

# WHO Position Paper on Rabies Vaccines - 6 August 2010

## Selected references

*When available, summaries are provided for references cited in the position paper and/or the accompanying grading tables.*

**Ajjan N, Pilet C. Comparative study of the safety and protective value, in pre-exposure use, of rabies vaccine cultivated on human diploid cells (HDCV) and of the new vaccine grown on Vero cells. *Vaccine*. 1989 Apr;7(2):125-8.**

The object of the study is to compare the tolerance and the efficacy of a new inactivated rabies vaccine grown on Vero cells (PVRV), with the vaccine cultivated on human diploid cells (HDCV), using the schedule recommended by WHO for pre-exposure on days 0, 7 and 21. Of students exposed to a risk of rabies at Alfort Veterinary School, 144 volunteers received either HDCV or PVRV vaccine. No student received a booster between the first blood sample before immunization on day 0 and the last antibody titration 21 months after the beginning of immunization. No serious side-effect occurred with either vaccine, although some vaccinees complained of redness, induration or local pain and, exceptionally, of fever. The study indicates the excellent immunogenicity of both HDCV and PVRV vaccines in all vaccinees. The geometric mean of antibody titres shows a higher titre in the PVRV group and a rapid decline in immune response with both vaccines, four months after the first injection, followed by a stabilization of the antibody level throughout the rest of the study. It should be noted, however, that very few individuals were found to be seronegative 21 months after the first injection.

**Ambrozaitis A, Laiskonis A, Balciuniene L, Banzhoff A, Malerczyk C. Rabies post-exposure prophylaxis vaccination with purified chick embryo cell vaccine (PCECV) and purified Vero cell rabies vaccine (PVRV) in a four-site intradermal schedule (4-0-2-0-1-1): an immunogenic, cost-effective and practical regimen. *Vaccine*. 2006 May 8;24(19):4116-21.**

Currently, two intradermal (ID) regimens for rabies post-exposure prophylaxis (PEP) are recommended by WHO and used in countries where approved by national authorities: the Thai Red Cross (TRC) two-site ID regimen and the eight-site ID regimen. Besides these WHO recommended schedules, a new economical four-site ID regimen was evaluated that reduces the cost of PEP by up to 80%, when compared with the standard intramuscular Essen regimen, reduces the number of visits required for the patients when compared with the TRC regimen, and is more convenient than the eight-site regimen. To determine the immunogenicity of the ID four-site PEP regimen (4-0-2-0-1-1), 180 healthy volunteers were randomized to receive 0.1 mL volumes of PCECV or PVRV administered ID over both left and right shoulders and both deltoid regions on day 0, both deltoid regions on day 7 and over one deltoid region on days 30 and 90. Regardless of the vaccine, every subject developed rabies virus neutralizing antibody (RVNA) titers above 0.5 IU/mL by day 14, as determined by rapid fluorescent focus inhibition test (RFFIT) using a homologous test system. Two weeks after the last dose of vaccine, RVNA titers were all above 0.5 IU/mL (day 104). Geometric mean titers were similar throughout the study period. Both vaccines were well tolerated. These results demonstrate that a new four-site ID PEP regimen is a cost-effective and convenient alternative to IM (Essen or Zagreb) or ID (TRC or eight-site) regimens, especially using a 1 mL vial of vaccine (PCECV).

**Anderson LJ, Sikes RK, Langkop CW, Mann JM, Smith JS, Winkler WG, Deitch MW. Postexposure trial of a human diploid cell strain rabies vaccine. J Infect Dis. 1980 Aug;142(2):133-8.**

A human diploid cell strain vaccine (HDCV) was evaluated in 90 persons treated after exposure to rabies, 21 of whom were bitten by proven rabid animals. Intramuscular doses of HDCV were given on days 0, 3, 7, 14, and 28, and human rabies immune globulin (HRIG) was given on day 0. Antibody to rabies virus was tested for by the rapid fluorescent focus inhibition test; adverse reactions were assessed from physician's forms. All 87 persons tested developed titers of greater than or equal to 0.5 international units (IU)/ml, with a maximum geometric mean titer of 15.0 IU/ml on day 42. One year after vaccination, all 33 persons tested had antibody to rabies virus. After one or more doses of vaccine, mild local or systemic reactions were reported in 19.0% and 21.4% of persons, respectively. No serious reactions occurred. The results show that this HDCV (plus HRIG) was safe and effective in eliciting antibody in postexposure prophylaxis of rabies. When it becomes available, it is recommended over the present treatment regimen.

**Bahmanyar M, Fayaz A, Nour-Salehi S, Mohammadi M, Koprowski H. Successful protection of humans exposed to rabies infection. Postexposure treatment with the new human diploid cell rabies vaccine and antirabies serum. JAMA. 1976 Dec 13;236(24):2751-4.**

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.

**Beran J, Honegr K, Banzhoff A, Malerczyk C. Potency requirements of rabies vaccines administered intradermally using the Thai Red Cross regimen: investigation of the immunogenicity of serially diluted purified chick embryo cell rabies vaccine. Vaccine. 2005 Jun 10;23(30):3902-7.**

To determine the minimum vaccine potency per intradermal dose required to elicit an adequate immune response using the Thai Red Cross (TRC) regimen (2-2-2-0-1-1), healthy volunteers received 0.1 mL volumes of PCECV containing decreasing amounts of antigen. Subjects also received HRIG to evaluate potential interference with antibody production. Results indicated that when each 0.1 mL intradermal dose of PCECV contained antigen corresponding to 0.32 IU per intramuscular dose, every subject had titers above 0.5 IU/mL by day 14. These results confirm that the current World Health Organization (WHO) recommendations for vaccine potency (2.5 IU per intramuscular dose) are sufficient for use in the Thai Red Cross intradermal regimen.

**Bourhy H, Rollin PE, Vincent J, Sureau P. Comparative field evaluation of the fluorescent-antibody test, virus isolation from tissue culture, and enzyme immunodiagnosis for rapid laboratory diagnosis of rabies. J Clin Microbiol. 1989 Mar;27(3):519-23.**

The rabies tissue culture infection test (RTCIT) and rapid rabies enzyme immunodiagnosis (RREID) were compared to the fluorescent-antibody test (FAT) with field specimens. At the French National Reference Center for Rabies, 15,248 specimens were analyzed by FAT and RTCIT, and 2,290 of those

specimens were also tested by RREID; 818 other specimens were tested by FAT and RREID in 12 laboratories located in Africa, Asia, and Latin America. The sensitivities and specificities of RREID and RTCIT were comparable. This study showed that both tests can be used as backup procedures to confirm FAT. RREID is also strongly recommended for epidemiological studies and for laboratories which are not equipped for performing FAT.

**Briggs DJ, Banzhoff A, Nicolay U, Sirikwin S, Dumavibhat B, Tongswas S, Wasi C. Antibody response of patients after postexposure rabies vaccination with small intradermal doses of purified chick embryo cell vaccine or purified Vero cell rabies vaccine. Bull World Health Organ. 2000;78(5):693-8.**

Although the introduction of tissue culture vaccines for rabies has dramatically improved the immunogenicity and safety of rabies vaccines, they are often prohibitively expensive for developing countries. To examine whether smaller doses of these vaccines could be used, we tested the safety and immunogenicity of purified chick embryo cell vaccine (PCECV) on 211 patients in Thailand with World Health Organization (WHO) category II and III exposures to rabies. The patients presented at two Thai hospitals and were randomized into three groups. Patients in Group 1 received 0.1 ml PCECV intradermally at two sites on days 0, 3, 7, and at one site on days 30 and 90. Group 2 was treated similarly, except that purified Vero cell rabies vaccine (PVRV) was used instead of PCECV. Group 3 received 1.0 ml PCECV intramuscularly on days 0, 3, 7, 14, 30 and 90. After 0, 3, 7, 14, 30 and 90 days serum was collected from the subjects and the geometric mean titres (GMTs) of rabies virus neutralizing antibody determined. After 14 days the GMT of 59 patients vaccinated intradermally with PCECV was equivalent to that of patients who received PVRV. Adverse reactions were more frequent in patients who received vaccines intradermally, indicating the reactions were associated with the route of injection, rather than the vaccine per se. We conclude that PCECV is a safe and highly immunogenic vaccine for postexposure rabies vaccination when administered intradermally in 0.1-ml doses using the two-site method ("2,2,2,0,1,1") recommended by WHO.

**Briggs DJ (2007). Human vaccines. In: Jackson AC, Wunner WH, eds. Rabies, 2nd ed. Amsterdam, Elsevier:505–516. Book chapter.**

**Brown D, Featherstone JJ, Fooks AR, Gettner S, Lloyd E, Schweiger M. Intradermal pre-exposure rabies vaccine elicits long lasting immunity. Vaccine, 2008; 26:3909–3912.**

A retrospective cohort study of current rabies antibody titres from adults who received pre-exposure immunisation administered intradermally between 1994 and 2005, examining the decay in titre over time relative to the interval since last dose, and the total dose received. Participants receiving at least 0.6 ml total dose intradermally of vaccine over at least two clinic visits and all with three clinic visits, were shown to have an adequate titre with measurable levels of antibody indicating sero-conversion above 0.5 IU/ml, irrespective of the time interval since the last dose. No clear relationship was found between time interval since immunisation and the level of antibody. Using a schedule that administers 0.6 ml of rabies vaccine over three clinic visits the boosting interval could be extended towards ten years; substantially cheaper than the standard licensed intramuscular dosing schedule.

