WHO Position Paper on Rabies vaccine. Selected references

General reviews and epidemiology


According to official WHO data more than 2.5 billion people are at risk in over 100 countries reporting the disease. Rabies mortality ranks ten in all infectious diseases worldwide. There are still about 50,000 to 60,000 human deaths annually although effective vaccines for post-exposure treatment are available. Most affected are the tropical countries in Africa, Asia, South America, and Oceania. The mortality figures range from about 0.001 per 100,000 for the US to 18 per 100,000 in Ethiopia. The vast majority (95-98%) of the 60,000 annual human death cases worldwide occur in canine (dog rabies) endemic regions with large stray dog population. Control of the disease is hampered by cultural, social and economic realities. In the rabies infested developing countries modern cell culture vaccines are hardly affordable. Dangerous neural tissue derived vaccines are still used. Three dose-saving treatment schedules have been developed: The reduced dose intramuscular 2-1-1 regimen, the two-site intradermal and the 8-site intradermal regimen. There is a critical shortage of human and purified equine rabies immunoglobulins, which are essential biologicals in the treatment of severe exposures.


Rabies is an acute, progressive, incurable viral encephalitis. The causative agents are neurotropic RNA viruses in the family Rhabdoviridae, genus Lyssavirus. Mammalian reservoirs include the Carnivora and Chiroptera, but rabid dogs still pose the greatest hazard worldwide. Viral transmission occurs mainly via animal bite, and once the virus is deposited in peripheral wounds, centripetal passage occurs towards the central nervous system. After viral replication, there is centrifugal spread to major exit portals, the salivary glands. The epidemiological significance of any host "carrier" state remains highly speculative. Although incubation periods average 1-3 months, disease occurrence days or years after exposure has been documented. Rabies should be suspected in patients with a concomitant history of animal bite and traditional clinical presentation, but a lack of such clues makes antemortem diagnosis a challenge. Pathogenetic mechanisms remain poorly understood, and current care entails palliative measures only. Current medical emphasis relies heavily on prevention of exposure and intervention before clinical onset. Prophylaxis encompasses thorough wound treatment, vaccine administration, and inoculation of rabies immunoglobulin. Although it is a major zoonosis, canine rabies can be eliminated, and application of new vaccine technologies permits significant disease control among wildlife species. Nevertheless, despite much technical progress in the past century, rabies is a disease of neglect and presents a modern public-health conundrum.

From 1 July 1987 to 31 December 1988, a total of 317 animals (91% of which were dogs) were confirmed to have rabies in Hermosillo, Mexico. The median age of rabid dogs was 1 year, 69% were male, and 98% were owned. The epizootic started in the southern areas of the city, rapidly involved the entire city, and persisted mainly in lower socioeconomic status areas. The area of the city and mean household size were significant predictor variables for the population density of rabid dogs around household clusters (Poisson linear regression, $P < 0.001$ and $P = 0.03$, resp). Approximately 2.5% of city residents were bitten by dogs in 1987, with the rate of reported dog bite injuries being positively correlated with mean household size and the proportion of households that owned dogs. Visits to the city health centre for evaluation of possible exposures to rabies increased by 135% after the start of the epizootic; approximately 273 per 100,000 city residents were administered a full or partial course of rabies post-exposure prophylaxis in 1987. Children were at greatest risk for exposures to rabies, accounting for 60% of all reported animal bite injuries evaluated at the health centre. Also they were more likely than older persons to have received bite injuries to the head, face, and neck (odds ratio = 21.6, 95% confidence interval = 5.4, 186.5).

**PIP:** Almost all cases of human rabies result from bites by rabid dogs. Controlling dog rabies is therefore crucial for humans. 317 animals, 91% of which were dogs, were confirmed to have rabies in Hermosillo, Mexico, from July 1, 1987, to December 31, 1988. The dogs were of median age 1 year, 69% were male, and 98% were owned. The epizootic started in the southern areas of the city, spread quickly through the city, and persisted largely in lower socioeconomic status areas. Approximately 2.5% of city residents were bitten by dogs in 1987, with the rate of reported dog bite injuries positively correlated with mean household size and the proportion of households which owned dogs. Visits to the city health center for evaluation of possible exposures to rabies increased by 135% after the start of the epizootic over which approximately 273 per 100,000 city residents were administered a full or partial course of rabies post-exposure prophylaxis in 1987. Comprising 60% of all reported animal bite injuries evaluated at the center, children were at greatest risk for exposures to rabies. Children were also more likely than older people to have received bite injuries to the head, face, and neck.


