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Review article

Rabies 2007: perspective from Asia

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Rabies remains a public health problem in many parts of the less developed world. Much is known about the virology, pathophysiology, epidemiology, and methods for control but this knowledge is not applied in many countries. Thailand has been on the frontline of efforts to conduct research in rabies for almost a century, starting with Dr. Leopold Robert from the Institute Pasteur of Paris. He was invited by the Thai King in 1913 to develop a research and production facility for rabies vaccine and snake antivenins which later became the Queen Saovabha Memorial Institute. Thai physicians, scientists and nurses, jointly with some notable expatriate colleagues, were then responsible for major advances in rabies vaccine development, rational application of pre- and postexposure prophylaxis and better understanding of immunology and pathophysiology of this dread disease. They not only discovered new scientific principles but also cost-benefit methods for their application and set the foundation for the work conducted in Thailand during the next two decades. Many concepts developed by Thai scientists have been incorporated into WHO and US-CDC rabies management guidelines. This is an overview of significant developments during the past two decades [1-8].

Keywords: Diagnosis, pathophysiology, prevention and vector control, rabies epidemiology, treatment.

Rabies is most commonly transmitted by bites from infected mammals. The rabies virus can infect any mammal. In man, it remains a fatal disease but some mammals are known to be able to recover from rabies. Lyssaviruses, can be found on all continents except Greenland, Antarctica and some isolated islands. Australia, previously considered rabies- free, harbors a Lyssavirus in fruit and insect-eating bats. This virus caused a fatal rabies-like illness in humans [9-12]. Regions with unsupervised dog populations present the greatest risk to man and canines that live in close proximity to humans are the principal vectors worldwide. Raccoons, skunks, foxes, wolves, bats and other wild carnivores are threats in some parts of the world but account only for a small portion of the large number of human deaths. Rabies reporting is unreliable in many endemic countries. The number of human deaths annually from rabies is unknown but thought to be well over 55,000. Nearly half of these are in children. Small children are less able to defend themselves against biting animals and are more likely to be bitten severely in high-risk body parts such as the face, head and hands [9]. Rabies is responsible for more deaths than polio, yellow fever, Japanese encephalitis, SARS and meningococcal meningitis each. India alone has over 20,000 estimated annual human rabies deaths and Pakistan approximately 5,000 [13]. The disease is amazingly not reportable in both countries. Rabies is emerging again in China which was virtually rabies-free during the reign of Mao Zedong. There were over 3,000 reported annual human cases between 1991-2005 and the trend is said to be increasing. Japan, Taiwan, Malaysia, Singapore and South Korea eliminated canine rabies decades ago. This was done by strict enforcement of vaccination rules and elimination of strays. Canine rabies is again emerging on the demilitarized border with North Korea. It is pathetic that no new Asian countries have been declared rabies free during the last two decades. Rabies can be transmitted through the inhalation of bat secretions, and through the transplantation of infected tissues. It is likely that there are still undetected bat Lyssaviruses in many parts of the world. Bats do not often interact with people but transmission to humans and pets has been documented in the Americas, Europe and Australia. Indigenous bat Lyssaviruses have now been identified in the Philippines, Thailand, Siberia, Central Asia,

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and Cambodia as well as in Australia [12, 15]. Britain, previously considered rabies- free, recently experienced a human death from a European bat Lyssavirus [16]. Bats have been shown to harbor several other viruses potentially dangerous to man and it must be noted that most bats are migratory animals.

Dogs, cats, and other vector animals are occasionally transported from rabies endemic regions to rabies- free ones. The recent introduction of rabies by Indonesian fishermen to rabies-free Flores Island (human population about one million) resulted in an ongoing rabies outbreak with over 100 human deaths [17]. Dogs remain the most important vector for transmission of rabies to humans. We know virtually all that is needed to eliminate canine rabies but cultural, political and economic barriers have prevented application of this knowledge. Sustained vaccination of over 70 % of the canine population can control rabies. In order to regularly vaccinate a large stray canine population, given their short lifespan and rapid reproduction rate, canine numbers must be reduced. This can only be done when societies and governments are motivated to enforce vaccination regulations and to reduce stray dog populations. This requires funding, legislation and energetic law enforcement [9].

