tibodies, even if attainable, may not lead to better protection [25]. One must not assume that the 8-site intradermal schedule is a replacement for RIG. A recent rabies-related death involving a 7-year-old Thai girl, who had received the 8-site schedule but no RIG after incurring multiple facial bites, emphasizes the importance of administering RIG in such cases. She died of encephalitic rabies (determined on the basis of clinical evidence) 15 days after having been bitten [30]. However, it is not known whether optimal treatment (including RIG injected into wounds) would have saved her life.

Administration of PEP after exposure is essential, and travelers should seek medical attention immediately. If the traveler later returns home to complete the PEP regimen (or will not be staying at one location long enough to finish the original series), a treatment record with names and contact numbers should be obtained. In the event that the same vaccine is not available at the next destination, a different WHO-recommended vaccine can be used. If there is a change in the route or schedule of administration, this should be the exception. In cases in which such a change has occurred or cannot be avoided, antibody levels should be monitored, if possible [31].

It is impossible to predict who will be completely free of risk in their travels and who should receive expensive preexposure vaccination before leaving. At least 2 studies have been published on the cost-effectiveness of preexposure vaccination [32, 33]. Although cost is a factor, important noneconomic issues are also a necessary part of the process for deciding whether to administer a preexposure series. Preexposure vaccination has long been used for selected populations, such as scientists working with rabies virus, veterinarians, zoologists, cave explorers, international aid workers, missionaries, diplomats, soldiers, and Peace Corps volunteers, as well as others who may come in contact with the virus or with rabid animals as part of their vocation or travel itineraries. We know of only 1 rabies-related death in a person who had received preexposure vaccination with a tissue-culture vaccine. She received 3 injections of HDCV intradermally while taking chloroquine malaria prophylaxis, but she did not receive the recommended booster vaccine after having been bitten by her dog in Africa [34]. Because chloroquine may cause interference with the immune response [22], the WHO recommends that preexposure treatment should be administered intramuscularly when a patient is receiving malaria prophylaxis concurrently [22]. The decision about whether to recommend preexposure vaccination must be based on a review of the traveler's itinerary and planned activities. Tourists staying in hotels and traveling in tour buses are less likely to experience a dog bite. Those who intend to backpack, run on urban or rural roads, or visit temples harboring unvaccinated dogs are at increased risk. Children are more likely than adults to be bitten by animals, and the bites they receive tend to be more severe [2, 8, 23]. Preexposure vaccination with any of the WHO-recommended tissue-culture products (HDCV, PVRV, or PCECV) is given as 1 full dose intramuscularly or 0.1 mL intradermally on days 0, 7, and 21 or 28 [22]. The intradermal route is not being used in the United States, because single-dose 0.1-mL ampules are no longer available. Current WHO-recommended tissue-culture rabies vaccines are among the most immunogenic biological agents known. Intradermal and intramuscular vaccine will produce memory cells that usually last for decades in healthy hosts. They are capable of inducing a rapid anamnestic response after administration of boosters [35–40].

Rabies-exposed individuals who have previously been vaccinated with a tissue-culture rabies vaccine should receive 2 intramuscular or intradermal vaccine injections given on days 0 and 3 [14, 22, 29]. RIG is not required for patients who have been previously vaccinated and are later exposed to rabies [22]. Subjects who have received preexposure vaccination need only to receive boosters and do not require RIG, which is in limited supply worldwide. This is a powerful argument for preexposure vaccination for travelers at risk of an exposure.

Encountering a case of rabies in a human in a country believed to be "rabies free" is a rare but not unheard of occurrence, as evidenced by the recent rabies-related death in Scotland [41]. Early diagnosis is imperative. Although physicians experienced with rabies in humans are generally able to diagnose encephalitic (furious) rabies, paralytic rabies (approximately one-third of all cases) can be difficult to identify [2, 10]. It resembles Guillain-Barré syndrome (GBS), and patients with GBS and patients with rabies who are in coma require sophisticated tests for accurate diagnosis [2, 10, 11]. The best specimens to test include CSF, urine, saliva, corneal imprints, and neck skin biopsy specimens, which must include hair follicles [2]. Saliva, urine, and CSF samples and tear secretions can be tested by RT-PCR or nucleic acid sequence–based amplification (NASBA). Serum and CSF specimens can be tested for the presence of IgM and IgG antibodies (in previously unvaccinated individuals), but the test results are often not positive when the patient first presents [2, 42]. Performance of RT-PCR or NASBA on several sequential samples of saliva, urine, and CSF will result in the best diagnostic yield for patients suspected of having rabies. Secretion of virus is intermittent even in CNS and saliva [10, 20, 21]. Rapid clinical and, where available, laboratory diagnosis is important to prevent potential exposure to the health care team and to reduce the anxiety and costs associated with avoidable PEP treatment. Comfort care should be the management goal for patients with rabies [43]. Invasive procedures and even respiratory support should be avoided in virtually every case. Liberal use of barbiturates and intravenous morphine are best for relief of terrifying attacks of anxiety, agitation, and respiratory spasms.

Despite our understanding of the epidemiology and prevention of rabies, we are still confronted with this horrific disease.

Travelers must be made aware that even regions considered to be "rabies free" are vulnerable if rabies is introduced. One example occurred in 1997 in Flores, Indonesia. This small, historically rabies-free island near Timor has a population of 1.4 million. Rabies was introduced when fishermen imported 3 dogs (at least 1 of which was incubating rabies) from rabies-endemic Sulawesi [44, 45]. Within 2 years, at least 101 people died of rabies. As a first response to control the spread of rabies, ~500,000 of the estimated 800,000 local dogs were killed. This massive canine culling failed to eliminate rabies on Flores, and human deaths were still reported in 2001. Sadly, canine rabies is still present on the island today. An islandwide dog-vaccination campaign may have prevented the spread of the disease when it was first discovered almost 6 years ago. Without vigilant surveillance and some contingency planning, the tragic events in Flores could repeat themselves in other rabies-free regions, such as neighboring Bali.

The limited supply of RIG is a critical problem that needs to be addressed on a global scale. Established international ERIG manufacturers must be encouraged to continue production and worldwide distribution of affordable ERIG. Countries where rabies is endemic that have technical expertise should consider production of their own RIG. With government motivation, technology transfer, and funding, well-equipped blood banks could make HRIG. Snake antivenin plants could use established technology to produce purified ERIG. One example is the Thai Red Cross, which has been manufacturing HRIG from serum obtained from unpaid donors for >1 decade and is currently upgrading its snake antivenin plant to make purified ERIG. New ERIG manufacturing facilities have appeared in China and have started to export their products. India is producing their own tissue-culture rabies vaccines (PCECV) and exporting to several Asian countries, including Thailand. Technology for production of monoclonal rabies antibodies (MAbs) is known, and reports indicate that a cocktail of MAbs is more effective in neutralizing virus than is HRIG or ERIG. Selected MAbs could someday replace RIG altogether [46, 47]. Travelers and expatriates will, however, continue to be at risk of exposure to rabies in countries where canine rabies is endemic, until effective dog population–control and vaccination measures are inaugurated. For these programs to be successful, we will need to learn more about dog ecology and to promote responsible dog ownership. Travel medical advisors must evaluate the travel plans of clients visiting countries where canine rabies is endemic and explain various options for protection against this disease. Before leaving home, every traveler should be informed that exposures to potentially rabid animals can have deadly consequences if not treated promptly and appropriately. They should carry contact information for reliable institutions that can be consulted in the event of a medical emergency abroad. Additional help can be found on several Web sites, including http://www.cdc.gov/travel, http://www.fitfortravel.scot.nhs.uk/, http://www.who.int/ith, http://www.who.int/emc/diseases/zoo/rabies.html, and http://www.who.int/disease-outbreak-news/n1997/may/n6may1997a.html.

Is there any good news about rabies? Yes. Expatriates and travelers moving to rabies-free countries with their dogs and cats may no longer have to quarantine their pets for many months. Many rabies-free countries now allow dogs and cats to be imported if they have positive identification by an implanted "microchip," a valid rabies-vaccination certificate, and documentation of an acceptable antibody level from an accredited laboratory [48]. Such travelers should contact the respective consular missions for instructions before departure. In addition, the WHO is currently intensifying efforts to make governments aware of the need to institute active and effective rabies-control measures, and it has appointed an international task force to focus on rabies prevention in Asia.

## Dedication

This essay is dedicated to the memory of Dr. Arthur King, a friend who devoted his life to the study of rabies. He passed away in June 2002. His counsel and humor will be greatly missed by his colleagues worldwide.

## References


1. World Health Organization. Annex table II. In: World health report. Geneva: World Health Organization, 2000:183.
2. Rupprecht CE, Hanlon CA, Hemachudha T. Rabies re-examined. Lancet Infect Dis 2002; 2:327–43.
3. World Health Organization. World survey of rabies no. 34. Document WHO/CDS/CSR/APH/99.6. Geneva: World Health Organization, 1999.
4. Krebs JW, Noll HR, Rupprecht CE, et al. Rabies surveillance in the United States during 2001. J Am Vet Med Assoc 2002; 221:1690–701.
5. Bhanganada K, Wilde H, Sakolsataydorn P, et al. Dog bite injuries at a Bangkok teaching hospital. Acta Tropica 1993; 55:249–55.
6. Weiss HB, Friedman DI, Coben JH. Incidence of dog bite injuries treated in emergency departments. JAMA 1998; 279:51–3.
7. Nixon J, Pearn J, McGarn F. Dog bite injuries to children: potential rabies threat to Australia. Med J Aust 1980; 1:175–6.
8. Tepsumethanon S, Tepsumethanon T, Wilde H. Risk of rabies after mammalian bites in Thai children. J Med Assoc Thai 2002; 85:77–80.
9. Warrell DA, Warrell MJ. Rabies. In: HP Lambert, ed. Infections of the central nervous system. Philadelphia: BC Decker, 1991:317–28.
10. Rupprecht CE, Hanlon CA, Hemachudha T. Rabies re-examined. Lancet Infect Dis 2002; 2:337–53.
11. Jackson AC, Wunner WH, eds. Rabies. Amsterdam: Academic Press, 2002.
12. World Health Organization. Strategies for the control and elimination of rabies in Asia: report of an interregional consultation. Document WHO/CSR/EPT/2002.8. Geneva: World Health Organization, 2002.
13. Ayele W, Fekadu M, Zewdie B, et al. Immunogenicity and efficacy of Fermi-type nerve tissue rabies vaccine in mince and in humans undergoing post-exposure prophylaxis for rabies in Ethiopia. Ethiop Med J 2001; 39:313–21.
14. World Health Organization. Rabies vaccines. Wkly Epidemiol Rec 2002; 77:109–19.

15. Wilde H, Tipkong P, Khawplod P. Economic issues in rabies treatment. J Travel Med 1999; 6:238–42.
16. Wilde H, Tipkong P, Khawplod P. Economic issues in postexposure rabies treatment. J Travel Med 1999; 6:238–42.
17. Pastoret PP, Brochier B. Epidemiology and control of fox rabies in Europe. Vaccine 1999; 17:1750–4.
18. Hanlon CA, Niezgoda M, Hamir AN, et al. First North American field release of a vaccinia-rabies glycoprotein recombinant virus. J Wildl Dis 1998; 34:228–39.
19. Perera MA, Harischandra PA, Wimalaratne O, et al. Feasibility of canine oral rabies vaccination in Sri Lanka: preliminary report. Ceylon Med J 2000; 45:61–4.
20. Wacharapluesadee S, Hemachudha T. Nucleic-acid sequencing based amplification in the rapid diagnosis of rabies. Lancet 2001; 358:892–3.
21. Wacharapluesadee S, Hemachudha H. Urine samples for rabies RNA detection in the diagnosis of rabies. Clin Infect Dis 2002; 34:874–5.
22. World Health Organization Expert Committee on Rabies. Eighth report. Technical series 824. Geneva: World Health Organization, 1992.
23. Wilde H, Sirikawin S, Subcharoen A, et al. Failure of post-exposure treatment of rabies in children. Clin Infect Dis 1996; 22:228–32.
24. 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.
25. Khawplod P, Wilde H, Tepsumethanon S, et al. Prospective immunogenicity study of multiple intradermal injections of rabies vaccine (an effort to obtain early immune response without using immunoglobulin). Clin Infect Dis 2002; 35:1562–5.
26. Wilde H, Thipkong P, Sitprija V, Chaiyabutr N. Heterologous antisera and antivenins are essential biologicals: perspectives on a worldwide crisis. Ann Intern Med 1996; 125:233–6.
27. Wilde H. What are today's orphaned vaccines? Clin Infect Dis 2001; 33:648–50.
28. Thisayakorn U, Pancharoen C, Wilde H. Immunological and virological evaluation of HIV-1 infected children after rabies vaccination. Vaccine 2001; 19:1534–7.
29. World Health Organization. Recommendations on rabies postexposure treatment and the correct technique of intradermal immunization against rabies. Document EMC.ZOO 96.6. Geneva: World Health Organization, 1996.
30. Sriaroon C. Rabies in a Thai child treated with the 8-site post-exposure regimen without rabies immune globulin. Vaccine (in press).
31. Martinez L, ed. International travel and health. Geneva: World Health Organization, 2002.
32. Bernard KW, Fishbein DB. Pre-exposure rabies prophylaxis for travelers: are the benefits worth the cost? Vaccine 1991; 9:833–6.
33. LeGuerrier P, Pilon PA, Deshaies D, Allard R. Pre-exposure rabies prophylaxis for the international traveler: a decision analysis. Vaccine 1996; 14:1075–76.
34. Bernard KW, Fishbein DB. Rabies virus. In: Mandel GL, ed. Principles and practice of infectious diseases. London: Churchill Livingston, 1995: 1527–42.
35. Thraenhart O, Krenzfelder M, Hillebrandt I, et al. Long-term humoral and cellular immunity after vaccination with cell culture rabies vaccine in man. Clin Immunol Immunopathol 1994; 71:287–92.
36. Briggs DJ, Schwenke JR. Longevity of rabies antibody titre in recipients of human diploid cell rabies vaccine. Vaccine 1992; 10:125–9.
37. Strady A, Lang J, Lienard M, et al. Antibody persistence following preexposure regimens of cell-culture rabies vaccine: 10-year follow-up and proposal for a new booster policy. J Infect Dis 1998; 177:1290–5.
38. Jaijaroensup W, Limsusanno S, Khawplod P, et al. Immunogenicity of rabies postexposure booster injections in subjects who had previously received intradermal preexposure vaccination. J Travel Med 1999; 6: 234–7.
39. Khawplod P, Benjavongkulchai M, Limusanno S, et al. Four-site intradermal boosters in previously rabies vaccinated subjects. J Travel Med 2002; 9:153–5.
40. Briggs DJ, Dreesen DW, Nicolay U, et al. Purified chick embryo cell culture rabies vaccine: interchangeability with human diploid cell culture rabies vaccine and comparison of one versus two-dose postexposure booster regimen for previously immunized persons. Vaccine 2000; 19:1055–60.
41. Holie E. Diagnosis of lyssavirus infection confirmed in case of human rabies in Scotland. Eurosurveillance Weekly. 28 November 2002:48.
42. Morrill P, Niezgoda M, Rupprecht C. Use of serology in human rabies diagnosis [abstract 73]. In: Program and abstracts of the XII International Meeting on Research Advances and Rabies Control in the Americas (Oaxaca, Mexico). Washington, DC: Pan American Health Organization, 2002:52.
43. Jackson AC, Warrell MJ, Rupprecht CE, et al. Management of rabies in humans. Clin Infect Dis 2003; 36:60–3.
44. Bingham J. Report on a consultation for the Office International des Epizootie (OIE) to investigate an outbreak of rabies on the island of Flores, Indonesia. Paris: OIE Document, 2000.
45. Wilde H. WHO technical advisory mission on intradermal rabies vaccination. Rabies outbreak: Flores Island, Indonesia. Report to Division of Zoonotic Diseases. Geneva: World Health Organization, 2000.
46. Hanlon CA, Niezgoda M, Morrill PA, et al. The incurable wound revisited: progress in human rabies prevention? Vaccine 2001; 19: 2273–9.
47. Rabies monoclonal antibody cocktail for rabies post exposure treatment: report of a WHO consultation. Geneva: World Health Organization, 2002.
48. Briggs DJ, Schweitzer K. Importation of dogs and cats to rabies-free areas of the world. Vet Clin North Am Small Anim Prac 2001; 31. 575–83.

## Search strategy and selection criteria

We have followed international publications on rabies for longer than 15 years. Data for this review were identified by searches of Medline (PubMed) and references supplied from older relevant review articles; many pieces were identified through searches of our own extensive files. Consideration for inclusion in the reference list included all relevant sources by topic. Only papers published in English were reviewed. Search terms included "rabies", "magnetic resonance imaging", and "encephalitis".

of unvaccinated rabies patients tested within 1–26 days of disease onset.[13,15] Antibody-positive serum samples can be obtained within 9 days after onset (three of six within the first 3 days). However, analysis of 102 samples from 39 patients in the USA and 16 in France since 1960 showed that serum antibody generally developed if the patient survived longer than 8 days (six of 43 between days 1 and 8 compared with 34 of 59 from day 9). Antibody appeared in the CSF later (none of 19 between days 1 and 8 and ten of 28 on day 9).[5,43] Nevertheless, out of 27 Thai patients, none had detectable rabies-virus antibody in the CSF.[13,15]

Rabies-virus antigen in the neural innervation of hair follicles can be demonstrated by the fluorescent-antibody technique on frozen sections of the skin from the nape of the neck. Though sensitive, this technique may not be practicable in all settings, because of the requirement for a cryostat for preparation of ideal frozen tissue sections.[5,43] Early studies suggested that the proportion of positive results should increase as the disease progressed,[50] but the results of another study did not confirm this idea. Test sensitivity was 82% (five of six) when it was done within 4 days compared with 60% (six of ten) between days 5 and 8.[43] Both corneal and salivary impressions for detection of rabies-virus antigen may be unreliable, because of differences in technique and interlaboratory variation in interpretation.

Brain biopsy, although not practicable, yields high sensitivity.[14] Nevertheless, false-negative results may occur when biopsy at the frontotemporal region is done during the first days of illness. Occasional differential occurrence of viral antigen may be overcome by molecular detection, but only with much attention paid to strategies for proper primer design and extraordinary care taken to preclude cross-contamination.[84,85]

In addition to CNS tissue, saliva, CSF, tears, skin biopsy samples, and urine may be sources for detection of rabies-virus RNA by reverse-transcription PCR or nucleic-acid sequence-based amplification.[5,6,43,85,86] Serial samples should be tested, because not all are positive, owing to intermittent shedding of virus.

## Management

Treatment is purely symptomatic, to lessen the degree of agitation and to comfort the patient and family. Fear of rabies is universal among health-care personnel, resulting in poor nursing care. Attending nurses and physicians who routinely care for patients with rabies may need pre-exposure vaccination, and other staff should receive postexposure treatment only if a true exposure occurs despite precautions. Past efforts to prevent fatal outcome have failed, with no spontaneous recoveries. A handful of survivors are known, essentially pre-exposure or postexposure treatment failures, with major sequelae.[13,15,34] Attempted treatments include interferon and antiviral drugs such as ribavirin, vidarabine, aciclovir, and inosine pronobex, intrathecal and systemic high-dose administration of human rabies immunoglobulin, steroids, and antithymocyte globulin; none has been successful.[13]

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#### Authors' contributions

All authors contributed equally to all parts of the text.

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We have no financial and personal relationships with other people or organisations that could inappropriately influence our work.

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#### References

1 World Health Organization. Strategies for the control and elimination of rabies in Asia. Geneva: WHO, 2001.
2 World Health Organization. World survey of rabies number 34 (for the year 1998). Geneva: WHO, 2000. http://www.who.int/emc/diseases/zoo/wsr98/HTML_version/wsr98index.html, accessed May 2002.
3 World Health Organization. Rabies control in Asia. Proceedings of the fourth international symposium organised by the Merieux foundation and WHO. Paris: John Libbey Eurotext, 2001.
4 Rupprecht CE, Hanlon CA, Hemachudha T. Rabies re-examined. *Lancet Infect Dis* 2002; 2: 337–53.
5 Noah DL, Drenzek CL, Smith JS, et al. Epidemiology of human rabies in the United States, 1980 to 1996. *Ann Intern Med* 1998; 128: 922–30.
6 Centers for Disease Control And Prevention. Epidemiology of human rabies. http://www.cdc.gov/ncidod/dvrd/rabies, accessed May 2002.
7 Hanna JN, Carney IK, Smith GA, et al. Australian bat lyssavirus infection: a second human case, with a long incubation period. *Med J Aust* 2000; 172: 597–99.
8 Fraser GC, Hooper PT, Lunt RA, et al. Encephalitis caused by a Lyssavirus in fruit bats in Australia. *Emerg Infect Dis* 1996; 2: 327–31. http://www.cdc.gov/ncidod/EID/index.htm, accessed May 2002.
9 Hemachudha T. Rabies. In: Vinken PJ, Bruyn GW, Klawans HL, eds. Handbook of clinical neurology, revised series. Amsterdam: Elsevier Science Publishers, 1989: 383–404.
10 Sitthi-Amorn C, Jiratanavattana V, Keoyoo J, Sonpunya N. The diagnostic properties of laboratory tests for rabies. *Int J Epidemiol* 1987; 16: 602–05.
11 Morimoto K, Patel M, Corisdeo S, et al. Characterization of a unique variant of bat rabies virus responsible for newly emerging human cases in North America. *Proc Natl Acad Sci USA* 1996; 93: 5653–58.
12 Hemachudha T, Phuapradit P. Rabies. *Curr Opin Neurol* 1997; 10: 260–67.
13 Hemachudha T. Human rabies: clinical aspects, pathogenesis and potential therapy. *Curr Top Microbiol Immunol* 1994; 187: 121–43.
14 Hemachudha T, Phanuphak P, Sriwanthana B, et al. Immunologic study of human encephalitic and paralytic rabies: preliminary report of 16 patients. *Am J Med* 1988; 84: 673–77.
15 Hemachudha T, Mitrabhakdi E. Rabies. In: Davis LE, Kennedy PGE, eds. Infectious diseases of the nervous system. Oxford: Butterworth-Heinemann, 2000: 401–44.
16 Smith JS, Fishbein DB, Rupprecht CE, Clark K. Unexplained rabies in three immigrants in the United States: a virologic investigation. *N Engl J Med* 1991; 324: 205–11.
17 Hemachudha T, Chutivongse S, Wilde H. Latent rabies. *N Engl J Med* 1991; 324: 1890–91.
18 Huang CC, Liu CC, Chang YC, Chen CY, Wang ST, Ye TF. Neurologic complications in children with enterovirus 71 infection. *N Engl J Med* 1999; 341: 936–42.
19 Solomon T, Dung NM, Kneen R, Gainsborough M, Vaughn DW, Khanh VT. Japanese encephalitis. *J Neurol Neurosurg Psychiatry* 2000; 68: 405–15.
20 Goh KJ, Tan CT, Chew NK, et al. Clinical features of Nipah virus encephalitis among pig farmers in Malaysia. *N Engl J Med* 2000; 342: 1229–35.
21 Hemachudha T, Tirawatnpong S, Phanthumchinda K. Seizures as the initial manifestation of paralytic rabies. *J Neurol Neurosurg Psychiatry* 1989; 52: 808–10.
22 Tirawatnpong S, Hemachudha T, Manutsathit S,