**CDC (1993). Recommendations of the Advisory Committee on Immunization Practices (ACIP): use of vaccines and immune globulins in persons with altered immunocompetence. Morbidity and Mortality Weekly Report, 42(RR-4):1–18.**

**CDC (1993). Recommendations of the Advisory Committee on Immunization Practices (ACIP): use of vaccines and immune globulins in persons with altered immunocompetence. Morbidity and Mortality Weekly Report, 42(RR-4):1–18.**

**CDC (2006). General recommendations on immunization: recommendations of the Advisory Committee on Immunization Practices (ACIP). Morbidity and Mortality Weekly Report, 55(RR-15):1–48.**

**Charanasri U, Meesomboon V, Kingnate D, Samuthananon P, Chaeychomsri W. Intradermal simulated rabies postexposure prophylaxis using purified chick embryo rabies vaccine. J Med Assoc Thai. 1992 Nov;75(11):639-43.**

The antibody responses of 65 volunteers receiving an i.d. regimen (0.1 ml given at two different sites on days 0, 3, 7 and 0.1 ml given at one site on days 30 and 90) were compared with a control group of 35 volunteers receiving the standard i.m. regimen. By day 14, seroconversion was observed in all vaccinees in both groups. Geometric mean titers remained higher than 0.5 IU/ml throughout the study period. At the end of the observation period on day 365, antibodies persisted in all subjects. The multisite i.d. PCEC regimen has been proved as immunogenic as the standard i.m. regimen. Both regimens were well tolerated. Thus, it would be the effective and cheapest available rabies postexposure treatment using tissue culture vaccine.

**Chutivongse S, Wilde H, Benjavongkulchai M, Chomchey P, Punthawong S. Postexposure rabies vaccination during pregnancy: effect on 202 women and their infants. Clin Infect Dis. 1995 Apr;20(4):818-20.**

This prospective study of 202 pregnant Thai women who received postexposure treatment for rabies with a tissue culture-derived rabies vaccine and human or equine rabies immune globulin revealed an adverse reaction rate similar to that seen among nonpregnant Thai patients who received the same treatment. Tissue culture-derived rabies vaccines as well as immune globulins are safe to use for postexposure prophylaxis during pregnancy. Such treatment should never be withheld or delayed if the patient possibly was exposed to rabies.

**Chutivongse S, Wilde H, Supich C, Baer GM, Fishbein DB. Postexposure prophylaxis for rabies with antiserum and intradermal vaccination. Lancet. 1990 Apr 14;335(8694):896-8.**

The Thai Red Cross intradermal postexposure rabies treatment schedule was prospectively assessed in 100 Thai patients severely bitten by proven rabid animals. It consists of 0.1 ml of purified Vero cell rabies vaccine containing more than 2.5 IU of rabies antigen per 0.5 ml of reconstituted vaccine given intradermally at two sites on days 0, 3, and 7, followed by one 0.1 ml injection on days 30 and 90. The commercial vaccine used had an antigen content of 3.17 IU per 0.5 ml ampoule. Purified equine or human rabies immuno-globulin was also given on day 0 to patients with severe exposures. As much of the immunoglobulin as possible was infiltrated around the wounds. All patients were followed for 1

year post exposure. There were no deaths; the efficacy of the regimen was 100%. Antibody titre determination in a randomly selected subgroup showed seroconversion in all 10 patients.

**Chutivongse S, Supich C, Wilde H. Acceptability and efficacy of purified vero-cell rabies vaccine in Thai children exposed to rabies. *Asia Pac J Public Health*. 1988;2(3):179-84. (No abstract).**

**Coleman PG, Fèvre EM, Cleaveland S. *Emerg Infect Dis*. 2004 Jan;10(1):140-2. Estimating the public health impact of rabies. *Emerg Infect Dis*. 2004 Jan;10(1):140-2.**

Rabies is a fatal, preventable zoonosis, but it is not effectively controlled throughout much of the developing world. The impetus for control is hampered by a lack of awareness of its true impact. We estimate a disability-adjusted life year (DALY) score for rabies to quantify the disease impact relative to other diseases to set priorities for public health interventions.

**Crepin P, Audry L, Rotivel Y, Gacoin A, Caroff C, Bourhy H. Intravital diagnosis of human rabies by PCR using saliva and cerebrospinal fluid. *J Clin Microbiol*. 1998 Apr;36(4):1117-21.**

An optimized reverse transcription (RT)-PCR protocol for the intravital detection of rabies virus genomic RNA was tested with clinical samples obtained from 28 patients suspected of having rabies, 9 of whom were confirmed to have had rabies by postmortem examination. RT-PCR using saliva combined with an immunofluorescence assay performed with skin biopsy samples allowed detection of rabies in the nine patients.

**Dacheux L, Reynes JM, Buchy P, Sivuth O, Diop BM, Rousset D, Rathat C, Jolly N, Dufourcq JB, Nareth C, Diop S, Iehlé C, Rajerison R, Sadorge C, Bourhy H. A reliable diagnosis of human rabies based on analysis of skin biopsy specimens. *Clin Infect Dis*. 2008 Dec 1;47(11):1410-7.**

**BACKGROUND:** The number of human deaths due to rabies is currently underestimated to be 55,000 deaths per year. Biological diagnostic methods for confirmation of rabies remain limited, because testing on postmortem cerebral samples is the reference method, and in many countries, sampling brain tissue is rarely practiced. There is a need for a reliable method based on a simple collection of nonneural specimens. **METHODS:** A new reverse-transcription, heminested polymerase chain reaction (RT-hnPCR) protocol was standardized at 3 participating centers in Cambodia, Madagascar, and France. Fifty-one patients from Cambodia, Madagascar, Senegal, and France were prospectively enrolled in the study; 43 (84%) were ultimately confirmed as having rabies. A total of 425 samples were collected from these patients during hospitalization. We studied the accuracy of the diagnosis by comparing the results obtained with use of biological fluid specimens (saliva and urine) and skin biopsy specimens with the results obtained with use of the standard rabies diagnostic procedure performed with a postmortem brain biopsy specimen. **RESULTS:** The data obtained indicate a high specificity (100%) of RT-hnPCR and a higher sensitivity ( $\geq 98\%$ ) when the RT-hnPCR was performed with skin biopsy specimens than when the test was performed with fluid specimens, irrespective of the time of collection (i.e., 1 day after the onset of symptoms or just after death). Also, a sensitivity of 100% was obtained with the saliva sample when we analyzed at least 3 successive samples per patient. **CONCLUSIONS:** Skin biopsy specimens should be systematically collected in cases of encephalitis of unknown origin. These samples should be tested by RT-hnPCR immediately

to confirm rabies; if the technique is not readily available locally, the samples should be tested retrospectively for epidemiological purposes.

**Dietzschold B, Li J, Faber M, Schnell M. Concepts in the pathogenesis of rabies. *Future Virol.* 2008 Sep;3(5):481-490.**

Rabies is a zoonotic disease that remains an important public health problem worldwide and causes more than 70,000 human deaths each year. The causative agent of rabies is rabies virus (RV), a negative-stranded RNA virus of the rhabdovirus family. Neuroinvasiveness and neurotropism are the main features that define the pathogenesis of rabies. Although RV pathogenicity is a multigenic trait involving several elements of the RV genome, the RV glycoprotein plays a major role in RV pathogenesis by controlling the rate of virus uptake and trans-synaptic virus spread, and by regulating the rate of virus replication. Pathogenic street RV strains differ significantly from tissue culture-adapted RV strains in their neuroinvasiveness. Whereas street RV strains are highly neuroinvasive, most tissue culture-adapted RV strains have either no or only limited ability to invade the CNS from a peripheral site. The high neuroinvasiveness of pathogenic street RVs is, at least in part, due to their ability to evade immune responses and to conserve the structures of neurons. The finding that tissue culture-adapted RV strains replicate very fast and induce strong innate and adaptive immune responses opens new avenues for therapeutic intervention against rabies.

**Dobardzic A, Izurieta H, Woo EJ, Iskander J, Shadomy S, Rupprecht C, Ball R, Braun MM. Safety review of the purified chick embryo cell rabies vaccine: Data from the Vaccine Adverse Event Reporting System (VAERS), 1997-2005. *Vaccine.* 2007 May 22;25(21):4244-51.**

On October 20, 1997, the U.S. Food and Drug Administration (FDA) licensed Purified Chick Embryo Cell (PCEC, RabAvert) vaccine against rabies in humans following clinical trials demonstrating safety and efficacy. From October 1997 through December 2005, the Vaccine Adverse Event Reporting System (VAERS) received 336 reports of adverse events (AEs) following vaccination with PCEC vaccine in the U.S.; there were no death reports. Serious events, including 20 hospitalizations and 13 neurological events, were described in 24 (7%) reports. There was no pattern among the 13 neurological AEs suggesting a plausible relationship to vaccination. A total of 20 AEs, 3 serious, were classified as possible anaphylaxis. There were 312 non-serious AEs (93%). Nineteen reports (6%) described that the vaccination series was discontinued because of non-serious AEs. Most reported AEs are non-serious and consistent with pre-licensure safety data. The rabies risk must be carefully considered before vaccine discontinuation.

**Dreesen DW, Bernard KW, Parker RA, Deutsch AJ, Brown J. Immune complex-like disease in 23 persons following a booster dose of rabies human diploid cell vaccine. *Vaccine.* 1986 Mar;4(1):45-9.**

Following a routine 0.1 ml booster dose of Merieux rabies human diploid cell vaccine (HDCV), administered intradermally, 23(10.2%) of 226 persons had signs and symptoms compatible with an immune complex-like disease. The disease had its onset from 3-13 days after the injection, lasted 1-5 days, and consisted primarily of urticaria (78.3%), macular rash

(65.2%), angioedema (39.1%), and arthralgia (17.4%). None of the cases were considered severe, and all recovered with no sequelae. There were significant differences in attack rates between men (78.3% of all cases) and women, and between those receiving vaccinations on different days. Similar reactions have been reported following intramuscular booster doses of HDCV. Since the Merieux HDCV is used worldwide, physicians administering HDCV must be aware of these adverse reactions and warn patients. Appropriate therapy should be instituted as warranted by severity of reactions.