Fig. 1 Furious dog.

Fig. 2 Child's ear.

Clinical diagnosis of rabies in dogs and humans

Rabies presents itself in one of two forms, 'furious' or "dumb", but atypical presentations are also seen. They are more common in bat origin viruses. Diagnosis of canine and feline rabies is not difficult when it is of the furious form. Irritability, aggression, increased salivation, indiscriminate biting with damaged and inflamed oral structures suggest rabies. The dumb or paralytic form of rabies (approximately 30 % in dogs) presents diagnostic challenges.The clinical picture is similar to other infections such as canine distemper. Euthanasia and histological examination of the animal's brain is not always available and it is best to start post-exposure prophylaxis (PEP) immediately in a possibly exposed human. A free interactive computer program (*www.soonak.com/rabies*) can aid in the clinical diagnosis of canine rabies [18].

The "furious" human form is characterized by prodromal symptoms manifesting as a feeling of impending doom and pain or abnormal sensation in or near the bite site. This is seen in 70 % of cases. Other symptoms are vague such as anxiety, fever, headache, muscular aching, even diarrhea. This is followed by the neurological phase consisting of alternating intervals of agitation, aggression and coherent calmness. Hydrophobic and aerophobic spasms of the neck and diaphragm may occur intermittently and may not appear together. Autonomic dysfunction may start early but usually becomes prominent in the neurological phase with excessive salivation, fluctuating blood pressure, urinary incontinence, cardiac arrythmias, pupillary dysfunctions and neurogenic pulmonary edema. Hallucinations and seizures are rare in dog-related cases but are seen in bat rabies. Within a few days, coma ensues with respiratory failure, leading to rapid demise unless life is prolonged by cardio-pulmonary support. One third of the human (and canine) cases present themselves in the paralytic form resembling the Guillain-Barre syndrome which is difficult to diagnose without experience and sophisticated laboratory help. Phobic spasms can be seen in only half of paralytic cases. Survival time is longer than in the furious form (11 versus 5 days). The paralytic form of rabies in humans is not due to different virus characteristics. We have found that the same dog can cause the furious form in one victim and paralytic manifestations in another. The clinical signs and symptoms appear to be due to different host responses and are caused largely by immune dysfunction of peripheral nerves and delay in invasion of the brain. In the advanced phase, the histo-pathology is virtually identical [19]. Rabies must be considered in any patient presenting an unclear encephalopathy. An acute psychosis, use of illicit drugs, medications or alcohol may mislead clinicians. Rapid deterioration to coma suggests rabies. Constant rigidity of muscles is the hall mark of tetanus. Acute hepatic porphyria can be excluded by appropriate tests. A history of recent animal encounters (which may be absent in cryptic bat related cases or unsupervised small children), fever, muscular paralysis with preserved consciousness and intact sensory function, urinary incontinence, percussion myoedema, inspiratory spasms and rapid progression to respiratory failure suggest paralytic rabies [20-22]. Rabies awareness is inadequate worldwide. This became evident in recent transplantation-related disasters in Germany and the United States. One donor was diagnosed as drug abuse psychosis and the other as having had drug intoxication and a possible subarachnoid hemorrhage. The history that one had just returned from India and having experienced a dog bite, in the German case, and that the American victim had been bitten by a bat, was not elicited or disregarded. The two patients were used as tissue donors for 10 recipients of which seven died of rabies [23, 24]. One recipient, who had received rabies vaccine years previously, survived the transplant and developed a huge accelerated neutralizing antibody response demonstrating long-lasting immune memory from rabies vaccine.

