

New Knowledge and New Controversies in Rabies

Henry Wilde, M.D.*,
Prapimporn Shantavasinkul, M.D.*,
Thiravat Hemachudha, M.D.*,
Veera Tepsumethanon, D.V.M.**,
Boonlert Lumlertacha, D.V.M.**,
Supaporn Wacharapluesadee, Ph.D.*,
Terapong Tantawichien, M.D.**,
Visith Sitprija, M.D.**,
Supawat Chutivongse, M.D.**,
Praphan Phanuphak, M.D., Ph.D.*

ABSTRACT

Our knowledge of rabies as a human disease has advanced rapidly since the advent of molecular biology and the work of dedicated teams of medical and veterinary scientists who devote a major part of their professional life to study this ancient disease. We present a short overview of what new has emerged from the Southeast Asian perspective. It is now high time to implement this knowledge and support national rabies eradication projects. (*J Infect Dis Antimicrob Agents* 2009;26:63-74.)

INTRODUCTION

New evidence-based knowledge provided us with the information that we need to control, if not even eliminate, canine rabies worldwide. The dangerous and poorly immunogenic nerve tissue-derived vaccines are now only used in very few localities, notably Pakistan and Bangladesh. We have had effective and safe tissue culture vaccines for humans and dogs for at least four decades. New locally manufactured ones are

undergoing World Health Organization (WHO) pre-acceptance studies in India and China, and this may help reduce periodic shortages and costs. Abbreviated lower dose and less expensive administration schedules have been extensively tested and are now in daily use in many countries. Rabies is, nevertheless, again expanding into regions that have previously been rabies free and showing an increased prevalence in other parts of the world.

*Division of Research and Development and WHO Collaborating Centers for Research and Training on Viral Zoonoses, and the Queen Saovabha Memorial Institute, Thai Red Cross Society, Bangkok 10330, Thailand.

**Faculty of Medicine, Chulalongkorn University, and the Queen Saovabha Memorial Institute, Thai Red Cross Society, (a WHO Collaborating Center for Research in Rabies Control), Bangkok 10330, Thailand.

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Reprint request: Prapimporn Shantavasinkul, M.D., Queen Saovabha Memorial Institute, Thai Red Cross Society, Rama IV Road, Bangkok 10330, Thailand.

E-mail: sprapimporn@gmail.com

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Pathophysiology

Much has been learned about the pathogenesis of rabies and why there are two forms including furious and paralytic rabies. Paralytic rabies has been shown not to be due to a different virus or sites of inoculation but to different mechanisms as yet unknown. A weakness in paralytic rabies is caused by peripheral nerve dysfunction and not by that of anterior horn cells, as seen in furious cases who have subclinical anterior horn cell dysfunction, as in the poliomyelitis-like syndrome due to Japanese encephalitis virus, West Nile virus, or Enterovirus 71. Such peripheral nerve dysfunction may be due to a humoral and complement activation process or cellular immunity mediation.¹⁻³

Long incubation periods are not explained by virus latency in the nervous system. Electrophysiological studies of the nerves and muscles indicated that the virus reaches certain sites that produce characteristic signs of rabies (such as paresthesia or local prodromal symptoms at the bite site) a few days before these dysfunctions can be electrophysiologically detected. Magnetic resonance imaging (MRI) of the brain showed abnormal signals a few days before the patient exhibited aggression and phobic spasms. Such long incubation periods, of weeks, months to years, can be explained by the latency at the bite site, most likely within muscle cells. Virus then becomes activated by unknown mechanisms. It is also not known whether this activation continues to emerge in periodic pattern or only once and then enter into the free nerve endings. Hence, it is important to maintain immune protection, of both humoral and cellular arms, for at least one year after start of post-exposure prophylaxis (PEP). Memory cells are of crucial importance when there is a re-exposure.

Diagnosis and Management of Human Rabies Exposures

A clinical diagnosis of rabies in humans is well described in current literature. It depends on awareness of this disease, clinical observation and appropriate utilization of increasingly available specific and sensitive tests. Computed tomogram (CT) has no value in diagnosing rabies. There are no early abnormalities detected by CT. Rabies does not produce brain swelling or hemorrhages unless there are complications such as hypoxia, superimposed injury, or electrolyte imbalance. MRI is only of value in potentially excluding other causes of CNS encephalitides and by suggesting rabies. A recent work by Hemachudha and colleagues in rabies infected dogs showed that MRI disturbances correlate with the degree of immune responses in the brain and not with viral load. Such immune responses wane once the animals become comatose. Keeping rabies-infected humans or animals alive with the hope that immunity may develop later, may save a rare life (albeit turn the victim into the vegetative state). There may, however, be hope if antibody in the cerebrospinal fluid (CSF) can be detected at the very early clinical stage when the patient is still arousable and has mustered his own immunological defenses at the start of his clinical illness. If one encounters such a patient, it may be worth to initiate intensive life-support efforts allowing endogenous mechanisms to defeat the virus.¹⁻³