Shuangshoti S, Phanthumchinda K, Phanuphak P. Regional distribution of rabies viral antigen in central nervous system of human encephalitic and paralytic rabies. *J Neurol Sci* 1989; 92: 91–99.
23 Phuapradit P, Manatsathit S, Warrell MJ, Warrell DA. Paralytic rabies: some unusual clinical presentations. *J Med Assoc Thai* 1985; 68: 106–10.
24 Hemachudha T, Phanthumchinda K, Phanuphak P, Manutsathit S. Myoedema as a clinical sign in paralytic rabies. *Lancet* 1987; 1: 1210.
25 Pleasure SJ, Fischbein NJ. Correlation of clinical and neuroimaging findings in a case of rabies encephalitis. *Arch Neurol* 2000; 57: 1765–69.
26 Brass DA. Rabies in bats: natural history and public health implications. Connecticut: Livia Press, 1994.
27 Nelson DA, Berry RG. Fatal rabies associated with extensive demyelination. *Arch Neurol* 1993; 50: 317–23.
28 Allworth A, Murray K, Morgan J. A human case of encephalitis due to a lyssavirus recently identified in fruit bats. *Commun Dis Intell* 1996; 20: 504.
29 Metze K, Feiden W. Rabies virus ribonucleoprotein in the heart. *N Engl J Med* 1991; 324: 1814–15.
30 Kureishi A, Xu LZ, Wu H, Stiver HG. Rabies in China: recommendations for control. *Bull World Health Organ* 1992; 70: 443–50.
31 Hattwick MA, Weiss TT, Stechschulte CJ, Baer GM, Gregg MB. Recovery from rabies: a case report. *Ann Intern Med* 1972; 76: 931–42.
32 Porras C, Barboza JJ, Fuenzalida E, Adaros HL, Oviedo AM, Furst J. Recovery from rabies in man. *Ann Intern Med* 1976; 85: 44–48.
33 Centers for Disease Control. Rabies in a laboratory worker. *MMWR Morb Mortal Wkly Rep* 1977; 26: 183–84.
34 Alvarez L, Cashat M, Arellano E, Baer GM. A new phenomenon in rabies: partial recovery in children treated prompty with potent vaccine. In: Proceedings of the 7th international meeting on advances towards rabies control in the Americas. CDC, Atlanta, GA, USA, 1996, 78.
35 Baer GM, Shaddock JH, Quirion R, Dam TV, Lentz TL. Rabies susceptibility and acetylcholine receptor. *Lancet* 1990; 335: 664–65.
36 Hattwick MAW. Human rabies. *Public Health Rev* 1974; 3: 229–74.
37 Debbie JG, Trimarchi CV. Prophylaxis for suspected exposure to bat rabies. *Lancet* 1997; 350: 1790–91.
38 Hattwick MAW, Gregg MB. The disease in man. In: GM Baer, ed. The natural history of rabies. New York: Academic Press, 1975: 281–303.
39 Constantine DG. Rabies transmission by air in bat caves. US Public Health Service publication 1967.
40 Tariq WU, Shafi MS, Jamal S, Ahmad M. Rabies in man handling infected calf. *Lancet* 1991; 337: 1224.
41 Fishbein DB, Robinson LE. Rabies. *N Engl J Med* 1993; 329: 1632–38.
42 Helmick CG, Tauxe RV, Vernon AA. Is there a risk to contacts of patients with rabies? *Rev Infect Dis* 1987; 9: 511–18.
43 Crepin P, Audry L, Rotivel Y, Gacoin A, Caroff C, Bourhy H. Intravitam diagnosis of human rabies by PCR using saliva and cerebrospinal fluid. *J Clin Microbiol* 1998; 36: 1117–21.
44 Lumbiganon P, Wasi C. Survival after rabies immunisation in newborn infant of affected mother. *Lancet* 1990; 336: 319.
45 Charlton KM. The pathogenesis of rabies and other lyssaviral infections: recent studies. *Curr Top Microbiol Immunol* 1994; 187: 95–119.
46 Lentz TL, Wilson PT, Hawrot E, Speicher DW. Amino acid sequence similarity between rabies virus glycoprotein and snake venom curaremimetic neurotoxins. *Science* 1984; 226: 847–48.
47 Hanham CA, Zhao F, Tignor GH. Evidence from the anti-idiotypic network that the acetylcholine receptor is a rabies virus receptor. *J Virol* 1993; 67: 530–42.
48 Lewis P, Fu Y, Lentz TL. Rabies virus entry at the neuromuscular junction in nerve-muscle cocultures. *Muscle Nerve* 2000; 23: 720–30.
49 Charlton KM, Nadin-Davis S, Casey GA, Wandeler AI. The long incubation period in rabies: delayed progression of infection in muscle at the site of exposure. *Acta Neuropathol (Berl)* 1997; 94: 73–77.
50 World Health Organization. 8th report of the WHO expert committee on rabies. Technical report series, no 824. Geneva: WHO, 1992.
51 Tsiang H. Interaction of rabies virus and host cells. In: Campbell JB, Charlton KM, eds. Rabies. Boston: Kluwer Academic Publishers, 1988: 67–100.
52 Tsiang H. Pathophysiology of rabies virus infection of the nervous system. *Adv Virus Res* 1993; 42: 375–412.
53 Tangchai P, Vejjajiva A. Pathology of the peripheral nervous system in human rabies: a study of nine autopsy cases. *Brain* 1971; 94: 299–306.
54 Minguetti G, Hofmeister RM, Hayashi Y, Montano JA. Ultrastructure of cranial nerves of rats inoculated with rabies virus. *Arq Neuropsiquiatr* 1997; 55: 680–86.
55 Kucera P, Dolivo M, Coulon P, Flamand A. Pathways of the early propagation of virulent and avirulent rabies strains from the eye to the brain. *J Virol* 1985; 55: 158–62.
56 Coulon P, Derbin C, Kucera P, Lafay F, Prehaud C, Flamand A. Invasion of the peripheral nervous systems of adult mice by the CVS strain of rabies virus and its avirulent derivative AvO1. *J Virol* 1989; 63: 3550–54.
57 Shankar V, Dietzschold B, Koprowski H. Direct entry of rabies virus into the central nervous system without prior local replication. *J Virol* 1991; 65: 2736–38.
58 Lentz TL, Chester J. Synaptic vesicle recycling at the neuromuscular junction in the presence of a presynaptic membrane marker. *Neuroscience* 1982; 7: 9–20.
59 Cameile P DE, Takei K. Molecular mechanisms in synaptic reside endocytosis and recycling. *Neuron* 1996; 16: 481–86.
60 Tuffereau C, Benejean J, Blondel D, Kieffer B, Flamand A. Low-affinity nerve-growth factor receptor (p75NTR) can serve as a receptor for rabies virus. *EMBO J* 1998; 17: 7250–59.
61 Thoulouze MI, Lafage M, Schachner M, Hartmann U, Cremer H, Lafon M. The neural cell adhesion molecule is a receptor for rabies virus. *J Virol* 1998; 72: 7181–90.
62 Etessami R, Conzelmann KK, Fadai-Ghotbi B, Natelson B, Tsiang H, Ceccaldi PE. Spread and pathogenic characteristics of a G-deficient rabies virus recombinant: an in vitro and in vivo study. *J Gen Virol* 2000; 81: 2147–53.
63 Kelly RM, Strick PL. Rabies as a transneuronal tracer of circuits in the central nervous system. *J Neurosci Methods* 2000; 103: 63–71.
64 Raux H, Flamand A, Blondel D. Interaction of the rabies virus P protein with the LC8 dynein light chain. *J Virol* 2000; 74: 10212–16.
65 Jacob Y, Badrane H, Ceccaldi PE, Tordo N. Cytoplasmic dynein LC8 interacts with lyssavirus phosphoprotein. *J Virol* 2000; 74: 10217–22.
66 Lopez A, Miranda P, Tejada E, Fishbein DB. Outbreak of human rabies in the Peruvian jungle. *Lancet* 1992; 339: 408–11.
67 Warner CK, Zaki SR, Shieh WJ, et al. Laboratory investigation of human deaths from vampire bat rabies in Peru. *Am J Trop Med Hyg* 1999; 60: 502–07.
68 Sheikh KA, Jackson AC, Ramos-Alvarez M, et al. Paralytic rabies: immune attack on nerve fibers containing axonally transported viral proteins. *Neurology* 1998; 50 (suppl 4): 183–84.
69 Piyasirisilp S, Schmeckpeper BJ, Chandanayingyong D, Hemachudha T, Griffin DE. Association of HLA and T-cell receptor gene polymorphisms with Semple rabies vaccine-induced autoimmune encephalomyelitis. *Ann Neurol* 1999; 45: 595–600.
70 Ceccaldi PE, Marquette C, Weber P, Gourmelon P, Tsiang H. Ionizing radiation modulates the spread of an apathogenic rabies virus in mouse brain. *Int J Radiat Biol* 1996; 70: 69–75.
71 Sugamata M, Miyazawa M, Mori S, Spangrude GJ, Ewalt LC, Lodmell DL. Paralysis of street rabies virus-infected mice is dependent on T lymphocytes. *J Virol* 1992; 66: 1252–60.
72 Weiland F, Cox JH, Meyer S, Dahme E, Reddehase MJ. Rabies virus neuritic paralysis: immunopathogenesis of nonfatal paralytic rabies. *J Virol* 1992; 66: 5096–99.
73 Camelo S, Lafage M, Lafon M. Absence of the p55 kD TNF-alpha receptor promotes survival in rabies virus acute encephalitis. *J Neurovirol* 2000; 6: 507–18.
74 Lafon M, Lafage M, Martinez-Arends A, et al. Evidence for a viral superantigen in humans. *Nature* 1992; 358: 507–10.
75 Dumrongphol H, Srikiatkhachorn A, Hemachudha T, Kotchabhakdi N, Govitrapong P. Alteration of muscarinic acetylcholine receptors in rabies viral-infected dog brains. *J Neurol Sci* 1996; 137: 1–6.
76 Dietzschold B, Morimoto K, Hooper DC. Mechanisms of virus-induced neuronal damage and the clearance of viruses from the CNS. *Curr Top Microbiol Immunol* 2001; 253: 145–55.
77 Li Z, Feng Z, Ye H. Rabies viral antigen in human tongues and salivary glands. *J Trop Med Hyg* 1995; 98: 330–32.
78 Deresiewicz RL, Thaler SJ, Hsu L, Zamani AA. Clinical and neuroradiographic manifestations of eastern equine encephalitis. *N Engl J Med* 1997; 336: 1867–74.
79 Nash D, Mostashari F, Fine A, et al. The outbreak of West Nile virus infection in the New York city area in 1999. *N Engl J Med* 2001; 344: 1807–14.
80 Hemachudha T, Griffin DE, Giffels JJ, Johnson RT, Moser AB, Phanuphak P. Myelin basic protein as an encephalitogen in encephalomyelitis and polyneuritis following rabies vaccination. *N Engl J Med* 1987; 316: 369–74.
81 Laothamatas J, Hemachudha T, Tulayadechanont S, Mitrabhakdi E. Neuroimaging in paralytic rabies. *Rama Med J* 1997; 20: 141–48.
82 Awasthi M, Parmar H, Patankar T, Castillo M. Imaging findings in rabies encephalitis. *Am J Neuroradiol* 2001; 22: 677–80.
83 Kalita J, Misra UK. Comparison of CT scan and MRI findings in the diagnosis of Japanese encephalitis. *J Neurol Sci* 2000; 174: 3–8.
84 Kamolvarin N, Tirawatnpong T, Rattanasiwamoke R, Tirawatnpong S, Panpanich T, Hemachudha T. Diagnosis of rabies by polymerase chain reaction with nested primers. *J Infect Dis* 1993; 167: 207–10.
85 Wacharapluesadee S, Hemachudha T. Nucleic-acid sequence based amplification in the rapid diagnosis of rabies. *Lancet* 2001; 358: 892–93.
86 Wacharapluesadee S, Hemachudha T. Urine as a source of rabies RNA detection in the diagnosis of human rabies. *Clin Infect Dis* 2002; 34: 874–75.

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Brief report

# Rabies in a Thai child treated with the eight-site post-exposure regimen without rabies immune globulin

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**Abstract**

We report the case of a 7-year-old Thai girl that was bitten by a dog. She received prompt wound care followed by eight-site intradermal post-exposure rabies schedule using purified chick embryo vaccine. Treatment followed WHO recommendations for desperate situations where no rabies immune globulin (RIG) is available. The patient died 15 days later with classical symptoms and signs of encephalitic rabies.


*Keywords:* Rabies; Accelerated post-exposure treatment failure; Lack of rabies immune globulin

This 7-year-old girl was tying her shoelaces on 8 March 2002, ready to go to school, when she was attacked by the neighbor's unvaccinated 3-month-old puppy. She incurred a 1 cm deep scratch above her left eyebrow, as well as a 3 cm transdermal abrasion of her left cheek. She also had a superficial abrasion of her left arm. Further inquiries revealed that the mother of the puppy had died recently of an undiagnosed illness. Prior to the attack, the puppy was noted to run and bite objects aimlessly, hitting trees and other object. It went into a coma and died naturally the day following the attack. It was not laboratory examined.

The grandmother took the child to the nearby Prachinburi Provincial Hospital within 20 min of the attack. Post-exposure treatment was rendered almost immediately after arrival at the hospital. It included irrigation of the wounds and application of betadine solution. There was no rabies immune globulin (RIG) available in Prachinburi and the staff followed the WHO and Thai Ministry of Public Health guidelines for treatment of a rabies exposure when no RIG is available [1]. They instituted the eight-site intradermal post-exposure schedule using purified chick embryo vaccine manufactured in India by Chiron Corporation of Germany (batch 667, potency 9.5 IU/ml, expiry date July 2005). On day 0, she was given eight intradermal injections of 0.1 ml vaccine at eight different sites by a nurse experienced in this technique [1]. She also received amoxycillin and a tetanus toxoid booster injection. The second course of intradermal injections was administered at four intradermal sites on 15 March at which time the patient appeared well. She was then scheduled for the recommended 0.1 ml one-site boosters on days 29 and 90 [1]. On 19 March, 11 days after having been bitten, the patient complained of headache and became febrile. Her grandmother took her to the village health station and she was given more oral amoxycillin. That evening she vomited and remained febrile. On 20 March, the patient's consciousness became clouded but she still could respond and answer questions. She was taken back to the Prachinburi Provincial Hospital where she was admitted with a temperature of 39.4 °C. She was started on intravenous ampicillin and was isolated. The admitting resident doctor did considered rabies in the differential diagnosis. On the following morning, she complained of severe pain over her left ear, the same site as her dog bites. An ENT consultant examined her and found a normal ear canal. However, there was evidence of a lower motor neuron left facial nerve palsy. He then ordered a CT of the mastoid area and head. It was unremarkable. During the

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afternoon and evening, she developed restlessness, spasms of both arms in response to air currents and people walking into the room. She also developed visual hallucinations and agitation, alternating with periods of normal behavior. She refused to eat and drink. A spinal puncture was performed on 22 March and was unremarkable (clear, RBC 45, protein 46, WBC 40, CSF glucose 64, serum glucose 121).

A CSF sample was sent to a reference laboratory in Bangkok for rabies PCR but was lost in transit. Routine laboratory studies revealed Hgb 12.4, HCT 38, WBC 11,400, PMN 75, Ly 15, Mono 7, urinalysis WBC 20, RBC 5–10, platelets 303,000, electrolytes were normal, BUN 10, creatinine 0.6. Late that night, she became comatose and was transferred to the Chonburi Regional Hospital. There, she was incubated but died on 23 March 2002 of circulatory failure 15 days after being attacked by a dog. Unfortunately, no autopsy was performed and no serum, skin or other samples were available for laboratory confirmation of rabies. Like most rabies patients in endemic regions, the diagnosis was based on the history (dog bite, prodrome), classical symptoms of fever, headache, alternating levels of consciousness, aerophobia and hydrophobia. Rabies post-exposure treatment failures with optimal management (good wound care, administration of potent tissue culture vaccine, injection of wounds with RIG) are very rare but have been reported [2]. Current WHO recommendations for severe (WHO Category III) exposures recommend administration of RIG as soon as possible but not later than day 7 after a post-exposure vaccine series had been started [1,3]. Unfortunately, this proved not feasible in this girl. This case is, to the best of our knowledge, the first treatment failure where an accelerated intradermal regimen was administered without immune globulin. It is, however, impossible to know whether this child would have survived if treated with rabies immune globulin according to optimal WHO guidelines [1]. To determine whether accelerated intradermal schedules, administered without immune globulin, provide any added protection over the conventional five-dose intramuscular or Thai Red Cross intradermal post-exposure regimens if no RIG is available, would be a most difficult task. One would have to select a sizable population with severe dog bites from laboratory proven rabid animals, and obtain ethics committee approval to treat without RIG [1]. To do such a study, is only possible in a location where RIG is unavailable. The current WHO recommendations concerning management of severely rabies exposed patients without RIG [1] need to be revised so that they can not be misinterpreted as being a substitute for bite-site administration of immunoglobulin in a patient with a severe rabies exposure [1,4]. Such a revision might, for example, state that accelerating vaccine administration without RIG represents an unproven and desperate effort where there is no possibility of obtaining RIG. Until the prevention of rabies in dogs is successful, energetic efforts to make affordable RIG or alternatives such as combinations of monoclonal antibodies must continue.

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## References

[1] WHO. Recommendations on rabies post-exposure treatment and the correct technique of intradermal immunization against rabies. No. 21. WHO/EMS/ZOO 96.6. Geneva: WHO; 1996.

[2] Hemachudha T, Mitrabhakdi E, Wilde H, et al. Additional reports of failure to respond after rabies exposure in Thailand. Clin Infect Dis 1999;28:143–4.

[3] 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.

[4] Khawplod P, Wilde H, Tepsumethanon S, Limusanno S, Tantawichien T, Chomchey P, 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(12):1562–5.

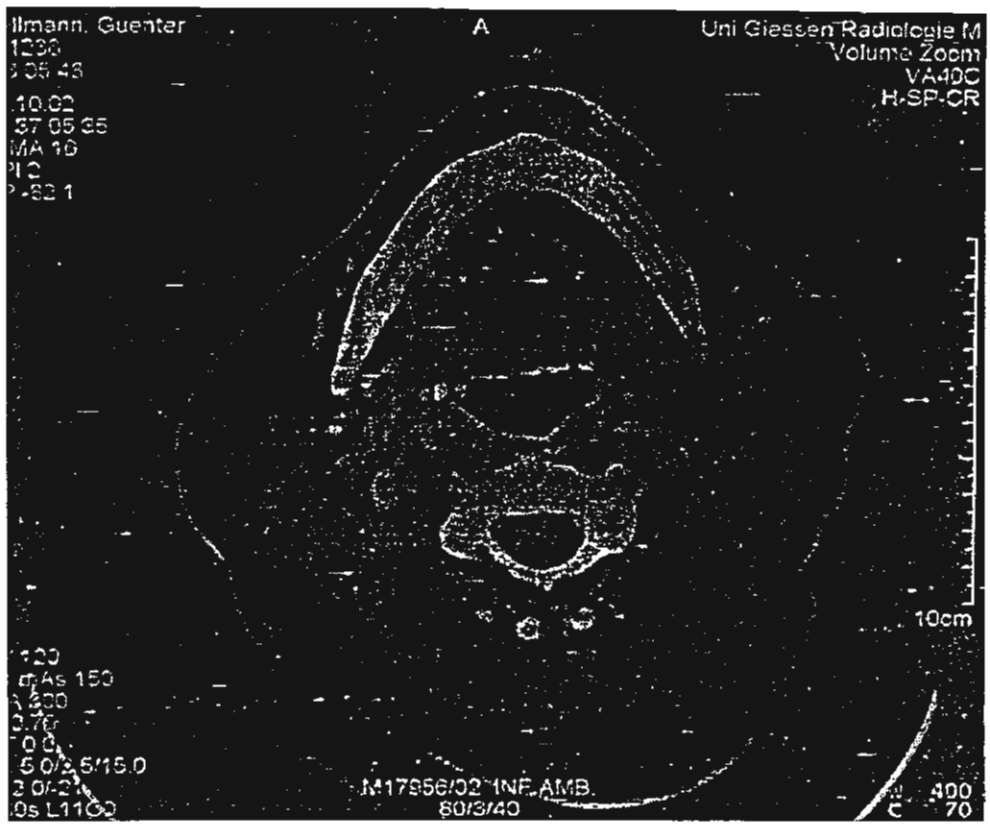


**Figure 1.** CT of the neck, demonstrating extensive accumulation of subcutaneous fat (i.e., "buffalo hump")

was a good response to antiretroviral therapy, the drugs were not changed.

The occurrence of OSA in HIV-infected patients was already investigated in a cohort of 134 subjects [2]. In that study, 12 patients were found to have OSA. Among these patients, all but 1 had adenotonsillar hypertrophy underlying the OSA. In our patient, otorhinolaryngological examination did not reveal any specific abnormalities. Obesity, which is the most important risk factor for the development of OSA, was also not present in the patient under discussion. Male sex and advanced age might have contributed to the emergence of OSA in the present patient. However, we suggest that the sleep-disordered breathing was mainly due to HIV-associated lipodystrophy, with extensive accumulation of adipose tissue around the neck and pharynx (i.e., "buffalo hump"). To the best of our knowledge, such an association has not yet been reported in the literature.

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### References

1. Sullivan PS, Dworkin MS. Adult and adolescent spectrum of HIV disease investigators: prevalence and correlates of fatigue among persons with HIV infection. J Pain Symptom Manage 2003; 25:329–33.
2. Epstein LJ, Strollo PJ Jr, Donegan RB, Delmar J, Hendrix C, Westbrock PR. Obstructive sleep apnea in patients with human immunodeficiency virus (HIV) disease. Sleep 1995; 18: 368–76.

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## Does Contact with Urine and Blood from a Rabid Dog Represent a Rabies Risk?

SIR—Outward spread of rabies virus from the CNS leads to infection of almost all organs, including infection of the heart, lung, gastrointestinal tract, bone marrow, cornea, neck tissues, kidneys, and salivary glands [1–4]. This has allowed clinical diagnosis on the basis of biopsies of the nape of the neck and, more recently, on the basis of detection of rabies virus RNA in saliva and urine samples [1, 5]. Infected

tissues can be a source of infection in person-to-person transmission, as reported with corneal transplants [1]. It is feared that exposure to blood and urine may also result in transmission. If this is, in fact, the case, the finding of preferential involvement of highly perfused renal cortex and renal medulla tissues would support the existence of viremia in rabies.

We report that the blood and urine of dogs infected with rabies was not infective at the time of death. Rabies virus RNA was predominantly found in urinary bladder tissue, thus supporting the idea of neural spread of the virus to the urinary bladder.

Four rabies-infected dogs quarantined in the Queen Saovabha Memorial Institute were included in this study. At the time of natural death, samples of urine and blood (obtained by bladder and heart puncture), as well as samples of kidney, ureter, and urinary bladder tissue, and nerve tissue supplying the bladder, were collected. Except for the samples of blood and urine, all samples were stored at −80°C until examination. Two milliliters of urine, 100 uL of whole blood, and 100 mg of each tissue specimen were examined for the presence of rabies virus RNA by nucleic acid sequence–based amplification, as described elsewhere [6]. Urine and blood samples were also subjected to rabies virus isolation in mouse neuroblastoma cells.