**OBJECTIVE:** To describe the epidemiological characteristics of rabies in Delhi in 1998. **METHODS:** Analysis of the records of hydrophobia cases admitted to the Infectious Diseases Hospital, Delhi (IDH) in 1998. **RESULTS:** About 46 percent (99/215) of the hydrophobia cases admitted to the IDH in 1998 belonged to Delhi. The remaining came from the adjoining states, both urban and rural areas. In Delhi
residents, overall hospitalization rate was 0.81 per 100,000 population. It was significantly higher in 5-14 year old than in other age groups and in males than in females (p <0.0009). Cases occurred round the year. Almost 96 percent cases (206/215) gave history of animal exposure, 13 days to 10 years (median 60 days) before hospitalization. Majority (195/206) had class III exposure. Animals involved were stray dog (193/206 = 90 percent), pet dog, cat, jackal, mongoose, monkey and fox. Most of cases were never vaccinated (78 percent) or inadequately vaccinated (22 percent); only 1 percent each received appropriate wound treatment, or rabies immunoglobulin. CONCLUSIONS: Rabies is a major public health problem in Delhi. Its incidence is significantly higher in 5-14 year old children than in other age groups. The results indicate the need to educate the community and health care workers about the importance of immediate and adequate post-exposure treatment and to start an effective control program for dogs, the principal vector of rabies.


The existing literature on the economics of rabies and its control can be characterised as a poorly documented set of cost estimates with insufficient information to allow replication of the analyses. Most articles have numerous 'violations' of the standard recommended procedures for assessing the burden of disease and the cost and benefits of interventions. Per capita costs are often crudely extrapolated from small to large populations without allowing for geographic differences in incidence. Furthermore, most studies do not distinguish between financial charges and true economic costs, and only a few articles contain a multiyear framework, complete with discounting of future costs and benefits. With the exception of the increase in average incidence of postexposure prophylaxes (PEPs) in Asia, the average incidences of both human-rabies cases and PEPs in Africa, the Americas and Europe have not changed significantly over time. There are, however, large differences between countries within a region and regional averages can conceal notable changes in incidences over time for a given country. The largest number of human-rabies cases occur in developing countries due to the low levels of vaccination among dogs, the high cost of biologicals for PEP and problems of availability. The costs (1995 values) of PEP range from $US1707 per person in Massachusetts, US, to $US2.50 for a complete series of vaccinations (without immunoglobulin) using sheep-derived vaccines in Karachi, Pakistan. Most studies which reported the cost of PEP, however, provided only direct medical costs and did not consider indirect costs such as lost productivity due to death, permanent disability or time spent while receiving medical care. Given the expense of controlling rabies in dogs and wildlife, there is an urgent need to develop a cheaper human-rabies vaccine or further refine the 'low-dose' PEP regimes. PEP is often given unnecessarily, and experience with expert consultations systems and algorithms has shown that the rate, and therefore total cost, of PEP can be significantly reduced. However, because it may be difficult to identify lesions from a bite by a bat, algorithms may be of less value when dealing with possible exposure to bat rabies. Using US prices and values, only 2 individuals per 1000 possible contacts have to be at risk from bat rabies in order for it to be economically justifiable to give PEP to all those potentially exposed to bat rabies. With regard to pre-exposure vaccination, routine use of pre-exposure has generally not been shown to be cost effective.
Laboratory diagnosis


