a modified multiple eight-site series of PEP consisting of eight intradermal injections on each of days 0, 3, 7, 14 and 30. All subjects responded with titres above 0.5 IU/mL (64). PEP administered to children that were exposed to rabies while receiving immunosuppressive therapy after solid organ transplants, was also reported to be successful in all patients (65).

**Infants and the elderly:** The immune response to rabies vaccine in infants and the elderly, without specific immunosuppressive conditions, is reported to be adequate. In a report published that reviewed two studies examining the immune responses in subjects of various ages, a reduction was observed in the level of RVNA after vaccination in older individuals (66). In one of the reported studies, the immune response of 260 subjects between the age of 11 to 25 that received a six-dose PEP regimen was compared to patients receiving the same regimen that were above the age of 50. In this study, 52% of the adults above 50 years of age had significantly lower RVNA titres after PEP compared to the younger cohort (67). In another study involving 875 patients aged between 2–74 years of age that received either PEP or PrEP, no significant difference in the production of RVNA compared to either age or sex was reported (68). The immune response to rabies PEP was also reported to be highly immunogenic in children with confirmed malnutrition, between Grade I and Grade IV (41).

**Patients taking chloroquine for anti-malarial treatment:** The administration of rabies vaccine by the ID route has been reported to produce reduced titres in patients taking chloroquine for anti-malarial treatment and, for this reason, vaccines should be administered to this group of patients using the IM route (13,69,70).

**Pregnant women:** Rabies PEP is not counterindicated for pregnant women and is immunogenic, safe and highly efficacious in this population (13). Rabies PEP should never be withheld from pregnant women as it is a life-saving vaccine. No reported risk of abortion, and no other harm to the fetus, has been reported due to administration of PEP with CCV in pregnant women (71,72).
3. Duration of immunity after immunization

3.1 Development of immunity

The development of immunological memory after immunization with CCVs is a critical component in the establishment of long-lasting immunity against rabies in humans (3). Of the millions of persons that have received CCVs, less than a handful of vaccination failures have been reported, all of which occurred in developing countries, and most of which involved deviations from the WHO recommended PEP protocol (25,28). Although one human death has been reported in a person that was previously vaccinated with a CCV and subsequently exposed to a rabid puppy (70), this patient did not seek, nor was she given the WHO recommended PEP booster series after the exposure occurred. The results of several clinical trials and retrospective studies have been published providing evidence that CCVs provide long-lasting immunity to rabies.

3.2 Duration of rabies virus-neutralizing antibody

The measurement of RVNAs is the most convenient method of confirming an immunological response after rabies PrEP or PEP. The relationship between the number of doses a patient receives during the initial vaccination (PrEP or PEP) and the longevity of circulating RVNA has been examined in several studies. In one retrospective study, a Kaplan-Meier survival analysis was used to evaluate the longevity of antibody in 875 patients that received either a primary three-dose (IM or ID) PrEP series or a five-dose IM PEP series of human diploid cell vaccine (HDCV) (68). The study reported no significant difference between the number of doses of vaccine a patient received and the length of time after initial vaccination that RVNA could be detected. In this study, no booster dose of vaccine was administered after the primary series, and blood samples from patients were tested at various time intervals up to nine years after primary vaccination. Circulating RVNA was detectable for a longer period of time in patients that were vaccinated IM as opposed to patients that had been vaccinated by the ID route, with approximately 80% of patients that had received vaccination by the IM route still having detectable RVNA titres nine years after primary vaccination.
The longevity of the humoral immune response was also evaluated in 18 patients that had received their primary series of PrEP or PEP using HDCV or purified chick embryo cell vaccine (PCECV) from two to 14 years previously (73). The patients in this study did not receive a booster vaccination between their initial series and the subsequent drawing of blood that was analyzed for the presence of RVNA. All patients in the study had detectable RVNA titres up to 14 years after having received their initial vaccination. In another study, levels of RVNA were evaluated in 58 patients that received, more than five years previously, PEP using HDCV, purified Vero cell rabies vaccine (PVRV), purified duck embryo cell vaccine (PDEV) or PCECV by either the Essen IM or Thai Red Cross ID regimen (74). All patients had detectable RVNA at the time their blood was drawn. In a more recent study examining the longevity of antibody and the effect of booster vaccination in 118 patients aged 16 to 78 years and vaccinated five to 21 years previously with either HDCV or PVRV, all patients had detectable antibody titres when they were tested prior to being given a booster dose of vaccine (75).