Rabies virus could not be isolated from all urine and blood samples. Rabies virus RNA could be recovered from urine samples (obtained from 4 of the 4 dogs), and could also be recovered from bladder (4 of 4), bladder trigone (4 of 4), urethral sphincter (4 of 4), nerve (4 of 4), ureter (3 of 4), renal pelvis (3 of 4), renal medulla (1 of 4), and renal cortex tissues (1 of 4). No rabies virus RNA could be recovered, however, from any of the blood samples obtained from the 4 dogs.

Human-to-human transmission of rabies once a patient with rabies has been admitted to a hospital has always been a serious concern. Other than the report of transmission via corneal transplantation, there are no reliable reports of such transmission [1]. Although rabies virus has been isolated from urine sediment after centrifugation (in 1 of 8 samples) [7], and although the detection of rabies virus RNA in urine samples is as accurate a diagnostic tool as its detection in saliva samples [5], we failed to demonstrate the infectivity of urine. We used uncentrifuged urine to infect neuroblastoma cells. Our findings should therefore be more relevant to the spread of the virus under natural conditions than if we had used centrifuged urine. Failure to detect and isolate rabies virus and its RNA from blood samples supports the previous report that, of 18 patients with rabies, none had virus isolated from their blood samples [7].

Our study suggests that neural spread of the virus to the bladder is the primary event in the progression of infection, with a subsequent propagation of the virus to renal structures. Rabies virus antigen was not found in samples of kidney tissue obtained from 3 human patients with rabies, as described in an earlier report [2]. Although the demonstration of rabies virus antigen or RNA in samples of tissue or biological fluids may help diagnosis [2, 3, 8], it does not necessarily indicate that such a virus is viable.

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### References

1. Hemachudha T, Laothamatas J, Rupprecht CE. Human rabies: a disease of complex neuropathogenetic mechanisms and diagnostic challenge. Lancet Neurol 2002; 1:101–9.
2. Jackson AC, Ye H, Phelan CC, et al. Extraneural organ involvement in human rabies. Lab Invest 1999; 79:945–51.
3. Debbie J, Trimarchi CV. Pantropism of rabies virus in free-ranging rabid red fox, *Vulpes fulva*. J Wildl Dis 1970; 6:500–6.
4. Ray NB, Ewalt LC, Lodmell DL. Rabies virus replication in primary murine bone marrow macrophages and in human and murine macrophage-like cell lines: implications for viral persistence. J Virol 1995; 69:764–72.
5. Wacharapluesadee S, Hemachudha T. Urine samples for rabies RNA detection in the diagnosis of rabies in humans. Clin Infect Dis 2002; 34:874–5.
6. Wacharapluesadee S, Hemachudha T. Nucleic-acid sequence based amplification in the rapid diagnosis of rabies. Lancet 2001; 358:892–3.
7. Helmick C, Tauxe, RV, Vernon, AA. Is there a risk to contacts of patients with rabies? Rev Infect Dis 1987; 9:511–8.
8. Jogai S, Radotra BD, Banerjee AK. Rabies viral antigen in extracranial organs: a post-mortem study. Neuropathol Appl Neurobiol 2002; 28: 334–8.

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## Use of Active Surveillance Cultures to Control Vancomycin-Resistant *Enterococcus*

SIR—Although most data on the efficacy of active surveillance for control of vancomycin-resistant *Enterococcus* (VRE) colonization come from outbreak settings [1–6], Calfee et al. [7] now report that active surveillance controlled VRE colonization rates at a university hospital for a 5-year period, and a recently published guideline from the Society for Healthcare Epidemiology of America [8] goes further than existing Hospital Infection Control Practices Advisory Committee recommendations for VRE control [9] and strongly recommends that every hospital implement a program of active surveillance for VRE.

Why, then, haven't more hospitals already implemented active surveillance programs for VRE control? Several obstacles to implementation exist: (1) current screening techniques for VRE involve the use of culture, which has limited sensitivity [10] and requires 48–72 h to perform (during which time, unless all patients are

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Case Report

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# Rabies encephalitis following fox bite – histological and immunohistochemical evaluation of lesions caused by virus

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**Key words**
fox bite rabies – neuropathology – electron microscopy – immunostaining – viral genome

**Abstract.** Rabies caused by fox bite is uncommon, most cases being caused by bite of rabid dogs (95%). We report a 45-year-old lady with rabies encephalomyelitis caused by bite of a rabid wild fox (Vulpes vulpes), a species prevalent in the Deccan plateaus of Central India. Though foxes are known to be susceptible to rabies, literature on the pathological changes caused by fox bite rabies in humans is scarce. Unlike the mild histological alterations described in canine rabies, a florid encephalitic process evolved in fox bite rabies, in our case, with intense microglial reaction, neuronophagia and perivascular inflammatory infiltrates despite clinical manifestation as a paralytic rabies. Immunostaining using polyclonal antibodies to the rabies viral nucleocapsid antigen and to the whole virion demonstrated high viral load within neurons with extensive spread along dendritic arborization and axonal tracts. Genomic sequence analysis demonstrated close homology with canine virus strain with only minor variations.

## Introduction

Rabies continues to be an important public health problem in developing countries. More than 4,000 mammalian species are susceptible to rabies, but the domestic dog remains the most important reservoir for transmission to humans by biting [Dutta 1999]. In a study carried out at the Infectious Diseases Hospital, Delhi, North India, the animal bites recorded to transmit rabies were most commonly stray dogs (90% of cases) followed by cat, Jackal, mongoose, monkey and fox [Singh et al. 2001]. There are regional differences in the animals responsible for the bite, depending on the habitat, community practices and veterinary practice. In an analysis of the profile and characteristics of animal bites in India (n = 869) from various regions, apart from dog and cat, other animals such as cow, monkey, horse, pig and camel were responsible for spread of rabies [Bhargava et al. 1996]. Sporadic reports of rabies infection transmitted by the bite of mongoose, leopard and wolf has been reported from India [Rathod et al. 1997]. Occurrence of rabies in foxes is reported from Europe, Southern Canada, USA and Arctic regions, Arab countries, Nepal, Pakistan, Tibet and India and is recognized as a successful rabies reservoir [Niezgoda 2002]. Foxes are also susceptible to experimental infection. Most of our understanding of the pathogenesis of rabies has emerged from experimental animals, using attenuated fixed strains of rabies virus [Jackson 2002]. This scenario does not represent the natural occurrence where humans and animals are infected with virulent street strains of virus by inoculation in subcutaneous tissue and skeletal muscle. The available literature on neuropathology of human rabies reflects the spectrum of changes in brain, spinal cord and peripheral nerves resulting from dog bite, while neuropathological changes caused by other animal bites are not on record. Study of the neuropathology of rabies resulting from the bite of wild animals, will provide an insight into the effect of strain variation and virulence on the progression of the disease.

Keeping this in mind, we report a case of human rabies transmitted by the bite of a fox from the forests of Deccan Plateau, South In-

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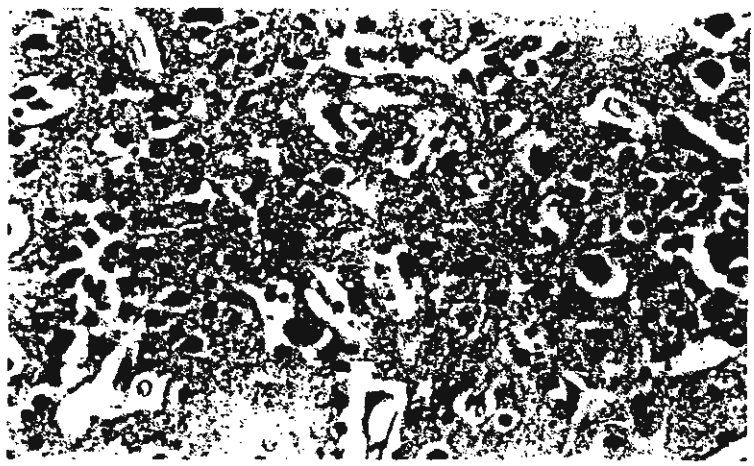

Figure 1. Florid encephalitis with microglial reaction, especially around degenerating neurons in the cerebral cortex; HE × 160.

dia. The neuropathology and neuroanatomic distribution of the viral antigen are described. Though clinically presenting as paralytic rabies, florid microglial reaction reflecting an encephalitic pathology and large amount of viral antigen in the neurons, fiber tracts and astrocytes was prominent.

## Case report

A 45-year-old lady presented to the Neurological services of the Nizam Institute of Medical Sciences, Hyderabad, Central India, with moderate grade of fever and vomiting for the past 6 days. On the same evening of onset of fever, she developed weakness of right upper limb, dysarthria, dysphagia and diplopia. The following day the weakness progressed to involve left upper limb and both lower limbs. On third day of illness, she became stuporous and the quadriparesis worsened. She was admitted to this secondary care hospital on the sixth day of illness. She did not have headache or seizures during the course of illness.

Three weeks prior to onset of symptoms, the patient was bitten on the face and forearm by a wild fox in a forest, where she went to collect firewood. The forest is arid with wild shrubs and trees, located close to Hyderabad, a city located in Deccan Plateau, Central part of India. Wound dressing was done and 4 doses of anti-rabies vaccine Verorab (vero cell-line culture vaccine) was administered intramuscularly by the local medical practitioner, but she did not receive any post-exposure anti-rabies immunoglobulins, due to non-availability.

At admission the patient was stuporous, opening eyes to pain. There were no bite marks. The pulse was 84/min, respiration was rapid and shallow, BP 118/80 mmHg. On neurological examination, the patient was unconscious, pupils were normal in size and reactive, dolls eye movement was absent, and the ocular fundi were normal. She had generalized hypotonia, quadriplegia, areflexia and equivocal plantar response. There were no signs of meningeal irritation. She did not have hydrophobia/aerophobia any time during the course of the illness. In view of history of canine bite, clinical diagnosis of paralytic rabies or a remote possibility of acute disseminated encephalomyelitis following vaccination was considered. The routine hematological and biochemical parameters were within normal limits. Chest x-ray showed bilateral non-homogenous opacities suggestive of bronchopneumonia. MRI carried out on the second day of admission revealed bilateral symmetrical hyperintensities in thalamus, basal ganglia, mid brain, pons, and medulla oblongata on T2 W and Flair images. Immunofluorescent test on corneal impression smears for rabies viral Ag was negative. She required mechanical ventilatory support and succumbed to the disease on 11th day of admission.

## Material and methods

A complete autopsy to examine the brain and visceral organs was performed (with informed consent of close relatives) 10 hours post mortem, adopting the prescribed precautions. Removal of spinal cord and peripheral nerves was not permitted.

The brain and organs were immersion-fixed in 10% formalin and tissue blocks were paraffin-embedded. Hematoxylin-eosin stains were performed on 6 μ thick sections. Immunohistochemical staining was performed by a standard indirect immunoperoxidase method, to demonstrate the rabies viral antigen. Two polyclonal antibodies were used for staining.

Antibody raised against the whole rabies virus in horse (Central Research Institute, Kasauli, Himachal Pradesh, India).

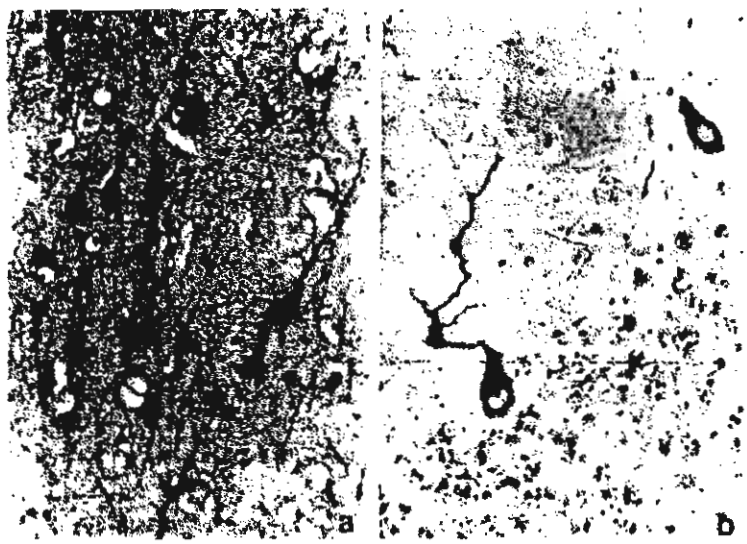

Figure 2a,b. Pyramidal cells of the temporal cortex (a), cerebellar Purkinje and some granule neurons, (b) show granular and globular viral antigen.

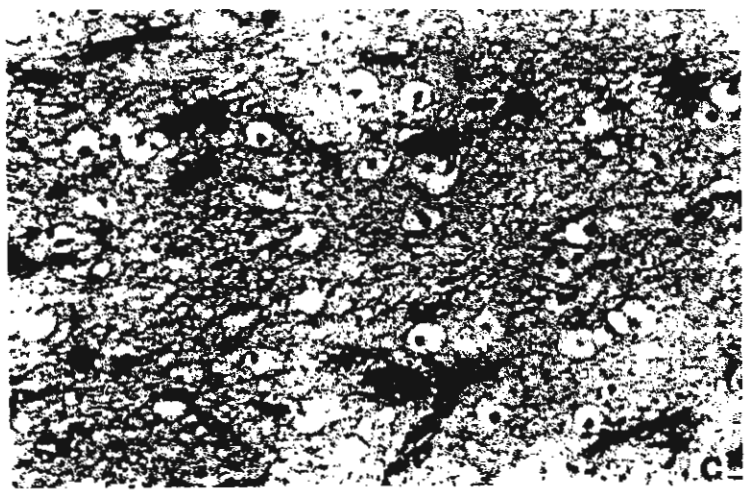

Figure 2c. Astrocytes in the white matter.

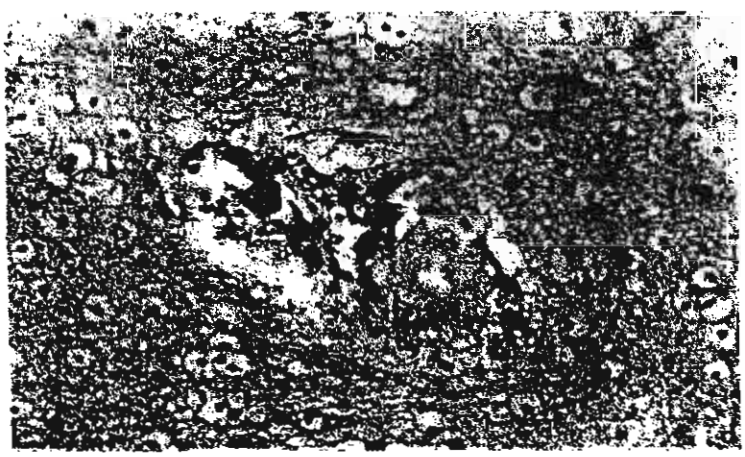

Figure 2d. Immunoperoxidase stain with polyclonal antibody to rabies viral nucleocapsid (a,b × 100, c,d × 180).

Antibody to rabies viral nuclear capsid protein raised in rabbit (in house).

The specificities of the antibodies were tested on blots and on confirmed cases of rabies encephalitis. The immunostain was visualized by appropriate secondary antibodies tagged with HRP and DAB/$H_2O_2$ as the chromogen. Carrying out the staining procedure in similar way but skipping the primary antibody constituted the negative control, while brain sections from confirmed cases of human and canine rabies were used as positive controls, and a section from a normal brain served as negative control. Wherever necessary, large paraffin sections were used to map the topographical distribution of antigen.

For immunoelectron microscopy (IEM) osmium-free LR white resin embedding and araldite embedding for conventional electron-microscopic method was utilized. Formaldehyde-fixed tissue bits were processed according to the protocol of Pelco International, USA. The fixed tissues were washed in phosphate buffer pH 7.4 and partially dehydrated in 50% and 70% alcohol. Impregnation was done in 2 : 1 (resin: 70% alcohol) for 1 hour and 100% LR white resin, overnight on a rotator. The tissues were embedded in flat mould and polymerized at 65 °C/24 hours. Ultrathin sections cut at 10 nm on Leica ultra-cut UCT, were collected on nickel grid (300 mesh) and subjected to immunolabeling, as described by Matsubara et al. [1996] using polyclonal antibody to rabies viral nucleoprotein (1 : 1,000) with (10 nm) immunogold-conjugated goat antirabbit (BBI International, 1 : 50 dilution) as secondary antibody. Sections were counterstained with uranylacetate and lead citrate, to examine under Jeol CX II Tem.

Ultra-thin sections embedded in LR white and araldite were stained with uranylacetate and lead citrate and examined under Jeol CX II Tem. For conventional method, tissue embedded in araldite was sectioned for EM examination and stained with uranylacetate lead citrate. The grid was examined under 60 kV Jeol 100 CX microscope.

Sequence analysis of the rabies viral genome was carried out from paraffin-embedded tissues from frontal cortex and cerebellum employing a recent technique developed by Hemachudha et al. [2003]. For comparison, viral genome sequence from frontal cortex of human rabies caused by dog

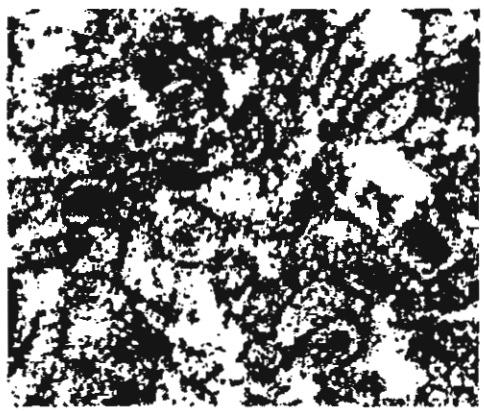

Figure 3. Electron micrograph of Negri body showing longitudinally and transversely cut rhabdovirus with central dense core of ribonucleoprotein and outer glycoprotein membrane; × 48,000.

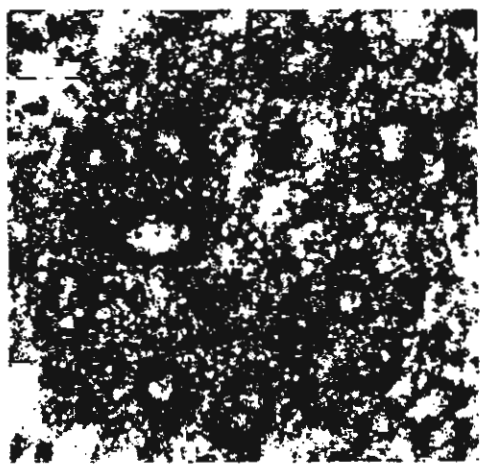

Figure 4. Immunoelectron micrograph showing dense labeling of the Negri body including the viral particles by antibody to viral nucleocapsid; × 58,000.

bite and canine rabies (1 case each) from the same geographical local were analyzed.

## Results

The brain was edematous and revealed focal areas of congestion on sectioning. No gross pathology was evident in the visceral organs. On histological examination, the cerebellar Purkinje cells had multiple intracytoplasmic eosinophilic Negri bodies, while occasional neurons in the cerebral cortex, diencephalic nuclei and some of the lower cranial nerve nuclei had similar inclusions. The striking feature were dense perivascular lymphocytic and histiocytic cuffing in the white matter in many areas and sparse meningeal inflammation. Florid microglial reaction with neuronophagia was noted in lower layers of insular cortex (Figure 1), around cerebellar Purkinje cells, the whole length of cerebellar dentate nucleus, around the pigmented neurons of substantia nigra and locus ceruleus, the pontine nuclei, the raphe and reticular nuclei of brainstem, III, V, IX and XII cranial nerve nuclei and the inferior olivary nucleus in medulla oblongata. Many of these neurons, on routine hematoxylin-eosin stain failed to show Negri bodies. The cortical white matter, the internal capsule, the medial decussating fibers in medulla oblongata and cerebellar white matter had focal zones of demyelination, astrocytosis and axonal spheroids indicating axonal deafferentation. The microglial reaction and neuronophagia noted indicated diffuse encephalitic process, a feature unusual in paralytic rabies.

The antibody to total viral protein including the glycoprotein demonstrated granular and globular intracytoplasmic masses within the neurons, spreading along its dendritic arborization and axonal tracts over long distances. The neuronal soma of cerebellar dentate nucleus, the inferior olivary nucleus, the raphe and reticular nuclei, the lower cranial nerve nuclei were densely and uniformly labeled, reflecting high viral antigen load. The immunolabeling by the antibody to viral nuclear capsid revealed more discrete and punctate staining of the neuronal soma, dendritic arborization and axons. The labeling was intense in cingulate gyrus, insular cortex, cerebellum, hippocampus, thalamic nuclei and the cranial nerve nuclei in brainstem, similar to labeling by the antibody to total viral protein (Figure 2a,b). The caudate nucleus was labeled sparsely in contrast to putamen. In cerebellar white matter and corona radiata, many of the astrocytes (Figure 2c) and occasional oligodendroglia were labeled, while none of the glial elements were stained in corpus callosum or internal capsule. Many of the perivascular histiocytes and monocytes were labelled (Figure 2d), while the perivascular microglia were labeled sparsely by the antibody to viral nucleocapsid.

In the cerebral cortex, the immunolabeling was more intense, highlighting the neurons of lower layers, while the crest of the gyri revealed neuropil labeling similar to synaptic staining. The sections through the heart failed

to reveal features of myocarditis. In the adrenal medulla, occasional cells were labeled by the antibody to total virus, but no Negri bodies or inflammation was noted.

On electronmicroscopy, within the neuronal cytoplasm 70 – 80 nm wide and 150 – 180 nm long bullet shaped or truncated double membrane-bound rabies viral particles were seen, surrounded by granular matrix (Figure 3). On transverse section, the central dense core was surrounded by small particles, corresponding to spikes enclosed in a membrane. On immunoelectron microscopy, the polyclonal antibody to nucleocapsid labeled the granular matrix around the capsid of the viral particles (Figure 4). In the neurons, the rough endoplasmic reticulum was abundant and dilated. The viral particles were easily found within dendritic processes. Membrane budding of the virus was not discernable due to suboptimal fixation.

Sequencing of the viral genome isolated from the frontal cortex and cerebellum from human rabies caused by fox bite, showed 93% homology with canine virus strain isolated from a rabid dog brain with minor variation in the nucleotidyl sequence and only 81% homology with the viral genome isolated from brain of a human bitten by a rabid dog.

## Discussion

The clinical progression and pathological manifestation of rabies transmitted by a rabid wild fox in this case was rapid and florid, with relatively short incubation period. The fate of the fox which transmitted the virus, and the circumstances of how the fox contracted the disease are not known. It is more likely that dogs that are carriers attack the foxes and wolves when they stray into their territory for food. This is quite common in Indian villages bordering forests.

In the biology of rabies infection, foxes are recognized to be highly susceptible, the incubation period varying from 4 days to 15 months, the average being 2 – 3 weeks, inversely related to viral dose [Blancou et al. 1991]. Most foxes secrete the virus in saliva, on an average 3 – 4 logs, the quantity sufficient to ensure infectious chain of transmission. Though strain variation in the rabies in foxes in different geographical zones in the west is recognized, these data are not available from India.