The importance of vigorous wound cleansing with soap and an antiseptic agent has been documented decades ago, but is often completely neglected in rabies endemic countries. It, alone, can reduce the infection by as much as 40 percent.⁴ This must be followed by wound infiltration with a rabies immune globulin and a WHO recommended rabies vaccine series. The introduction of reduced dose intradermal regimens has opened the door to a significant reduction of the cost of post-exposure prophylaxis (PEP) which has aided the abolition of dangerous and poorly immunogenic

nerve tissue derived products in many poor countries.

Current WHO approved regimens are:

- 1) The so called “Gold Standard” Essen regimen consisting of one full dose intramuscular injection (into lateral thigh or deltoid region) of a tissue culture vaccine on days 0,3,7,14, and 28.⁵
- 2) The abbreviated Zagreb regimen (1 full intramuscular dose at two sites on day 0 and one each on days 7 and 21).⁶
- 3) The Oxford 8-site intradermal regimen (8 intradermal injections of 0.1 mL at 8 different body sites on day 0, at 4 sites on day 7, and at one site each on days 28 and 90).⁷
- 4) The Thai Red Cross intradermal schedule (injections of 0.1 mL at two sites on days 0, 3, 7, and 28).⁸

Many studies have documented the immunogenicity and efficacy of the two WHO approved intradermal PEP regimens (Oxford and Thai Red Cross). Studies have also shown that the intradermal route induces at least equivalent or greater humoral immunity as well as an earlier cellular response.

Hemachudha and colleagues (unpublished data) recently confirmed that the intradermal route induces a predominant Th2 response, as compared to predominant Th1 response induced by intramuscular injections of rabies vaccine. This might be due to its ability to activate the abundant dendritic cells at the epidermis and dermis. The results acquired from a cytokine protein array study also showed that processed viral antigen reached regional lymph nodes earlier than with the intramuscular route.

Recently, an as yet unpublished study by scientists at the Thai Red Cross, indicated that it might be possible to develop a one-week post-exposure intradermal regimen that would reduce travel time and costs as well as drop-outs for rural victims with rabies exposures who can often ill afford travel expenses.

(Khawplod, unpublished data). Our group and previous studies by Thraenhart and colleagues, long suspected that the first 3 injections on days 0, 3, and 7 are the most important ones. To prove the immunogenicity and safety, this new approach will require additional larger human trials. One reason why there is insistence on documenting evidence for the presence of active immunity for at least one year post exposure, is because it is likely that the virus can slowly multiply at the bite site and switch between silent and activation stages. This may be the explanation for the occasional very long incubation periods.⁹

There have been suggestions that the Oxford 8-site intradermal regimen, which results in higher antibody levels on day 14 than the three other WHO recognized regimens, can be used effectively when the immune globulin is not available. This is not true, as the 8-site regimen does not result in consistently and significantly earlier antibody responses. This leaves the “window period” intact where the virus has enough time to enter nerve endings.¹⁰⁻¹¹

The introduction of the reduced vaccine dose intradermal schedule requires that one lyophilized ampoule (of 0.5 or 1.0 mL) has to be reconstituted into the liquid state and, according to the manufacturers instructions, must then be used within one working day. This limits the use of the intradermal regimen to clinics that see more than one patient daily and can thus take advantage of the remaining vaccine for an other patient within the same day. Khawplod and colleagues have shown that storing such vaccine, remaining after use on one patient, under sterile conditions in a clinic refrigerator will not cause significant loss of antigen over one week.¹² A study then revealed that, using such an ampoule for one patient for at least the days 0, 3, and 7 injections (using vaccine diluted in 1.0 mL) or for days 0 and 3 (with vaccine diluted in 0.5 mL) allows the economical use of the intradermal regimen in smaller

clinics. This practice has not yet been approved by WHO, but several studies have shown safety and immunogenicity.¹³

The importance of using rabies immunoglobulins (of human or equine origin) has been shown to be life-saving, if applied as soon as possible after exposures and up to one week later if vaccine alone had been started.¹⁴⁻¹⁶

However, the immunoglobulin must be injected into and around the wounds. Whatever goes into muscle or fat elsewhere will not produce a significant circulating level to neutralize the virus at the bite sites.^{4-5,17-19}