Finally, a study conducted in 29 travellers who had received their initial PreP using HDCV by the ID route reported long-lasting immunity in patients that had received their primary vaccination between two and >10 years previously (76).

In addition to published data delineating the extended duration of circulating RVNA in patients that received only a primary PrEP or PEP vaccination series without an additional booster vaccination at one year post-primary vaccination, studies have reported long-lasting RVNA in patients that have received a primary series of PrEP followed by one booster one year later. In one study, 312 subjects were followed for 10 years after having received either a 2-dose or 3-dose PrEP regimen, with either HDCV or PVRV, and one booster dose of vaccine one year later (77). The results indicate that approximately 96% of all subjects that received the 3-dose PrEP regimen followed by one dose of vaccine one year later still had measurable RVNA 10 years after having received their initial series. Similar results were reported in another study where 10 subjects that had received their initial PrEP series with PCECV 14 years earlier were administered a booster dose one year later (78).

In another study conducted in 72 Vietnamese children, half of the children received a 3-dose series of a combined diphtheria, tetanus, whole cell pertussis and inactivated poliomyelitis vaccine (DTP-IPV) along with three doses of PVRV given at two and four months and one year, and the other half of the children received only DTP-IPV (33). Results from this study indicate that rabies vaccines had no effect on the long-term antibody levels of diphtheria and poliomyelitis, and the majority of children continued to have measurable RVNA titres throughout the five-year follow-up study. Similarly, a study was conducted in 200 Thai children that were vaccinated with PCECV in either a 2- or 3-dose IM or ID PreP regimen concommitent with Japanese encephalitis vaccine (JEV), followed by a booster dose of PCECV either IM or ID (as per the original route of vaccination) one year later, and a booster dose of JEV (79). Three years after primary vaccination, all children that received their initial PrEP series by the IM route or that received a 3-dose PrEP ID regimen still had detectable RVNA.
3.3 Anamnestic response

Two of the arguments in favour of administering PrEP to persons at risk of contracting rabies are that if these persons are subsequently exposed to rabies:

1) a short series of booster vaccinations will elicit a rapid anamnestic response thus reducing the number of doses of vaccine and visits required for a full PEP;
2) RIG is not required (13).

Several published clinical trials provide data confirming that a previously vaccinated person will respond to one or more booster doses of rabies vaccine even if the initial series of PrEP or PEP was administered several years previously; regardless of whether the initial vaccination regimen was administered IM or ID; regardless of whether they are boosted using the ID or IM route, and independent of whether the previously vaccinated person has detectable RVNA or not (74,75,80,81,82). A three-year study conducted in 194 subjects who initially received one, two, or three doses of HDCV administered either by the IM or ID route, and were boosted six to 24 months later with one dose of HDCV administered by the ID or IM route, reported the highest titres and longest lasting antibodies in the subjects that had received an initial 3-dose vaccination series (ID or IM) (80). All subjects in this study, regardless of whether they had received one additional dose of vaccine ID or IM, had an anamnestic response when boosted 6, 12, or 24 months later.