It has been suggested that different rabies virus variants associated with particular vectors may be responsible for different clinical manifestation [Hemachudha 2002]. Recent studies by Hemachudha et al. showed that analysis of glycoprotein (G), nucleoprotein (N) and phosphoprotein (P) genes of rabies virus from encephalitic and paralytic rabies demonstrated only minor nucleotide differences, suggesting that clinical manifestation of rabies is not explained solely by the associated rabies virus variant. Sequencing of the virus from the brain tissue in the present case showed 93% homology with canine virus strain, with only minor variation in the nucleotidyl sequence. Further evaluation on a greater number of samples taken from within and around the same geographic local with construction of a phylogenetic tree is essential for confirmation, as the genetic sequences may differ within the same species and at different time points in the disease process and evolution. The knowledge will permit recognition of reservoir host, natural history of the virus in the reservoir and the virulence pattern, both in relation to human beings and other domesticated animals of economic importance.

Under natural conditions of rabies infection by the street virus strain, the neuropathological changes in the CNS are mild. This led to the concept that the morbidity and mortality in rabies results from neuronal dysfunction rather than neuronal cell death. However, in the present case, the microglial reaction and neuronophagia was florid, with severe encephalitis process. Similarly, the viral load was high and spread was extensive. It is not clear if this phenomenon is related to viral strain or amplification of virus in fox akin to amplification of Japanese encephalitis virus in pigs, enhancing the morbidity and mortality [Rodrigues 1984]. Like arboviral infection, in rabies also cytokine/lymphokine-mediated enhanced inflammatory reaction could be contributing to the spread of virus disrupting the blood-brain barrier and facilitating the targeting of virus to critical areas. Cardiac involvement does not appear to be the cause for death in this case, but related to involvement of reticular formation, raphe nuclei and thalamic neurons.

In a random study of fox rabies in Karnataka, the mortality has been found to be very high, with relatively short incubation period, unlike canine rabies (personal communication, Dr. N. Suresh, Retired Deputy Director, Institute of Animal Health and Veterinary Biologicals, Bangalore). Though in the West, fox rabies has been almost eradicated by prophylactic animal vaccination strategies; in India, similar attempts are not in practice, exposing the domestic animals to the risk of infection with devastating consequences to the community.

Rabies virus glycoprotein is now recognized to exert very important influence on the viral entry and distribution of virus infection in the nervous system [Yan et al. 2002]. Sparse immunostaining with antibody to total virus, including glycoprotein and heavy labeling by antibody to nucleocapsid of rabies virus in this case suggests that, after gaining entry to the brain, the virus has replicated rapidly to involve wide interconnected anatomical areas. The viral strain transmitted by fox and its biological properties that cause pathologically florid encephalitic picture yet clinically presenting as paralytic rabies, needs to be elucidated for better understanding of the phenomenon.

## References


*Bhargava A, Deshmukh R, Ghosh TK, Goswami A, Prasannaraj P, Marfatia SP, Sudarshan MK* 1996 Profile and characteristics of animal bites in India. J Assoc Physicians India *44:* 37-38

*Blancou J, Aaubert MFA, Artois M* 1991 Fox rabies. In: *Baer GM (ed)* The Natural History of Rabies (2nd ed). CRC Press, Boca Raton, pp 257-290

*Dutta JK* 1999 Human rabies in India: epidemiological features, management and current methods of prevention. Trop Doct *29:* 196-201

*Hemachudha T, Wacharapluesadee S, Lumlertdaecha B, Orciari LA, Rupprecht CE, La-Ongpant M, Juntrakul S, Denuangboripant J* 2003 Sequence analysis of rabies virus in humans exhibiting encephalitic and paralytic rabies. J Infect Dis *188:* 960-966

*Hemachudha T, Laothamatas J, Rupprecht CE* 2002 Human rabies: a disease of complex neuropathogenetic mechanism and diagnostic challenges. Lancet *1:* 101-109

*Jackson AC* 2002 Pathogenesis. In: *Jackson AC, Wunner WH (eds)* Rabies (1st ed). Academic Press, San Diego, pp 245-282

*Matsubara A, Laake JH, Davanger S, Usami S, Otterson OP* 1996 Organization of AMPA receptor subunits at a glutamate synapse. A quantitative immunogold analysis of hair cell synapses in the rat organ of cortis. J Neurosci *16:* 4457-4467

*Niezgoda M, Hanton CA, Rupprecht CE* 2002 Animal rabies. In: *Jackson AC, Wunner WH (eds)* Rabies (2nd ed). Academic Press, London, pp 163-218

*Rathod NJ, Salunke S, Barviskar VR* 1997 Clinical profile of wolf bite cases in Jalgaon district. J Assoc Physicians India *45:* 866-867

*Rodrigues FM* 1984 Epidemiology of Japanese encephalitis in India: a brief overview. In: *Indian Council of Medical Research: Proceedings of the National Conference on Japanese Encephalitis. New Delhi, pp 1-9*

*Singh J, Jain DC, Bhatia R, Ichpujani RL, Harit AK, Panda RC, Tewari KN, Sokhey J* 2001 Epidemiological characteristics of rabies in Delhi and surrounding areas. Ind Paediatr *38:* 1534-1560

*Yan X, Mohankumar PS, Dietzschold B, Schnell MJ, Fu ZF* 2002 Rabies virus glycoprotein determines the distribution of different rabies virus strains in the brain. J Neurovirol *8:* 345-352

picion of a nosocomial infection among patients with IE and recent hospitalization could justify the empirical use of vancomycin as part of the initial empirical antimicrobial regimen. However, the authors do not provide data to support this conclusion. We believe that more sound evidence is needed before a broader definition of nosocomial IE should be accepted and IE management modified accordingly.


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### References


1. Ben-Ami R, Giladi M, Carmeli Y, Orni-Wasserlauf R, Siegman-Igra Y. Hospital-acquired infective endocarditis: should the definition be broadened? Clin Infect Dis 2004; 38:843–50.
2. Moreillon P, Que YA. Infective endocarditis. Lancet 2004; 363:139–49.
3. Durack DT, Lukes AS, Bright DK. New criteria for diagnosis of infective endocarditis: utilization of specific echocardiographic findings. Duke Endocarditis Service. Am J Med 1994; 96:200–9.
4. Moreno F, Crisp C, Jorgensen JH, Patterson JE. Methicillin-resistant *Staphylococcus aureus* as a community organism. Clin Infect Dis 1995; 21: 1308–12.
5. Cookson BD. Methicillin-resistant *Staphylococcus aureus* in the community: new battlefronts, or are the battlefronts lost? Infect Control Hosp Epidemiol 2000; 21:398–402.
6. Naimi TS, LeDell KH, Como-Sabetti K, et al. Comparison of community- and health care–associated methicillin-resistant *Staphylococcus aureus* infection. JAMA 2003; 290:2976–84.



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**Clinical Infectious Diseases 2004; 39:1084–5**



## Reply to Cicalini et al.

Sir—We welcome the effort made by Cicalini et al. [1] to validate our observations [2] in a different population. However, before one concludes that their results contradict our own, several points should be considered. Cicalini et al. [1] state that 4 of the patients with cases included in the broadened definition of hospital-acquired infective endocarditis (IE) had been discharged from the hospital within the previous 6 months. However, it is unclear whether the other 5 patients—whose cases were included because the patients had undergone invasive procedures (i.e., surgery, dental work, endoscopy, and dialysis)—underwent these procedures during hospitalization or as outpatients. In our study, patients who underwent invasive procedures as outpatients were assigned to the true community-acquired IE group: 10 (20%) of 49 episodes of true community-acquired IE were associated with such procedures [2]. Therefore, it is possible that only 4 of the patients described by Cicalini and colleagues had cases that fit our broadened definition of hospital-acquired IE. Because the individual associations of bacterial isolates with specific patients are not provided, the distribution of bacterial species among these 4 patients is unknown. However, the small number of recently hospitalized patients, as well as the high proportion of culture-negative IE episodes (3 of 9 episodes), preclude a meaningful assessment of the bacteriological and clinical characteristics of this group. Finally, we hypothesized that IE in recently hospitalized patients reflects the prevalence of bacterial isolates in the discharging medical institution. Therefore, the characteristics of hospital-acquired IE (as traditionally defined) in the relevant medical institution should also be considered when assessing the significance of recent hospitalization. These data are not reported by Cicalini et al. [1].

Currently, there is no well-established definition of hospital-acquired IE. On the basis of our observations, we proposed that patients who had been discharged from the hospital ≤6 months before the onset of their symptoms should be considered to have hospital-acquired infections. However, we agree with Cicalini et al. [1] that a widely applicable definition should be based on data from diverse epidemiological settings. We hope that our observations will encourage other researchers to examine local data, so that this emerging medical problem can be better addressed.


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### References


1. Cicalini S, Puro V, Angeletti C, Proietti MF, Petrosillo N. Broadened definition for hospital-acquired infective endocarditis. Clin Infect Dis 2004; 39:1084–5 (in this issue).
2. Ben-Ami R, Giladi M, Carmeli Y, Orni-Wasserlauf R, Siegman-Igra Y. Hospital-acquired infective endocarditis: should the definition be broadened? Clin Infect Dis 2004; 38:843–50.



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## Antemortem Diagnosis of Human Rabies

Sir—We previously reported the use of nucleic acid sequence-based amplification (NASBA) for the detection of rabies virus RNA in samples of saliva, CSF, and/or urine obtained during life from 8 patients infected with rabies [1, 2]. Here, we summarize the results of NASBA in correlation with the clinical onset of symptoms of the disease.

From September 1998 through March 2004, we collected and tested 58 specimens from 23 rabies patients (20 with furious

and 3 with paralytic rabies). Samples collected included: 27 saliva, 14 CSF, 15 urine, and 1 tear specimen; and extracted hairs from 1 patient. All patients had been bitten by stray dogs. Postmortem brain samples from all patients were positive for rabies virus by either fluorescence antibody testing or mouse inoculation; all samples were also positive for rabies virus by either NASBA or RT-PCR. All samples, except the first 4 specimens, which were frozen, stored, and examined retrospectively [1], were kept at 4°C for 24–48 h until examined for the presence of the rabies nucleocapsid gene. In 21 of 23 patients, we identified rabies RNA in specimens obtained on the first day of hospitalization. Specimens collected within 3 days after clinical onset yielded the highest number of positive results with saliva samples having the highest rate of positivity (7 of 8 specimens), followed by CSF (4 of 6) and urine (2 of 5). The sensitivity of all specimen types dropped after 3 days; however, saliva remained the most practical and reliable source for virus detection (11 of 15 specimens positive during days 4–6 after onset and 1 of 2 positive during days 7–9). The test sensitivity for urine (3 of 9 specimens positive for rabies virus RNA) and CSF (2 of 7) was comparable during days 4–6. Test results for 2 saliva specimens, 1 CSF specimen, and 1 urine specimen obtained during days 10–12 were all negative. Of particular interest were the test results for hairs extracted from 1 patient obtained 4 days after onset of symptoms. Fifty hair samples were extracted from this patient instead of excising skin with hair follicles from the nape of the neck [3]. We were able to demonstrate the presence of rabies RNA in the ends of the hair follicles.

Negative results were obtained exclusively from tests performed on samples collected sequentially from 2 patients with paralytic rabies. Samples tested from the first patient included saliva, CSF, and urine collected on day 11 after onset and saliva collected on day 12. Samples tested from the second patient included CSF and urine collected on day 4, saliva and urine collected on days 5 and 6, and tears collected on day 7. For a third patient with paralytic rabies, results from a CSF sample collected on day 3 were positive, but results for saliva samples collected on days 3 and 7 were both negative.

In summary, we conclude that molecular methods, although useful and extremely sensitive, may not always give positive results for patients with rabies. This may be due to the intermittency of virus shedding, the timing of sample collection, and the type of specimens collected. Moreover, the extent the clinical type of rabies (particularly paralytic rabies and cases with atypical features) [4] influences the outcome of laboratory results remains to be determined. We strongly urge that specimens be collected simultaneously from several sources and examined; they should include saliva, urine, and CSF. Sample collection should be repeated until a diagnosis is confirmed [5]. Postmortem examination should also be conducted in all suspected cases of rabies and other encephalitides, regardless of the results of antemortem examination.


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## References


1. Wacharapluesadee S, Hemachudha T. Nucleic-acid sequence based amplification in the rapid diagnosis of rabies. Lancet 2001; 358: 892–3.
2. Wacharapluesadee S, Hemachudha T. Urine samples for rabies RNA detection in the diagnosis of rabies in humans. Clin Infect Dis 2002; 34:874–5.
3. Crepin P, Audry L, Rotivel Y, Gacoin A, Caroff C, Bourhy H. Intravitam diagnosis of human rabies by PCR using saliva and cerebrospinal fluid. J Clin Microbiol 1998; 36:1117–21.
4. Hemachudha T, Laothamatas J, Rupprecht CE. Human rabies: a disease of complex neuropathogenetic mechanisms and diagnostic challenges. Lancet Neurol 2002; 1:101–9.
5. Trimarchi C, Smith J. Diagnostic evaluation. In: Jackson A, Wunner WH, eds. Rabies. Amsterdam: Academic Press, 2002:307–49.



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## Infection by Drug-Resistant *Streptococcus pneumoniae* Is Not Linked to Increased Mortality

SIR—We congratulate Dr. Aspa and colleagues and the Pneumococcal Pneumonia in Spain Study Group [1] for their large, labor-intensive study of 638 cases of community-acquired pneumonia due to *Streptococcus pneumoniae*. We take issue with only one point made in an otherwise excellent study: "The impact of drug-resistant *S. pneumoniae* on morbidity and mortality is still controversial" [1, p. 795]. The authors underestimate the potency of their own findings when they claim that the issue is controversial. If it is controversial, the authors have provided additional support for the numerous authorities on pneumococcal infection who claim that drug resistance is an artifact of the NCCLS guidelines, with few clinical implications. Their major finding was that in vitro resistance to macrolides and β-lactam agents did not result in increased morbidity or increased mortality—a finding that has been reiterated in >20 peer-reviewed articles, including our own [2].

The authors cited the Centers for Disease Control and Prevention (CDC; Atlanta, GA) study [3] in which mortality was significantly associated with an MIC of penicillin of ≥4 μg/mL, and they suggested that high-level resistance may be associated with an adverse outcome. In fact, the CDC study did not attempt to correlate discordant therapy with out-

**In Press**

Difference in Neuropathogenetic Mechanisms in Human Furious and Paralytic Rabies

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**Abstract**

Whereas paralysis is the hallmark for paralytic rabies, the precise pathological basis of paralysis is not known. It is unclear whether weakness results from involvement of anterior horn cells or of motor nerve fibers. There is also no conclusive data on the cause of the neuropathic pain which occurs at the bitten region, although it has been presumed to be related to sensory ganglionopathy. In this study, six laboratory-proven rabies patients (3 paralytic and 3 furious) were assessed clinically and electrophysiologically. Our data suggests that peripheral nerve dysfunction, most likely demyelination, contributes to the weakness in paralytic rabies. In furious rabies, progressive focal denervation, starting at the bitten segment, was evident even in the absence of demonstrable weakness and the electrophysiologic study suggested anterior horn cell dysfunction. In 2 paralytic and 1 furious rabies patients who had severe paresthesias as a prodrome, electrophysiologic studies suggested dorsal root ganglionopathy. Postmortem studies in 2 paralytic and 1 furious rabies patients, who had local neuropathic pain, showed severe dorsal root ganglionitis. Intense inflammation of the spinal nerve roots was observed more in paralytic rabies patients. Inflammation was mainly noted in the spinal cord segment corresponding to the bite in all cases, however, central chromatolysis of the anterior horn cells could be demonstrated only in furious rabies patient. We conclude that differential sites of neural involvement and possibly different neuropathogenetic mechanisms may explain the clinical diversity in human rabies.

Keywords: rabies; Guillain-Barre syndrome; paralysis; demyelination; sensory ganglionopathy; anterior horn cell; anti-ganglioside antibody

Abbreviations: DRG = dorsal root ganglia, NCS= nerve conduction study; CV= conduction velocity; CMAP=compound muscle action potential; SNAP= sensory nerve action potential; EMG=electromyography; LLN= lower limit of normal; ULN= upper limit of normal; PMCB = partial motor conduction block

## Introduction

Rabies remains a major human threat in countries where rabies is endemic. The worldwide number of rabies deaths as estimated by the World Health Organization survey in 1998 appeared to be between 35,000 to 50,000 annually with the highest incidence in Asia [1,2]. Approximately 70 patients die of rabies each year in Thailand [3]. Two distinct clinical rabies syndromes, furious and paralytic rabies, have been recognized in humans [2,4]. Limbic symptoms dominate the clinical picture in the former, whereas paralysis of lower motor neuron type dominates the latter. Weakness of extremities is usually not observed in furious rabies until very late in the course of the disease, with the exception of rare patients where weakness can be found in the bitten extremity during the early stage. In paralytic rabies, pure motor weakness of all limbs and respiratory muscles and absence of deep tendon reflexes are cardinal features with relative sparing of consciousness.

The neural pathway of rabies virus spread has been demonstrated in experimental models [5]. Following a delay at the muscle [6], rabies virus reaches the central nervous system (CNS) by retrograde axoplasmic flow [5,7-10]. Replication may occur again in dorsal root ganglia (DRG) and anterior horn cells [5,11,12]. It is presumed that viral replication in the DRG can then be recognized and attacked by immune effectors, resulting in prodromal neuropathic symptoms at the bite site observed in humans [13,14]. Once in the CNS, the exact pathway of virus propagation is not known [15,16]. Postmortem examination in human rabies patients showed the brainstem and spinal cord as preferential sites of virus infection regardless of clinical types, as determined by immunohistochemistry [17]. Predilective involvement of brainstem, hippocampus and hypothalamus was noted on magnetic resonance imaging (MRI) examination in both clinical forms of rabies[18]. The site of neural involvement responsible for clinical weakness in paralytic rabies remains unknown. Segmental demyelination of peripheral nerve with or without axonal degeneration has been previously reported in postmortem studies of paralytic rabies [19]. Such demyelinating changes were not noted in patients with furious rabies [20]. Dorsal root ganglionitis was found in both clinical forms [19,20].

Although postmortem studies have the advantage of examining the human disease directly, they only give a snap shot of the situation at death. In order to clarify the mechanisms responsible for neurological deficits in human rabies, we performed electrodiagnostic studies in 3 paralytic and 3 furious rabies patients. Three patients underwent autopsy: 2 paralytic and 1 furious. Our results suggest that the peripheral nerve is mainly involved in paralytic rabies and a dorsal root ganglionopathy contributes to the local neuropathic symptoms.

## Materials and Methods

Between August 1997 and May 1999, six rabies patients were admitted to Chulalongkorn University Hospital. These patients received standard care and ventilatory support as indicated. No aggressive attempts to resuscitate these patients were made once signs of circulatory insufficiency appeared. All patients had a definite history of dog bites. The diagnosis of rabies was also confirmed at autopsy on brain impression smear by fluorescent antibody test or by mouse inoculation test and polymerase chain reaction or nucleic acid sequence based amplification technique [2,21].

Electrodiagnostic studies were done (E.M.) with a Clarke-Davis Advantage EMG system (London, Ontario, Canada). Motor nerve conduction studies were performed on median, ulnar, deep peroneal and tibial nerves bilaterally and sensory nerve conduction studies were performed orthodromically in bilateral median and ulnar nerves and antidromically in bilateral sural and radial nerves utilizing surface electrodes with conventional surface recording techniques. Skin temperature was monitored and controlled at higher than $32^0$ C. All patients underwent electromyography (EMG) of the selected proximal and distal limb, facial, paraspinal and diaphragmatic muscles. Differentiation between neuronopathy and axonal and demyelinating neuropathy was considered by various electrodiagnostic parameters and the overall pattern of nerve conduction and EMG abnormalities [22-24]. Determination of demyelination of peripheral nerve was based on previously proposed criteria

[25,26].

Sera collected on the first day of admission from all patients were also assayed for anti-ganglioside antibodies as previously described [27]. IgG and IgM antibody activities against gangliosides GM1, GD1a, GalNAc-GD1a, GD1b, GT1a, and GQ1b were investigated.

In three patients (2 paralytic and 1 furious), spinal cords, spinal nerve roots and DRG were removed for postmortem pathological examination. All specimens were fixed in 10% formalin, and embedded in paraffin wax. Haematoxylin and eosin stain was used as a primary staining method. Luxol-fast-blue stain and immunohistochemical staining for CD3 and CD20 cells were performed in spinal nerve root specimens of one paralytic patient.

## Results

The characteristics of patients with these two forms of rabies were summarized in Table 1. Only Patient 2 received appropriate post-exposure prophylaxis [3]. Anti-ganglioside antibodies were negative in all patients tested.

### Paralytic Rabies

#### Patient 1

A 59 year-old woman who had a dog bite on her left leg presented with 8 days of bilateral dull aching leg pain. This was followed shortly by progressive proximal muscle weakness of all extremities. Two days prior to admission, she had difficulty swallowing and urinary retention and later became obtunded. On day 12 after clinical onset, muscle strength of the upper and lower limbs was graded 2 and 4 out of 5 in the proximal and distal muscles respectively. She required ventilatory support. Deep tendon reflexes were absent. Laboratory testing was unremarkable except for severe hyponatremia (111 mmol/litre). After hyponatremia had been corrected, she became alert for 12 hours but subsequently lapsed into a coma. She survived for 13 days.

Electrodiagnostic studies were performed on day 12 post onset. Sensory nerve conduction studies showed a length-dependent decrease in SNAP amplitudes with concurrent reduction in conduction velocities in all sensory nerves tested (Table 1A). Motor nerve conduction studies showed features of multifocal demyelination with reduced conduction velocities. (Table 2) All F-waves were absent. EMG showed fibrillations and positive sharp waves in the proximal and distal muscles of all limbs.

#### Patient 2

This 72-year-old woman who had a dog been bitten on her left cheek presented with 6 days of fever and severe aching and paresthesias of the left face followed by progressive ascending weakness of the limbs. She then developed high-grade fever, urinary retention and fluctuating mental state. She became comatose. On examination (20 days post-onset), she had areflexic flaccid quadriplegia. Laboratory tests were unremarkable. She died 21 days after onset.

Nerve conduction studies performed on the day of admission showed absent median, ulnar and radial sensory potentials bilaterally while sural responses were normal. Motor nerve conduction studies showed markedly reduced distally evoked CMAP amplitudes in all the nerves studied. F-waves were absent. Markedly reduced motor conduction velocities in a patchy distribution were present in bilateral median and bilateral ulnar nerves (Table 2). EMG showed widespread fibrillations and positive sharp waves in all the limb, facial, truncal and diaphragmatic muscles.

Pathological examination showed moderate to severe lymphocytic infiltration in both the spinal nerve roots and dorsal root ganglia. Aggregation of small lymphocytes and some plasma cells were observed in and around the ganglion cells and nerve fibers. Such inflammation also involved the perineurium. The degree of inflammation in the spinal nerves appeared to be greater in the cervical and thoracic segments as compared to the cauda equina. The spinal gray matter was inflammed and undergoing necrosis. Perivascular cuffing by lymphocytes

along with microglial proliferation were noted. Anterior horns cells were depleted, but no central chromatolysis was observed in the remaining nerve cells.