The relative safety of modern purified equine rabies immune globulins (ERIG) has been documented in many studies. However, mild to moderate and rarely more severe serum sickness reactions must be anticipated. They occur in 1-6 percent of recipients, when highly purified products were used and can be easily managed by using reassurance of the patient, antihistamines, and NSAIDs. They usually occur one week after administration and last for several days. The vaccine series is continued during this symptomatic period, and steroids are avoided. Even infected bite wounds can be infiltrated with the immune globulin after a proper wound care and a use of appropriate antibiotics.²⁰

Studies have also demonstrated that the skin test, being performed as an ancient ritual when equine products are used for rabies exposures or snake bites, does not predict serum sickness or anaphylaxis. ERIG has been used under close supervision for severe bite wounds, where the human immune globulin was not available and without any serious events.²¹⁻²²

The animal bite clinic at the Queen Saovabha Memorial Institute has seen only two cases of anaphylaxis among over 150,000 patients that received ERIG since the immune globulins were routinely used

at that institution after 1986. Both had negative skin tests to ERIG and were treated as outpatients on site by the clinic staff trained in managing adverse reactions to heterologous sera and other pharmaceuticals.¹⁴ The equine products are far less expensive than the human ones. Several new manufacturers in canine rabies endemic countries, where human rabies immune globulin is either not available or unaffordable, are now undergoing WHO pre-acceptance studies. The Thai Red Cross Institute is one of these.

Thailand is in the midst of an epidemic with the human immunodeficiency virus (HIV) and many children infected at birth are being actively followed at virtually every Thai medical center. Children represent 50 percent of animal bite victims, and are usually more severely bitten than adults. More children die worldwide of rabies than adults. Not surprisingly, several local studies showed that subjects with a low CD4 count also have a poor and even no detectable antibody response to rabies vaccination.²³ Successful PEP for an HIV patient with a severe exposure may rest largely on good wound cleansing and infiltration of all wounds with rabies immune globulin (RIG). A complete vaccine series must, nevertheless, follow.

Emergency room staff in canine rabies endemic countries often encounter pregnant patients who have had a rabies exposure and who are very anxious about potential harm to mother and fetus from rabies vaccine and immunoglobulin. Chitvongse and colleagues have shown, in a large prospective study of 202 mothers-to-be, that PEP did no harm to both mother and child. The babies were followed-up for one post-partum year, and all complications were compared to a normal matched control group, showing no differences in risks. Rabies has no contraindication to PEP.²⁴

We still encounter patients in Asia who had remote PEP using the Semple or suckling mouse

brain vaccines. A study showed that the antibody response to booster injections in such patients are unpredictable and probably depend on the immunogenicity of the Semple or suckling mouse brain vaccine that they had received. It may be a vigorous response or none at all. Such patients must therefore be treated the same way as a patient who had never received any rabies vaccine.²⁵

Pre-exposure rabies vaccination simplifies PEP. Previously rabies vaccinated individual need only booster vaccination when re-exposure occurs. RIG is not required for such a case, even in severe rabies exposure (WHO category III). WHO recommends two booster vaccinations with cell culture vaccine on days 0 and day 3 by intramuscular or intradermal method. Boosters are highly effective, and have been proved to induce an anamnestic (accelerated) immune response.^{5,26-27} The four-site intradermal booster regimen, given 0.1mL of any tissue culture vaccine one each on the arms and thighs, can be used safely in a previously vaccinated individual. It induced higher immunogenicity up to day 360 than the standard intramuscular booster regimen and requires only one visit.²⁸⁻³⁰ This regimen was used safely in over 5,000 patients at Queen Saovabha Memorial Institute since 1998.

Rabies diagnosis in humans and animals in vivo is valuable as it may allow making better management decisions and reduce possible exposures to health-care personnel and family members. It should also exclude or identify a potentially treatable cause rather than suspected rabies. Having a definitive diagnosis at hand, also allows better planning of the clinical management.³¹⁻³³ No patient with a progressive neurological syndrome is a candidate for tissue transplantation. This has been demonstrated in a recent series of tragic transplant origin rabies cases occurring in developed Western countries with little experience

with rabies.³⁴ Rabies virus is excreted intermittently in the saliva of dogs and man, and may be demonstrable one day and not the next. The mechanism for this is still unknown. A negative result is therefore of no help and requires repeat testing.³⁵ Furthermore, the secretion of the virus may occur in several biological fluids other than saliva and CSF, including urine.^{31-33,36} Hair follicles, not necessarily from the nape of neck, can be another source to detect rabies RNA.³⁷ Quick test kits for rabies RNA in the saliva have been developed and, at least one such kit is already being marketed to private practicing veterinarians for a rapid diagnosis in Asia.³⁸ We consider such tests dangerous as a false negative test may discourage providing early PEP. Testing brain tissue is still the “gold standard” for diagnosing rabies.³⁹⁻⁴⁰