**Dutta JK. Adverse reactions to purified chick embryo cell rabies vaccine. *Vaccine*. 1994 Nov;12(15):1484. (No abstract)**

**Fishbein DB, Dreesen DW, Holmes DF, Pacer RE, Ley AB, Yager PA, Sumner JW, Reid-Sanden FL, Sanderlin DW, Tong TC, et al. Human diploid cell rabies vaccine purified by zonal centrifugation: a controlled study of antibody response and side effects following primary and booster pre-exposure immunizations. *Vaccine*. 1989 Oct;7(5):437-42.**

Systemic allergic reactions following booster immunizations have complicated rabies pre-exposure prophylaxis with the human diploid cell rabies vaccine licensed in the US (conventional HDCV). We conducted two studies comparing an HDCV purified by zonal centrifugation to conventional HDCV. In a study of primary pre-exposure immunization, volunteers received one of four regimens: three 1.0-ml intramuscular (i.m.) or 0.1-ml intradermal (i.d.) doses of conventional or purified HDCV over 28 days. Although volunteers vaccinated i.m. had significantly greater rabies neutralizing antibody titres (VNA) 49 days, 91 days and 26 months after immunization began than volunteers vaccinated i.d. ( $p$  less than 0.005- $p$  less than 0.05), there were no significant differences between vaccines. In a study of booster immunizations, 77 volunteers immunized with conventional HDCV 2 years earlier received a 0.1-ml i.d. booster with either conventional or purified HDCV. VNA was significantly greater with the conventional HDCV on days 7 and 28 after booster, but not on day 365. A moderate or severe reaction was reported by 5 (13%) of the 40 persons who received boosters with conventional HDCV, versus none of 37 who received the purified HDCV ( $p = 0.03$ ). Purified HDCV appears to be preferable to conventional HDCV for booster vaccination.

**Franka R, Wu X, Jackson FR, Velasco-Villa A, Palmer DP, Henderson H, Hayat W, Green DB, Blanton JD, Greenberg L, Rupprecht CE. Rabies virus pathogenesis in relationship to intervention with inactivated and attenuated rabies vaccines. *Vaccine*. 2009 Nov 27;27(51):7149-55.**

Despite progress in vaccine development in the past century the mechanisms behind immune responses elicited by rabies biologics or via natural infection remain largely unknown. In this study, we compared protection elicited by standard, early, or delayed prophylaxis with a reduced number of vaccine doses using inactivated and live-attenuated vaccines. Two-month-old Syrian hamsters, 4-week-old ICR mice or adult rhesus macaques were inoculated with canine rabies virus variants. Thereafter, prophylaxis was initiated 6h, 1, 2, 3, 4, 5, 6 or 7 days post-exposure (p.e.). One or several

doses of inactivated (HDCV), or reverse genetically attenuated (live), or gamma-irradiated (inactivated)-ERAG333 vaccines were administered intramuscularly. The dynamics of virus spread were measured over time in the rodent models. Rabies virus reached the spinal cord at day 4 and brain at day 6 p.e. All hamsters succumbed in groups in which live ERAG333 was delayed until days 5 and 6 p.e. However, 78%, 44%, 56% and 22% of hamsters survived when one dose of live ERAG333 was administered 6h, 1, 2, 3, and 4 days p.e., respectively. Similarly, 67% survived when inactivated ERAG333 was administered at 24h p.e. All hamsters succumbed when standard prophylaxis (the Essen regimen) was delayed until days 3-6, but 67% and 33% of hamsters survived when PEP began 1 or 2 days p.e., respectively. Macaques were protected by one dose of attenuated ERAG333 at 24h p.e. The highly attenuated (live) and inactivated ERAG333 vaccines elicited potent protective immune responses, even when prophylaxis initiation was delayed. When 2-5 doses of commercial vaccine and HRIG were administered according to the Essen scheme, 89-100% of the animals survived. Reduced vaccine schedules provided efficacious intervention, regardless of the total number of vaccine doses administered.

**Gherardin AW, Scrimgeour DJ, Lau SC, Phillips MA, Kass RB. Early rabies antibody response to intramuscular booster in previously intradermally immunized travelers using human diploid cell rabies vaccine. J Travel Med. 2001 May-Jun;8(3):122-6.**

**BACKGROUND:** Postexposure treatment (PET) of travelers who may have had a potential rabies exposure is simpler, safer, and cheaper if the traveler is preimmunized. Preimmunization can be done with human diploid cell rabies vaccine (HDCV) administered intramuscularly or intradermally. Some authorities, however, are now advocating that travelers vaccinated by the intradermal (ID) route should be treated as if they are not immunized. A particular concern raised is that travelers who have received pre-exposure rabies vaccination intradermally, may have a delayed response to postexposure boosters. This study is designed to elucidate whether a single intramuscular (IM) HDCV booster will provoke an early (day 5) immune response in individuals given pre-exposure ID HDCV. **METHODS:** Twenty-nine travelers who had received a course of three 0.1 mL ID HDCV between 12 and 24 months previously were given a single 1.0 mL IM booster of HDCV. Rabies antibody levels were compared 5 days later to those before the booster. **RESULTS:** Twenty-five of the 29 subjects (86%) showed an adequate rise in virus neutralizing antibody (VNA) titer 5 days after booster. Nine of the 29 subjects (31%) had inadequate antibody levels prior to the simulated postexposure booster. Five days after the postexposure booster, 27 of 29 (93%) had adequate antibody levels. The other 2 travelers were subsequently shown to have adequate VNA levels when tested 4 and 6 weeks later, respectively. **CONCLUSION:** For travelers who were given pre-exposure ID HDCV vaccination within the last 2 years and received one IM postexposure booster dose of HDCV, most mounted an adequate early immune response. This data does not support a change in current recommendations for rabies PET in this group. Further research to ascertain the duration of protection of pre-exposure ID rabies immunization is required.

**Glück R, Wegmann A, Germanier R, Keller H, Dettwiler HU, Wandeler AI. Confirmation of need for rabies immunoglobulin as well as post-exposure vaccine. Lancet. 1984 Nov 24;2(8413):1216-7. (No summary)**

**Hampson K, Dobson A, Kaare M, Dushoff J, Magoto M, Sindoya E, Cleaveland S. Rabies exposures, post-exposure prophylaxis and deaths in a region of endemic canine rabies. PLoS Negl Trop Dis. 2008;2(11):e339. Epub 2008 Nov 25.**

**BACKGROUND:** Thousands of human deaths from rabies occur annually despite the availability of effective vaccines following exposure, and for disease control in the animal reservoir. Our aim was to assess risk factors associated with exposure and to determine why human deaths from endemic canine rabies still occur. **METHODS AND FINDINGS:** Contact tracing was used to gather data on rabies exposures, post-exposure prophylaxis (PEP) delivered and deaths in two rural districts in northwestern Tanzania from 2002 to 2006. Data on risk factors and the propensity to seek and complete courses of PEP was collected using questionnaires. Exposures varied from 6-141/100,000 per year. Risk of exposure to rabies was greater in an area with agropastoralist communities (and larger domestic dog populations) than an area with pastoralist communities. Children were at greater risk than adults of being exposed to rabies and of developing clinical signs. PEP dramatically reduced the risk of developing rabies (odds ratio [OR] 17.33, 95% confidence interval [CI] 6.39-60.83) and when PEP was not delivered the risks were higher in the pastoralist than the agro-pastoralist area (OR 6.12, 95% CI 2.60-14.58). Low socioeconomic class and distance to medical facilities lengthened delays before PEP delivery. Over 20% of rabies-exposed individuals did not seek medical treatment and were not documented in official records and <65% received PEP. Animal bite injury records were an accurate indicator of rabies exposure incidence. **CONCLUSIONS:** Insufficient knowledge about rabies dangers and prevention, particularly prompt PEP, but also wound management, was the main cause of rabies deaths. Education, particularly in poor and marginalized communities, but also for medical and veterinary workers, would prevent future deaths.

**Hanlon CA, Kuzmin IV, Blanton JD, Weldon WC, Manangan JS, Rupprecht CE. Efficacy of rabies biologics against new lyssaviruses from Eurasia. *Virus Res.* 2005 Jul;111(1):44-54.**

New causative agents of rabies continue to emerge as shown by the recent description of four novel lyssaviruses from bats in Eurasia, Aravan (ARAV), Khujand (KHUV), Irkut (IRKV), and West Caucasian bat virus (WCBV). The effect of rabies vaccination prior to exposure to these new lyssaviruses was investigated in two animal models (i.e., Syrian hamsters and ferrets). The hamsters were vaccinated intramuscularly with a commercial human or veterinary vaccine or with an experimental vaccinia-rabies glycoprotein recombinant vaccine. At 5 weeks after vaccination, animals were challenged with ARAV, KHUV, IRKV, or WCBV, or with a traditional rabies virus of dog/coyote origin. Previously vaccinated and rabies-naïve ferrets were also challenged with the four new isolates. In addition, the combined effect of rabies immunoglobulin and vaccine after exposure to the four isolates was investigated in hamsters using commercially available human products or an experimental monoclonal antibody. Results showed reduced protection with pre-exposure vaccination and with conventional rabies post-exposure prophylaxis against all four new bat viruses. In general, protection was inversely related to the genetic distance between the new isolates and traditional rabies viruses. For example, the WCBV is the most divergent of these lyssaviruses, and neither pre-exposure vaccination nor conventional post-exposure prophylaxis provided significant protection. The potential impact of these new lyssaviruses on human and domestic animal health and the impact on the putative bat reservoir populations will require further field and laboratory investigation.

**Hemachudha T, Wacharapluesadee S, Mitrabhakdi E, Wilde H, Morimoto K, Lewis RA. Pathophysiology of human paralytic rabies. *J Neurovirol.* 2005 Feb;11(1):93-100.**

Furious rabies is a well-recognized clinical disorder in humans but the paralytic form is not as easily identified. The mechanisms responsible for the weakness and longer survival periods are not clear. Several hypotheses have been proposed, including rabies virus variants associated with a particular vector, location of wounds, incubation period, influence of prior rabies vaccination, and virus localization in the central nervous system (CNS). However, none of these have been substantiated. Regarding molecular analyses of rabies viruses isolated from both furious and paralytic rabies patients,

only minor genetic variations with no specific patterns in glyco- (G), phospho- (P), and nucleoprotein (N) sequences have been identified and arginine 333 in G protein was present in all samples. Regional distribution of rabies virus antigen in rabies patients whose survival periods were 7 days or less and magnetic resonance imaging (MRI) of the CNS indicated brainstem and spinal cord as predilection sites regardless of clinical presentations. There are clinical, electrophysiological, and pathological indications that peripheral nerve dysfunction is responsible for weakness in paralytic rabies whereas in furious rabies, even in the absence of clinical weakness, abundant denervation potentials with normal sensory nerve conduction studies and proximal motor latencies suggest anterior horn cell dysfunction. The lack of cellular immunity to rabies virus antigen accompanied by an absence of cerebrospinal fluid (CSF) rabies neutralizing antibody in most paralytic rabies patients may argue against role of an immune response against rabies virus-positive axons. Aberrant immune responses to peripheral nerve antigen, in particular those mediated by one or more cellular-dependent mechanisms, may be involved as is supported by the absence of putative anti-ganglioside antibodies commonly found in immune-mediated peripheral nerve diseases. Longer survival period in paralytic rabies may possibly be related to currently unidentified mechanism(s) on neuronal gene expression, required for virus transcription/replication and for maintaining neuronal survival.