**Patient 3**

This 34-year-old man who had a dog bite on his right ankle presented with 2 days of severe itching and piloerection on his right leg. These started around the bitten site, extended upward to his right groin and progressed to his left leg by the time he was admitted. Examination performed on day 3 of his illness revealed normal motor strength. Reflexes were 1+ in the right lower limb and 2+ in the other limbs. Hypoesthesia to pin was present on the right leg in a circumferential fashion up to his right groin. By day 4, paraparesis was noted and reflexes were absent in both lower limbs and barely elicitable in the upper limbs. On day 6, facial diaparesis and bulbar dysfunction were noted and he was later intubated. He became agitated on the following day and died on day 9.

Electrodiagnostic studies were performed on 4 occasions. (Table 2) On day 3, there was markedly prolonged right peroneal F-wave latency (86 msec vs 53 msec on the left). The right H-reflex showed prolonged latency as compared to the left (34.2 msec vs 31.1msec). Motor and sensory conduction studies were otherwise normal. On day 4, there was a 40% reduction in bilateral sural amplitudes compared to day 3 and unobtainable and the right deep peroneal F-wave was unobtainable while the left was normal. The third study on day 6 showed absent bilateral sural sensory potentials and absent peroneal and tibial F-waves. Conduction studies including F-wave latencies in the upper limbs remained normal. Even on the last study, one day before he died, no abnormal spontaneous activity was seen on EMG examination.

Pathological examination revealed heavy lymphocytic infiltration, most remarkably in the dorsal root ganglia (Fig.1A), adjacent dorsal and and ventral nerve roots and cauda equina. The majority of these lymphocytes were CD3-positive T cells. The nerve roots were infiltrated by lymphocytes (Fig.1B). Mild to moderate inflammatory infiltrates were noted in the gray matter of the lumbar segment of spinal cord and the conus, while minimal inflammation was noted in a more rostral segments. Perivascular cuffing by lymphocytes and occasional microglial nodules were observed. The anterior horn cells were intact.

**Furious Rabies**

**Patient 4**

A 50-year-old man who had a dog bite on the left wrist presented with 3 days of severe aching pain and paresthesias on his left hand and arm. Initial examination showed only diminished to absent reflexes in the left upper limb. He exhibited noctural agitation but remained calm during the day. Difficulty in handling objects with his left hand was noted 5 days after the onset. There was a moderately impaired propioceptive sense of the left hand and hypoesthesia to pin up to the left elbow. By day 6, pain was no longer present. Proprioceptive and vibratory senses of the left hand were markedly impaired. Mild weakness of the left wrist flexors and extensors and intrinsic muscles of the left hand was also found. He became more persistently agitated and died on day 7.

Electrodiagnostic studies were done on 3 occasions (Table 1). The first study on day 3 showed normal and symmetric sensory and motor nerve conduction studies including F-waves in the upper and lower limbs. The only abnormality was the presence of abundant fibrillations and positive sharp waves in left C5-C8 limb and cervical paraspinal muscles. On day 5, there was approximately 50% reduction of SNAP amplitudes in the left upper limb nerves compared to the values from the first study and to the values obtained on the right. By day 6, there was a further reduction of SNAP amplitudes on the left upper extremity along with progression of fibrillations and positive sharp waves involving bilateral C5-T7 limb and paraspinal muscles. Motor nerve conduction studies, including the F-waves, remained normal.

**Patient 5**

This 65 year-old man who had a dog bite on his lower lip presented with 2 days of dysphagia and hydrophobia followed by itching on his right face. On examination, hypoesthesia to pin was present along the entire right trigeminal distribution and the right C2-3 dermatomes. The rest of neurologic examination was normal. He became severely agitated and died on the following day.

Electrodiagnostic studies performed on day 2 after onset showed normal sensory nerve conduction studies. Motor nerve conduction studies including F-wave latencies were normal but showed reduced CMAP amplitudes in the right median and ulnar nerves compared to the left (5.52 mV vs 10.89 mV and 4.76 mV vs 9.6 mV in the median and ulnar nerves respectively). H-reflexes to gastrocnemius- soleus were normal and symmetric. EMG showed abnormal spontaneous activities (fibrillations and positive sharp waves) in the limb and paraspinal muscles innervated by right C3-C8 and also in the right facial musculature.

**Patient 6**

A 60 year-old who had a dog bite on his right ankle presented with 2 days of fever, severe itching on the right leg and phobic spasms. Neurologic examination was normal. He then became severely agitated and died 2 days later. Nerve conduction studies performed on day 3 after the onset showed normal and symmetric sensory nerve conduction studies. Motor nerve conduction studies were normal except for reduced distally evoked CMAP amplitudes of the right deep peroneal and tibial nerves compared to the left (6.4 mV vs 10.0 mV and 2.95 mV vs 5.74 mV in the deep peroneal and tibial nerves respectively). F-wave latencies and H-reflexes were normal and symmetric. EMG showed abnormal spontaneous activities (fibrillations and positive sharp waves) in the right L2-S1 limb and paraspinal muscles.

Pathological examination showed marked lymphocytic infiltration in the dorsal root ganglia. Mild degree of inflammation was observed in the dorsal and ventral nerve roots. Moderate mononuclear inflammatory cell infiltrates were present in the spinal gray matter of the thoracic and lumbar levels and, to the lesser extent, in the cervical segment. Perivascular cuffing by lymphocytes and microglial proliferation, with occasional microglial nodules, were observed. Some of the anterior horn cells demonstrated central chromatolysis (Fig. 1C).

**Discussion**

This study suggests that electrodiagnostic studies, particularly if done serially, can provide insights as to the basis of weakness and local neuropathic symptoms in human rabies patients. Loss of motor and, in some cases, sensory fibers occurred predominantly in the initially affected regions and spread to other regions of the body. Also, local neuropathic pain is associated with dorsal root ganglionopathy. Electrophysiologic and pathological evidence of peripheral nerve dysfunction was present in all 3 paralytic rabies patients. Pathologic studies showed inflammation of spinal nerve roots but intact anterior cells .Incontrast, furious rabies patients had eletrophysiological and pathological evidence of anterior horn cell dysfunction. Our previous studies showed that rabies virus localization on autopsy or MRI pattern of involvement or specific rabies virus variant did not predict whether a patient was to have paralytic or furious rabies [2,17,18,28]. The findings of peripheral nerve, rather than anterior horn cell involvement in paralytic rabies, suggests that another mechanism other than direct viral infection may be involved in the paralysis. The clinical resemblance between paralytic raibe and Guillain-Barre syndrome (GBS) raises the possibility that immune-mediatd mechanism, not direct viral infection, may be involved in the pathogenesis of weakness in paralytic rabies, although antiganglioside antibodies appeared to be negative in sera of all our patients.

In Patient 1, there were features of peripheral nerve demyelination (reduction of conduction velocities to < 70% of lower limit of normal (LLN) in the left median and bilateral ulnar nerves; partial motor conduction block (PMCB) in the axilla to elbow segment in the left median nerve; prolonged distal motor latencies to > 150% of upper limit of normal (ULN) in the left ulnar and left tibial nerves and; absent F-wave latencies in all the 7 tested nerves). [29-33]. There was also evidence of a length-dependent sensory neuropathy or ganglionopathy. Patient 2 had severely reduced motor evoked amplitudes accompanied by EMG findings of widespread denervation, making differentiation between neuropathy and neuronopathy difficult. However, such severe reduction in conduction velocities and the marked prolongation of distal latencies were unlikely to be explained by axonal loss alone [23]. In patient 3, sequential nerve conduction studies showed a progressive loss of motor and sensory amplitudes while no denervation potentials were observed. The patient died in 9 days, a time when fibrillations and/or positive sharp waves may not be seen. Although all the distal motor latencies were near the ULN and the conduction velocities were reduced, such abnormalities were not in a demyelinating range [25, 26]. The separation between axonal and demyelinating neuropathy, therefore, could not be made with certainty. However, the early abnormalities in late responses in patient 3 reflected the predilection of the disease process for the proximal nerve segments and suggested a primary demyelinating disorder.

In furious rabies, sensory and motor nerve conduction studies were normal, yet, abundant denervation potentials were clearly evident primarily in the bitten limb during the early stage of clinical illness. F-wave and H-reflex latencies were normal and symmetric in all 3 patients (Patients 4, 5, 6). In addition, sequential studies in Patient 4 showed that these denervation potentials spread to the contralateral limb and subsequently to more rostral and caudal segments. These suggested an acute motor fiber loss, probably at the anterior horn cell level. The presence of anterior horn cells with central chromatolysis in our postmortem study indicated intrinsic neuronal defect or interruption of the axons in close proximity to the cell bodies, thus in keeping with the above electrophysiological findings.

Preferential infection of lower motor neurons is known to occur not only with rabies but also with poliovirus and flavivirus infections of the Japanese and tick-borne encephalitic complex, including West Nile virus [34,35]. EMG findings of abnormal spontaneous activities have been demonstrated on the paralyzed limb muscles of patients who presented with asymmetric flaccid paralysis in several studies [34-37]. Of interest is that profuse denervation potentials were found in the bitten segment of our furious rabies patients even in the absence of clinical weakness and occurred very early, suggesting that the denervation preceded clinical presentation.

Local prodromal symptoms are likely to be explained by dorsal root ganglionopathy. This is based on the presence of typical pain character, the progressive decline in SNAP amplitudes (Patients 3 and 4) or absence of sensory potentials in the bitten segments, not in a length-dependent fashion (Patient 2), and pathological findings of dorsal root ganglionitis (Patients 2, 3, 6). Sensory nerve conduction studies in the other 2 furious cases (Patients 5 and 6) were normal. These, however, were not assessed serially and the normality of sensory amplitudes could be found in the early phase of sensory neuronopathy [38]. In Patients 3 and 4, SNAP amplitudes were also preserved in the initial studies and progressive reduction in SNAP amplitude was noted after the first examination.

The only other comparable report of electrophysiologic study in human rabies was limited. recently reported.[39] They tended to show reduced h motor amplitudes and denervation and could not be characterized further.

A prior histopathological study performed in 11 paralytic rabies patients also suggested peripheral nerve demyelination as a prime pathological change [19]. Such demyelination was absent in cases of furious rabies [20]. In the 17 peripheral nerve specimens studied of the paralytic patients [19], there was mild to moderate loss of myelinated nerve fibres in 11 nerves. Segmental demyelination and remyelination was present in 16 teased nerve preparations. Axonal loss of variable degree was present in only 4 cases and Wallerian-like degeneration in teased single fibers was noted in 6 nerves. In 9/17 nerves, the primary abnormality was segmental demyelination and remyelination . In none of these cases was Wallerian-like degeneration seen as the only pathological feature. Although our postmortem examination in paralytic rabies patients was incomplete as no teased preparation and plastic section were available, marked inflammatory infiltration of the spinal nerve roots was demonstrated. When combined with the electrodiagnostic data, our findings suggested that peripheral nerves roots were primarily involved in all these paralytic rabies cases. Abnormalities of the spinal gray matter on pathological evaluation in patient 2 were consistent with terminal stage of the disease, as previously described in our recent MRI study on rabies patients [18]. Pathological features of dorsal root ganglionitis have also been demonstrated both in paralytic and furious cases [19,20], as in our patients who had local neuropathic symptoms and electrophysiological evidence of sensory ganglionopathy. Like most of the inflammatory diseases of the nervous system, T-cells predominated in the reaction.

We hypothesize that an immune phenomenon may be responsible for nerve injury in paralytic rabies. The anterior horn cell loss in furious rabies may be a direct effect of the virus. An antibody-mediated complement dependent attack against viral particles in the axons has been suggested in one Chinese paralytic rabies patient initially diagnosed as AMAN. Viral protein and Wallerian-like degeneration were found to be more abundant in the ventral than the dorsal nerve roots [40]. This antibody dependent process could also explain our case with furious rabies who developed paralysis of all limbs soon after the intravenous administration of human rabies immune globulin [41]. There may also be more than one mechanisms involved, since neutralizing antibody to rabies virus could not be demonstrated in the CSF of our paralytic patients. We have shown that

some paralytic rabies patients may lack the ability to produce antibody as evidenced by diminished circulating B cells and IL-6 levels. Fewer paralytic rabies patients had rabies neutralizing antibody in the sera [14,42].

In summary, this is the first study in which electrophysiologic features of human rabies have been investigated in detail. In paralytic rabies, evidence of peripheral nerve demyelination or axonal degeneration has been demonstrated by electrophysiological study. Changes suggesting anterior horn cell dysfunction were present in furious rabies patients, both electrophysiologically and pathologically. The exact causal relationship between host response to both rabies virus and nerve antigens and the development of different clinical manifestations await further studies.

## References


[1] Organization WH World survey of rabies number 34 (for the year 1998). in. Geneva: WHO, 2000

[2] Hemachudha T, Laothamatas J, Rupprecht CE. Human rabies: a disease of complex neuropathogenetic mechanisms and diagnostic challenges. Lancet Neurol 2002;1: 101-9.

[3] 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.

[4] Hemachudha T. Rabies. In: Klawans HL, editor. Handbook of clinical neurology. Viral Disease. Amsterdam: Elsevier Science Publishers, 1989. pp. 383-404.

[5] Tsiang H. Pathophysiology of rabies virus infection of the nervous system. Adv Virus Res 1993;42: 375-412.

[6] Charlton KM, Nadin-Davis S, Casey GA, Wandeler AI. The long incubation period in rabies: delayed progression of infection in muscle at the site of exposure. Acta Neuropathol (Berl) 1997;94: 73-7.

[7] Dean DJ, Evans, W.M., McClure, R.C. Pathogenesis of rabies. Bull WHO 1963;29: 803-11.

[8] Baer GM, Shantha TR, Bourne GH. The pathogenesis of street rabies virus in rats. Bull WHO 1968;38: 119-25.

[9] Tsiang H. Evidence for an intraaxonal transport of fixed and street rabies virus. J Neuropathol Exp Neurol 1979;38: 286-99.

[10] Bulenga G, Heaney, T. Post-exposure local treatment of mice infected with rabies with two axonal flow inhibitors, colchicine and vinblastine. J Gen Virol 1978;39: 381-5.

[11] Lycke E, Tsiang H. Rabies virus infection of cultured rat sensory neurons. J Virol 1987;61: 2733-41.

[12] Tsiang H, Lycke E, Ceccaldi PE, Ermine A, Hirardot X. The anterograde transport of rabies virus in rat sensory dorsal root ganglia neurons. J Gen Virol 1989;70: 2075-85.

[13] Yamamoto T, Ohtani S. Ultrastructural localization of rabies virus antigens in infected trigeminal ganglion of hamsters. Acta Neuropathol (Berl) 1978;43: 229-33.

[14] Hemachudha T. Human rabies: clinical aspects, pathogenesis, and potential therapy. Curr Top Microbiol Immunol 1994;187: 121-43.

[15] Charlton KM. The pathogenesis of rabies and other lyssaviral infections: recent studies. Curr Top Microbiol Immunol 1994;187: 95-119.

[16] Charlton KM, Casey GA, Wandeler AI, Nadin-Davis S. Early events in rabies virus infection of the central nervous system in skunks (Mephitis mephitis). Acta Neuropathol 1996;91: 89-98.

[17] Tirawatnpong S, Hemachudha T, Manutsathit S, Shuangshoti S, Phanthumchinda K, Phanuphak P. Regional distribution of rabies viral antigen in central nervous system of human encephalitic and paralytic rabies. J Neurol Sci 1989;92: 91-9.

[18] Laothamatas J, Hemachudha T, Mitrabhakdi E, Wannakrairot P, Tulayadaechanont S. MR imaging in human rabies. AJNR Am J Neuroradiol 2003;24: 1102-9.

[19] Chopra JS, Banerjee AK, Murthy JMK, Pal SR. Paralytic rabies: A clinicopathological study. Brain 1980;103: 789-802.

[20] Tangchai P, Vejjajiva A. Pathology of the peripheral nervous system in human rabies: a study of nine autopsy cases. Brain 1971;94: 299-306.

[21] Wacharapluesadee S, Hemachudha T. Nucleic-acid sequence based amplification in the rapid diagnosis of rabies. Lancet 2001;358: 892-3.

[22] Kimura J Electrodiagnosis in diseases of nerve and muscles: Principles and practice., ed 3rd. New York: Oxford University Press, 2002

[23] Comblath DR, Kuncl RW, Mellits ED, Quaskey SA, Clawson L, Pestronk A, et al. Nerve conduction studies in amyotrophic lateral sclerosis. Muscle Nerve 1992;15: 1111-5.

[24] Comblath DR, Chaudhry, V. Electrodiagnostic evaluation of the peripheral neuropathy patient. In: Mendell JR, Kissel, J.T., Comblath, D.R., editor. Diagnosis and management of peripheral nerve disorders. New York: Oxford University Press, 2001. pp. 30-7.

[25] Comblath DR. Electrophysiology in Guillain-Barre syndrome. Ann Neurol 1990;27 Suppl: S17-20.

[26] Asbury AK, Comblath DR. Assessment of current diagnostic criteria for Guillain-Barre syndrome. Ann Neurol 1990;27 Suppl: S21-4.

[27] Yuki N, Tagawa, Y., Irie, F., Hirabayashi, Y., Handa, S. Close association of Guillain-Barre syndrome with antibodies to minor monosialogangliosides GM1b and GM1 alpha. J Neuroimmunol 1997;74: 30-4.

[28] Hemachudha T, Wacharapluesadee S, Lumlertdaecha B, Orcian LA, Rupprecht CE, La-Ongpant M, et al. Sequence analysis of rabies virus in humans exhibiting encephalitic or paralytic rabies. J Infect Dis 2003;188: 960-6.

[29] McDonald WI. The Effects of Experimental Demyelination on Conduction in Peripheral Nerve: A Histological and Electrophysiological Study. I. Clinical and Histological Observations. Brain 1963;86: 481-500.

[30] Brown WF, Feasby TE. Conduction block and denervation in Guillain-Barre polyneuropathy. Brain 1984;107 ( Pt 1): 219-39.

[31] Mills KR, Murray NM. Proximal conduction block in early Guillain-Barre syndrome. Lancet 1985;2: 659.

[32] Comblath DR, Mellits ED, Griffin JW, McKhann GM, Albers JW, Miller RG, et al. Motor conduction studies in Guillain-Barre syndrome: description and prognostic value. Ann Neurol 1988;23: 354-9.

[33] Albers JW, Kelly JJ, Jr. Acquired inflammatory demyelinating polyneuropathies: clinical and electrodiagnostic features. Muscle Nerve 1989;12: 435-51.

[34] Johnson RT, Comblath DR. Poliomyelitis and flaviviruses. Ann Neurol 2003;53: 691-2.

[35] Li J, Loeb JA, Shy ME, Shah AK, Tselis AC, Kupski WJ, et al. Asymmetric flaccid paralysis: a neuromuscular presentation of West Nile virus infection. Ann Neurol 2003;53: 703-10.

[36] Leis AA, Stokic DS, Polk JL, Dostrow V, Winkelmann M. A poliomyelitis-like syndrome from West Nile virus infection. N Engl J Med 2002;347: 1279-80.

[37] Glass JD, Samuels O, Rich MM. Poliomyelitis due to West Nile virus. N Engl J Med 2002;347: 1280-1.

[38] Griffin JW, Cornblath DR, Alexander E, Campbell J, Low PA, Bird S, et al. Ataxic sensory neuropathy and dorsal root ganglionitis associated with Sjogren's syndrome. Ann Neurol 1990;27: 304-15.

[39] Nathwani D, McIntyre PG, White K, Shearer AJ, Reynolds N, Walker D, et al. Fatal human rabies caused by European bat Lyssavirus type 2a infection in Scotland. Clin Infect Dis 2003;37: 598-601.

[40] Sheikh KA, Jackson AC, Ramos-Alvarez M, Li CY, Ho TW, Asbury AK, et al. Paralytic rabies: Immune attack on nerve fibres containing axonally transported viral proteins (Abstract). Neurology 1998;501: 183.

[41] Hemachudha T, Sunsaneewitayakul B, Mitrabhakdi E, Suankratay C, Laothamathas J, Wacharapluesadee S, et al. Paralytic complications following intravenous rabies immune globulin treatment in a patient with furious rabies. Int J Infect Dis 2003;7: 76-7.

[42] Sriwanthana B, Hemachudha T, Griffin DE, Manutsathit S, Tweardy D, Phanuphak P. Lymphocyte subsets in human encephalitic and paralytic rabies. Acta Neurol Scand 1989;80: 287-9.

**Figure Legend**

**Pathology of the dorsal root ganglia, nerve roots and spinal cord in human rabies.** In both paralytic and furious forms, the dorsal root ganglia are infiltrated by numerous lymphocytes (A). There are also infiltrating lymphocytes noted in the spinal nerve roots (B). Anterior horn cells with central chromatolysis are found only in the furious form (C). (Figures A, B from Patient 3, and C from Patient 6, haematoxylin and eosin stain).

**Table 1: Patient data and study details**

| Patient No./Age (y)/ Sex | Bite Site | Incubation Period | Survival Period (days) | Date of Electro-physiologic Study After Clicinal Onset | CSF | rabies antibody |
|---|---|---|---|---|---|---|
| **Paralytic rabies** | | | | | | |
| 1/59/F | L.leg | 3 mo | 13 | Day 12 | 150 mononuclear cells/ cu.mm. protein 600 mg/dl | undetectable (serum and CSF) |
| 2/72/F* | L.cheek | 3 wk | 21 | Day 20 | 862 red cells; 25 mononuclear cells/cu.mm.; protein 41mg/dl | 27.26IU/ml (serum) (CSF-absent) |
| 3/34/M | R.leg | 2 mo | 9 | Day 3<br>Day 4<br>Day 6<br>Day 8 | acellular; protein 70 mg/dl | not done |
| **Furious rabies** | | | | | | |
| 4/50/M | L.wrist | 7 wk | 7 | Day 3<br>Day 5<br>Day 6 | 2 mononuclear cells; protein 40mg/dl | not done |
| 5/65/M | Lower lip | 1 mo | 3 | Day 2 | not done | not done |
| 6/60/M | R. ankle | 1 mo | 4 | Day 3 | not done | not done |

All cases except Patients 1 and 2 had electrophysiologic studies done while they were fully conscious

Local prodrome was present in all except Patient 1. Phobic spasms were observed in 2 out of 3 furious cases (Patients 5 and 6) and appeared very late in one paralytic case (Patient 3).