Brain tissue-based vaccines


Semple rabies vaccine is composed of rabies virus-infected sheep or goat brain inactivated with phenol and is administered daily after exposure for 14-21 days. Semple rabies vaccine-induced autoimmune encephalomyelitis (SAE) has clinicopathological findings of demyelination similar to experimental autoimmune encephalomyelitis (EAE) caused by injection of central nervous system tissue or purified myelin proteins into experimental animals and frequently studied as a model for the human demyelinating disease, multiple sclerosis (MS). T-cell-mediated immune responses play a major role in induction of EAE, and antibody responses enhance disease severity. We studied the antibody responses to myelin basic protein (MBP) in 24 Thai patients with SAE and 77 control individuals to define the linear epitopes in human MBP that are encephalitogenic. Antibody levels were assessed by ELISA using native human MBP or synthetic MBP peptides of 20 amino acids. The major B-cell epitope was MBP61-80 and a minor epitope was MBP106-140 in SAE while in MS the major B-cell epitope is MBP84-96. MBP61-80-specific IgG1 and IgG3 levels were significantly higher in patients than controls while IgG2 and IgG4 were not. The data support the hypothesis that autoreactive Th1 cells induce SAE. The difference in B-cell epitope recognition may be due to differences in the genetic backgrounds of the populations studied or may reflect underlying differences in the pathogenesis of SAE and MS.

Tissue culture/cell culture-based vaccines

Forty-five persons severely bitten by rabid dogs and wolves in Iran were treated after exposure with a new rabies vaccine produced in cultures of human diploid cells. All except one also received one injection of rabies immune serum. This treatment, in contrast to past experience with other vaccines, resulted in protection of all individuals against rabies. Thus, almost a century after the postexposure treatment of humans was initiated, an effective tool for protecting man against rabies has finally been developed.


Recent studies suggest that human diploid cell rabies vaccine (HDCV) may not always produce acceptable titers after intradermal (id) preexposure prophylaxis. To stimulate accidental deviation from the recommended route of administration and to determine the immunogenicity of smaller-than-recommended doses of HDCV, we injected each of 154 persons either intramuscularly (im) with 100%, 10%, or 3% of the standard im dose of vaccine or id with 10%, 3% or 1% of the standard im dose. Seroconversion (titers of antibody greater than or equal to 1:11) was found in all subjects at 49 and 90 days after vaccination. Titers were higher for subjects receiving 100% of the recommended dose im than for those receiving 10% of this dose id (P less than .01); these titers in turn were higher than those from persons receiving smaller doses (P less than .05). Persons receiving 10% or 3% of the standard im dose had lower titers on day 49 than did those receiving the same dose id (P less than .05).


The equivalence and interchangeability of Purified Chick Embryo Cell Culture Rabies Vaccine (PCECV) to Human Diploid Cell Culture Rabies Vaccine (HDCV) and the immunogenicity of a reduced post-exposure regimen with PCECV was investigated. Statistical analyses revealed no difference (P</=0.05) between the geometric mean titers (GMT) on day 49 of subjects that received PCECV or HDCV. In Year 2, subjects were boosted with one or two dose(s) of PCECV. No significant difference (P</=0.05) was detected between the GMT of the two groups on days 7 and 365 post-booster. Subjects that received HDCV initially developed an adequate anamnestic response to PCECV. On day 21 post-booster, the GMT of subjects that received two boosters was higher (151.6 IU/ml) than those that received one booster (120.9 IU/ml). However, this difference may not be clinically significant.