Laboratory diagnosis

Histological or molecular examination of the brain is the "gold standard" for diagnosing rabies. Antemortem laboratory diagnosis of rabies in animals is not recommended since distribution of virus in organs may vary and shedding in saliva, urine and spinal fluid is intermittent [25]. Postmortem brain examination is by demonstration of rabies antigen using the direct fluorescent antibody test (DFA), immunohistochemistry or by molecular methods. There were no false-negative results in a prospective study of 8,987 brain impression smears carried out by an experienced laboratory [26]. Brain tissue, dried on filter paper, can be kept at room temperature for many days for rabies virus RNA detection [27]. To be of clinical value, results must be rapidly available, sensitive and specific since they will contribute to evidence-

based post exposure prophylaxis decisions for managing patients.In contrast to the above-mentioned methods, detection of classical Negri bodies (by Sellers stain) in histopathological specimens is neither sensitive nor specific [28]. Knowledge of the genetic sequences of rabies virus is useful for epidemiological surveillance and the study of transmission dynamics and should be obtained wherever possible [29]. A recent doctoral thesis project, using a genetically modified virus that fluoresces in green, allowed modification of the established DFA test without the use of the expensive commercial conjugate [30]. This procedure needs further larger controlled study before it can be applied in clinical practice but appears promising.

Rapid antemortem laboratory diagnosis in humans is important for formulating ethical and rational management decisions concerning patients with rabies [31]. Using RT-PCR and other molecular techniques can be performed with results known within a day. Saliva, cerebrospinal fluid, urine, hair follicles and tears should be tested simultaneously owing to the intermittency of virus secretion. Negative results require repeat testing when there is a clinical suspicion of rabies. [32-35]. CT is of virtually no diagnostic value in rabies. Carefully carried out MRI can be helpful and is the subject of several recent studies that analyzed viral and cytokine distribution in relation to MRI abnormalities. Results are variable and closely related to the time when MRI is performed. MRI performed early in the clinical course may be normal. Significant findings appear late [19, 20]. In vivo brain biopsy is now rarely done in rabies patients but should be able to demonstrate the virus using DFA or molecular techniques. The authors do not recommend using tissue donors who have unclear neurological disease. If, however, such a donor is still considered, multiple brain samples should be examined prior to using any tissue for transplants. Brain necropsy can be done via the superior orbital fissure using a kidney or liver biopsy needle. It is invaluable when a complete necropsy can not be performed. A VDO demonstration can be seen in *www.soonak.com.*

Postexposure prophylaxis (PEP)

Postexposure prophylaxis (PEP) requires immediate vigorous cleansing of bite wounds with flowing water and soap, preferably under pressure. This is followed by application of a viricidal agent (iodophore, benzalkonium chloride, hydrogen peroxide, 70 % alcohol or, equally effective and least expensive, Dakins solution). Washing with water may decrease the size of the virus inoculum, while soap and antiseptic agents denature the virus and prevent invasion. This is followed by risk evaluation. This often involves a game of "Russian Roulette" since, even though PEP carries few risks of serious adverse reactions, it can be painful and is expensive [36]. In countries with a well developed public health care system and few if any rabies risk factors, it is often decided to observe a responsible animal or to euthanize and examine it by a competent laboratory. This takes time and can result in delayed PEP. In a canine endemic country with a large stray dog population, it is a different story. The risk is high and the threshold for PEP is and should be much lower. Monkeys, cats, other domestic and agricultural mammals are all potential accidental hosts as they live in close proximity to dogs and bats. Rats have only been found very rarely infected but they are quite large in many Asian cities and theoretically able to survive an attack by a rabid dog or cat. PEP consists of thorough wound care followed by administration of a course of tissue culture vaccine (see **Table 1**). It is important to understand that it takes 7-10 days for a significant level of vaccine-induced natural antibody to form. This may leave time for the virus to invade peripheral nerves. Once inside nerve cells, virus is not reached by anti-rabies antibody and can travel through nerve cells centrally to the brain. Passive immunity must therefore be provided as soon as possible after the bite. This is done by aggressively injecting anti-rabies immunoglobulin (RIG) into and around the bite wounds to neutralize virus [9]. Immunoglobulin, if injected intramuscularly at a distant site from the wound, is probably useless [37]. A vaccine series is then started to induce active immunity by endogenous antibody production. Consultation with an infectious disease expert is advised when unusual problems are encountered. Post- exposure rabies prophylaxis is expensive and often not properly performed. Prevention through vector control should be our first goal for the future [38].