*received appropriated postexposure prophylaxis

**Table 1A Sensory nerve conduction studies in Patient 1**

| Nerve Stimulated | Stimulation Site | Recording Site | DL (ms) | | Distance (mm) | | CV (m/s) | | Amplitude (μV) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | L | R | L | R | L | R | L | R |
| Sural | Post lower-leg | Lat malleolus | **N/R** | **N/R** | **N/R** | **N/R** | **N/R** | **N/R** | **N/R** | **N/R** |
| Median | 2nd digit | Wrist | 3.2 | 2.8 | 115 | 115 | 35.4 | 41.1 | **7.14** | 8.30 |
| Ulnar | 5th digit | Wrist | 2.5 | 2.4 | 90 | 90 | 36.2 | 37.5 | **5.26** | 6.69 |
| Radial | Rad Forearm | Wrist | 2.1 | 2.0 | 90 | 90 | 42.1 | 45.0 | 23.2 | 25.20 |

Normal Values : Amplitude > 9μV
Conduction velocity - lower limb > 39 m/s, upper limb > 49 m/s

N/R = no response
DL = onset latency
CV = conduction velocity

**Table 1B Motor nerve conduction studies in Patient 1**

| Nerve Stimulated | Stimulation Site | Recording Site | DL (ms) | | CV (m/s) | | Amplitude (mv) | | F-wave Latency (msec) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | L | R | L | R | L | R | L | R |
| Deep peroneal | Ankle | Ext digit brev | **N/R** | 4.6 | **N/R** | N/A | **N/R** | **0.59** | **N/R** | **R/A** |
| Deep peroneal | Fib head | Ext digit brev | 8.0 | 12.8 | | 32.3 | | **0.55** | | |
| Deep peroneal | Pop fossa | Ext digit brev | 1.0 | 15.8 | | 33.3 | | **0.44** | | |
| Tibial | Ankle | Abd hal | 11.0 | 7.4 | N/A | N/A | 0.20 | 0.72 | R/A | R/A |
| Median | Wrist | Thenar eminence | **5.0** | 4.5 | N/A | N/A | 3.67 | 4.24 | R/A | R/A |
| Median | Elbow | Thenar eminence | 11.4 | 10.0 | **38.4** | 40.9 | 3.24 | 3.78 | | |
| Median | Axilla 1 | Thenar eminence | 15.7 | 12.5 | **21.2** | **36.8** | 1.42 | 3.76 | | |
| Median | Axilla 2 | Thenar eminence | 18.0 | 13.6 | **25.6** | 54.5 | 1.04 | 3.63 | | |
| Median | Axilla 3 | Thenar eminence | 20.4 | 15.0 | **30.5** | 51.4 | 0.98 | 3.46 | | |
| Median | Erb' s point | Thenar eminence | N/R | 16.9 | **N/R** | 58.9 | **N/R** | 3.08 | | |
| Ulnar | Wrist | Hypothenar emin | **5.21** | 3.3 | N/A | N/A | 2.43 | 3.53 | R/A | R/A |
| Ulnar | Dist elbow | Hypothenar emin | 11.2 | 10.3 | **30.2** | **25.7** | 2.23 | 2.98 | | |
| Ulnar | Prox elbow | Hypothenar emin | 14.3 | 13.5 | **32.4** | **29.7** | 2.15 | 2.95 | | |
| Ulnar | Axilla 1 | Hypothenar emin | 16.5 | 15.2 | 40.6 | 47.5 | 1.98 | 2.98 | | |
| Ulnar | Axilla 2 | Hypothenar emin | 19.1 | 17.2 | **33.5** | 43.5 | 1.17 | 2.67 | | |
| Ulnar | Erb' s point | Hypothenar emin | 21.5 | 19.1 | 50.4 | 62.0 | 0.98 | 2.28 | | |

| Normal Values : | DL(ms) | CV(ms) | Amplitude(mv) | F(ms) |
|---|---|---|---|---|
| Peroneal | < 5.6 | > 39 | > 2 | < 56 |
| Tibial | < 6.1 | > 39 | > 2 | < 56 |
| Median | < 4.3 | > 49 | > 4 | < 32 |
| Ulnar | < 3.5 | > 49 | > 4 | < 33 |

DL = Distal latency
CV = Conduction velocity
N/R = no response
N/A = not applicable
R/A = response absent

**Table 2A Sensory nerve conduction studies in Patient 2**

| Nerve Stimulated | Stimulation Site | Recording Site | DL (ms) | | Distance (mm) | | CV (m/s) | | Amplitudes (μv) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | L | R | L | R | L | R | L | R |
| Sural | post lower-leg | Lat melleolus | 2.1 | 2.2 | 110 | 110 | 51.2 | 50.0 | 11.21 | 10.16 |
| Median | 2nd digit | wrist | N/R | N/R | N/R | N/R | N/R | N/R | N/R | N/R |
| Ulnar | 5th digit | wrist | N/R | N/R | N/R | N/R | N/R | N/R | N/R | N/R |
| Radial | Rad Forearm | wrist | N/R | N/R | N/R | N/R | N/R | N/R | N/R | N/R |

Normal Values : Amplitude > 9μv
Conduction velocity – lower limb > 39 m/s, upper limb > 49 m/s

N/R = no response
DL = onset latency
CV = conduction velocity

**Table 2B Motor nerve conduction studies in Patient 2**

| Nerve Stimulated | Stimulation Site | Recording Site | DL (ms) | | CV (m/s) | | Amplitude (mv) | | F-wave Lat (ms) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | L | R | L | R | L | R | L | R |
| Deep peroneal | Ankle | Ext dig Brev | 5.8 | 5.9 | N/A | N/A | 0.28 | 0.07 | R/A | N/A |
| | Fib head | Ext dig Brev | 16.9 | 12.4 | 30.7 | 40.0 | 0.27 | 0.07 | | |
| | Pop fossa | Ext dig Brev | 19.1 | 15.3 | 35.8 | 34.5 | 0.18 | 0.05 | | |
| Tibial | Ankle | Abd hal | 6.9 | 6.5 | N/A | N/A | 0.38 | 0.21 | R/A | R/A |
| | Pop fossa | Abd hal | 20.2 | 17.9 | 32.3 | 37.8 | 0.24 | 0.17 | | |
| Median | Wrist | Thenar eminence | **6.5** | 4.4 | N/A | N/A | 0.87 | 0.51 | R/A | R/A |
| | Elbow | Thenar eminence | 13.5 | 12.05 | **32.5** | **30.7** | 0.68 | 0.34 | | |
| | Axilla | Thenar eminence | 17.1 | 14.6 | **30.4** | 40.4 | 0.43 | 0.27 | | |
| | Sup clav notch | Thenar eminence | 21.3 | 19.8 | 50.1 | 50.2 | 0.31 | 0.18 | | |
| Ulnar | Wrist | | 4.3 | 4.0 | N/A | N/A | 0.42 | 0.68 | R/A | R/A |
| | Dist elbow | Hypothenar emin | 7.8 | 7.81 | 52.9 | 48.5 | 0.38 | 0.57 | | |
| | Prox elbow | Hypothenar emin | 9.8 | 10.0 | 47.5 | 44.2 | 0.28 | 0.41 | | |
| | Axilla | Hypothenar emin | 12.8 | 12.6 | **28.3** | **30.8** | 0.25 | 0.37 | | |
| | Sup clav notch | Hypothenar emin | 18.1 | 19.0 | 37.7 | 40.4 | 0.19 | 0.28 | | |

| Normal Values : | DL(ms) | CV(m/s) | Amplitude | F(ms) |
|---|---|---|---|---|
| Peroneal | < 5.6 | > 39 | > 2 | < 56 |
| Tibial | < 6.1 | > 39 | > 2 | < 56 |
| Median | < 4.3 | > 49 | > 4 | < 32 |
| Ulnar | < 3.5 | > 49 | > 4 | < 33 |

DL = Distal latency
CV = Conduction velocity
N/A = not applicable
R/A = response absent

**Table 3A Serial sensory nerve conduction studies in Patient 3**

| Nerve Stimulated | Stimulation Site | Recording Site | Day | DL (ms) | | CV (mls) | | Amplitude (μv) | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | L | R | L | R | L | R |
| Sural | Post lower-leg | Lat malleolus | 3 | 2.1 | 2.0 | 52.3 | 55.0 | 27.84 | 23.35 |
| | | | 4 | 2.6 | 2.3 | 42.3 | 47.8 | **17.19** | **16.34** |
| | | | 6 | **NR** | **NR** | **NR** | **NR** | **NR** | **NR** |
| | | | 8 | **NR** | **NR** | **NR** | **NR** | **NR** | **NR** |
| Median | 2nd digit | wrist | 3 | | 2.8 | | 50.0 | | 19.44 |
| | | | 4 | | 2.8 | | 50.0 | | 18.80 |
| | | | 6 | | 2.7 | | 51.9 | | 19.42 |
| | | | 8 | | 2.8 | | 50.2 | | 18.32 |
| Ulnar | 4th digit | wrist | 3 | | 2.2 | | 50.0 | | 18.50 |
| | | | 4 | | 2.3 | | 52.2 | | 18.02 |
| | | | 6 | | 2.2 | | 50.0 | | 16.95 |
| | | | 8 | | 2.2 | | 51.4 | | 17.02 |

Normal Values: Amplitude > 9μv
Conduction velocity – lower limb > 39 m/s, upper limb > 49 m/s

N/R = no response
DL = onset latency
CV = conduction velocity

**Table 3B Serial motor nerve conduction studies in Patient 3**

| Nerve Stimuiated | Stimulation Site | Recording Site | Day | DL (ms) | | Amplitude (mv) | | F-wave Latency (msec) | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | L | R | L | R | L | R |
| Deep peroneal | Ankle | Ext digit brev | 3 | 5.7 | 5.6 | 3.57 | 2.31 | 53.0 | 86.0 |
| | | | 4 | 5.7 | 5.7 | 3.59 | 2.55 | 57.6 | R/A |
| | | | 6 | 5.8 | 5.8 | 2.35 | 1.29 | **R/A** | R/A |
| | | | 8 | 5.8 | 5.8 | 2.24 | 1.05 | **R/A** | N/A |
| Tibial | Ankle | Abd hal | 3 | 5.3 | 5.8 | 8.34 | 4.16 | 54.3 | 52.8 |
| | | | 4 | 5.8 | 5.9 | 7.70 | 4.16 | 52.5 | 53.4 |
| | | | 6 | 5.8 | 6.0 | 3.89 | 2.35 | **R/A** | R/A |
| | | | 8 | 5.9 | 6.0 | 3.05 | **1.45** | **R/A** | R/A |

**Note** Segmental stimulation was performed on each motor nerve but only values from distal stimulation were shown in this table.

| Normal Values : | DL(ms) | CV(m/s) | Amplitude (mv) | F(ms) |
|---|---|---|---|---|
| Peroneal | < 5.6 | > 39 | > 2 | < 56 |
| Tibial | < 6.1 | > 39 | > 2 | < 56 |
| Median | < 4.3 | > 49 | > 4 | < 32 |
| Ulnar | < 3.5 | > 49 | > 4 | < 33 |

DL = Distal latency
R/A = response absent
N/A = not applicable

**Table 4 Serial sensory nerve conduction studies in Patient 4**

| Nerve Stimulated | Stimulation Site | Recording Site | Day | DL (ms) | | CV (m/s) | | Amplitudes (μv) | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | L | R | L | R | L | R |
| Sural | post lower leg | Lat malleolus | 3 | 2.1 | 2.0 | 52.3 | 55.5 | 24.62 | 26.33 |
| | | | 5 | 2.1 | 2.0 | 51.7 | 54.8 | 26.13 | 26.24 |
| | | | 6 | 2.1 | 2.1 | 52.7 | 53.4 | 22.41 | 24.31 |
| Median | 2nd digit | Wrist | 3 | 2.5 | 2.3 | 50.0 | 54.3 | 22.44 | 25.41 |
| | | | 5 | 2.65 | 2.3 | 47.2 | 54.6 | 11.62 | 25.83 |
| | | | 6 | 2.8 | 2.5 | 44.3 | 50.2 | **5.7** | 18.42 |
| Ulnar | 4th digit | Wrist | 3 | 2.0 | 1.9 | 52.5 | 55.6 | 15.8 | 17.23 |
| | | | 5 | 2.5 | 2.0 | 42.4 | 51.4 | **6.61** | 14.52 |
| | | | 6 | 2.7 | 2.2 | 38.7 | 48.3 | **4.63** | 12.31 |
| Radial | Rad forearm | Wrist | 3 | 1.9 | 1.8 | 61.0 | 63.8 | 30.42 | 37.21 |
| | | | 5 | 2.3 | 2.1 | 51.2 | 56.5 | 12.12 | 28.14 |
| | | | 6 | 2.8 | 2.4 | 41.2 | 49.2 | **8.33** | 15.24 |

Normal Values : Amplitude > 9μv
Concuction velocity – lower > 39 m/s, upper limb > 49 m/s

N/R = no response
DL = onset latency
CV = conduction velocity

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*Mini-Review*

# Pathophysiology of human paralytic rabies

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**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.** *Journal of NeuroVirology* **(2005) 11, 1–8.**

**Keywords:** axonopathy; demyelination; encephalitis; magnetic resonance imaging; paralysis; rabies; RNA virus

## Introduction

Furious rabies has been recorded since antiquity (Rupprecht and Hemachudha, 2004) but the paralytic form of the disease was not recognized until much later. It was initially recorded in 1887 (Gamaleia, 1887) but not widely identified until decades later (Chopra *et al*, 1980; Pawan, 1939). Paralytic rabies continues to be confused with Guillain-Barré syndrome (GBS) and related disorders, treatable autoimmune diseases of peripheral nerves. Misdiagnoses of rabies has led to human-to-human transmission

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through corneal, liver, and kidney transplants from donors who were thought to have GBS or stroke (Jackson, 2002; Lanska, 1992; *MMWR*, 2004). Many rabies patients misdiagnosed as GBS have undergone plasma exchange (Hemachudha *et al*, 2002).

Survival time in paralytic rabies is longer than in furious rabies (Hemachudha *et al*, 2002). We have also demonstrated that dysfunction of peripheral nerves, not anterior horn cells, is responsible for weakness in paralytic rabies (Mitrabhakdi *et al*, 2004).

In order to improve the diagnosis of human rabies, there is a need to better recognize paralytic rabies patients. This article summarizes the distinct clinical features associated with paralytic rabies as compared to GBS and furious rabies. Data on the electrophysiological and pathological features and magnetic resonance imaging (MRI) findings are also reviewed. In addition, we present hypotheses regarding the pathophysiologic mechanisms in paralytic rabies patients.

## Clinical features

*Furious and paralytic rabies*

Clinical data from 115 Thai rabies patients (furious 80, paralytic 35) were examined and compiled from our published reports between 1988 and 2004. These data revealed longer survival periods in the paralytic rabies group (11 days versus 5.7 days in furious group). Cardinal features of furious rabies, fluctuating consciousness, hydro- or aerophobia and inspiratory spasms, signs of autonomic dysfunction, were seen in all Thai furious rabies patients. In noncanine rabies endemic areas, such as in North America, where bats are the principle vector of rabies, clinical expression may be variable (Hemachudha and Phuapradit, 1997).

Only one or two classical signs of rabies, or even none, may be seen during the whole clinical course in paralytic rabies. Consciousness was preserved until the preterminal phase. Phobic spasms were reported in only half of our confirmed paralytic rabies patients (Hemachudha and Rupprecht, 2004). Weakness was the initial manifestation in paralytic rabies, whereas this was noted only when furious rabies patient approached coma.

*Paralytic rabies and Guillain-Barre syndrome*

There are some unique clinical features associated with GBS and paralytic rabies, but some can overlap. This to the extent that they may be indistinguishable clinically (Hemachudha, 1989; Kissel *et al*, 2001; Mitrabhakdi *et al*, 2004) (Table 1).

All three main subtypes of GBS, in which weakness is predominant, result from an immune-mediated process directed against Schwann cells and myelin or axolemma of motor and sensory fibers (Griffin *et al*, 1996; Hafer-Macko *et al*, 1996a, 1996b; McKhann *et al*, 1993) (Table 1).

**Table 1** Clinical features of paralytic rabies and GBS

| | |
|---|---|
| Paralytic rabies | Fever, local prodrome in 1/3*, phobic spasms in 1/2 |
| | Rare distal paresthesias, percussion myoedema |
| | Ascending weakness, may start at bitten limb |
| | Average survival period = 11 days (versus 6 days in furious rabies) |
| AIDP** | 2/3 have antecedent viral or bacterial infections |
| | Symptoms begin with pain and paresthesias in 1/2 |
| | Ascending weakness in 90%, descending weakness initially at the arms in 10%, 80% recovery in 6 months |
| AMAN*** | Commonly preceded by diarrhea from *C. Jejuni* |
| | Abrupt onset of weakness, quadriplegia, respiratory failure |
| | Recovery pattern similar to or better than AIDP |
| AMSAN**** | Commonly preceded by diarrhea from *C. Jejuni* |
| | Minimal sensory symptoms |
| | Abrupt onset of weakness, quadriplegia, respiratory failure, may have ophthalmoparesis |
| | Longer recovery period as compared to AIDP and AMAN |

*In dog-related cases; 2/3 in bat-related cases.
**AIDP = acute inflammatory demyelinating polyradiculoneuropathy.
***AMAN = acute motor axonal neuropathy.
****AMSAN = acute sensory axonal neuropathy.

Similarities with GBS and paralytic rabies are not seen in paralysis associated with other viral infections such as flaviviruses, poliovirus, and West Nile virus where anterior horn cell involvement has been documented by electrophysiologic and magnetic resonance studies (Gorson and Ropper, 2001; Leis *et al*, 2002; Li *et al*, 2003; Solomon *et al*, 1998).

Clinical similarities of GBS and paralytic rabies raise questions whether both share the same neuroanatomical involvement and whether mechanisms responsible for weakness in paralytic rabies are immunologic in nature as has been reported for GBS in association with *Campylobacter jejuni*, *Mycoplasma pneumoniae*, cytomegalovirus, and Epstein-Barr virus (Ogawara *et al*, 2000).

## Pathophysiology of paralytic rabies

*Neuroanatomical base for weakness*

Symptoms and signs in paralytic and furious rabies are indicative of derangement of spinal cord (anterior horn cell) or peripheral nerve in the former and cerebral functions in the latter. Although MRI is useful in aiding diagnosis, MRI fails to explain the clinical diversity in human rabies (Laothamatas *et al*, 2003). This is also true in the case of distribution of rabies virus antigen and inflammatory reactions in the CNS (Tirawatnpong *et al*, 1989). Evidence supporting peripheral nerve dysfunction is based on electrophysiologic studies, the result of which is in accord with peripheral nerve pathology.

*MRI in human rabies:* MRI clearly demonstrated anterior horn cell involvement in paralytic patients

associated with flaviviruses, poliovirus, and West Nile virus (Gorson and Ropper, 2001; Leis *et al*, 2002, 2003; Li *et al*, 2003; Sejvar *et al*, 2003a, 2003b; Solomon *et al*, 1998, 2000a, 2000b). Asymmetric flaccid paralysis with or without signs of meningoencephalitis as well as reversible paraparesis or monoparesis can be seen in association with West Nile virus infection (Leis *et al*, 2003).

In human rabies of both forms, spinal cord gray matter and anterior horn cell were involved in axial gradient-echo T2-weighted images; however, lateral and posterior columns were also affected (Laothamatas *et al*, 2003). Furthermore, MRI findings of the brain were similar in five rabies patients (two furious and three paralytic) and demonstrated as ill-defined, mild hyperintensity changes in the brain stem, hippocampi, hypothalami, deep and subcortical white matter, and deep and cortical gray matter on T2-weighted images (Laothamatas *et al*, 2003). Such findings were evident as early as day 3 after onset and even when consciousness remained intact. Only when the patients became comatose that contrast-enhanced lesions could be demonstrated in brainstem, hypothalami, and spinal nerve roots.

Neither the distribution of rabies virus antigen nor inflammation can explain MRI abnormalities and clinical symptomatology. Of seven patients studied, four (three furious and one paralytic) who had survival times of 7 days or less had a greater amount of antigen-positive neurons in brain stem and spinal cord by avidin-biotin immunoperoxidase staining (Tirawatnpong *et al*, 1989). Hippocampi and hypothalami contained a very minimal amount of rabies antigen. It was not until day 8 after clinical onset that rabies virus antigen disseminated throughout the whole neuraxis, including deep and cortical gray matter. Inflammation was scant in all cases in relation to the amount of viral antigen and survival time. It was not limited to spinal cord in paralytic cases. Inflammation, when present, was usually found in the brain stem and/or spinal cord.

It is also intriguing regarding the pathological basis of MRI changes in the white matter (Laothamatas *et al*, 2003). Extensive demyelination has been reported in one furious rabies patient (Nelson and Berry, 1993), but demyelination in the brain was not observed in another histopathologic series (Chopra *et al*, 1980; Tangchai *et al*, 1970; Tirawatnpong *et al*, 1989). Whether this is caused by alteration of actin-based cytoskeleton mediated by rabies virus nucleocapsid remains to be determined (Ceccaldi *et al*, 1997).

Hyperintense lesions on T2-weighted MRI scans are related primarily to increased water content and thus cannot distinguish between inflammation, edema of vasogenic or cytotoxic origin, demyelination, wallerian degeneration, and axonal loss as well as disruption of cellular or blood brain/nerve barrier integrity (Chard *et al*, 2002; Zivadinov and Bakshi, 2004). Therefore, MRI may not show a reliable correlation with clinical disablitiy. In a disease with a high degree of variability of clinical signs and symptoms such as rabies, newer techniques are required to elucidate mechanisms underlying tissue damage.

*Electrophysiologic features in paralytic rabies:* Electrophysiologic studies showed evidence of peripheral nerve dysfunction in all three paralytic rabies patients with findings indistinguishable from demyelinating and axonal GBS variants. (Mitrabhakdi *et al*, 2004).

Dysfunction of peripheral nerve was suggested by findings of multifocal demyelination along with length-dependent sensory neuropathy in one patient; severe reduction in conduction velocities and marked prolongation of distal latencies in another patient; and progressive loss of motor and sensory amplitudes without accompanied denervation potentials in the third patient who also had early abnormalities in late response indicative of proximal nerve segment involvement during sequential examinations on days 3, 4, 6, and 8 after clinical onset.

Local neuropathic pain is also likely to be due to dorsal root ganglionopathy based on the evidence of absence or progressive decline in sensory nerve action potential amplitudes in the bitten segment.

As opposed to the electrodiagnostic findings in paralytic rabies, the sensory and motor nerve conduction studies, including late responses in three furious patients, were normal. Abundant denervation potentials were evident primarily in the bitten limb even before clinical weakness appeared. This suggests an acute motor fiber loss, probably at the anterior horn cell level. Recent studies also showed that rabies virus–infected rat spinal cord motoneurons resist cytolysis, and apoptotic process is delayed in these neurons as compared to hippocampus cells (Guigoni and Coulon, 2002). Hence, all of these may suggest peripheral nerve dysfunction as being responsible for weakness in paralytic rabies.