Another study reported that an anamnestic response occurred in 76 individuals initially vaccinated with HDCV by the ID route and then boosted two years later with one ID dose of HDCV (81). The anamnestic response occurred in all individuals, regardless of whether the subjects had a detectable antibody titre just prior to the administration of the booster, or not. Similar results were reported in a study where 29 travellers were initially vaccinated with a 3-dose ID HDCV regimen and boosted with one IM dose of HDCV 12 to 14 months later (82). In this study, all persons developed an anamnestic response even though some did not have detectable titres at the time that they were boosted. In another study, the immune response of 57 patients vaccinated for PEP, either by the 5-dose Essen IM regimen or the ID Thai Red Cross regimen, were evaluated for a subsequent anamnestic response after receiving a booster vaccination (74). In this study, patients were vaccinated 5–10 years previously with HDCV, PCECV, PVRV or PDEV, and titres were evaluated after patients were boosted with two ID doses of PDEV. All patients developed an anamnestic response after boosters were administered, and there was no significant difference in the antibody level in patients that had received vaccination 5–10 years earlier and those that had been vaccinated more than 10 years previously. In another study, the immunological response was examined in 118 patients that had received primary PEP or PrEP with HDCV or PVRV five to 21 years earlier and were boosted with two ID doses of PVRV to determine if they would mount an anamnestic response (75). In this study, all patients vaccinated up to 21 years previously developed an immunological response with no significant difference in the level of titres in patients that received PrEP or PEP, nor in the length of time since their initial vaccination was administered.
3.4 Timeliness of routine booster vaccination

Due to the fact that rabies is virtually 100% fatal once clinical symptoms are evident, and because until recently no long-term immunity studies were available, the recommendations for timely routine booster doses of rabies vaccine after a primary series has varied from one to five years. However, several recently-published clinical trials have shown that individuals that have received an initial 3- to 5-dose series of rabies CCVs will have long-term immunity lasting for decades (74, 75, 76, 82). These published data indicate that individuals that received their primary series up to 21 years previously will elicit a good anamnestic response after booster vaccination.

As mentioned earlier, individuals vaccinated with a CCV will respond to a booster vaccination regardless of whether the vaccinated individual had measurable antibody present or not at the time that the booster was administered (81, 82, 83). A recent survival case in which a human patient that had been unknowingly given a transplanted liver from a donor that was later diagnosed as having rabies provides an indication as to the efficacy of rabies vaccines (84). The patient that received the infected liver survived, whereas the recipients of the two kidneys and pancreas from the infected donor died of rabies encephalitis within three weeks following transplant. Further investigation revealed that the liver recipient had received rabies vaccination as a child.
4. Techniques to measure the immune response

4.1 Choosing the test to fit the purpose

Numerous assays are available to test for the presence of rabies virus in the tissues of infected mammals, and to confirm evidence of a humoral or cellular immune response after exposure to rabies viral antigens (85–89,96). Ultimately, the intended purpose of an assay and the precision of the data required should be the determining factors when choosing a testing procedure. For example, confirming herd immunity after oral vaccination in animals generally does not require the same level of accuracy as does evaluating the immunogenicity of a new rabies vaccine for humans, or when serology testing is employed as part of the diagnostic workup for human rabies patients (90,91,92). The importance of the level of quality assurance associated with conducting any assay for evaluating an immune response for diagnoses of a patient, or after immunization, or for identifying rabies antigen in tissue samples, cannot be overstated, due to the consequences associated with a misdiagnosis (93). To summarize, the sensitivity and specificity of an assay, the accuracy and precision required by the investigator or clinician, the laboratory facilities that are available, and the purpose of the data to be collected all need to be critically evaluated before testing of a sample’s status is initiated (92).

4.2 Virus neutralization assays

Virus-neutralization assays are among the most widely-used methods to detect the presence of antibody to the rabies virus. RVNAs are not only responsible for protection against rabies, the presence of RVNA in serum is seen as a reliable indicator of active immunization after vaccination (13,89). The rapid fluorescent focus inhibition test (RFFIT) and the fluorescent antibody virus neutralization (FAVN) test are both in vitro virus-neutralization assays. Both the RFFIT and the FAVN test are equivalent when conducted under good laboratory practices and both are considered to be the most efficient methods for accurate measurement of RVNA (13,90,94,95). A third virus-neutralization assay, the mouse neutralization test (MNT), is an in vivo method to measure RVNA that is still utilized in some laboratories that lack the capacity to conduct in vitro tests (96). Methodologies for all three virus-neutralization assays are published elsewhere (85,86,96,97,98). Virus-neutralization assays are valuable tools that can confirm the presence of protective antibodies specifically targeted against rabies virus, but they are also highly complex tests to perform and must be conducted by experienced personnel in a high containment facility (92,93). It is advisable for diagnostic laboratories performing either one or both of these types of RVNA assays to participate in an established quality assurance programme (92).
4.3 Binding assays