**Hemachudha T, Wacharapluesadee S. Antemortem diagnosis of human rabies. Clin Infect Dis. 2004 Oct 1;39(7):1085-6.**

**Horman JT, Rullán JV, Myers RA, Bond JO, Israel E, Joseph JM. Antibody response after a two-year intradermal booster of rabies human diploid cell vaccine. J Am Vet Med Assoc. 1987 Jul 15;191(2):185-7.**

A study was undertaken to evaluate the effectiveness of a low dose of rabies human diploid cell vaccine administered intradermally for preexposure booster inoculation. Seventy-six volunteers received a 0.1-ml dose of rabies human diploid cell vaccine intradermally, approximately 2 years after their primary series. Though only 25 (32.9%) had a titer less than 0.5 IU/ml before the booster, all had a postbooster titer of greater than or equal to 4.0 IU/ml 3 weeks later. Five of 73 (6.8%) reported an allergic reaction after the booster.

**Jackson AC. Human disease. In: Jackson AC, Wunner AH, eds. Rabies, 2nd ed. London, Elsevier Academic Press, 2007:309–340. Book chapter**

**Jaiaroensup W, Lang J, Thipkong P, Wimalaratne O, Samranwataya P, Saikasem A, Chareonwai S, Yenmuang W, Prakongsri S, Sitprija V, Wilde H. Safety and efficacy of purified Vero cell rabies vaccine given intramuscularly and intradermally. (Results of a prospective randomized trial). Vaccine. 1998 Oct;16(16):1559-62.**

**OBJECTIVES:** To determine adverse reactions as a result of pre- and post-exposure rabies vaccination, using the conventional intramuscular, and reduced dose intradermal regimens and purified Vero cell rabies vaccine. **DESIGN:** A prospective and randomized study of patients exposed to rabies and of subjects in need of pre-exposure rabies vaccination. **SETTING:** A metropolitan rabies control center in a canine rabies endemic country. **PATIENTS:** 1198 subjects were recruited between May, 1994 and March, 1996. They were divided into four groups. Patients with suspected or proven rabies exposures were given the vaccine intramuscularly using the conventional regimen, or intradermally

using the World Health Organization approved Thai Red Cross schedule. Human or equine rabies immune globulin was administered where indicated. Pre-exposure and post-exposure vaccine recipients were divided randomly into two groups each and given the vaccine either by the intramuscular or intradermal schedules. MEASUREMENTS: All local and systemic adverse reactions were recorded and statistically analyzed. RESULTS: Pruritus at injection sites was the only significant local reaction. It was more common in the intradermal groups. Low-grade fever, the only significant adverse systemic event, was more common in the intramuscular groups and was noted in 8% of all subjects. Eighty-four patients bitten by proven rabid animals were found to be alive and well 3 years later. Forty-four of these had received the intramuscular and 40 the intradermal postexposure regimens with human or equine immune globulin injected into wounds on the first day of treatment. CONCLUSIONS: Purified Vero cell rabies vaccine is safe, carries a very low adverse reaction rate and is effective in preventing rabies in severely exposed subjects when used with human or equine rabies immune globulin.

**Kamoltham T et al. (2009). Rabies PrEP in children: anamnestic immune response to PCECV booster doses up to 5 years after primary vaccination. In: Proceedings of the second Rabies in Asia Conference, Hanoi, 9–11 September 2009 (available at: [www.rabiesinasia.org](http://www.rabiesinasia.org)).**

**Khawplod P, Wilde H, Chomchey P, Benjavongkulchai M, Yenmuang W, Chaiyabutr N, Sitprija V. What is an acceptable delay in rabies immune globulin administration when vaccine alone had been given previously? *Vaccine*. 1996 Apr;14(5):389-91.**

Rabies immune globulins (RIG) are not always available. Rabies-exposed patients often present to medical centers, particularly in canine rabies infested regions, after a vaccine series has been started without immune globulin administration. It is known that rabies immune globulin can result in suppression of the neutralizing antibody response which usually yields detectable antibodies by day 7. We have shown that it can be administered with a delay of up to 5 days after the start of vaccine treatment without significant antibody suppression within the first month. This study utilized the WHO approved multisite Thai Red Cross intradermal postexposure regimen. Effective use of rabies immune globulin in severe and multiple wounds, particularly in small children, may require dilution of the RIG in normal saline to provide a volume adequate for infiltration of all wounds.

**Knobel DL, Cleaveland S, Coleman PG, Fèvre EM, Meltzer MI, Miranda ME, Shaw A, Zinsstag J, Meslin FX. Re-evaluating the burden of rabies in Africa and Asia. *Bull World Health Organ*. 2005 May;83(5):360-8.**

OBJECTIVE: To quantify the public health and economic burden of endemic canine rabies in Africa and Asia. METHODS: Data from these regions were applied to a set of linked epidemiological and economic models. The human population at risk from endemic canine rabies was predicted using data on dog density, and human rabies deaths were estimated using a series of probability steps to determine the likelihood of clinical rabies developing in a person after being bitten by a dog suspected of having rabies. Model outputs on mortality and morbidity associated with rabies were used to calculate an improved disability-adjusted life year (DALY) score for the disease. The total societal cost incurred by the disease is presented. FINDINGS: Human mortality from endemic canine rabies was estimated to be 55 000 deaths per year (90% confidence interval (CI) = 24 000-93 000). Deaths due to rabies are responsible for 1.74 million DALYs lost each year (90% CI = 0.75-2.93). An additional 0.04 million DALYs are lost through morbidity and mortality following side-effects of

nerve-tissue vaccines. The estimated annual cost of rabies is USD 583.5 million (90% CI = USD 540.1-626.3 million). Patient-borne costs for post-exposure treatment form the bulk of expenditure, accounting for nearly half the total costs of rabies. CONCLUSION: Rabies remains an important yet neglected disease in Africa and Asia. Disparities in the affordability and accessibility of post-exposure treatment and risks of exposure to rabid dogs result in a skewed distribution of the disease burden across society, with the major impact falling on those living in poor rural communities, in particular children.

**Lang J, Cetre JC, Picot N, Lanta M, Briantais P, Vital S, Le Mener V, Lutsch C, Rotivel Y. Immunogenicity and safety in adults of a new chromatographically purified Vero-cell rabies vaccine (CPRV): a randomized, double-blind trial with purified Vero-cell rabies vaccine (PVRV). *Biologicals*. 1998 Dec;26(4):299-308.**

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 Mérieux 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  $\geq$  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 rabies 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.

**Laothamatas J, Hemachudha T, Mitrabhakdi E, Wannakrairot P, Tulayadaechanont S. MR imaging in human rabies. *AJNR Am J Neuroradiol*. 2003 Jun-Jul;24(6):1102-9.**

**BACKGROUND AND PURPOSE:** Whether human rabies of different forms, encephalitic (furious) and paralytic (dumb), share similar MR imaging patterns is unknown. We assessed the diagnostic value of MR imaging in both forms of the disease and compared the clinical and neuroimaging findings. **METHODS:** Three patients with paralytic and two with encephalitic rabies were examined during preserved or deteriorated levels of consciousness. Six MR examinations of the brain, three of the spinal cord, and one of the brachial plexus were performed with a 1.5-T superconducting magnet. **RESULTS:** No difference was noted between the MR findings in both clinical forms of human rabies. Nonenhancing, ill-defined, mild hyperintensity changes in the brain stem, hippocampi, hypothalami, deep and subcortical white matter, and deep and cortical gray matter were demonstrated on T2-weighted images in the noncomatose patients with rabies. Enhancement along the brachial plexus of the bitten arm was noted in one patient with encephalitic rabies who at that time had only local neuropathic pain symptoms. Enhancement with gadolinium-based contrast material was seen at the hypothalami, brain stem nuclei, spinal cord gray matter, and intradural cervical nerve roots only when the patients became comatose. **CONCLUSION:** Both forms of human rabies share a similar MR imaging pattern. Such pattern and the lack of enhancement in a noncomatose patient with suspected encephalitis may differentiate rabies from other viral encephalitides.

**Lembo T, Niezgoda M, Velasco-Villa A, Cleaveland S, Ernest E, Rupprecht CE. Evaluation of a direct, rapid immunohistochemical test for rabies diagnosis. Emerg Infect Dis. 2006 Feb;12(2):310-3.**

A direct rapid immunohistochemical test (dRIT) was evaluated under field and laboratory conditions to detect rabies virus antigen in frozen and glycerol-preserved field brain samples from northwestern Tanzania. Compared to the direct fluorescent antibody test, the traditional standard in rabies diagnosis, the dRIT was 100% sensitive and specific.