One thousand three hundred and seventy-five (1375) persons, who were vaccinated against Rabies with Purified Chick Embryo Cell (PCEC) vaccine from 1984 to 1993, were included in this ten-year longitudinal study, conducted to observe the consistency, immunogenicity, inocuity, safety and efficacy of PCEC vaccine under controlled trial and field conditions. The study period was divided into three phases. Phases I and II covered the premarketing controlled trial and Phase III the post-marketing serosurveillance study of the vaccine. During Phase I, fifteen healthy volunteers were given a pre-exposure regime of vaccine on Day 0, 7 and 21, and the rest 15, simulated post-exposure regime on Day 0, 3, 7, 14, 30 and 90. All the subjects had satisfactory antirabies antibody response with mean titres, of 7.08 and 5.72 I.U./ml respectively, and minimal side reactions. In the Phase II, from 1984-85, 56 persons with proven rabid animal bites were given post-exposure vaccination and all had satisfactory antibody titres with mean titre of 4.45 I.U./ml after 6th dose of vaccine and with minimal side reactions. 19 to 36 months follow up after vaccination revealed no vaccine failures. In the Phase III post-marketing field study conducted from 1985 to 1993, 1289 persons reported to our Centre for consultation and antirabies antibody titre estimation following PCEC vaccination. One thousand two hundred and fifty-two (1252) persons took post-exposure vaccination following bites by rabid animals, contact with an hydrophobia patient and 37 high risk personnel took pre-exposure vaccination. (ABSTRACT TRUNCATED AT 250 WORDS)


Three hundred and nine (309) persons, vaccinated against rabies with Purified Vero-cell Rabies (PVR) vaccine from 1991-1995, were included in this five-year longitudinal study. This study was conducted to observe the consistency, immunogenicity, inocuity, safety and efficacy of this vaccine under field conditions. All the 309 persons attended our centre after taking post-exposure vaccination following bites by suspected rabid animals or contact with hydrophobia patients for antirabies antibody titre estimation. The vaccine was very well tolerated by vaccinees with only 7 per cent, complaining of mild to moderate side reactions. On an average, every year 70-100 vaccinees reported at this centre after PVR vaccination. The epidemiological characteristics of rabies based on above data are also discussed in this paper. Serological response, i.e., antirabies antibody titre following vaccination in all these persons were found to be satisfactory with mean antibody titre of 4.25 I.U./ml.


Recent improvements in chromatographic purification procedures have made it possible to develop a new chromatographically purified rabies vaccine (CPRV) by further purifying the current rabies vaccine prepared from Vero-cell culture (Verorab; Pasteur Merieux Connaught). The immunogenicity and safety of primary
immunization, followed by a booster at one year, with CPRV was compared to that of the purified Vero cell vaccine (PVRV) in a randomized, double-blind study carried out at four veterinary schools in France. A total of 330 healthy, male and female, first-year veterinary students, aged at least 18 years and who required pre-exposure rabies prophylaxis, were enrolled in this study. Included subjects were randomly assigned either CPRV (*n* = 163) or PVRV (*n* = 167) to be given as a primary immunization series of three intramuscular injections (D0, D7, D28), followed by a booster after 1 year (D365). Blood samples for serological analysis were taken at D0 (before first injection), D28, D42, D180, D365 (before booster) and D379. All subjects developed a strong immune response to the primary series, and at D42, all subjects had seroconverted for rabies neutralizing antibody (serum titre ≥ 0.5 IU/ml). The rabies virus-neutralizing antibody GMT value at D42 in the CPRV group (23.0 IU/ml) was non-inferior to that in the PVRV group (29.6 IU/ml), according to a one-sided non-inferiority test. While antibody titres tended to decrease over the period of follow-up, at D365 (before booster), 97.5% subjects in the CPRV group and 98.8% of subjects in the PVRV group remained seroconverted. After booster, although the antibodies antibody GMT value in the CPRV group was lower than that in the PVRV group, all subjects in both groups were seroconverted, and the difference is probably not clinically important. The incidence of local and systemic reactions tended to decrease with each dose during the primary immunization series, followed by a slight increase after booster (significant time-effect in an exploratory logistic regression analysis). Although mild or moderate local reactions tended to be more frequent after injection with CPRV compared to PVRV, systemic reactions were reported less often (significant group-effects in exploratory logistic regression analyses). One serious adverse event possibly related to vaccine occurred during this study (severe asthenia after the third dose of PVRV). This comparative study in healthy young adults demonstrates that the new chromatographically purified rabies vaccine is as immunogenic as PVRV, and seems to be associated with fewer systemic reactions.