Serum antibodies are usually negative in newly admitted rabies patients. There have been discrepant results when comparing the occurrence of antibodies in serum and CSF in patients associated with dog (in Thailand) and bat variants (in the Americas). In the case of dog variants, antibody appears unpredictably in dogs (early or late). In humans, early central nervous system (CNS) antibody appears only in very rare cases and never in our series of over 40 canine-origin patients where the virus was searched for using molecular methods. We have not had any experience with bat rabies cases in Thailand but, based on experience elsewhere in the world, it is logical that bat-associated cases be managed with intensive life support, hoping for an early immune responses to develop. This is particularly true if the patient has CSF neutralizing antibodies.

Post-exposure treatment failures

We have long assumed that rabies post-exposure treatments, carried out to WHO standards, will prevent the disease in all. A recent collection of human rabies

deaths, where apparently all had been done according to WHO recommendations, weakens this belief. Such cases are, however, very rare and should encourage us even more to follow WHO standards in caring for rabies exposed subject.⁴¹⁻⁴⁴

Pre-exposure vaccination

The possibility of providing pre-exposure rabies vaccination to all children in highly endemic regions has been discussed at many conferences and at WHO Rabies Expert Committee meetings. It has been shown to be safe and effective in several studies. It is the high cost of such an effort that has prevented it from being implemented. Canine endemic areas are also those with many other demands on scarce health-care funds and extended program of immunization (EPI) rabies vaccine plans have low priority.⁴⁵⁻⁴⁶

Curing human rabies

Many centers have tried to cure a patient with rabies. Ribavirin, interferon, and RIG had been administered intravenously and even intrathecally. Human partial or transient survivors from proven rabies have been reported. Virtually all remained severely incapacitated or died soon after a recovery due to debilitating illnesses that resulted from severe CNS damage from the virus.⁴⁷⁻⁴⁸ Our institution treated one still arousable young female with a total of 900 mL of human RIG intravenously to no avail. She had high serum neutralizing antibodies but none ever appeared in her CSF. This documented an intact blood/brain barrier in her case. She died of multisystem failure.⁴⁹

The survival of an unvaccinated teenager in the United States using sedatives to create a brain wave burst suppression stage as well as a cocktail of antiviral agents (ribavirin, ketamine, and amantadine) has become known as the Wisconsin protocol. ([http://](http://www.hpa.org.uk/web/HPAwebFile/HPAweb_C/1230105639592)

www.hpa.org.uk/web/HPAwebFile/HPAweb_C/1230105639592).

This has raised much publicity and hope that rabies may yet be curable. At least 12 attempts, one at our hospital, to repeat this method failed.⁵⁰ The latest victim treated using the Wisconsin protocol was from an African country. He had apparently started to show some neurological responses when he died of "malnutrition".⁵¹ The young patient that survived in Wisconsin had an unusual presentation in that she had antibody in both CSF and serum shortly after the onset of symptoms, and that no virus could be isolated and characterized at any time during her hospitalization.⁵² It is likely that she had developed early antibody that neutralized the virus. Treatment, other than high quality intensive care, may have had little to add to the favorable outcome. The extensive media cover of this survival case suggested that rabies in humans is a curable disease and ignited an ongoing controversy. A similar case, in a 6-year-old boy in Ohio, was reported previously. He had been vaccinated after a bat bite and was supported in an intensive care unit without intubation and antiviral agents. He also made a complete recovery and was found to have antibodies in the serum and CSF shortly after admission. No virus could be isolated during the entire clinical course.⁵³

Our institution will provide energetic respiratory and circulatory support for rabies patients that have CSF rabies antibody on admission and are still arousable. We would not use brain burst suppression deep anesthesia or unproven antiviral agents. Other human rabies patients will continue to receive a comfort care only.⁵⁴ If there is continuing advocacy for use of the Wisconsin Protocol on all rabies patients one should support carrying out a controlled study in suitable primates at a center experienced in dealing with human rabies patients.