**Lin FT, Lina N. Developments in the production and application of rabies vaccine for human use in China. Trop Doct. 2000 Jan;30(1):14-6. (No summary)**

**Malerczyk C, Briggs DJ, Dreesen DW, Banzhoff A. Duration of immunity: an anamnestic response 14 years after rabies vaccination with purified chick embryo cell rabies vaccine. J Travel Med. 2007 Jan-Feb;14(1):63-4. (No abstract)**

**Meslin FX, Kaplan MM, Koprowski H. Laboratory techniques in rabies, 4th ed. Geneva, World Health Organization, 1996. Book chapter.**

**Morris J, Crowcroft NS. Pre-exposure rabies booster vaccinations: a literature review. Dev Biol (Basel). 2006;125:205-15.**

In Europe, more attention is turning towards human infection with European bat lyssaviruses (EBLVs). Following the death of a bat conservationist from EBLV in Scotland, in 2002, the Department of Health in the United Kingdom (UK) recommended that all bat workers receive prophylactic rabies vaccination. This systematic literature review aims to review the evidence base for current UK policy on rabies booster vaccination. Ten papers met the inclusion criteria and were

reviewed. Most of the papers were prospective cohort studies with follow-up ending after the first booster vaccination. One year after a three dose intramuscular primary rabies vaccination course, 87.9-100 % of participants had a rabies antibody level  $\geq$  0.5 IU/ml, before the first booster. It may, therefore, be prudent for the UK to reduce its current recommended interval, primary course to first booster, from two years to one year. More research, with longer follow-up, is required to enable recommendations on subsequent boosters to be made.

**Naraporn N et al. (1999). Immune response to rabies booster vaccination in subjects who had postexposure treatment more than 5 years previously. Journal of Travel Medicine, 6:134–136. No abstract**

**Nicholson KG, Turner GS, Aoki FY. Immunization with a human diploid cell strain of rabies virus vaccine: two-year results. J Infect Dis. 1978 Jun;137(6):783-8.**

Antibody responses following primary vaccination with 1.0 ml of intramuscularly (im) or 0.1 ml of intradermally (id) administered human diploid cell rabies virus vaccine were observed for two years. Three primary doses of vaccine were given to 77 volunteers on days 0, 28, and 56. An antibody response was detected in all vaccinees after a single dose; at one month, the response in the group that received vaccine id was identical to that in the group that was given vaccine im, although only 1/10th of the dose of vaccine was used. After the second and third doses, the antibody responses were higher with the primary im regimen; this difference was significant at two, three, and 12 months when the geometric mean titers of antibody were twofold higher for im than for id vaccination. The antibody responses to a booster dose of vaccine administered to randomly grouped volunteers by the subcutaneous or id route at six, 12, or 24 months were similar irrespective of the method of primary immunization but were greater with increasing intervals between primary and booster doses.

**Pakamat Khawplod et al (2002) Four-Site Intradermal Postexposure Boosters in Previously Rabies Vaccinated Subjects, J travel Med, 9:153-155. No summary.**

**Pappaioanou M, Fishbein DB, Dreesen DW, Schwartz IK, Campbell GH, Sumner JW, Patchen LC, Brown WJ. Antibody response to preexposure human diploid-cell rabies vaccine given concurrently with chloroquine. N Engl J Med. 1986 Jan 30;314(5):280-4.**

We conducted a randomized controlled trial to evaluate the antibody response of freshman veterinary students to intradermal human diploid-cell rabies vaccine administered concurrently with chloroquine, a drug frequently used for chemoprophylaxis against malaria. Fifty-one students who had not been vaccinated against rabies were enrolled: 26 received 300 mg of chloroquine base per week (the recommended dose for malaria prophylaxis); 25 did not receive chloroquine and served as controls. All subjects received 0.1 ml of rabies vaccine intradermally on days 0, 7, and 28. Chloroquine was administered weekly to the treatment group, beginning nine days before the first dose of vaccine and continuing until day 48. The mean rabies-neutralizing antibody titer for the chloroquine group was significantly lower than that for the control group on each day of testing--i.e., day 28 ( $P = 0.0094$ ), day 49 ( $P = 0.0008$ ), and day 105 ( $P = 0.0002$ )--although both groups had neutralizing antibody titers on days 49 and 105, according to the criteria of the Centers for Disease Control. The blood concentrations of chloroquine and desethylchloroquine (the major metabolite of chloroquine, which also has antimalarial properties) were negatively associated with log antibody titers. These results indicate that

chloroquine taken in the dose recommended for malaria prophylaxis can reduce the antibody response to primary immunization with intradermal human diploid-cell rabies vaccine.

**Phanuphak P, Khawplod P, Sirivichayakul S, Siriprasomsub W, Ubol S, Thaweepathomwat M. Humoral and cell-mediated immune responses to various economical regimens of purified Vero cell rabies vaccine. Asian Pac J Allergy Immunol. 1987 Jun;5(1):33-7.**

Purified Vero cell rabies vaccine (PVRV) is a new effective but inexpensive tissue culture rabies vaccine for human use. We investigated if the cost of immunization with PVRV could be further reduced by intradermal immunization. Fifty-eight subjects with low-risk exposure to rabies were randomized into 4 groups to receive full-dose (0.5 ml) intramuscular injection of PVRV on days 0, 3, 7, 14 and 28 or 4, 2 or 1 intradermal injections of PVRV (0.1 ml) on days 0, 3, and 7, followed by another intradermal injection on day 28. Neutralizing antibodies and specific cell-mediated response (CMIR) were sequentially followed up to day 36. The antibody levels in the intradermal groups increased with the number of injection sites and the levels achieved by the 2-site i.d. regimen were not significantly different from those obtained by the full-dose i.m. even though only 1/3 of the amount of PVRV was used. Specific CMIR occurred 1 week sooner in the 2 and 4-site i.d. regimens than the full-dose i.m. We therefore recommended that our 2-site i.d. regimen of PVRV should be further tested with a view to substituting it for the more expensive full-dose i.m. regimen in order to further reduce the cost of rabies prophylaxis particularly in the developing countries.

**Plotkin SA, Koprowski H, Rupprecht CE. Rabies vaccines. In: Plotkin SA, Orensterin WA, Offit PA, eds. Vaccines, 5<sup>th</sup> edition, Saunders Elsevier, 2008, 687-714.**

**Prapimporn Shantavasinkul et al, Queen Saovabha Memorial Institute, Thai Red Cross Society - WHO Collaborating Centre for Research on Rabies Pathogenesis and Prevention-, Bangkok, Thailand in Report of the WHO Consultation on Human and Animal Rabies Prevention and Control, 7-9 October 2009, Annecy, France.**

**Quiambao BP, Dytioco HZ, Dizon RM, Crisostomo ME, Laot TM, Teuwen DE. Rabies post-exposure prophylaxis in the Philippines: health status of patients having received purified equine F(ab')(2) fragment rabies immunoglobulin (Favirab). PLoS Negl Trop Dis. 2008 May 28;2(5):e243.**

**BACKGROUND:** Recommended treatment for severe rabies exposure in unvaccinated individuals includes wound cleaning, administration of rabies immunoglobulins (RIG), and rabies vaccination. We conducted a survey of rabies treatment outcomes in the Philippines. **METHODS:** This was a case series involving 7,660 patients (4 months to 98 years of age) given purified equine RIG (pERIG) at the Research Institute for Tropical Medicine (Muntinlupa, Philippines) from July 2003 to August 2004 following Category II or III exposures. Data on local and systemic adverse reactions (AR) within 28 days and biting animal status were recorded; outcome data were obtained by telephone or home visit 6-29 months post-exposure. **RESULTS:** Follow-up data were collected for 6,464 patients. Of 151 patients with laboratory-confirmed rabies exposure, 143 were in good health 6-48 months later, seven could not be contacted, and one 4-year-old girl died. Of 16 deaths in total, 14 were unrelated to rabies exposure or treatment. Two deaths were considered PEP failures: the 4-year old girl, who had multiple deep lacerated wounds from a rabid dog of the nape, neck, and shoulders requiring suturing on the day of exposure, and an 8-year-old boy who only received rabies PEP on the day of exposure.

**CONCLUSIONS:** This extensive review of outcomes in persons with Category III exposure shows the recommended treatment schedule at RITM using pERIG is well tolerated, while survival of 143 laboratory-confirmed rabies exposures confirms the intervention efficacy. Two PEP intervention failures demonstrate that sustained education and training is essential in rabies management.

**Quiambao BP, Dimaano EM, Ambao C, Davis R, Banzhoff A, Malerczyk C. Reducing the cost of post-exposure rabies prophylaxis: efficacy of 0.1 ml PCEC rabies vaccine administered intradermally using the Thai Red Cross post-exposure regimen in patients severely exposed to laboratory-confirmed rabid animals. Vaccine. 2005 Feb 25;23(14):1709-14.**

Purified chick embryo cell rabies vaccine (PCECV) administered as 0.1 ml intradermally according to the Thai Red Cross (TRC) regimen could reduce the cost of PEP by up to 84% when compared to the traditional five-dose Essen regimen. To confirm the efficacy of 0.1 ml of PCECV using the TRC regimen, a clinical trial was conducted in 113 patients presenting with category III exposures from confirmed rabid animals at two bite referral centres in the Philippines. Patients were monitored monthly for 1 year after exposure. PCECV was well tolerated, no vaccine-related serious adverse events occurred and all patients were alive 1 year after their initial exposure.

**Ranney M, Partridge R, Jay GD, Rozzoli DE, Pandey P. Rabies antibody seroprotection rates among travelers in Nepal: "rabies seroprotection in travelers". J Travel Med. 2006 Nov-Dec;13(6):329-33.**

**BACKGROUND:** Rabies preexposure immunization is recommended for international travelers who are at risk for exposure to rabid animals, especially in areas where postexposure treatment may be limited. Rabies antibody seroprotection rates among international travelers has not been previously investigated. **OBJECTIVE:** To assess preexisting rabies seroprotection among travelers presenting to a health clinic in Nepal. **METHODS:** A prospective convenience sample of international travelers evaluated at a health center in Kathmandu, Nepal during a 2-month period. Subjects were eligible for inclusion if they had received rabies preexposure vaccination within the previous 5 years. Demographic information and vaccination records of rabies preexposure prophylaxis were obtained. Consenting subjects provided serum for rabies antibody measurement measured using the rapid fluorescent focus inhibition test. A dilution greater than or equal to 1:5 (0.5 IU/mL) was considered positive. Data were analyzed using chi-squares and two-sample t-tests with unequal variances. **RESULTS:** A total of 43 patients consented to enroll. Complete data were available for 38 patients. Subjects had received human diploid cell vaccine (HDCV) or purified Vero cell rabies vaccine (PVRV) vaccine, either via the intradermal or intramuscular route. All patients had adequate antibody titers except one, who had a titer below 0.5 IU/mL. There was no statistically significant relationship between antibody titer and type of vaccine, route of administration, time since vaccination, number of vaccinations, or patient age. **CONCLUSIONS:** Among 38 travelers to Nepal who had received documented preexposure rabies HDCV or PVRV vaccination series, 37 demonstrated adequate titers of  $> \text{or } = 0.5 \text{ IU/mL}$  and would be considered boostable if exposed to rabies virus. One traveler had a titer of  $< 0.5 \text{ IU/mL}$ . Type of vaccine, method of administration, number of vaccinations, and time since vaccination did not influence rabies antibody titer. Rabies vaccination with HDCV and PVRV vaccine was effective in stimulating adequate seroprotection in this sample of travelers.