Delay in starting PEP must be avoided at all cost. Rabies incubation periods may be as short as a few days, or as long as years. This depends on the site of the bite (distance to the CNS and presence of many peripheral nerve endings), unknown host factors and the size of the inoculum [31]. **Table 1** summarizes the approach to rabies-exposed patients. It is best to initiate PEP unless immediate necropsy of the

responsible animal excludes rabies. There are no reliable in vivo tests on the animal that would eliminate rabies. It is best to start PEP and, if the animal is observed and later found free of the virus, the vaccine series can be discontinued. There are no contraindications to rabies PEP [9, 22]. It has been used safely in pregnant mothers and their infants [39]. An infected wound can be injected safely with RIG as long as antibiotics are also used [40]. A vaccine history in the responsible dog is not an absolute justification for not providing PEP to a bite victim, unless the vaccination has been thoroughly documented and more than one annual dose had been reliably administered [41, 42]. The observation that a dog bite was provoked has limited value in excluding rabies [43].

The rabies expert committee of WHO has recognized four postexposure rabies treatment schedules. They are:

1) The "Gold Standard" Essen regimen consists of one intramuscular full dose injection on days 0, 3, 7, 14 and 28. (5 clinic visits) [9].

2) The Zagreb or 2-1-1 regimen consists of two full dose intramuscular injections at different sites on day 0 and one each on days 7 and 21 (3 clinic visits) [44].

3) The Thai Red Cross Intradermal Regimen consists of two injections of 0.1 mL of any WHO recognized tissue culture vaccine at two different lymphatic drainage sites on days 0, 3, 7 and 28 (4 clinic visits). It was first developed in 1984 and consisted of 5 clinic visits. It was modified and clinic visits are now reduced to 4 by omission of the original day 90 booster dose and doubling of the day 28 dose [45, 46].

4) The Oxford Intradermal or 8-site intradermal regimen consists of one injection of 0.1 mL of any WHO recognized tissue culture vaccine at 8 different body sites on day 0, at 4 sites on day 7, and at one site on days 28 and 90 [47] (5 clinic visits).

Intramuscular injections of vaccine must be administered in the deltoid or lateral thigh regions avoiding fat. Intradermal vaccines are injected into arms or legs at different lymphatic drainage sites [47] and in the case of the Oxford Regimen, also into the abdominal and intrascapular regions. Just like with BCG vaccination and tuberculin testing, the appearance of a "bubble" at the injection site demonstrates successful intradermal and not subdermal administration. If the "bubble" has not appeared, simply repeat the injection nearby. Reduced-dose intradermal PEP (schedules 3 and 4) significantly decrease the cost of vaccination and have been used at rabies control clinics in several countries for nearly two decades. Many studies have shown equivalent immunogenicity and efficacy of the four regimens. There is even some experimental evidence that intradermal rabies vaccination induces an earlier cellular immune response than the intramuscular schedules [48].