Recent improvements in chromatographic purification procedures have made it possible to develop a new chromatographically purified rabies vaccine (CPRV) by further purifying the current rabies vaccine prepared from Vero-cell culture (PVRV) (Verorab; Pasteur Merieux Connaught). The immunogenicity and effectiveness of post-exposure rabies prophylaxis with this new vaccine were evaluated in a two-stage clinical trial conducted in the Philippines. In both study stages, post-exposure treatment consisted of five injections of vaccine [(D)ays 0, 3, 7, 14, 28], together with a dose of rabies immunoglobulin (RIG) of equine or human origin on D0. In stage 1, 231 subjects with low-risk rabies exposure (WHO category I or II), and who had a negative ERIG skin test, were treated with either CPRV (*n* = 114) or PVRV (*n* = 117). By D14, all subjects in each group had achieved rabies antibody titres over ten times that recommended by the WHO as indicating seroconversion (≥ 0.5 IU/ml). The kinetics of the immune response to vaccination were very similar in the two groups, and at D28, the immunogenicity of CPRV was equivalent to that of PVRV (one-sided equivalence test). Following these positive results, 132 subjects with severe rabies
exposure were included in the second stage of this trial. All were scheduled to receive four vaccine doses with CPRV. After D14, only those 57 patients with confirmed rabies exposure (animal with positive FA test) and seven patients for whom rabies exposure could not be excluded (animal lost or not tested) completed the treatment and were followed for one year to assess survival. After 1 year, 62 patients treated for confirmed or possible severe rabies exposure had been examined and were still alive. Two patients contacted by letter and telephone confirmed good health 7 and 16 months after exposure. No severe local or systemic reactions were reported in either stage of the study, and no treatment-related serious adverse event occurred. This two-stage clinical trial attests to the safety and satisfactory immunogenicity of CPRV in post-exposure rabies treatment, and confirms the effectiveness of a new rabies vaccine in patients with severe confirmed exposure.

**Schedules, storage and administration**


Neutralising antibody responses to six post-exposure regimens of human diploid cell strain rabies vaccine with or without human rabies immune globulin (HRIG) were studied in 98 patients. The total amount of vaccine used was 22-34% of that required by conventional regimens. Vaccine was given at multiple sites intradermally or subcutaneously with or without adjuvant. Antibody was detectable within 7 days of the first dose in all subjects only in the groups given 0.1 ml intradermally at 8 sites. From day 14 onwards all groups showed an excellent antibody response; there was little difference between the various regimens. Suppression of the response to 8-site intradermal vaccination by a large dose of HRIG could be prevented by giving the second dose of vaccine on day 7 rather than day 14.


These revised recommendations of the Advisory Committee on Immunization Practices update the previous recommendations on rabies prevention (MMWR 1991;40[No.RR-3]:1-14) to reflect the current status of rabies and antirabies biologics in the United States. This report includes new information about a human rabies vaccine approved for U.S. use in 1997, recommendations regarding exposure to bats, recommendations regarding an observation period for domestic ferrets, and changes in the local administration of rabies immune globulin.

An alternative strategy for pre-exposure rabies vaccination to the institutional recommendations of the World Health Organization and the Centers for Disease Control and Prevention is proposed based on recent long-term follow-up of post-vaccinal seroconversion rates. The alternative strategy uses the same primary series (i.e. vaccination in the deltoid area on D0, D7, and D28), but is completed by a scheduled booster vaccination at D365. The frequency of recommended subsequent booster injections depends on the serological test results obtained by a RFFIT on D379 and 3 years later. The objective of this study was to compare the efficiency of the two pre-exposure strategies. A cost-minimization analysis was carried out to compare the two rabies pre-exposure vaccination and serological test strategies based on the data from two published studies on the long-term evolution of the immunity achieved using the different recommendations. For a theoretically equivalent immunogenicity, the cost of the alternative strategy ranged from 1.7 to 5.2 times lower than that of the institutional recommendations. A sensitivity analysis confirmed the robustness of the results. The alternative strategy should be validated externally under field conditions. This approach would compare its real efficiency to the institutional recommendations.