**Rosanoff E, Tint H. Responses to human diploid cell rabies vaccine: neutralizing antibody responses of vaccinees receiving booster doses of human diploid cell rabies vaccine. Am J Epidemiol. 1979 Sep;110(3):322-7.**

Rabies neutralizing antibody levels were determined before and after administration of a booster-dose of Wyeth rabies vaccine (WRV) in persons immunized earlier with either duck embryo vaccine (DEV) or with WRV. Virtually all those receiving an initial 3-dose regimen of WRV (0, 7 and 21--28 days) still had neutralizing antibody one year later, but there was a decline in titer from 10--50 IU per ml at 35 days to about 1--3 IU. Only one-half of those receiving DEV as the primary vaccine had even detectable antibody one year later. All volunteers responded anamnesticly to a single WRV booster given 8--12 months after either primary vaccine. Those given WRV initially had much higher antibody levels than those given DEV, but after the WRV booster antibody levels in all vaccinees remained high, even one year later.

**Rupprecht CE, Briggs D, Brown CM, Franka R, Katz SL, Kerr HD, Lett S, Levis R, Meltzer MI, Schaffner W, Cieslak PR. Evidence for a 4-dose vaccine schedule for human rabies post-exposure prophylaxis in previously non-vaccinated individuals. *Vaccine*. 2009 Nov 27;27(51):7141-8.**

After exposure, human rabies is preventable by prompt application of post-exposure prophylaxis. Historically, the total number of rabies vaccine doses administered during human prophylaxis has decreased, as modern biologics have improved and scientific knowledge has grown. A review of the literature on rabies virus pathogenesis, experimental animal studies, clinical trials, epidemiological surveillance, and economic analyses was conducted to determine the potential utility of reducing the current 5-dose intramuscular series of human rabies vaccine administered in the United States. Based upon the available evidence, a reduced schedule of cell-culture rabies vaccine, administered on days 0, 3, 7, and 14, given in conjunction with rabies immune globulin, was supported and recommended by the United States Advisory Committee on Immunization Practices.

**Rupprecht CE, Barrett J, Briggs D, Cliquet F, Fooks AR, Lumlertdacha B, Meslin FX, Müller T, Nel LH, Schneider C, Tordo N, Wandeler AI. Can rabies be eradicated? *Dev Biol (Basel)*. 2008;131:95-121.**

Rabies, an acute progressive encephalitis, is an ancient zoonosis. Its distribution encompasses all continents, except Antarctica. Agents consist of at least 11 species or genotypes of rhabdoviruses, in the Genus *Lyssavirus*. Susceptible natural hosts include all mammals. Primary reservoirs reside in the Orders Carnivora and Chiroptera. A plethora of variants, maintained by a diversity of abundant hosts, presents a challenge to a strict concept of true eradication. Globally, the domestic dog remains the most significant species for viral transmission, responsible for millions of suspect human exposures and tens of thousands of fatalities. As such, this single major target provides an ideal opportunity for focused intervention programmes in humane disease prevention and control, driven by laboratory-based surveillance and guided via modern epidemiological insights. Historically, substantial technical progress throughout the 20th century led to the development of safe, affordable and efficacious animal and human vaccines, resulting in declining disease burdens in selected developed and developing countries. Regional and local disease resurgence occurs, due in part to a combination of political and economic instability, environmental perturbations, and shifting government priorities. Society must recall that despite the recent recognition of other important emerging infectious diseases, none exceed the case fatality rate of rabies. Given the clear relevance of rabies in public health, agriculture, and conservation biology, substantive international progress must continue towards enhanced public awareness, human rabies

prevention, wildlife rabies control, and canine rabies elimination, with renewed collaborative vigour.

**Sabchareon A, Chantavanich P, Pasuralertsakul S, Pojjaroen-Anant C, Prarinyanupharb V, Attanath P, Singhasivanon V, Buppodom W, Lang J. Persistence of antibodies in children after intradermal or intramuscular administration of preexposure primary and booster immunizations with purified Vero cell rabies vaccine. *Pediatr Infect Dis J.* 1998 Nov;17(11):1001-7.**

**BACKGROUND:** The use of intradermal (i.d.) injections of purified Vero cell rabies vaccine (PVRV) for preexposure prophylaxis has not been well-established. We studied the safety and immunogenicity of i.d. and intramuscular (i.m.) PVRV injections for primary and booster preexposure immunizations. **METHODS:** One of two rabies preexposure PVRV regimens comprising three doses of either 0.1 ml i.d. or 0.5 ml i.m. administered during 28 days was assigned at random to 190 school children. One booster dose was given 1 year later either i.d. or i.m., according to their initial randomization group. Serologic results were available from 155 (82%) children at 1 year after primary immunization and 118 (62%) children at 2 years after booster. **RESULTS:** Although children vaccinated i.d. had significantly lower rabies-neutralizing antibody titers after primary immunization as well as after booster than children vaccinated i.m. ( $P < 0.001$  for all time points), there were no significant differences in the percentages of children with adequate titers ( $>$  or  $= 0.15$  IU/ml) between the i.d. and i.m. groups after both primary and booster immunizations. Mild local reactions were more frequent after i.d. vaccination. Mild or moderate systemic reactions were infrequent and similar after i.d. and i.m. vaccinations. Fever and headache were reported by  $<$  or  $= 6\%$ . The reactions after booster were not different from those of post-primary immunization. **CONCLUSIONS:** Purified Vero cell rabies vaccine appears to be safe and immunogenic for primary and booster preexposure immunizations. An i.d. PVRV preexposure regimen should be useful especially for rabies-endemic countries with low per capita income.

**Sehgal S, Bhattacharya D, Bhardwaj M. Ten year longitudinal study of efficacy and safety of purified chick embryo cell vaccine for pre- and post-exposure prophylaxis of rabies in Indian population. *J Commun Dis.* 1995 Mar;27(1):36-43.**

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, innocuity, 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).

**Sehgal S, Bhattacharya D, Bhardwaj M. Clinical evaluation of purified vero-cell rabies vaccine in patients bitten by rabid animals in India. J Commun Dis. 1994 Sep;26(3):139-46.**

Fifty-five individuals bitten by rabid animals were administered purified vero-cell rabies vaccine (PVRV) at WHO Collaborative Centre for Rabies Epidemiology for South-East Asia at National Institute of Communicable Diseases, Delhi to test its immunogenicity, innocuity, safety and clinical efficacy. Fifty-two (94.5 per cent) of these individuals underwent complete course of treatment. Sera samples collected prior to the commencement of treatment showed all these persons to be sero-negative for antibody against Rabies virus. However mean titre of 2.44 I.U./ml, 7.76 I.U./ml and 10.77 I.U./ml were detected after third, fourth and sixth injections, respectively of PVRV. Persistence of protective titres of this antibody could be demonstrated even after 15 months of treatment. Of 327 inoculations, local and general reactions were observed after 10.6 per cent inoculations. All these cases were followed up for periods between 7 and 25 months and were, alive and healthy till the end of observation period, thereby proving the efficacy of the vaccine in preventing rabies.

**Shantavasinkul P, Tantawichien T, Wacharapluesadee S, Jeamanukoolkit A, Udomchaisakul P, Chattranukulchai P, Wongsaroj P, Khawplod P, Wilde H, Hemachudha T. Failure of rabies postexposure prophylaxis in patients presenting with unusual manifestations. Clin Infect Dis. 2010 Jan 1;50(1):77-9.**

We report an atypical case of paralytic rabies presenting with trismus followed by limb weakness, areflexia, ophthalmoparesis, and bilateral ptosis. Atypical presentations and history of rabies postexposure prophylaxis led to delayed diagnosis. Nucleocapsid and glycoprotein genes of rabies viruses from the patient's and biting dog's brains were of identical sequences.

**Strady A, Lang J, Lienard M, Blondeau C, Jaussaud R, Plotkin SA. Antibody persistence following preexposure regimens of cell-culture rabies vaccines: 10-year follow-up and proposal for a new booster policy. J Infect Dis. 1998 May;177(5):1290-5.**

Subjects (n = 312) received either the human diploid cell rabies vaccine (HDCV) or the purified Vero cell rabies vaccine (PVRV) according to either two-injection (days 0 and 28) or three-injection (days 0, 7, and 28) primary regimens. They received a booster injection at 1 year. Rabies antibody levels were measured after the primary series and the booster and then each year for the next 10 years. The results confirm the superior long-term immunogenicity of the three-injection over the two-injection protocol. HDCV and PVRV in three doses were equally immunogenic. A booster injection at 1 year provides long-term seroconversion (titer > or = 0.5 IU/mL). Antibody titers 2 weeks after the 1-year booster allowed prediction of long-term immunity. Good responders, with titers > or = 30 IU/mL, were protected for at least 10 years. An algorithm for differentiation between good responders and poor responders with respect to vaccine booster strategies is proposed.