The ELISA is the most frequently used binding assay available, with numerous published protocols and professionally marketed ELISA kits available to detect rabies antibodies (99–103). The specificity of the ELISA is dependent upon the choice of the target antigen used in the test — whole virus or purified viral proteins. Antibodies detected in an ELISA do not necessarily have a neutralizing function (92). Published reports indicate that cross-reactivity, potentially leading to false positives, may increase in ELISA assays that employ whole virus rather than purified G as the target antigen (99,103). Several studies have been published comparing results from serum samples tested by various ELISA techniques, and by the RFFIT or FAVN test, with mixed results (89,102,104,105). Newer ELISA protocols and ELISA kits with increased specificity, such as the PLATELLA™ RABIES II, have been reported to have better correlation with the RFFIT and FAVN test. For example, in comparative testing with the RFFIT, the sensitivity and specificity of the PLATELLA™ RABIES II ELISA kit was reported to be approximately 95% (102). Additionally, a competitive ELISA has been developed, using highly-purified G as the target antigen, that reportedly will bind neutralizing-related antibodies (103). In the development of this competitive ELISA, 4350 canine serum samples were tested in a comparative study using the FAVN test where the results indicated that there were no false positives or negatives and that there was a correlation between serological titre results as determined by the ELISA and FAVN test of 96.2% (103). The remaining 3.8% of serum samples tested had titres above the level of 8.0 IU/mL when assayed by both testing methods, and the serological titre results from both tests were more divergent at high titre levels.

4.4 Measuring cell-mediated immunity

Assays to measure a cell-mediated immune response are usually used for research purposes only because they are more complicated to perform on a routine basis than serological assays. Detection of a cell-mediated immune response is commonly achieved by measuring an increase in lymphocyte proliferation using a [H³]thymidine assay. Methodologies for [H³]thymidine assays are published elsewhere (36,73). Newer assays have been developed to measure cell-mediated immunity that utilize cell-tracking dyes in conjunction with flow cytometry and are able to quantify the response of specific types of lymphocytes to rabies antigen (36,106).
5. Innocuity and efficacy of rabies biologicals

The development and widespread use of rabies biologicals prepared on cell culture have dramatically increased the safety and efficacy of PEP (23). Failures of rabies PEP have been reported in some patients in developing countries, but in most of these cases some deviation was reported from the WHO recommended PEP protocol (25,28,57). Generally, the reasons associated with ‘treatment’ failures (where there was a failure to follow the correct PEP protocol) include: delays in seeking treatment; lack of, or improper primary wound care; lack of, or improper administration of RIG; suturing wounds without infiltrating with RIG, or poor quality rabies vaccines (25,28,107). The number of ‘true’ PEP failures (where a patient died despite receiving the correct PEP protocol in a timely manner) are very low when compared to the millions of doses of CCVs administered globally each year (25,107). Short incubation periods of less than one week have been reported in patients that have severe head wounds, such as in patients that have sustained brachial-lexus injuries from dog bites (20). In one paper that examined case records from 15 human rabies patients reported worldwide, it was concluded that seven patients received PEP in a timely and appropriate manner, and still died of rabies (28). The paper discusses potential reasons for these failures, including the possibility that a small unidentified wound may have been overlooked, that perhaps one or more of the patients may have had an underlying immunosuppressive condition, that the biologicals used to treat these patients were of low potency, or perhaps that the treatment protocols were misrepresented. In any case, all of the treatment failures occurred after dog bites, and the paper stresses the fact that, on rare occasions treatment failures may occur, even with CCVs and RIGs.