WHO recognized tissue culture rabies vaccines have excellent safety records. Adverse reactions are minor and equivalent to those seen with vaccines against polio, mumps and measles. They are far less reactogenic than tetanus toxoid. Transient erythema, discomfort and itching at injection sites as well as mild regional lymphadenopathy (particularly with ID regimens which stimulate the cellular immune response) are reported with rabies vaccine injections. Mild transient fever, headache and malaise are also seen. Human diploid cell rabies vaccine (HDCV), and rarely other tissue culture vaccines, may cause serum sickness-like reactions in individuals that had a prior series and are later given frequent boosters. These are not due to the antigen but from immune complexes, usually between serum albumin and propiolactone [49]. Vaccines alone will protect the vast majority of rabies exposed patients, but it is not possible to predict who will die if not also given passive immunization using immunoglobulin injected into and around the bite wounds. Patients with facial, head and hand bites are at the highest risk of death and they represent a priority group if immunoglobulin is in short supply [50]. Patients often present to a clinic after having received vaccine without immunoglobulin. It has been shown that RIG can be administered without significant suppression of the immune response up to 7 days after the first vaccine dose had been given [51].

The original equine or sheep serum-derived antisera had a deserved bad reputation for serum sickness and anaphylaxis [52]. Second generation highly purified equine anti-rabies immunoglobulins (ERIG) have an acceptable safety margin causing only 1-7 % serum sickness reactions depending on the product, equine protein content and batch [52]. An effort was later made to reduce the serum sickness and local reaction rate by further purification and splitting of the IgG antibody using pepsin digestion. However, there is controversy whether splitting the IgG by pepsin digestion does also reduce efficacy. Human rabies immunoglobulin (HRIG) is in short supply, very expensive and therefore not available

where it is needed the most. A new equine product has been chromatography purified, pepsin digested and heat inactivated. It is safe but its efficacy has been challenged [53]. Several new manufacturers of ERIG have emerged in Thailand, India, South America and China. Some are currently undergoing WHO pre-approval studies and may soon appear on the international market. Unfortunately, all appear to be pepsin digested split IgG products that have a shorter half-life than the former purified ERIG that was not pepsin digested. However, they are the only RIG products presently available in most of the canine rabies endemic world and thus must remain an essential biological that is used when RIG is indicated. Serum sickness usually appears one week after ERIG is administered and can be managed by using analgesics, antihistamines and good communication with the patient. Steroids are contraindicated as they will suppress the immune response to the vaccine. Using purified ERIG products in Bangkok, we encountered only two cases of anaphylaxis among more than 150,000 ERIG recipients. Both occurred within 30 minutes of receiving the ERIG and could be managed as outpatients. Transient local injection site reactions are common, usually appear within hours and are best managed by antihistamines and reassurance of the patient. However, facilities that use any equine serum products, antibiotics and other parenteral products likely to cause rare anaphylaxis must have a protocol and regularly update their emergency procedures. Monoclonal rabies antibody technology has emerged and was found safe and effective in animal experiments. It is now undergoing human studies. It will take time for such products to become commercially available worldwide [54].

Since brain tissue derived Semple and suckling mouse brain (SMB) vaccines are still being used in some countries, one can expect to encounter patients who have rabies exposure but had prior vaccination with Semple or SMB. How do you handle such a patient? A study has shown that antibody responses in such patients are unpredictable. The vaccine batch may have been of high or zero potency [55]. There is at least one vaccine manufacturer in Asia who has consistently made a Semple vaccine with little or no potency [56]. WHO and The Thai Red Cross therefore recommend that patients who had been treated with nerve tissue derived vaccine previously, be dealt with as if they have never been rabies vaccinated [55].

Lack of funding for PEP is a major problem for physicians and nurses in poor developing countries. It is worse for those working outside large cities and in village health centers that have to manage canine bites in patients who do not have the funds to travel to a nearby medical center where they can receive inexpensive intradermal vaccination. The Thai Red Cross intradermal schedule has reduced the cost of a PEP treatment by close to 70 percent over the standard intramuscular regimens. However, using the intradermal regimen, where only one new bite victim or less is seen daily, makes the use of splitting a vaccine ampoule for more than one patient impossible unless the lyophilized vaccine is stored after reconstitution. Several studies have demonstrated that tissue culture vaccine can be reconstituted and used on the same patient for the day 0, 3 and 7 injections as long as it is held under sterile conditions in a refrigerator. The loss of potency over this one week is negligible. This method has been successfully applied in the field but is not WHO approved [57].