**Sudarshan MK, Mahendra BJ, Madhusudana SN, Narayana DH, Sanjay TV, Gangaboraiah, Anandagiri MS. Assessing the relationship between antigenicity and immunogenicity of human rabies vaccines. Results of a meta-analysis. Hum Vaccin. 2005 Sep-Oct;1(5):187-90.**

A meta-analysis was done to study the relationship between antigenicity and immunogenicity of human rabies vaccines. The data of ten cell culture human rabies vaccine studies conducted at a single

centre during 1993-2004 were used in the study. The vaccines studied included Purified Chick Embryo Cell Vaccine (Kaketsuken, Japan and Rabipur, India), Purified Vero cell Rabies Vaccine (Verorab, France), Human Diploid Cell Vaccine (MIRV, France and Rabivax, Adsorbed and Lyophilized, India) and Rhesus Diploid Rabies Vaccine (adsorbed, USA). Interestingly, it was revealed that an higher antigenicity of rabies vaccines viz. potency of  $> \text{ or } = 5$  IU per single intramuscular dose did not result in significantly higher immunogenicity, as measured by rabies virus neutralizing antibody (RVNA) titers in the vaccinees, both on day 14 ( $t = 0.42$ ,  $p > 0.66$ , GMR = 1.06, 95% CI of GMR = 0.82, 1.37) and day 90 ( $t = 0.80$ ,  $p > 0.43$ , GMR = 1.15, 95% CI of GMR = 0.74, 1.14). However, as there are no reports of meta-analysis of cell culture human rabies vaccine trials, to confirm this observation the authors recommend further studies in this regard.

**Suntharasamai P, Chaiprasithikul P, Wasi C, Supanaranond W, Auewarakul P, Chanthavanich P, Supapochana A, Areeraksa S, Chittamas S, Jittapalapongsa S, et al. A simplified and economical intradermal regimen of purified chick embryo cell rabies vaccine for postexposure prophylaxis. *Vaccine*. 1994 May;12(6):508-12.**

Healthy volunteers were randomized to receive either intradermal purified chick embryo cell rabies vaccine (PCEC) alone (0.1 ml at each of two sites on days 0, 3 and 7, and at one site on days 28 and 90) ( $n = 81$ ), or intradermal PCEC with one dose of human rabies immunoglobulin (HRIG) intramuscularly at 20 IU kg<sup>-1</sup> on day 0 ( $n = 52$ ). Neutralizing antibody (NAB) was detectable in every volunteer, in both groups, from day 14 up to day 365. The peak NAB occurred on day 28 in both groups. No significant suppressive effects of HRIG on NAB response were observed. Side-effects were mild and self-limiting. These preliminary results suggest that this simplified low-dose intradermal regimen could be an alternative schedule in rabies postexposure prophylaxis, resulting in lower overall costs.

**Suntharasamai P, Warrell MJ, Warrell DA, Viravan C, Looareesuwan S, Supanaranond W, Chanthavanich P, Supapochana A, Tepsumethanon W, Pouradier-Duteil X. New purified Vero-cell vaccine prevents rabies in patients bitten by rabid animals. *Lancet*. 1986 Jul 19;2(8499):129-31.**

The protective effect of a new, potentially economical tissue-culture rabies vaccine, purified vero-cell rabies vaccine (PVRV), was tested in 106 patients bitten by animals with proven rabies. 0.5 ml PVRV was given intramuscularly on days 0, 3, 7, 14, 28, and 91; 47 patients with severe exposure were also given 20 IU/kg human rabies immune globulin (HRIG). All patients are alive and well after 1 year. Side-effects of treatment were negligible. Rabies neutralising antibody (greater than or equal to 1.6 IU) was demonstrated on day 14 and persisted for 1 year in every case. There was no significant suppression of the antibody response by HRIG. If the untreated mortality is 15%, PVRV is 81% efficient in protecting patients against rabies encephalitis (95% confidence limit). PVRV is likely to replace human diploid-cell strain vaccine as the most widely used tissue-culture rabies vaccine.

**Suwansrinon K, Wilde H, Benjavongkulchai M, Banjongkasaena U, Lertjarutorn S, Boonchang S, Suttisri R, Khowplod P, Daviratanasilpa S, Sitprija V. Survival of neutralizing antibody in previously rabies vaccinated subjects: a prospective study showing long lasting immunity. *Vaccine*. 2006 May 1;24(18):3878-80.**

Physicians dealing with potential rabies exposures and travel medicine are frequently asked how long previous pre- or post-exposure rabies vaccination induced immunity persists. We therefore carried out

a prospective study on 118 rabies vaccine recipients who had received pre- or post-exposure regimens with tissue culture rabies vaccines by intramuscular or intradermal schedules 5-21 years previously. Rabies neutralizing antibody was detectable in the sera of all subjects on day 0. They then received one intradermal 0.1 mL booster injection on days 0 and 3. Neutralizing antibody determination was carried out on days 5, 7 and 14. All except one subject showed an accelerated antibody response following the two booster injections. Vaccination with a WHO recognized tissue culture rabies vaccine evokes long lasting immunity. This study supports current recommendations that immunity is long lasting and that boosters without immunoglobulin are sufficient even when prior vaccination was longer than 5 years previously.

**Suwansrinon K, Jaijareonsup W, Wilde H, Benjavongkulchai M, Sriaroon C, Sitprija V. Sex- and age-related differences in rabies immunoglobulin hypersensitivity. *Trans R Soc Trop Med Hyg.* 2007 Feb;101(2):206-8.**

We retrospectively reviewed 72132 patients who had received rabies immunoglobulin between 1987 and 2005 at the Queen Saovabha Memorial Institute, Bangkok. Purified equine rabies immunoglobulin (ERIG) was given to 42965 (59.56%) patients and human rabies immunoglobulin (HRIG) to 29167 (40.44%) patients. A total of 812 patients from both groups (1.13%) reported adverse reactions; among those who had received ERIG, 43.13% were male and 56.87% were female, and among those who had received HRIG, 34.62% were male and 65.38% were female. Females were at higher risk of exhibiting ERIG or HRIG hypersensitivity than males ( $P < 0.01$ ). None of the reactions was life-threatening. Serum sickness-like reactions to ERIG and HRIG were rare under the age of 10 years (0.05 and 0.01% among recipients in that age group).

**Tantawichien T, Benjavongkulchai M, Limsuwan K, Khawplod P, Kaewchompoo W, Chomchey P, Sitprija V. *Clin Infect Dis.* 1999 May;28(5):1100-3. Antibody response after a four-site intradermal booster vaccination with cell-culture rabies vaccine.**

The current World Health Organization recommendation for booster vaccination of previously immunized individuals with potential exposure to rabies is two doses of vaccine intramuscularly or intradermally on days 0 and 3. We report responses to two types of postexposure treatment of healthy individuals who had received preexposure rabies vaccination 1 year previously. Group A individuals received four intradermal doses (one-fifth of the diluent volume of vaccine per dose) on day 0, and group B individuals received two intramuscular doses on days 0 and 3. Immunogenicity of the two booster regimens was assessed by titrating the amount of neutralizing antibody (Nab). We found that the booster doses of vaccine produced remarkable responses in all subjects. Nab titers of  $> \text{or} = 0.5$  IU/mL (acceptable antibody level for protection against rabies) were detected in all subjects on day 14, and they were shown to be consistently high 1 year after the booster vaccination. We also found that the Nab titers for group A were significantly higher (two- to eightfold) than those for group B on days 5, 14, 150, and 360 after the initial booster vaccination ( $P < .05$ ). Our study shows that the four-site intradermal booster regimen with use of one-fifth of the diluent volume of cell-culture rabies vaccine on day 0 is associated with a significantly higher antibody response than is the conventional booster regimen for subsequent postexposure rabies treatment of individuals who have received preexposure rabies vaccination with cell-culture rabies vaccine 1 year previously.

**Tanterdtham S, Chaiprasithikul P, Yuthavong K, Wasi C. *J Med Assoc Thai.* 1991 Nov;74(11):498-501. Follow-up of protective antibody level after post-exposure vaccination with**

**purified tissue culture rabies vaccine (PCEC) small doses intradermally. J Med Assoc Thai. 1991 Nov;74(11):498-501.**

Twenty-nine vaccinees, 18 males, 11 females, aged 2-61 years (median 12 years) received PCEC intradermally for post-exposure prophylaxis during February to May 1989. Twenty-one cases received 4 sites of 0.1 ml ID on days 0, 3, 7 and 1 site ID on days 28 and 90. Four cases received 4 sites ID on days 0, 3, 7 and 1 site on days 14 and 28. The other 4 cases got the different schedules of ID by the poor compliance. Blood specimens were taken from these vaccinees at 2, 6 or 7, 9, 12 and 16 months after the initial dose. Neutralizing antibodies were measured by standard mouse neutralization test used WHO rabies antiserum as reference. All 65 tested sera showed positive neutralizing antibody. The GMT of antibodies at months 2, 6 or 7, 9, 12 and 16 were 17.68, 2.01, 1.56, 0.88 and 0.56 IU/ml respectively. Mild itching at injection sites was reported in 3 cases and low fever with malaise in 1 case.

**Thraenhardt O, Kreuzfelder E, Hillebrandt M, Marcus I, Ramakrishnan K, Fu ZF, Dietzschold B. Long-term humoral and cellular immunity after vaccination with cell culture rabies vaccines in man. Clin Immunol Immunopathol. 1994 Jun;71(3):287-92.**

To determine the duration of anti-rabies immunity, peripheral blood of 18 vaccinees was obtained between 2 and 14 years after immunization. Peripheral blood mononuclear cells (PBMC) and serum were tested for the presence of either rabies virus-specific antibodies or rabies antigen-specific proliferation. Neutralizing immunoglobulin class G anti-rabies virus antibodies could be detected in sera of all vaccinees, but not in 18 age- and sex-matched controls. Rabies antigen-induced proliferation of PBMCs from vaccinees was significantly higher than that of controls. The anti-rabies T and B cell response showed no time-dependent pattern. These results suggest the induction of a long-term immunity after rabies immunization according to pre- and post-exposure schedules with inactivated cell culture vaccines against rabies.