*Pathology:* Histopathological study performed on 11 paralytic rabies patients suggested peripheral nerve demyelination as the prime change (Chopra *et al*, 1980). Mild-to-moderate loss of myelinated nerve fibres was reported in 11 of 17 nerves examined; segmental demyelination and remyelination in 16 teased nerve preparations; axonal loss of a variable degree was present in 4 cases; and wallerian-like degeneration in teased single fibres was noted in 6 nerves (Chopra *et al*, 1980). In 9 nerves, the primary abnormality was segmental demyelination and remyelination or myelinated nerve fibre loss, either singly or in combination. In none of these cases was wallerian-like degeneration seen as the only pathological feature. All spinal nerve studies showed evidence of wallerian-like degeneration as well as segmental demyelination (Chopra *et al*, 1980). Such

**Table 2** Pathological features of paralytic rabies and GBS

| | |
|---|---|
| Paralytic rabies | 1. Primarily segmental demyelination and remyelination, occasionally accompanied by wallerian-like degeneration<br>2. T cell–mediated immune attack and/or antibody-mediated complement attack at rabies virus positive axon (or peripheral nerve antigen?)<br>3. Marked inflammatory infiltration of spinal nerve roots by T cell, dorsal root ganglionitis in segment of sensory prodrome |
| AIDP | 1. Demyelination of nerve roots and peripheral nerve fibers<br>2. Antibody-mediated complement attack at Schwann cells, plasmalemma, especially in nerve roots followed by macrophage-mediated demyelination<br>3. Cellular infiltrations not consistent. When present, found predominantly in nerve roots<br>4. Axonal degeneration in severe case |
| AMAN | 1. Axonal degeneration affecting large myelinated fibers of ventral roots<br>2. Antibody-mediated complement attack at axonal plasmalemma and macrophage recruitment to nodal region<br>3. Lack of significant lymphocytic infiltration<br>4. Most axons recover from injury without undergoing wallerian-like degeneration |
| AMSAN | 1. Severe axonal degeneration of myelinated and unmyelinated nerve fibers of nerve roots, with subsequent extension to peripheral nerves<br>2. Antibody-mediated complement attack at axonal plasmalemma and macrophages recruitment to the nodal region<br>3. Lack of significant lymphocytic infiltration<br>4. Extensive wallerian-like degeneration in nerve roots Peripheral nerves are involved later |

demyelination was absent in the case of furious rabies (Tangchai and Vejjajiva, 1971).

Our recent histopathological examination of two paralytic and one furious rabies patients agrees with previous reports (Mitrabhakdi *et al*, 2004) (Table 2). All of them had local neuropathic symptoms. In both paralytic cases, moderate to severe degree of lymphocytic infiltrations, mainly of CD3-positive T cells, was evident in dorsal and spinal nerve roots. The degree of inflammation appeared to be greater at the level of bitten segment. Inflammation of less intense degree was also seen in spinal cord gray matter. Anterior horn cells appeared intact in one and depleted in another but no central chromatolysis was observed in the remaining cells. Only mild inflammation of the spinal nerve roots at all levels was observed in furious rabies patient who had a bite at right ankle. Moderate mononuclear inflammatory cell infiltrates were present in the spinal gray matter of thoracic and lumbar levels and, to a lesser extent, in the cervical cord. Some of the anterior horn cells demonstrated central chromatolysis. Dorsal root ganglionitits was found in all cases.

Inflammation and demyelination of the spinal nerve roots and peripheral nerve, therefore, are characteristic findings in paralytic rabies (Chopra *et al*, 1980; Mitrabhakdi *et al*, 2004). Although inflammation was also evident in spinal cords of these patients, this may not be a constant finding. As mentioned previously, spinal cord inflammation was scant in all four furious and in three paralytic rabies patients in relation to viral antigen and survival time. It was also not limited to the spinal cord in paralytic cases (Tirawatnpong *et al*, 1989).

Nevertheless, it is not known when inflammation and peripheral nerve demyelination take place. If these occur during centrifugal spread of the virus transport, functional derangement of the peripheral nerve as evidenced by electrodiagnostic test may not be necessarily explained by such pathology.

### *Immunologic mechanisms as critical factors in determining survival period and clinical manifestations in human rabies*

*Immunologic parameters in human furious and paralytic rabies:* The term "early death" phenomenon has been coined to emphasize the immune role in accelerating deaths in rabies-infected animals (Blancou *et al*, 1980; Prabhakar and Nathanson, 1981). Immunosuppressed animals show a delay in death (Ceccaldi *et al*, 1996; Iwasaki *et al*, 1977; Smith *et al*, 1982; Tignor *et al*, 1974). Studies in animals with accelerated death indicate that paralysis is a CD4 and CD8 T cell–dependent immunopathologic phenomemon. Foot pad inoculation of a temperature-sensitive variant of the CVS or with the Evelyn-Rokitnicky-Abelseth strain induced paralysis with severe necrosis or degeneration of myelinated motor neurons of the spinal cord in immunocompetent mice (Iwasaki *et al*, 1977; Weiland *et al*, 1992). Street rabies virus–infected T lymphocyte–deficient (nude) mice developed hind limb paralysis after receiving passive transfer of spleen cells (with T cells) from normal immunocompetent mice (Sugamata *et al*, 1992). Perivascular infiltrates included CD8+ and CD4+ T lymphocytes and Mac-1+ macrophage microglial cells.

In human rabies, six of nine furious rabies patients had T-cell immunity to rabies virus based on *in vitro* lymphocyte stimulation technique, whereas none of seven paralytic patients had such response (Hemachudha, 1994; Hemachudha *et al*, 1993, 1988). In another study, more furious patients had raised soluble interleukin-2 (IL-2) receptor (sIL2R) (12/22 versus 1/6 paralytic) and IL-6 levels (5/22 versus 0/6), whereas sCD8 levels were rarely elevated in both groups (Hemachudha *et al*, 1993). Lack of T-cell responses to rabies virus in paralytic rabies is not explained by excessive cortisol production or by a pan-immunosuppressive process. Although serum cortisol levels were elevated in human rabies patients, their levels were comparable among both forms (Hemachudha, 1994). Antigen-specific cell-mediated immune response suppression in mice infected with pathogenic lyssaviruses has

been reported (Perrin *et al*, 1996). There were no differences in total T-cell and T suppressor/cytotoxic–cell numbers in four furious and three paralytic patients, although B-cell numbers were diminished exclusively in all three paralytic cases (Sriwanthana *et al*, 1989). Cellular reactivity against antigens other than rabies, such as myelin basic protein, could still be demonstrated in some of furious and paralytic rabies patients (Hemachudha *et al*, 1988).

Although there is a general agreement that immunopathologic mechanisms may accelerate death and influence the clinical outcome in both human rabies and animal models, specific virus variant itself may be entirely responsible for the outcome and any accompanying immunological findings could be merely epiphenomenon. Rabies glycoprotein is crucial for neutralizing antibody production and initiation of cellular immunity response. Differences in glycoprotein (G) may affect G protein–receptor interactions, nicotinic acetylcholine receptor at bite site, P75 neurotrophin receptor, and glycolipid or ganglioside central nervous system (CNS) receptors. Minor variations of the G protein, such as an amino acid substitution of arginine at position 333, may affect neuroinvasiveness by the use of different neuronal pathways (Wunner, 2002). Pathogenicity of different rabies virus variants inversely correlates with apoptosis and rabies virus glycoprotein expression based on study in infected primary neuron cultures (Morimoto *et al*, 1999). Modifying the dynein light chain binding site to rabies virus capsid P protein can reduce the efficiency of the peripheral spread of certain rabies virus (Mebatsion, 2001).

Comparison of a 1-432-, a 1575-, and a 894-nucleotide region from the rabies virus N, G, and P protein genes of samples obtained from two furious and two paralytic human patients associated with canine rabies virus of genotype 1 showed only minor nucleotide differences (Hemachudha *et al*, 2003b). Deduced amino acid patterns of N protein were identical among both human and canine samples that belonged to the same geographic origin, regardless of clinical forms. All differences in the amino acid of G protein were found outside the ectodomain, in the signal peptide and transmembrane and endodomains. None were in an interactive region with receptors known responsible for virus pathogenicity, nor did they lie in an immunodominant G domain. Moreover, a single rabid dog transmitted furious rabies to one patient and paralytic rabies in another (Hemachudha *et al*, 1988).

Both the involvement of peripheral nerves in paralytic rabies and the unexplained aggression with extreme excitability in furious rabies, despite a similar virus distribution in the CNS and nearly identical MRI patterns in the brain, argue against the existence of specific variants, and instead, suggest a participation of host factors. The degree of functional impairment of the muscarinic acetylcholine receptor in the brains of rabid dogs does not correlate with the virus distribution and virus load (Dumrongphol *et al*, 1996).

In human rabies, proinflammatory cytokines might also affect, directly or indirectly, the levels of neurotrophins, growth factors, neurotransmitters, and neurotoxins in the brain, via the activation of glia, neurons, and vascular and immune cells (Hemachudha *et al*, 2002; Tomonaga, 2004). It is not known to what extent that the initiation of immune response and amplification of cytokine cascade in rabies-infected brain (Hemachudha *et al*, 2002) can influence rabies virus modulation of host gene expression (Prosniak *et al*, 2001). Differences in the host neuronal gene expression patterns may be important in virus replication and spread in the CNS as well as neuronal survival (Prosniak *et al*, 2003). Perturbation of such cellular factors could also induce functional loss and/or abnormal activation of infected neural cells, leading to broad incoordination of the neural system (Tomonaga, 2004).

*Hypothetical mechanisms in human paralytic rabies:* Mechanisms involved in nerve injury could be mediated by rabies neutralizing antibody against rabies virus in axons. Neutralizing antibody was present as early as 3 days after onset of nonspecific symptoms (Kasempimolporn *et al*, 1991). Deposition of immunoglobulin G (IgG) and complement on rabies virus–positive axons was evident in a Chinese paralytic rabies patient (Sheikh *et al*, 1998). Viral protein and wallerian-like degeneration were found to be more abundant in the ventral than in dorsal nerve roots. Our recent case with furious rabies developed weakness of facial, limb, and neck flexor muscles 36 h after receiving intravenous human rabies immune globulin (Hemachudha *et al*, 2003a). However, there may be more than one mechanism involved because cerebrospinal (CSF) rabies neutralizing antibodies could not be demonstrated in this case as well as in another 30 rabies patients, 14 of whom were cases of paralytic rabies (Hemachudha and Mitrabhakdi, 2000; Laothamatas *et al*, 2003).

Auto-antibody against peripheral nerve antigen may be another mechanism. Vivid enhancement of the ventral and dorsal nerve roots could be demonstrated in two cases of paralytic rabies (Laothamatas *et al*, 2003). Such enhanced nerve roots can also be found in other conditions and in almost all of classical GBS patients (Gorson *et al*, 1996).

Molecular mimicry has been accepted for classical GBS and its variants. There appears to be a link between anti-glycolipid antibodies and many pathogens (Willison and Yuki, 2002). These autoantibodies were found associated with GBS and its variants and Bickerstaff's encephalitis (Odaka *et al*, 2003; Susuki *et al*, 2004; Willison and Yuki, 2002). None of our three furious and three paralytic rabies patients had anti-GM1, -GD1a, -GalNAc-GD1a, -GD1b, -GT1a, and -GQ1b ganglioside antibodies (Mitrabhakdi *et al*, 2004). Nevertheless, this does

not exclude the existence of other pathogenic autoantibodies.

It may be possible that antibody production in paralytic rabies may be inefficient. We have shown that some paralytic rabies patients had diminished circulating B cells and IL-6 levels (Hemachudha *et al*, 1993; Sriwanthana *et al*, 1989). Fewer paralytic rabies patients had serum rabies neutralizing antibody (Hemachudha and Phuapradit, 1997). Rabies patients with cellular reactivity to MBP did not have anti-MBP antibody (Hemachudha *et al*, 1988).

Other proposed factor for inducing weakness includes initiation of cellular-dependent mechanisms. Pathological examination of the peripheral nerve and spinal nerve roots indicated a more severe degree of inflammation in paralytic than in furious rabies patients (Chopra *et al*, 1980; Mitrabhakdi *et al*, 2004). Cellular infiltrates were characterized as T cells. Whether they are directed against rabies virus or peripheral nerve antigen is not known. Rabies virus, in the form of uncoated nucleocapsids or nascent ribonucleoprotein, migrates along peripheral nerve via fast-retrograde axonal transport (Mebatsion, 2001; Murphy, 1977; Wunner, 2002). Rabies phosphoprotein interacts with LC8 component of dynein light chain in the transportation process (Mebatsion, 2001). The whole rabies virion, or only a subviral fragment containing glycoprotein (G) molecule, may also be carried by means of vesicular cargo using attachment between rabies G protein and p75NTR (Mazarakis *et al*, 2001; Tuffereau *et al*, 2001). Peripheral administration of rabies G-pseudotyped equine infectious anemia virus vectors to the rat gastrocnemius muscle leads to gene transfer in motoneurons of lumbar spinal cord (Mazarakis *et al*, 2001). Therefore, peripheral nerve containing noninfectious rabies virus, or just a subfragment, can be attacked by immune cells. Persistence of neuroadapted Sindbis virus antigen promotes progressive CNS neuronal damage and demyelination despite clearance of infectious virus. This is mediated by CD4 T cells and macrophage/microglia cells (Kimura and Griffin, 2003). Lack of specific cellular immune response to rabies virus is found in most paralytic rabies patients (Hemachudha *et al*, 1988). Hence, nerve cell–derived antigen may be a primary target in paralytic rabies. A strict homology between self- (peripheral nerve antigen) and foreign (rabies virus) antigens may not be necessary for cross recognition according to the concept of degenerate T-cell receptors (Gran *et al*, 1999).

## References

Blancou J, Andral B, Andral L (1980). A model in mice for the study of the early death phenomenon after vaccination and challenge with rabies virus. *J Gen Virol* **50**: 433–435.

Ceccaldi PE, Marquette C, Weber P, Gourmelon P, Tsiang H (1996). Ionizing radiation modulates the spread of an apathogenic rabies virus in mouse brain. *Int J Radiat Biol* **70**: 69–75.

Ceccaldi PE, Valtorta F, Braud S, Hellio R, Tsiang H (1997). Alteration of the actin-based cytoskeleton by rabies virus. *J Gen Virol* **78**: 2831–2835.

Chard DT, Griffin CM, Parker GJ, Kapoor R, Thompson AJ, Miller DH (2002). Brain atrophy in clinically early relapsing-remitting multiple sclerosis. *Brain* **125**: 327–337.

Chopra JS, Banerjee A, Murthy JMK, Pal SR (1980). Paralytic rabies: a clinicopathological study. *Brain* **103**: 789–802.

Dumrongphol H, Srikiatkhachorn A, Hemachudha T, Kotchabhakdi N, Govitrapong P (1996). Alteration of muscarinic acetylcholine receptors in rabies viral-infected dog brains. *J Neurol Sci* **137**: 1–6.

Gamaleia N (1887). Etude sur la rage paralytique chez L'homme. *Ann Inst Pasteur (Paris)* **1**: 63–83.

Gorson K, Ropper A, Muriello M, Blair R (1996). Prospective evaluation of MRI lumbosacral nerve root enhancement in acute Guillain-Barré syndrome. *Neurology* **47**: 813–817.

Gorson KC, Ropper AH (2001). Nonpoliovirus poliomyelitis simulating Guillain-Barré syndrome. *Arch Neurol* **58**: 1460–1464.

Gran B, Hemmer B, Vergelli M, McFarland HF, Martin R (1999). Molecular mimicry and multiple sclerosis: degenerate T-cell recognition and the induction of autoimmunity. *Ann Neurol* **45**: 559–567.

Griffin JW, Li CY, Ho TW, Tian M, Gao CY, Xue P, Mishu B, Cornblath DR, Macko C, McKhann GM, Asbury AK (1996). Pathology of the motor-sensory axonal Guillain-Barré syndrome. *Ann Neurol* **39**: 17–28.

Guigoni C, Coulon P (2002). Rabies virus is not cytolytic for rat spinal motoneurons in vitro. *J NeuroVirol* **8**: 306–317.

Hafer-Macko C, Hsieh ST, Li CY, Ho TW, Sheikh K, Cornblath DR, McKhann GM, Asbury AK, Griffin JW (1996a). Acute motor axonal neuropathy: an antibody-mediated attack on axolemma. *Ann Neurol* **40**: 635–644.

Hafer-Macko CE, Sheikh KA, Li CY, Ho TW, Cornblath DR, McKhann GM, Asbury AK, Griffin JW (1996b). Immune attack on the Schwann cell surface in acute inflammatory demyelinating polyneuropathy. *Ann Neurol* **39**: 625–635.

Hemachudha T (1989). Rabies. In: *Handbook of clinical neurology viral disease*. Vinken P, Bruyn G, Klawans H (eds). Amsterdam: Elsevier Science Publishers, pp 383–404.

Hemachudha T (1994). Human rabies: clinical aspects, pathogenesis, and potential therapy. *Curr Top Microbiol Immunol* **187**: 121–143.

Hemachudha T, Laothamatas J, Rupprecht CE (2002). Human rabies: a disease of complex neuropathogenetic mechanisms and diagnostic challenges. *Lancet Neurol* **1**: 101–109.

Hemachudha T, Mitrabhakdi E (2000). Rabies. In: *Infectious diseases of the nervous system*. Davis L, Kennedy PGE (eds). Oxford: Butterworth-Heinemann, pp 401–444.

Hemachudha T, Panpanich T, Phanuphak P, Manatsathit S, Wilde H (1993). Immune activation in human rabies. *Trans R Soc Trop Med Hyg* **87**: 106–108.

Hemachudha T, Phanuphak P, Sriwanthana B, Manutsathit S, Phanthumchinda K, Siriprasomsup W, Ukachoke C, Rasameechan S, Kaoroptham S (1988). Immunologic

study of human encephalitic and paralytic rabies. Preliminary report of 16 patients. *Am J Med* **84**: 673–677.

Hemachudha T, Phuapradit P (1997). Rabies. *Curr Opin Neurol* **10**: 260–267.

Hemachudha T, Rupprecht C (2004). Rabies. In: *Principle of neurological infectious diseases*. Roos K (ed). New York: McGraw Hill, in press.

Hemachudha T, Sunsaneewitayakul B, Mitrabhakdi E, Suankratay C, Laothamathas J, Wacharapluesadee S, Khawplod P, Wilde H (2003a). Paralytic complications following intravenous rabies immune globulin treatment in a patient with furious rabies. *Int J Infect Dis* **7**: 76–77.

Hemachudha T, Wacharapluesadee S, Lumlertdaecha B, Orciari LA, Rupprecht CE, La-Ongpant M, Juntrakul S, Denduangboripant J (2003b). Sequence analysis of rabies virus in humans exhibiting encephalitic or paralytic rabies. *J Infect Dis* **188**: 960–966.

Iwasaki Y, Gerhard W, Clark HF (1977). Role of host immune response in the development of either encephalitic or paralytic disease after experimental rabies infection in mice. *Infect Immun* **18**: 220–225.

Jackson A (2002). Human disease. In: *Rabies*. Jackson A, Wunner WH (eds). Amsterdam: Academic Press, pp 219–244.

Kasempimolporn S, Hemachudha T, Khawplod P, Manatsathit S (1991). Human immune response to rabies nucleocapsid and glycoprotein antigens. *Clin Exp Immunol* **84**: 195–199.

Kimura T, Griffin DE (2003). Extensive immune-mediated hippocampal damage in mice surviving infection with neuroadapted Sindbis virus. *Virology* **311**: 28–39.

Kissel JT, Cornblath DR, Mendell JR (2001). Guillain-Barré syndrome. In: *Diagnosis and management of peripheral nerve disorders*. Mendell JR, Kissel JT, Cornblath DR, (eds). New York: Oxford University Press, pp 145–172.

Lanska DJ, (1992). Rabies virus, paralytic and classical. *Lancet* **339**: 809.

Laothamatas J, Hemachudha T, Mitrabhakdi E, Wannakrairot P, Tulayadaechanont S (2003). MR imaging in human rabies. *AJNR Am J Neuroradiol* **24**: 1102–1109.

Leis AA, Stokic DS, Polk JL, Dostrow V, Winkelmann M (2002). A poliomyelitis-like syndrome from West Nile virus infection. *N Engl J Med* **347**: 1279–1280.

Leis AA, Stokic DS, Webb RM, Slavinski SA, Fratkin J (2003). Clinical spectrum of muscle weakness in human West Nile virus infection. *Muscle Nerve* **28**: 302–308.

Li J, Loeb JA, Shy ME, Shah AK, Tselis AC, Kupski WJ, Lewis RA (2003). Asymmetric flaccid paralysis: a neuromuscular presentation of West Nile virus infection. *Ann Neurol* **53**: 703–710.

Mazarakis ND, Azzouz M, Rohll JB, Ellard FM, Wilkes FJ, Olsen AL, Carter EE, Barber RD, Baban DF, Kingsman SM, Kingsman AJ, O'Malley K, Mitrophanous KA (2001). Rabies virus glycoprotein pseudotyping of lentiviral vectors enables retrograde axonal transport and access to the nervous system after peripheral delivery. *Hum Mol Genet* **10**: 2109–2121.

McKhann GM, Cornblath DR, Griffin JW, Ho TW, Li CY, Jiang Z, Wu HS, Zhaori G, Liu Y, Jou LP, *et al* (1993). Acute motor axonal neuropathy: a frequent cause of acute flaccid paralysis in China. *Ann Neurol* **33**: 333–342.

Mebatsion T (2001). Extensive attenuation of rabies virus by simultaneously modifying the dynein light chain binding site in the P protein and replacing Arg333 in the G protein. *J Virol* **75**: 11496–11502.

Mitrabhakdi E, Hemachudha T, Shuangshoti S, Wannakrairot P, Susuki K, Laothamatas J (2004). Difference in neuropathogenetic mechanisms in human furious and paralytic rabies. *J Neurol Sci*, in press.

*MMWR* (2004). Investigation of rabies infection in organ donor and transplant recipients—Alabama, Arkansas, Oklahoma, and Texas, 2004. *MMWR Morb Mort Wkly Rep* **53**: 586–589.

Morimoto K, Hooper DC, Spitsin S, Koprowski H, Dietzschold B (1999). Pathogenicity of different rabies virus variants inversely correlates with apoptosis and rabies virus glycoprotein expression in infected primary neuron cultures. *J Virol* **73**: 510–518.

Murphy FA (1977). Rabies pathogenesis. *Arch Virol* **54**: 279–297.

Nelson DA, Berry RG (1993). Fatal rabies associated with extensive demyelination. *Arch Neurol* **50**: 317–323.

Odaka M, Yuki N, Yamada M, Koga M, Takemi T, Hirata K, Kuwabara S (2003). Bickerstaff's brainstem encephalitis: clinical features of 62 cases and a subgroup associated with Guillain-Barré syndrome. *Brain* **126**: 2279–2290.

Ogawara K, Kuwabara S, Mori M, Hattori T, Koga M, Yuki N (2000). Axonal Guillain-Barré syndrome: relation to anti-ganglioside antibodies and *Campylobacter jejuni* infection in Japan. *Ann Neurol* **48**: 624–631.

Pawan J (1939). Paralysis as a clinical manifestation in human rabies. *Ann Trop Med Parasitol* **33**: 21–29.

Perrin P, Tino de Franco M, Jallet C, Fouque F, Morgeaux S, Tordo N, Colle JH (1996). The antigen-specific cell-mediated immune response in mice is suppressed by infection with pathogenic lyssaviruses. *Res Virol* **147**: 289–299.

Prabhakar BS, Nathanson N (1981). Acute rabies death mediated by antibody. *Nature* **290**: 590–591.

Prosniak M, Hooper DC, Dietzschold B, Koprowski H (2001). Effect of rabies virus infection on gene expression in mouse brain. *Proc Natl Acad Sci USA* **98**: 2758–2763.

Prosniak M, Zborek A, Scott GS, Roy A, Phares TW, Koprowski H, Hooper DC (2003). Differential expression of growth factors at the cellular level in virus-infected brain. *Proc Natl Acad Sci USA* **100**: 6765–6770.

Rupprecht CE, Hemachudha T (2004). Rabies. In: *Infections of the central nervous system*. Scheld M, Whitley RJ, Marra C (eds). Philadelphia: Lippincott Williams and Wilkins, in press.