Treatment-failures

Failures of PEP have been recorded. Most are due to omissions or deviations from WHO recommendations [58].However, there are documented cases where all appeared to have been done correctly in a normal host including proper wound care, timely start of PEP, use of WHO recognized vaccine, HRIG or ERIG injected into and around bite wounds. A recent paper collected seven such cases [59]. We recently found one more case in the older literature. An eight year old American military dependent was treated with daily duck embryo vaccine and whole IgG anti-rabies serum that was injected into and around the wound. He developed furious rabies three months later and died. Autopsy confirmed the diagnosis by animal inoculation of brain tissue [60]. It is likely that an overwhelming viral load may have been injected into or close to peripheral nerve endings in these eight cases. Virus then propagated and advanced towards the CNS in an immune protected environment. It is not surprising that severely immune compromised subjects, particularly those with full blown AIDS and very low CD4 counts, will not develop antibodies to rabies vaccine PREP or PEP [61, 62]. Good wound care and diligent injection of immunoglobulin into and around the wounds may be life- saving in such a rabies exposed subject. A regular WHO approved PEP vaccine schedule must always be administered [63].

Pre-exposure vaccination (PREP)

Human and equine rabies immunoglobulins are not available in many rabies endemic regions and PEP is often not carried out to WHO standards [56, 64,65].

Pre-exposure prophylaxis (PREP) is therefore recommended for travelers to endemic countries, certain occupations that are likely to come in contact with infected animals and laboratory workers exposed to Lyssaviruses. One study from Thailand showed that 9% of tourists had canine contacts, suggesting that PREP for tourists to high risk regions should be encouraged. Recent studies have demonstrated that immunity following WHO recommended tissue culture vaccine injections is very long lasting. Neutralizing antibodies can be detected as long as two decades after completing a PREP or PEP series. Booster injections then result in an accelerated antibody response [66]. WHO recommends one intramuscular or intradermal booster injection on day 0 and 3 in an individual who experienced a possible rabies exposure after having had a reliable history of PREP or PEP with a WHO recognized tissue culture vaccine [9]. An alternate method is to administer intradermal injections of 0.1 mL of vaccine at four sites (deltoid and lateral thigh) at one sitting. This saves clinic costs and travel time and actually results in higher antibody titers than the conventional injections on days 0 and 3 [67]. Laboratory scientists, working with potentially rabid animals or with live virus, are still advised to have either periodic antibody titer determinations or a booster every 5 years [9]. Some diplomatic missions, non governmental organizations, military and UN teams recommend PREP for their staff when transferred to canine rabies-endemic countries.

Most tourists or business travelers visit rabies-endemic countries for less than two months. They are also reluctant to pay the 300-400 US dollars usually charged for a full three injection series of PREP by travel clinics in western countries. Often there is not enough time before departure to complete such a series. Recent studies have shown that PREP can be completed in one clinic visit at low cost with the expectation that there will be memory cells and an anamnestic response to boosters for at least 12 months [68]. This discovery may be of interest to military personnel or UN staff who may have to be dispatched on very short notice to high risk regions.

Many countries are failing to control canine rabies and harbor large stray and uncontrolled dog populations. The fact that children represent half the world's rabies deaths, led to suggestions to include rabies vaccine as part of routine childhood immunizations (EPI) in high risk regions. Cost-benefit considerations and priority of funds for other vaccinations have, however, prevented implementation [69, 70, 71]. Manufacture of a low cost liquid tissue culture rabies vaccine in a multiple dose vial with preservative is technically feasible and could allow EPI rabies vaccination in high risk areas. The high cost of having any vaccine approved by international regulatory organizations (FDAs, NRAs) may have prevented the development of such a product by one of the large international firms that currently make rabies vaccines. It would require a national government in a rabies endemic country to initiate plans for producing a tissue culture rabies vaccine in a multiple dose formulation that would be affordable for large scale public-sector use.