Local and systemic reactions have been recorded after the administration of CCVs in clinical trials (30,31,32,69). These studies generally reported local reactions, including pain, itchiness, redness and/or swelling at the site of injection in 35% to 45% of the enrolled subjects. Common systemic reactions, which are usually reported in 10% to 15% of subjects include fever, myalgia, malaise, headaches, dizziness, hives and rash (108).
6. Future prospects

The CCVs currently recommended by WHO are among the most efficacious vaccines to combat disease available in the world. Only a few human deaths have been reported in the literature in cases when WHO-recommended PEP protocols were strictly adhered to (25,28). In spite of this, rabies continues to kill tens of thousands of people and cost billions of US dollars annually (15). Most of the burden of rabies is felt by those living in poor countries that can least afford to provide adequate PEP treatment. Unfortunately also, rabies continues to spread to previously rabies-free areas and to cause human fatalities, for example, the recent introduction of canine rabies on to the island of Bali (109).

Although clinical rabies is preventable, even after exposure, a lack of educational awareness is one of the major reasons why humans exposed to bites from infected animals do not seek proper treatment after exposure (29). The cost of rabies biologicals, and the frequent necessity to travel long distances over extended periods to receive one of the recommended WHO PEP regimens, are also deterrents for persons exposed to rabid animals (110). Newer rabies biologicals, for example monoclonal antibodies targeted against rabies virus antigens that are currently undergoing clinical trials, or are being evaluated for clinical trials, will hopefully provide a wider global access to passive immunization at a reduced cost (111,112). Reduced-dose regimens for PEP and PrEP, that can be completed within one week, are currently under evaluation, and may also provide a solution to reduce the expense for patients that cannot afford to travel to clinics located outside their immediate area to receive multiple doses of rabies vaccine over extended periods of time (113). Molecular techniques are providing new concepts for the development of rabies vaccines, for example, subunit vaccines and safe modified-live viral vaccines, that could reduce the number of doses required for PEP and PrEP to one, and significantly lower the cost to protect an entire population (26,114,115). In addition, diagnostic tests, such as the dRIT, that are inexpensive, simple to use and capable of rapidly producing accurate data, will help facilitate surveillance in many poor settings. Finally, it is only through the introduction and embodiment of comprehensive rabies control strategies, including animal control programmes, PEP and PrEP for humans, educational programmes and financial commitment, and ultimately cooperation between public-health professionals, research scientists, laboratory technologists, not-for-profit organizations and government officials that rabies will eventually be controlled (13,19,116,117).
References


The World Health Organization has provided technical support to its Member States in the field of vaccine-preventable diseases since 1975. The office carrying out this function at WHO headquarters is the Department of Immunization, Vaccines and Biologicals (IVB).

IVB’s mission is the achievement of a world in which all people at risk are protected against vaccine-preventable diseases. The Department covers a range of activities including research and development, standard-setting, vaccine regulation and quality, vaccine supply and immunization financing, and immunization system strengthening.

These activities are carried out by three technical units: the Initiative for Vaccine Research; the Quality, Safety and Standards team; and the Expanded Programme on Immunization.

The Initiative for Vaccine Research guides, facilitates and provides a vision for worldwide vaccine and immunization technology research and development efforts. It focuses on current and emerging diseases of global public health importance, including pandemic influenza. Its main activities cover: i) research and development of key candidate vaccines; ii) implementation research to promote evidence-based decision-making on the early introduction of new vaccines; and iii) promotion of the development, evaluation and future availability of HIV, tuberculosis and malaria vaccines.

The Quality, Safety and Standards team focuses on supporting the use of vaccines, other biological products and immunization-related equipment that meet current international norms and standards of quality and safety. Activities cover: i) setting norms and standards and establishing reference preparation materials; ii) ensuring the use of quality vaccines and immunization equipment through prequalification activities and strengthening national regulatory authorities; and iii) monitoring, assessing and responding to immunization safety issues of global concern.

The Expanded Programme on Immunization focuses on maximizing access to high quality immunization services, accelerating disease control and linking to other health interventions that can be delivered during immunization contacts. Activities cover: i) immunization systems strengthening, including expansion of immunization services beyond the infant age group; ii) accelerated control of measles and maternal and neonatal tetanus; iii) introduction of new and underutilized vaccines; iv) vaccine supply and immunization financing; and v) disease surveillance and immunization coverage monitoring for tracking global progress.

The Director’s Office directs the work of these units through oversight of immunization programme policy, planning, coordination and management. It also mobilizes resources and carries out communication, advocacy and media-related work.