The canine and bat vectors

A clinical diagnosis of canine rabies first requires an awareness of this disease. The animal should be quarantined or euthanized and laboratory tested if there is any possibility of rabies. If this can be carried out rapidly and reliably, it can assure proper prophylactic treatment of exposed subjects and exclude costly PEP if the animal is found free of rabies. There are now reliable tests that can confirm rabies. A computer-based guideline for diagnosing rabies in canines and felines can be accessed on the web.³⁹ Although rabies is almost always fatal in canines, there are rare reports of survivors and asymptomatic rabies in dogs have been identified and even called “carriers dogs”.⁵⁵ These cases are very uncommon and with the animals not showing the picture and aggressive behavior of furious rabies, they are less likely to present a serious risk to man. Little is known, if and how long, such a dog (or cat) might excrete the virus in the saliva. Experiments by Baer and Rupprecht demonstrated that a significant number of dogs artificially infected with rabies will survive after being only mildly ill or virtually asymptomatic. Other studies in Ethiopia and Thailand have also confirmed survival from rabies in canines.⁵⁵⁻⁵⁷ More recent reports of large numbers of “asymptomatic secretor” dogs in China blamed them as a cause of re-emerging rabies in that country. These reports were proven false and not based on a good science.⁵⁸ We have also learned more about the clinical features and pathogenesis of canine rabies. The two clinical forms are present in both humans and dogs in a similar ratio (75% furious, 25% dumb) and with similar MRI findings. It must be noted that a dog attack, considered provoked, does not eliminate a possible rabies exposure, particularly in rabies endemic regions.⁵⁹ If the responsible dog is available for observation, PEP might still be indicated and can be stopped if the animal is still alive and well 10 days later.⁶⁰

Rabies viruses isolated from humans, pets, and wildlife in Thailand had N gene sequenced. All belonged to the same canine street virus origin. Although the mutation rates were not enormous, the nucleotide differences were still sufficient to construct a phylogeographic diagram. The viruses in Thailand could be classified into 2 genogroups, each consisting of many clades distributed along different geographical regions (although some were overlapping). By correlating with the transportation routes, socioeconomic status, and geographical barriers, gene flow direction followed rural to urban patterns and then disseminated by human-facilitated-mechanisms. Rabies outbreaks in the South have been traced to the virus in the Northeast. This may be explained as being similar to the incident on Flores island in Indonesia where fishermen brought asymptomatic rabies infected dogs from Sulawesi which then established itself throughout Flores. A similar tragedy later occurred on Anbon, Indonesia and more recently on Bali, all previously rabies free island provinces.⁶¹

Thailand is home to 120 species of fruit and insect-eating bats. At least 4 species are known to be infected by Lyssavirus. This has been demonstrated by cross neutralizing antibodies against Arawan, Khujand, and Irkut viruses. This also supports the fact that the old-world bats, such as in Thailand, are unlikely to be infected with Lyssavirus of genotype I. The detailed genetic information of this new Lyssavirus genotype in Thailand remains unknown. The study involved antibody assay and not genetic characterization. Bats in Thailand are conserved species, and it is illegal to kill bats. Despite of this knowledge, there have been no recognized human cases due to identified bat rabies variants. Bats do not interact much with humans, but cases have been reported from northern Europe and Australia. Bats harbor several other viruses which may have serious human implications. There have been at

least 78 viruses known to infect bats including Ebola, Hendra, Nipah, Dengue, and others to be yet identified. Many Thai villagers do eat bats and often collect bats outside caves where they are resting or have fallen to earth. Thus, bats must be considered a hidden or as yet not recognized human risk.⁶²

A controversy continues regarding the management of the huge number of canine vectors in many developing countries. They are responsible for over 90 percent of human cases. Mass dog culling was practiced in an outbreak on Flores Island, Indonesia. Over 100 human deaths are known to have been identified at the peak of the outbreak, and rabies is still there some 8 years later. Only sporadic dog vaccination, with delay and inadequate to stop transmission, was practiced despite of many communications from WHO urging compliance with preventive and eradication guidelines. The same tragedy was later repeated in Anbon and now on Bali, Indonesia.^{61,63}

Thailand managed to reduce the human rabies death toll from 179 in 1985 to 7 in 2008. This was done by public education and the availability of WHO level post-exposure treatment throughout most of the country. This is an expensive and not definitive way of tackling the problem. Cultural barrier and reduction of human rabies deaths have impaired motivation for an intensive countrywide effort to reduce the disease among the principal canine vectors. This, even though there is a history that it had been done successfully in neighboring Malaysia and Singapore and previously also in Taiwan, South Korea, and Japan. Rabies is still a major public health problem today in China, Mongolia, Indonesia, Philippines, Myanmar, Cambodia, Bangladesh, Nepal, India, Pakistan, Afganistan, and the newly independent former Soviet republics.⁶⁴