Management of human rabies patients

Treatment of human rabies was the subject of a 2002 Canadian and US-CDC sponsored conference in Toronto. The convened experts agreed that supportive and comfort care must be the first goal. There have been many past efforts to "cure" rabies using antiviral agents such a ribavirin, interferons, and intrathecal immunoglobulin [72]. One patient was treated with 900 mL of intravenous human rabies immunoglobulin to no avail. This patient did not show detectable antibody in the CSF; thus presenting convincing evidence that the blood- brain barrier remained intact [73]. Intensive curative efforts should be reserved for a time when promising new drugs become available. We have as yet no known proven antiviral agents against Lyssaviruses. However, the survival of a 15 year old girl, bitten by a bat in the USA, who had not received PEP, created hope and much media interest. Treatment consisted of intensive care and induced deep brain wave suppression coma with ketamine and benzodiazepine to lessen excitotoxicity as well as ribavarine and amantadine [74]. This patient was unusual in that she had neutralizing antibodies on admission in both serum and spinal fluid but no demonstrable viable virus or viral RNA could be identified. Her case was similar to the 6 year old other survivor who also had a bat bite and early antibodies in serum and spinal fluid. He had received PEP and was treated with supportive care only and made a full recovery [75]. His virus also could not be isolated. We suggest that these bat-

derived agents could have been of less virulence or that the two young patients were able to mount more rapid cellular or humoral immune responses that controlled their disease. There have now been six documented survivors of rabies. With the exception of the two cases discussed above, all had severe neurological sequele and antibodies in serum and CSF early in the course of their illness. No virus could be recovered from any of them [76, 77]. We treated one rabies patient using the coma induction regimen with ketamine and ribavarine. He never developed neutralizing antibodies and died on the eighth hospital day of multisystem failure. Ample virus was identified throughout his hospital course [78]. Approximately 25 % of our dog related human patients developed serum neutralizing antibodies regardless of the form of rabies. However, none had significant titers in CSF. Similar coma induction regimens have now been applied to ten other patients in the USA, Canada, Mexico, Europe and India; all without success.

Remaining problems

There are still many rabies questions for future researchers and public health officers to answer. Here are a few from the Southeast Asian perspective:

1. Continuing surveillance of rabies in vectors is essential for planning effective control measures. Several Asian countries do not have any effective infrastructure for evidence- based veterinary diagnosis of lyssavirus infection. Cultural barriers block performance of necropsies on canines in parts of Asia. These issues need being addressed.
2. A large number of uncontrolled dogs in many countries are the main threat to humans today and are responsible for most rabies deaths. Control measures are inhibited by cultural, religious and financial constraints. Dog population control will be essential to reducing and eliminating this threat. Culling of dogs is not an option in most countries and surgical castration requires human resources that are not often available. Well-tolerated testicular zinc injection will lead to permanent sterilization of male dogs. It can be done by sub-professional staff at low cost. However, one virile dog can fertilize many females and there is an obvious need for additional chemical, endocrine or immunological low cost sterilization technology for female canines [80]. Promoting vaccination alone, in efforts to control rabies in a large stray canine population, is doomed to failure. Population reduction must go hand in hand with a sustainable immunization program.
3. One injection of rabies vaccine for dogs will not confer lifelong immunity in a canine rabies- endemic region. These are the very regions where sustainable multi-injection rabies vaccination of the large number of uncontrolled dogs has been difficult to achieve. More potent vaccines that confer longer lasting immunity with one injection could mitigate this problem [82].
4. Lacking effective dog rabies control measures in many endemic regions, the feasibility of large scale pre-exposure rabies vaccination of high risk children must now be considered. This is only possible if low cost tissue culture vaccines become available. Introducing a multiple dose formulation human vaccine for public sector use might be one solution.
5. Exposure to bats is now the major cause of human rabies in North America and the only one in Australia. Bat rabies may be a worldwide phenomenon having been reported from Thailand, Cambodia, Australia and Siberia; though we have not yet identified a human case in Thailand. There is need to learn more about the epidemiology of bat viruses including rabies [81].
6. Biologicals such as tissue rabies culture vaccines, immunoglobulins and monoclonal antibody cocktails must be affordable to poor rabies endemic societies. This will require manufacture in endemic regions.
7. Human and equine rabies immunoglobulins are products that should have long been replaced by monoclonal cocktails. These are available and have been shown in many studies to be more effective and possibly less costly to produce. Their commercial production must be encouraged [79].
8. Most human rabies patients lack serum antibody on presentation and they almost invariably succumb before neutralizing antibody appears in the CNS. How does the virus manage to avoid surveillance by the immune system?
9. There have been a very small number of human survivors who did manage to develop early serum and CNS antibody and stop viral replication, allowing natural defense mechanisms to control the inflammation. What was the mechanism involved in this and could a better understanding of it provide us with new therapeutic tools?