WHO's reference protocol for post-exposure rabies vaccination advises five intramuscular injections on days 0, 3, 7, 14, and 30; in addition, rabies immunoglobulins (RIG) must be given to serious cases of exposure (grade III severity). Some studies indicate that these immunoglobulins suppress the immunogenicity of rabies vaccine when administered according to an alternative protocol of four injections (2-1-1) on days 0, 7, and 21, which was therefore not recommended for grade III exposures. To test this effect, we conducted a multicentre study in Indonesia using three groups of subjects. One group received only the Vero-cell rabies vaccine (PVRV, Verorab, usual commercial lot) according to the 2-1-1 schedule. The second and third groups received the same schedule of PVRV, plus either equine rabies immunoglobulins (ERIG, 40 IU/kg body weight) or human rabies immunoglobulins (HRIG, 20 IU/kg body weight). Our results confirmed the immunoglobulin suppressant effect, which was more pronounced with human than equine immunoglobulins. In both groups receiving immunoglobulins, the seroconversion rates did not reach 100% on day 28 and the geometric mean antibody titre was lower. Thus, WHO's recommendation in 1992 of the reference protocol plus immunoglobulins for severe cases is substantiated by these results in Indonesian subjects. If the 2-1-1 regimen is chosen by the treating physician and immunoglobulins are indicated, preference should be given to purified equine RIG, which also costs less than human RIG.

BACKGROUND: The prevention of rabies in Mexico continues to be an important goal for the health sector. Although the prevalence of this disease continues to fall, between 1990 and 1995 a total of 238 cases were registered (an average of 40 cases annually), with a mean annual incidence of 0.04 cases per 100,000 inhabitants and a mortality of almost 100%, so that it is important to rely on highly effective vaccines with few side effects. The objective of this work was to evaluate seroconversion and tolerance to the human diploid cell antirabies vaccine administered to individuals with a history of exposure to rabies, to compare these results with those reported in the literature for the Fuenzalida vaccine, a rabies vaccine produced in the brain tissue of suckling mice, and to find the role antirabies hyperimmune gamma globulin plays in the concentration of post-vaccination antibody concentrations. METHODS: An analytical transverse study was carried out in 40 children and adults with a history of rabies exposure who were given a complete, five-dose intramuscular schedule of the human diploid cell rabies vaccine. Subjects were followed daily, and local and systemic signs and symptoms were recorded. Two blood samples (at baseline and at the end of the vaccination schedule) were taken and antibody titers against rabies glycoprotein, using the ELISA technique, were measured. RESULTS: Adverse side effects produced by the human diploid cell antirabies vaccine, such as frequency of pain, erythema, itching, and regional adenopathy were fewer than those reported in the literature for the Fuenzalida vaccine (p < 0.05), and of induration and local pain (p < 0.05) in relation to the latter vaccine. All patients seroconverted, producing geometric mean antibody titers of 6.22 IU/mL, an arithmetic mean titer of 9.66 IU/mL with a SD of 9.1 IU/mL. The level of tolerance to the diploid cell vaccine was good and its adverse effects were minimal and fewer than those reported for the Fuenzalida rabies vaccine. Patients receiving the diploid cell vaccine plus antirabies hyperimmune gamma globulin developed higher antibody titers (measured by ELISA test) at the end of the vaccination schedule than those only receiving the vaccine. CONCLUSIONS: These results are important in order to achieve an adequate and opportune level of protection provided by prophylactic vaccines to patients with exposure to rabies.


The economical Thai Red Cross intradermal (TRC-ID) post-exposure rabies treatment schedule is now widely used in Asia. However, directives from WHO and manufacturers mandated that the vaccine be used within 8h after reconstitution of the freeze-dried product. This limits the use of TRC-ID to large animal bite clinics that see several rabies exposed patients daily. This study demonstrated that refrigerated purified chick embryo and Vero cell rabies vaccines can be stored safely for up to 7 days after reconstitution; allowing use of this treatment regimen in clinics that see few rabies exposed subjects. A large project applying this method in a Northern Thai canine rabies endemic province is now in place.
Some WHO documents on human rabies immunization


World survey on rabies, 1998, No 34, English. WHO/CDS/CSR/APH/99.6