Controlling the large owned and stray dog population in many parts of Asia must be an integral part of any rabies control measure. It has to be

accompanied by sustained rabies vaccination of at least 70 percent of the dog population. This is virtually impossible with the present population that has a short life span and rapid turn-over. Our team developed a locally manufactured inexpensive Zinc compound which, when injected intratesticularly (0.5 mL with a 29 Ga tuberculin or insulin needle) will permanently sterilize the dog. The procedure can be done by trained lay personnel at low cost. It is, however, only a partial solution for canine population reduction. One fertile male dog is able to impregnate several receptive females. Surgical sterilization of female dogs is specialist-labor intensive and costly. It has not yet succeeded in any of the developing canine endemic countries of Asia to reduce populations of stray dogs to a manageable level. The science for developing anti-fertility vaccines is available and needs to be tested as a method for reducing dog populations in canine rabies endemic countries.⁶⁵

CONCLUSIONS

This ancient disease now offers few mysteries. We know enough to act and start eradication of rabies in canine vectors. This, however, is now a cultural, political, and economical issue that has to be tackled by governments willing to actively support the medical and veterinary communities.

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References

1. Hemachudha T, Wacharapluesadee S, Laothamatas J, Wilde H. Rabies. *Curr Neurol Neurosci Rep* 2006;6:460-8.
2. Laothamatas J, Wacharapluesadee S, Lumlertdacha B, et al. Furious and paralytic rabies of canine origin: neuroimaging with virological and cytokine studies. *J Neurovirol* 2008;14:119-29.
3. Mitrabhakdi E, Shuangshoti S, Wannakrairo P, et al. Difference in neuropathogenetic mechanisms in human furious and paralytic rabies. *J Neurol Sci* 2005;238:3-10.
4. Dean DJ, Baer GM, Thompson WR. Studies on the local treatment of rabies-infected wounds. *Bull World Health Organ* 1963;28:477-86.
5. WHO Expert Consultation on rabies. *World Health Organ Tech Rep Ser* 2005;931:1-88.
6. Chutivongse S, Wilde H, Fishbein DB, Baer GM, Hemachudha T. One-year study of the 2-1-1 intramuscular postexposure rabies vaccine regimen in 100 severely exposed Thai patients using rabies immune globulin and Vero cell rabies vaccine. *Vaccine* 1991;9:573-6.
7. Warrell MJ, Warrell DA, Suntharasamai P, et al. An economical regimen of human diploid cell strain anti-rabies vaccine for post-exposure prophylaxis. *Lancet* 1983;2:301-4.
8. Phanuphak P, Khawplod P, Sirivichayakul S, Siriprasomsu 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;5:33-7.
9. Thraenhart O, Marcus I, Scheiermann N, et al. 3-1 scheme, a regimen of only two clinic visits with optimal antibody and interferon-induction. In: Thraenhart O, Koprowski H, Bogel K, Sureau P, eds. *Progress in Rabies Control*. Kent: Wells Medical, 1989: 536-47.
10. Khawplod P, Wilde H, Tepsumethanon S, et al. Prospective immunogenicity study of multiple intradermal injections of rabies vaccine in an effort to obtain an early immune response without the use of immunoglobulin. *Clin Infect Dis* 2002;35:1562-5.
11. Sriaroon C, Daviratansilpa S, Sansomranjai P, et al. Rabies in a Thai child treated with the eight-site post-exposure regimen without rabies immune globulin. *Vaccine* 2003;21:3525-6.
12. Khawplod P, Wilde H, Tantawichien T, et al. Potency, sterility and immunogenicity of rabies tissue culture vaccine after reconstitution and refrigerated storage for 1 week. *Vaccine* 2002;20:2240-2.
13. Kamoltham T, Khawplod P, Wilde H. Rabies intradermal post-exposure vaccination of humans using reconstituted and stored vaccine. *Vaccine* 2002;20:3272-6.
14. Wilde H, Chutivongse S. Equine rabies immune