Table 1. Guide for postexposure prophylaxis (Modified from WHO for rabies endemic regions).

Category (Type of contact with a suspected or confirmed domestic or wild animal or animal not available for observation)	Recommended Treatment
I Touching or feeding animal, licks over intact skin	No treatment if reliable history (a)
II Nibbling over uncovered skin, minor scratches or abrasions without bleeding, licks on broken skin euthanized and found negative for rabies by appropriate laboratory techniques.	Administer vaccine immediately (b) stop treatment if animal healthy after observation for 10 days; or if animal is
III Single or multiple transdermal bites or scratches, contamination of mucous membranes by saliva (licks) if animal is euthanized and found negative for rabies by appropriate laboratory techniques.	Administer rabies immunoglobulin and vaccine immediately (c). Stop treatment if animal remains healthy for 10 days; or

(a) A history obtained from a small child may be unreliable.
(b) If an apparently healthy dog or cat from a low rabies risk area is placed under close observation, it may be justified to delay post-exposure treatment. This observation period applies only to dogs and cats. Other domestic or wild animals should be euthanized and their tissues examined using appropriate laboratory techniques. An exception can be considered in the case of threatened or endangered species. Exposure to rodents, rabbits and hares seldom, if ever, requires specific anti-rabies treatment.
(c) Immunoglobulin is administered into and around the bite sites (see text). In the event of a mucous membrane exposure it is given by deep intramuscular injection (efficacy in this case is controversial)

Table 2. Current WHO recognized rabies vaccines.

This table lists current WHO recognized tissue and avian culture products, as well as older preparations derived from neuronal tissues that are no longer approved but still in use in several countries. Additional tissue and avian vaccines are now appearing from India, China and South America. Some of these are being evaluated by WHO and may be added to the list of recognized products. WHO has approved four postexposure vaccine schedules using tissue culture vaccines [3]:

HDCV	**France, Germany, Canada, *India***
PVRV	**France, *India, Columbia, China***
PCEC	**Germany, India, Japan**
PDEV	***India***
PHKC	*China, Russia, Central Asian Republics*
SMB	South America, Vietnam, Cambodia *(no longer WHO approval)*
Semple	India, Bangladesh, Nepal, Pakistan, Africa *(no longer WHO approval)*

Bold print is WHO recognized product.
Italic Bold print is recognized locally only.

HDCV=Human diploid cell vaccine; PVRV=Purified vero cell rabies vaccine; PCEC = Purified chick embryo cell vaccine; PDEV=Purified duck embryo vaccine; SMB=Suckling mouse brain vaccine; Semple = Semple sheep brain derived vaccine. Note: India has discontinuing production of Semple type vaccines. Switzerland has discontinued producing PDEV and transferred technology to India.

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