**Terapong Tantawichien et al (1999) Antibody Response After a Four-Site intradermal Booster vaccination with Cell-Culture Rabies Vaccine, CID, 28 May, 1100-1103 .**

**Thisyakorn U, Pancharoen C, Ruxrungtham K, Ubolyam S, Khawplod P, Tantawichien T, Phanuphak P, Wilde H. Safety and immunogenicity of preexposure rabies vaccination in children infected with human immunodeficiency virus type 1. Clin Infect Dis. 2000 Jan;30(1):218. No summary available.**

**Vodopija I, Baklajić Z, Vodopija R. Rabipur: a reliable vaccine for rabies protection. Vaccine. 1999 Mar 26;17(13-14):1739-41.**

Rabipur, a vaccine propagated on chick embryo fibroblasts, is one of the 'second generation' rabies vaccines produced by cell culture techniques. It compares in tolerance, immunogenicity and efficacy with the human diploid cell culture vaccines and is significantly more economical to be produced. It has proven to be an excellent vaccine, particularly when employed by the 2-1-1 schedule vaccination. This approach combines economy of vaccine with increased safety of treatment. Rabipur was investigated in all immunological parameters and can be recommended as a vaccine of choice for postexposure rabies treatment.

**Wacharapluesadee S, Hemachudha T. Ante- and post-mortem diagnosis of rabies using nucleic acid-amplification tests. Expert Rev Mol Diagn. 2010 Mar;10(2):207-18. Expert review of molecular diagnostics.**

Sensitivity, specificity and short turn-around time nucleic acid-amplification tests (NATs) have been steadily improving. NATs have been employed in the diagnosis of rabies to distinct different strains, as well as to identify new lyssaviruses. NATs have advantages over traditional methods, such as the direct fluorescence antibody test. They can be applied to fluid samples and brain tissue that is substantially decomposed. NATs can be used as an alternative method for confirmation or exclusion of the diagnosis in a suspected rabies patient. Real-time PCR methods are more favored than conventional reverse-transcription PCR methods by several laboratories. Second-round PCR, either nested or heminested, has been used for ante-mortem diagnosis to detect low levels of RNA. This review the details obstacles in making a diagnosis, how to properly utilize NATs (sample preparation, nucleic amplification techniques, amplification targets and primer design); and interprets the results obtained in recent studies.

**Wang XJ, Lang J, Tao XR, Shu JD, Le Mener V, Wood SC, Huang JT, Zhao SL. Immunogenicity and safety of purified Vero-cell rabies vaccine in severely rabies-exposed patients in China. Southeast Asian J Trop Med Public Health. 2000 Jun;31(2):287-94.**

The immunogenicity and safety of a purified Vero-cell rabies vaccine (PVRV, VERORAB; Aventis Pasteur, France) were evaluated in 171 patients treated for severe exposure to rabies (WHO category III contacts) at the Shandong Provincial Antiepidemic Station in Jinan and an EPI center in Ping Yin, China. Post-exposure treatment consisted of a single dose of equine rabies immunoglobulin (ERIG, 40 IU/kg body weight) on Day (D) 0, and intra-muscular administration of PVRV on D 0, 3, 7, 14 and 28. Antirabies antibody levels were evaluated on D 0, 7, 14, 28, 90 and 180 using the rapid fluorescent focus inhibition test. By D 14 all subjects had seroconverted ( $>$  or  $=$  0.5 IU/ml), with a geometric mean titer of 50.3 IU/ml. Antibody titers remained above the seroprotection threshold in all patients for 3 months, and in 98.2% of subjects for 6 months. All patients were still alive 6 months after the start of treatment. PVRV and ERIG were shown to be well tolerated and no serious adverse events were observed. Following PVRV administration, 12 patients (7.0%) had at least one local reaction (mostly pruritus, erythematous rash and pain). Fourteen patients (8.2%) developed local reactions at the site of ERIG administration. Twelve patients (7.0%) developed systemic reactions following post-exposure treatment, the most frequent of which were pruritus, rash and vertigo. This study demonstrates that PVRV is immunogenic and safe in Chinese patients treated according to WHO recommendations for severe rabies exposure.

**Warrell MJ, Nicholson KG, Warrell DA, Suntharasamai P, Chanthavanich P, Viravan C, Sinhaseni A, Chiewbambroongkiat MK, Pouradier-Duteil X, Xueref C, et al. Economical multiple-site intradermal immunisation with human diploid-cell-strain vaccine is effective for post-exposure rabies prophylaxis. Lancet. 1985 May 11;1(8437):1059-62.**

An economical post-exposure regimen of Mérieux human diploid-cell-strain vaccine (HDCSV) was compared with Semple vaccine (SV), the most widely used vaccine in Asia. 155 patients bitten by animals proved to be rabid received either conventional courses of SV (34 severe and 43 mild cases) or HDCSV, 0.1 ml intradermally, at eight sites on day 0, at four sites on day 7, and at one site on days 28 and 91 (36 severe and 42 mild cases). All severely bitten patients were given equine anti-rabies serum (EARS), 80 IU/kg on day 0. There were no deaths from rabies in either group. Follow-up was

97.5% at 1 year and 93% at 2 years. 88% of patients given HDCSV alone had detectable neutralising antibody on day 7 in contrast to 2% given SV alone. Antibody persisted until 1 year in all sera tested from HDCSV patients in contrast to only 48% of SV sera. The high dose of EARS resulted in pronounced suppression of response to HDCSV. There were no serious systemic side-effects but local side-effects were significantly more common in the SV group. The multiple-site intradermal HDCSV regimen was at least as effective as SV. The amount of HDCSV used was 30% of the conventional dose.

**WHO Consultation on Human and Animal Rabies Prevention and Control, 7-9 October 2009, Annecy, France.**

**WHO:** Recommendations for inactivated rabies vaccine for human use produced in cell substrates and embryonated eggs. Annex 2, 56th meeting of the Expert Committee on Biological Standardization, October 2005. Geneva, World Health Organization, 2005 (available at <http://www.who.int/biologicals/publications/trs/areas/vaccines/rabies/en/index.html>).

**WHO: GPV policy statement on vaccine quality.** Geneva, World Health Organization, 1996 (WHO/VSQ/GEN/96.02; available from the IVB documentation centre, World Health Organization, 1211 Geneva 27, Switzerland.

**Wilde H, Chomchey P, Punyaratabandhu P, Phanupak P, Chutivongse S. Purified equine rabies immune globulin: a safe and affordable alternative to human rabies immune globulin. Bull World Health Organ. 1989;67(6):731-6.**

Reported are the results of a retrospective study of 3156 patients who were treated at the Queen Saovabha Memorial Institute, Bangkok, with equine rabies immune globulin (ERIG). Only 51 patients (1.6%) exhibited serum-sickness-like reactions, none of which persisted for more than a week, and only 8 of these patients (15%) were treated with a short course of steroids. One patient, whose skin test was negative, had an immediate anaphylactic reaction to ERIG that responded to parenteral therapy with epinephrine and hydrocortisone sodium succinate. Serum-sickness-like reactions were more frequent among females and over 21-year-olds but were exceedingly rare (0.086%) among children under 10 years of age.

**Wilde H, Chutivongse S. Equine rabies immune globulin: a product with an undeserved poor reputation. Am J Trop Med Hyg. 1990 Feb;42(2):175-8. Queen Saovabha Memorial Institute of the Thai Red Cross Society, Bangkok.**

Four hundred nineteen patients exposed to rabies in Thailand were treated with equine rabies immune globulin (ERIG) manufactured by Sclavo of Italy, a product also licensed in the United States of America. They were followed for a minimum of 1 month after ERIG injection and rabies vaccine administration. Adverse serum sickness-like reactions were noted in 15 patients (3.58%). These were clinically acceptable and only 1 of these patients required corticosteroid therapy and short term hospitalization for serum sickness. ERIG is approximately 1/10 of the cost of human rabies immune globulin (HRIG), which is not generally available in developing countries. ERIG is a safe and underutilized essential biological when HRIG is not affordable or available.

**Wilde H. Failures of post-exposure rabies prophylaxis. Vaccine. 2007 Nov 1;25 (44):7605-9.**

Rabies remains a public health problem in many emerging countries. Virtually all is known that should enable us to eliminate this scourge by controlling the disease in canine populations and by diligent provision of WHO recommended post-exposure prophylaxis (PEP). Nevertheless, post-exposure prophylaxis failures do occur. Most common failures are due to deviations from WHO management recommendations and lack of essential biologicals. True failures, where all was done according to WHO recommendations, are fortunately extremely rare. Presented are seven such deaths. Other examples of common management deviations that resulted in deaths are also shown.

**Wilde H, Sirikawin S, Sabcharoen A, Kingnate D, Tantawichien T, Harischandra PA, Chaiyabutr N, de Silva DG, Fernando L, Liyanage JB, Sitprija V. Failure of postexposure treatment of rabies in children. Clin Infect Dis. 1996 Feb;22(2):228-32.**

Five failures of postexposure treatment of rabies in small children with multiple severe bites on the face and head are discussed. All had received rabies immune globulin and a potent tissue-culture vaccine. However, not all wounds had been infiltrated with immune globulin. Surgical closure prior to wound injection with immune globulin was performed in three cases. Another patient had wounds sutured after an intramuscular injection of immune globulin, without wound infiltration.

**Wilde H, Chomchey P, Punyaratabandhu P, Phanupak P, Chutivongse S. Purified equine rabies immune globulin: a safe and affordable alternative to human rabies immune globulin. Bull World Health Organ. 1989;67(6):731-6.**

Reported are the results of a retrospective study of 3156 patients who were treated at the Queen Saovabha Memorial Institute, Bangkok, with equine rabies immune globulin (ERIG). Only 51 patients (1.6%) exhibited serum-sickness-like reactions, none of which persisted for more than a week, and only 8 of these patients (15%) were treated with a short course of steroids. One patient, whose skin test was negative, had an immediate anaphylactic reaction to ERIG that responded to parenteral therapy with epinephrine and hydrocortisone sodium succinate. Serum-sickness-like reactions were more frequent among females and over 21-year-olds but were exceedingly rare (0.086%) among children under 10 years of age.

**WHO Expert Consultation on Rabies: first report. Geneva, World Health Organization, 2005 (WHO Technical Report Series, No. 931); available at [http://whqlibdoc.who.int/trs/WHO\\_TRS\\_931\\_eng.pdf](http://whqlibdoc.who.int/trs/WHO_TRS_931_eng.pdf)**

**WHO Expert Consultation on Rabies First Report. 2004 WHO Technical Report Series 931. Geneva Switzerland WHO, 2004, pp. 121.**