- globulin: a product with an undeserved poor reputation. *Am J Trop Med Hyg* 1990;42:175-8.
15. Chutivongse S, Wilde H, Supich C, BAER GM, Fishbein DB. Postexposure prophylaxis for rabies with antiserum and intradermal vaccination. *Lancet* 1990;335:896-8.
 16. Khawplod P, Wilde H, Chomchey P, et al. What is an acceptable delay in rabies immune globulin administration when vaccine alone had been given previously? *Vaccine* 1996;14:389-91.
 17. Lang J, Attanath P, Quiambao B, et al. Evaluation of the safety, immunogenicity, and pharmacokinetic profile of a new, highly purified, heat-treated equine rabies immunoglobulin, administered either alone or in association with a purified, Vero-cell rabies vaccine. *Acta Trop* 1998;70:317-33.
 18. Chomchay P, Khawplod P, Wilde H. Neutralizing antibodies to rabies following injection of rabies immune globulin into gluteal fat or deltoid muscle. *J Travel Med* 2000;7:187-8.
 19. Anderson D. WHO guidelines dealing with immunoglobulin use impede rabies prevention. *Asian Biomed* 2007;1:103-10.
 20. Wilde H, Bhangnada K, Chutivongse S, Siakasem A, Boonchai W, Supich C. Is injection of contaminated animal bite wounds with rabies immune globulin a safe practice? *Trans R Soc Trop Med Hyg* 1992;86:86-8.
 21. Vejjajiva A. Neurological sequelae of anti-rabic inoculation. *Proc Aust Assoc Neurol* 1968;5:367-70.
 22. Tantawichien T, Benjavongkulchai M, Wilde H, et al. Value of skin testing for predicting reactions to equine rabies immune globulin. *Clin Infect Dis* 1995;21:660-2.
 23. Pancharoen C, Thisyakorn U, Tantawichien T, Jaijaroen W, Khawplod P, Wilde H. Failure of pre- and postexposure rabies vaccinations in a child infected with HIV. *Scand J Infect Dis* 2001;33:390-1.
 24. 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;20:818-20.
 25. Khawplod P, Wilde H, Yenmuang W, Benjavongkulchai M, Chomchey P. Immune response to tissue culture rabies vaccine in subjects who had previous postexposure treatment with Semple or suckling mouse brain vaccine. *Vaccine* 1996;14:1549-52.
 26. Manning SE, Rupprecht CE, Fishbein D, et al. Human rabies prevention-United States, 2008: recommendations of the Advisory Committee on Immunization Practices. *MMWR Recomm Rep* 2008;57:1-28.
 27. Rabies vaccines. WHO position paper. *Wkly Epidemiol Rec* 2007;82:425-35.
 28. Tantawichien T, Benjavongkulchai M, Limsuwan K, et al. Antibody response after a four-site intradermal booster vaccination with cell-culture rabies vaccine. *Clin Infect Dis* 1999;28:1100-3.
 29. Khawplod P, Benjavongkulchai M, Limusanno S, et al. Four-site intradermal postexposure boosters in previously rabies vaccinated subjects. *J Travel Med* 2002;9:153-5.
 30. Tantawichien T, Tantawichien T, Supit C, Khawplod P, Sitprija V. Three-year experience with 4-site intradermal booster vaccination with rabies vaccine for postexposure prophylaxis. *Clin Infect Dis* 2001;33:2085-7.
 31. Wacharapluesadee S, Hemachudha T. Nucleic-acid sequence based amplification in the rapid diagnosis of rabies. *Lancet* 2001;358:892-3.
 32. Wacharapluesadee S, Hemachudha T. Rabies diagnosis in human. *J Med Assoc Thai* 2005;88:859-66.
 33. Wacharapluesadee S, Hemachudha T. Urine samples for rabies RNA detection in the diagnosis of rabies in humans. *Clin Infect Dis* 2002;34:874-5.
 34. Bronnert J, Wilde H, Tepsumethanon V, Lumlertdacha B, Hemachudha T. Organ transplantations and

- rabies transmission. *J Travel Med* 2007;14:177-80.
35. Saengseesom W, Mitmoonpitak C, Kasempimolporn S, Sitprija V. Real-time PCR analysis of dog cerebrospinal fluid and saliva samples for ante-mortem diagnosis of rabies. *Southeast Asian J Trop Med Public Health* 2007;38:53-7.
 36. Sitprija V, Sriaroon C, Lumlertdaecha B, et al. Does contact with urine and blood from a rabid dog represent a rabies risk? *Clin Infect Dis* 2003;37:1399-400.
 37. Hemachudha T, Wacharapluesadee S. Antemortem diagnosis of human rabies. *Clin Infect Dis* 2004;39:1085-6.
 38. Kang B, Oh J, Lee C, et al. Evaluation of a rapid immunodiagnostic test kit for rabies virus. *J Virol Methods* 2007;145:30-6.
 39. Lumlertdacha B, Kassawat S, Thepsumethanon V, Hemachudha T. Computer based program for rapid canine rabies diagnosis. *J Med Assoc Thai* 2005;88:1144-6.
 40. Tepsumethanon V, Lumlertdacha B, Mitmoonpitak C, Fagen R, Wilde H. Fluorescent antibody test for rabies: prospective study of 8,987 brains. *Clin Infect Dis* 1997;25:1459-61.
 41. Hemachudha T, Mitrabhakdi E, Wilde H, Vejabhuti A, Siripataravanit S, Kingnate D. Additional reports of failure to respond to treatment after rabies exposure in Thailand. *Clin Infect Dis* 1999;28:143-4.
 42. Wilde H, Sirikawin S, Sabcharoen A, et al. Failure of postexposure treatment of rabies in children. *Clin Infect Dis* 1996;22:228-32.
 43. Wilde H, Choomkasien P, Hemachudha T, Supich C, Chutivongse S. Failure of rabies postexposure treatment in Thailand. *Vaccine* 1989;7:49-52.
 44. Wilde H. Failures of post-exposure rabies prophylaxis. *Vaccine* 2007;25:7605-9.
 45. Chulasugandha P, Khawplod P, Havanond P, Wilde H. Cost comparison of rabies pre-exposure vaccination with post-exposure treatment in Thai children. *Vaccine* 2006;24:1478-82.
 46. Lang J, Feroldi E, Vien NC. Pre-exposure purified vero cell rabies vaccine and concomitant routine childhood vaccinations: 5-year post-vaccination follow-up study of an infant cohort in Vietnam. *J Trop Pediatr* 2009;55:26-31.
 47. Alvarez L, Fajardo R, Lopez E, et al. Partial recovery from rabies in a nine-year-old boy. *Pediatr Infect Dis J* 1994;13:1154-5.
 48. Jackson AC. Rabies therapy. In: Jackson AC, Wunner WH, eds. *Rabies*. Amsterdam: Academic Press, 2002:232.
 49. Hemachudha T, Sunsaneewitayakul B, Mitrabhakdi E, et al. Paralytic complications following intravenous rabies immune globulin treatment in a patient with furious rabies. *Int J Infect Dis* 2003;7:76-7.
 50. Hemachudha T, Sunsaneewitayakul B, Desudchit T, et al. Failure of therapeutic coma and ketamine for therapy of human rabies. *J Neurovirol* 2006;12:407-9.
 51. Rubin J, David D, Willoughby RE Jr, et al. Applying the Milwaukee protocol to treat canine rabies in Equatorial Guinea. *Scand J Infect Dis* 2009;41:372-5.
 52. Willoughby RE Jr, Tieves KS, Hoffman GM, et al. Survival after treatment of rabies with induction of coma. *N Engl J Med* 2005;352:2508-14.
 53. Hattwick MA, Weis TT, Stechschulte CJ, BAER GM, Gregg MB. Recovery from rabies. A case report. *Ann Intern Med* 1972;76:931-42.
 54. Wilde H, Hemachudha T, Jackson AC. Viewpoint: Management of human rabies. *Trans R Soc Trop Med Hyg* 2008;102:979-82.
 55. Fekadu M, Shaddock JH, Baer GM. Intermittent excretion of rabies virus in the saliva of a dog two and six months after it had recovered from experimental rabies. *Am J Trop Med Hyg* 1981;30:1113-5.
 56. Yasmuth C, Nelson KE, Laima T, Supawadee J, Thaiyanant P. Prevalence of abortive canine rabies

- in Chiang Mai, Thailand. *J Med Assoc Thai* 1983;66:169-75.
57. Yasmuth C, Roberts EC Jr, Doege TC. Rabies antibody in healthy dogs and vaccine response after MPA. *J Med Assoc Thai* 1974;57:131-4.
58. Zhang YZ, Fu ZF, Wang DM, et al. Investigation of the role of healthy dogs as potential carriers of rabies virus. *Vector Borne Zoonotic Dis* 2008;8:313-9.
59. Siwasontiwat D, Lumlertdacha B, Polsuwan C, Hemachudha T, Chutvongse S, Wilde H. Rabies: is provocation of the biting dog relevant for risk assessment? *Trans R Soc Trop Med Hyg* 1992;86:443.
60. Tepsumethanon V, Lumlertdacha B, Mitmoonpitak C, Sitprija V, Meslin FX, Wilde H. Survival of naturally infected rabid dogs and cats. *Clin Infect Dis* 2004;39:278-80.
61. Windiyaningsih C, Wilde H, Meslin FX, Suroso T, Widarso HS. The rabies epidemic on Flores Island, Indonesia (1998-2003). *J Med Assoc Thai* 2004; 87:1389-93.
62. Lumlertdacha B, Boongird K, Wanghongsa S, et al. Survey for bat lyssaviruses, Thailand. *Emerg Infect Dis* 2005;11:232-6.
63. Clifton M. More Bali rabies deaths [homepage on the Internet]. *Animal People*, April 2009 [cited 2009 May 18]. Available from: <http://www.animalpeoplenews.org>.
64. Wilde H, Khawplod P, Khamoltham T, et al. Rabies control in South and Southeast Asia. *Vaccine* 2005;23:2284-9.
65. Tepsumethanon V, Wilde H, Hemachudha T. Intra-testicular injection of a balanced zinc solution for permanent sterilization of dogs. *J Med Assoc Thai* 2005;88:686-9.