Sejvar JJ, Haddad MB, Tierney BC, Campbell GL, Marfin AA, Van Gerpen JA, Fleischauer A, Leis AA, Stokic DS, Petersen LR (2003a). Neurologic manifestations and outcome of West Nile virus infection. *JAMA* **290**: 511–515.

Sejvar JJ, Leis AA, Stokic DS, Van Gerpen JA, Marfin AA, Webb R, Haddad MB, Tierney BC, Slavinski SA, Polk JL, Dostrow V, Winkelmann M, Petersen LR (2003b). Acute flaccid paralysis and West Nile virus infection. *Emerg Infect Dis* **9**: 788–793.

Sheikh K, Jackson A, Ramos-Alvarez M, Li C, Ho T, Asbury A, Griffin J (1998). Paralytic rabies: Immune attack on nerve fibres containing axonally transported viral proteins [abstract]. *Neurology* **501**: 183.

Smith JS, McCelland CL, Reid FL, Baer GM (1982). Dual role of the immune response in street rabiesvirus infection of mice. *Infect Immun* **35:** 213–221.

Solomon T, Dung NM, Kneen R, Gainsborough M, Vaughn DW, Khanh VT (2000a). Japanese encephalitis. *J Neurol Neurosurg Psychiatry* **68:** 405–415.

Solomon T, Dung NM, Vaughn DW, Kneen R, Thao LT, Raengsakulrach B, Loan HT, Day NP, Farrar J, Myint KS, Warrell MJ, James WS, Nisalak A, White NJ (2000b). Neurological manifestations of dengue infection. *Lancet* **355:** 1053–1059.

Solomon T, Kneen R, Dung NM, Khann VC, Thuy TT, Ha DQ, Day NP, Nisalak A, Vaughn DW, White NJ (1998). Poliomyelitis-like illness due to Japanese encephalitis virus. *Lancet* **351:** 1094–1097.

Sriwanthana B, Hemachudha T, Griffin DE, Manutsathit S, Tweardy D, Phanuphak P (1989). Lymphocyte subsets in human encephalitic and paralytic rabies. *Acta Neurol Scand* **80:** 287–289.

Sugamata M, Miyazawa M, Mori S, Spangrude GJ, Ewalt LC, Lodmell DL (1992). Paralysis of street rabies virus-infected mice is dependent on T lymphocytes. *J Virol* **66:** 1252–1260.

Susuki K, Odaka M, Mori M, Hirata K, Yuki N (2004). Acute motor axonal neuropathy after Mycoplasma infection: evidence of molecular mimicry. *Neurology* **62:** 949–956.

Tangchai P, Vejjajiva A (1971). Pathology of the peripheral nervous system in human rabies: a study of nine autopsy cases. *Brain* **94:** 299–306.

Tangchai P, Yenbutr D, Vejjajiva A (1970). Central nervous system lesions in human rabies. *J Med Assoc Thailand* **53:** 471–488.

Tignor GH, Shope RE, Gershon RK, Waksman BH (1974). Immunopathologic aspects of infection with Lagos bat virus of the rabies serogroup. *J Immunol* **112:** 260–265.

Tirawatnpong S, Hemachudha T, Manutsathit S, Shuangshoti S, Phanthumchinda K, Phanuphak P (1989). Regional distribution of rabies viral antigen in central nervous system of human encephalitic and paralytic rabies. *J Neurol Sci* **92:** 91–99.

Tomonaga K (2004). Virus-induced neurobehavioral disorders: mechanisms and implications. *Trends Mol Med* **10:** 71–77.

Tuffereau C, Desmezieres E, Benejean J, Jallet C, Flamand A, Tordo N, Perrin P (2001). Interaction of lyssaviruses with the low-affinity nerve-growth factor receptor p75NTR. *J Gen Virol* **82:** 2861–2867.

Weiland F, Cox JH, Meyer S, Dahme E, Reddehase MJ (1992). Rabies virus neuritic paralysis: immunopathogenesis of nonfatal paralytic rabies. *J Virol* **66:** 5096-5099.

Willison HJ, Yuki N (2002). Peripheral neuropathies and anti-glycolipid antibodies. *Brain* **125:** 2591-2625.

Wunner W (2002). Rabies virus. In: *Rabies.* Jackson A, Wunner WH (eds). Amsterdam: Academic Press, pp 23–77.

Zivadinov R, Bakshi R (2004). Role of MRI in multiple sclerosis I: inflammation and lesions. *Front Biosci* **9:** 665–683.

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# Complex genetic structure of rabies virus in Bangkok, Thailand, and its surrounding provinces: implications for canine rabies control

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Short running title: Genetic sequencing of rabies virus isolates

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**Summary**

Dog vaccination and population management have been suggested as priorities in attempts at disease control in canine rabies-endemic countries. Budget limitations and the complexity of social, cultural and religious variables have complicated progress in the developing world. In Bangkok, Thailand, an intensive canine vaccination and sterilization programme has been in place since November 2002. Our objective was to determine if rabies virus could be mapped according to its genetic variations and geographical location on the small localized scale of Bangkok and its surrounding provinces. Phylogenetic characterization of 69 samples from Bangkok and from five neighbouring and two remote provinces, by limited sequence analysis of the rabies virus nucleoprotein gene, distinguished six different clades. Rabies viruses of four clades were intermixed in Bangkok and in the surrounding highly populated regions whereas the other two clades were confined to rural and less populated provinces. Such a complex pattern of gene flow, particularly in Bangkok, may affect the outcome of canine control programmes.

**KEYWORDS**

Rabies virus; Molecular epidemiology; Genetic sequencing; Canines; Vaccination; Thailand

## 1. Introduction

Thailand, like other canine rabies-endemic countries, relies heavily on rabies post-exposure prophylaxis and increased awareness of rabies in the general public to prevent rabies in humans. Over the last decade, the number of humans that have received post-exposure prophylaxis in Thailand has continually increased (for example, 93 641 cases in 1991, 183 815 in 1996 and 350 535 in 2001; Ministry of Public Health (MOPH), unpublished report). However, despite a vigorous campaign to increase awareness of rabies and post-exposure prophylaxis, the annual number of human rabies deaths in Bangkok rose from <5 between 1990 and 1994 to 6–8 between 1995 and 2001 (MOPH, unpublished report). Moreover, as more is understood about the pathogenesis and complex nature of the presentation of human rabies, it is clear that the number of human deaths attributed to rabies both in Bangkok and the whole country may be underreported (Hemachudha et al., 2002).

Of the six million dogs estimated to be living in Thailand, there are no reliable data indicating their relative population densities or distribution patterns in various regions throughout the country. However, in a survey conducted by the Bureau of National Statistics and the Bangkok Metropolitan Administration in 1999, it was estimated that approximately 630 000 dogs live in Bangkok (an area of 1565 km$^2$). In this survey, approximately 110 000 of the dogs living in Bangkok were considered to be ownerless (stray or feral) and the remainder to be owned or semi-restricted. When the dog population statistics collected in 1999 are compared to an earlier survey conducted in 1992, the stray dog population in Bangkok appears to have almost tripled in size over the 8-year period (approx. 40 000 out of a total of 360 000 in 1992 vs. 110 000 out of a

total of 630 000 in 1999). The latest survey, conducted in 2002, indicated that dog populations, both in Bangkok and countrywide, have continued to increase and are unlikely to level-off or decline in the near future. Moreover, a significant number of dogs living throughout the country, especially ownerless and semi-restricted dogs, are not vaccinated.

Dog vaccination and population management have been suggested as priorities to control and reduce the incidence of both animal and human rabies in canine rabies-endemic countries. Experience in Latin America has proven that vaccination of a critical percentage of dogs, to the order of 40–70%, at least in major urban areas, was sufficient to significantly interrupt canine rabies transmission and significantly reduce the number of human rabies deaths (PAHO, 2000). Due to the budget limitations of implementing a nationwide canine vaccination programme in Thailand accompanied by the complexity of social, cultural and religious variables, attempts to conduct a mass dog vaccination and sterilization programme has been limited to Bangkok. This programme was initiated in Bangkok in June 2002. As part of the programme, dogs that were vaccinated and sterilized were released at the location where they were initially captured in an attempt to prevent the potential influx of new dogs from neighbouring regions.

Assessment of the success of an animal vaccination and control programme can be measured in part by analysis of the temporal and spatial incidence of rabies in humans and animals after the programme has been implemented. However, such an analysis may not provide an accurate picture. For example, the number of samples submitted for testing to the rabies diagnostic laboratories in Bangkok has been declining over the last

decade (approx. 1300–1700 specimens per annum in 1994–1997 vs. 1000–1300 per annum in 1998–2001). However, the percentage of samples that were confirmed infected with rabies during this period remained unchanged, within the range of 23–30% (MOPH, unpublished report).

In order to examine another variable that could be a useful tool to measure the progress of animal rabies control programmes, we performed a preliminary genetic analysis of rabies virus isolates collected from animal and human cases in Bangkok and the surrounding area, as well as remote areas (for baseline data). Our objective was to determine if rabies virus could be mapped according to its genetic variations and geographical location on such a localized scale and thereby to determine if we could identify translocation of rabies-infected animals from one region of the country to another.

## 2. Materials and methods

Sixty-five animal (dog = 57, cat = 7 and hog badger = 1) and four human brain samples collected between 1998 and 2001 were analysed. All samples had tested positive for the presence of rabies virus antigen by the direct fluorescent antibody technique. Samples were originally submitted to different rabies diagnostic laboratories (Bangkok = 20, Nonthaburi = 9, Pathum Thani = 9, Nakhon Pathom = 8, Samut Prakan = 6, Samut Sakhon = 6, Kanchanaburi = 6 and Chaiyaphum = 5). All tissue samples were subjected to routine nucleotide (nt) sequencing protocols, as previously described (Hemachudha et al., 2003). A 301 nt sequence of the rabies virus nucleoprotein gene was analysed.

Phylogenetic trees were constructed using the neighbour-joining method, as implemented in the computer program PAUP* 4.0b10 (Swofford, 2002). Three additional sequences (Mokola virus, Pasteur virus and a Thai rabies virus sequence, THA8738) retrieved from the DNA database were also included. The Pasteur virus sequence served as the outgroup for the tree-rooting analyses. The Mokola virus sequence was added in order to confirm that there was no paraphyletic problem among Thai rabies sequences. Branch support values from bootstrap (BS) analysis (e.g. 1000 replicates) and the collection area and year of each sample were mapped on the tree. Although the neighbour-joining method is suitable enough for illustrating potential species relationships, maximum parsimony analysis was also performed. GenBank accession numbers of the nucleoprotein sequences in this study are **AY580089–AY580158**.

**3. Results**

All Thai rabies virus sequences formed a monophyletic group (Figure 1). Considering the BS values and maximum parsimony branch lengths, two putative groups were identified, i.e. group A (65% BS, 29 nt changes) and group B (82% BS, 31 nt changes). Moreover, six different clades of rabies virus were distinguished using the same criteria, though with weak supporting values in some clades. Clades I–III were members of group A and clades IV–VI were members of group B, with clades II and III and clades IV and V closely associated. The tree topology remained unchanged when either the Mokola virus sequence was excluded from or several other Asian rabies virus nucleoprotein sequences were added to the analysis (data not shown).

**4. Discussion**

Our study revealed a complex genetic structure of rabies virus on a localized scale, in Bangkok and its surrounding provinces, based on the existence of multiple clades in these regions. This suggests frequent localized gene flow in these highly populated areas. Although a relationship between a specific virus clade and a particular geographical location could not be easily mapped in the small-scale study areas, evidence for population subdivision was found in more rural communities such as Chaiyaphum (area H; Figure 2) and potentially in Kanchanaburi (area G; Figure 2).

An homogeneous population was found in Chaiyaphum (major members of clade VI) and Kanchanaburi (clade V) provinces, the two largest regions in this study, with areas of 12 778 and 19 483 km$^2$ respectively (Figure 2). These two provinces are not heavily populated (populations of 1 095 360 and 734 394 respectively; Bureau of National Statistics, unpublished report). The compartmentalization phenomenon can be explained by a bottleneck effect, complex relationships of dogs and humans in the same community, and relative dog population density related to local geographic features (Holmes et al., 2002; Smith, 2002). Although these two rural provinces are bordered in one or two directions by mountains, it remains doubtful whether this can create physical barriers to animal movement and lead to geographical isolation of the virus clades, especially of clade VI, from other territories. Rabies virus of similar clades was found along both banks of the Chaophya River which runs through Bangkok, Nonthaburi, Patum Thani and Samut Prakan (Figure 3). There are at least 10 main bridges connecting both sides. Similarly, other routes, such as roads and railways can facilitate movement of dogs into rural regions including Chaiyaphum and Kanchanaburi.

Viruses of clades I–IV were distributed randomly within Bangkok and its five neighbouring areas (Figure 2). These highly populated regions; Bangkok 1565 km² (population 6 355 144), Nonthaburi 622 km² (816 614), Patum Thani 1565 km² (677 649), Nakhon Pathom 2168 km² (815 122), Samut Prakan 1004 km² (1 028 401) and Samut Sakhon 872 km² (466 281) are smaller in size and overpopulated compared to Kanchanaburi and Chaiyaphum (Figure 2). These observations suggest that perpetuation of dog rabies variants in our rural study areas may rely on the degree of dependence of dogs on human care, as well as the density of dog and human populations. A higher degree of genetic diversity found in dogs living in urban communities may suggest a continuous movement of dog populations and is probably related to the movement of people and animals to more industrialized zones, like Bangkok, and away from rural areas (De Mattos et al., 1999; Susetya et al., 2003).

Movements of infected animals to unaffected areas may produce spillover occurrences, which may lead to sustainable outbreaks (Smith, 2002). Although there was no strong evidence supporting a rural-to-urban movement of viral strains in group A (clades I–III), the close relationships between clade V (rural) and clade IV (urban) of group B, and particularly the presence of two clade V viruses in urban areas (Samut Sakhon and Samut Prakan), may suggest that rural viruses have moved into urban areas. Further work needs to be done to confirm this transmission polarity hypothesis of the group B strains.

Rabies viruses associated with the cats and wild boar were also related to dogs, and were presumed to be a spillover from rabid dogs (Figure 1).

This preliminary study suggests that rabies virus clusters may be distinguishable in small selected areas with high population densities. This may be useful in terms of rabies control, for example, while clade VI and possibly clade V showed that Chaiyaphum (region H; Figure 2) and Kanchanaburi (region G; Figure 2) had less genetic divergence and could be considered to be geographically isolated from other study areas, all urban and overpopulated areas might appear as a 'sink' for the integration of rabid dogs, thus demanding more stringent dog control measures.

There were no reported human rabies deaths in Bangkok in 2002, and three deaths in 2003. This observation may indicate that an immunization campaign in only one overly populated and industrialized city like Bangkok when done inadequately and particularly without an additional immunization belt in surrounding areas, is unlikely to control dog rabies. An influx of unvaccinated dogs from the suburbs and rural areas either by migration with humans or on their own could potentially sustain a rabies outbreak.

In conclusion, rabies virus could be mapped according to its genetic variation. More studies need to be conducted in order to see whether translocation of rabies-infected animals from one region of the country to another can be traced using molecular biology techniques.

**Conflicts of interest statement**

The authors have no conflicts of interest concerning the work reported in this paper.

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**References**

De Mattos, C.C., De Mattos, C.A., Loza-Rubio, E., Aguilar-Setien, A., Orciari, L.A., Smith, J.S., 1999. Molecular characterization of rabies virus isolates from Mexico: implications for transmission dynamics and human risk. Am. J. Trop. Med. Hyg. 61, 587–597.

Hemachudha, T., Laothamatas, J., Rupprecht, C.E., 2002. Human rabies: a disease of complex neuropathogenetic mechanisms and diagnostic challenges. Lancet Neurol. 1, 101–109.

Hemachudha, T., Wacharapluesadee, S., Lumlertdaecha, B., Orciari, L.A., Rupprecht, C.E., La-ongpant, M., Juntrakul, S., Denduangboripant, J., 2003. Sequence analysis of rabies virus in humans exhibiting encephalitic or paralytic rabies. J. Infect. Dis. 188, 960–966.

Holmes, E.C., Woelk, C.H., Kassis, R., Bourhy, H., 2002. Genetic constraints and the adaptive evolution of rabies virus in nature. Virology 292, 247–257.

PAHO, 2000. Epidemiological surveillance of rabies in the Americas. Pan American Health Organization, Washington D.C.

Smith, J.S., 2002. Molecular epidemiology, in: Jackson, A.C., Wunner, W.H. (Eds), Rabies. Academic Press, Amsterdam, pp. 79–111.

Susetya, H., Sugiyama, M., Inagaki, A., Ito, N., Oraveerakul, K., Traiwanatham, N., Minamoto, N., 2003. Genetic characterization of rabies field isolates from Thailand. Microbiol. Immunol. 47, 653–659.

Swofford, D.L., 2002. PAUP*: Phylogenetic Analysis Using Parsimony (and Other Methods), version 4. Sinauer Associates, Sunderland, MA.

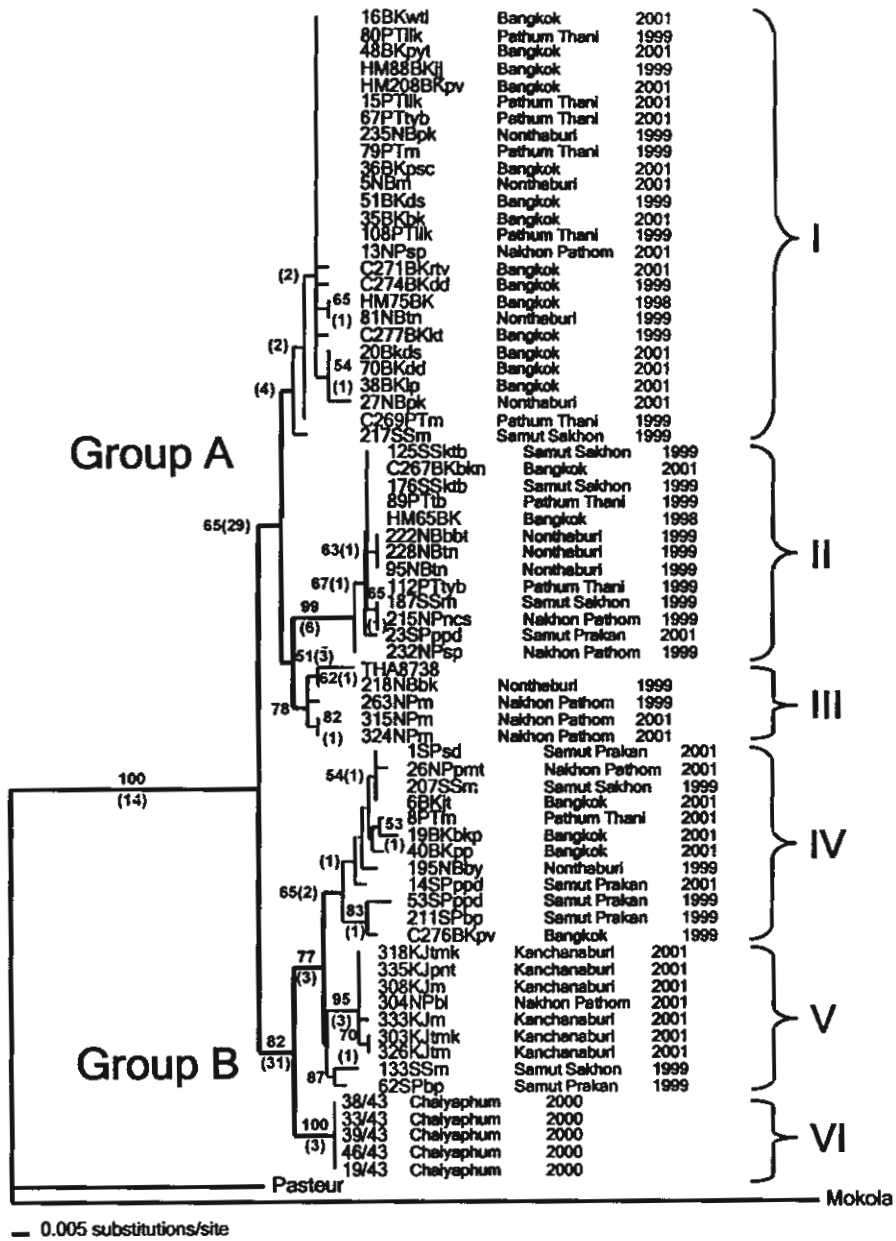
**Figure Legends**

**Figure 1.** Neighbour-joining tree for 301 bp of rabies virus nucleocapsid partial sequence data (bp 1101–1401) for 72 human and animal rabies virus isolates. Numbers along branches indicate >50% bootstrap supporting percentages of 1000 re-sampling replicates. Numbers within parentheses indicate character changes retrieved from maximum parsimony analysis. The collection area and year of each sample were also mapped on the neighbour-joining tree.

**Figure 2.** Distribution of rabies viruses of clades I–VI according to geographical location in Bangkok, Thailand and its surrounding provinces: (A) Pathum Thani, (B) Bangkok, (C) Samut Prakan, (D) Samut Sakhon, (E) Nakhon Pathom, (F) Nonthaburi, (G) Kanchanaburi and (H) Chaiyaphum.

**Figure 3.** Distribution of rabies virus in Bangkok, Thailand. The distribution of two samples could not be precisely localized.

**Figure**


Group A
Group B
I
II
III
IV
V
VI
16BKwtl Bangkok 2001
80PTlik Pathum Thani 1999
48BKpyt Bangkok 2001
HM88BKjl Bangkok 1999
HM208BKpv Bangkok 2001
15PTlik Pathum Thani 2001
67PTtyb Pathum Thani 2001
235NBpk Nonthaburi 1999
79PTm Pathum Thani 1999
36BKpsc Bangkok 2001
5NBm Nonthaburi 2001
51BKds Bangkok 1999
35BKbk Bangkok 2001
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13NPsp Nakhon Pathom 2001
C271BKrtv Bangkok 2001
C274BKdd Bangkok 1999
HM75BK Bangkok 1998
81NBtn Nonthaburi 1999
C277BKkt Bangkok 1999
20Bkds Bangkok 2001
70BKdd Bangkok 2001
38BKlp Bangkok 2001
27NBpk Nonthaburi 2001
C269PTm Pathum Thani 1999
217SSm Samut Sakhon 1999
125SSktb Samut Sakhon 1999
C267BKbkn Bangkok 2001
176SSktb Samut Sakhon 1999
89PTtb Pathum Thani 1999
HM65BK Bangkok 1998
222NBbbt Nonthaburi 1999
228NBtn Nonthaburi 1999
95NBtn Nonthaburi 1999
112PTtyb Pathum Thani 1999
187SSm Samut Sakhon 1999
215NPncs Nakhon Pathom 1999
23SPppd Samut Prakan 2001
232NPsp Nakhon Pathom 1999
THA8738
218NBbk Nontheburi 1999
263NPm Nakhon Pathom 1999
315NPm Nakhon Pathom 2001
324NPm Nakhon Pathom 2001
1SPsd Samut Prakan 2001
26NPpmt Nakhon Pathom 2001
207SSm Samut Sakhon 1999
6BKjt Bangkok 2001
8PTm Pathum Thani 2001
19BKbkp Bangkok 2001
40BKpp Bangkok 2001
195NBby Nonthaburi 1999
14SPppd Samut Prakan 2001
53SPppd Samut Prakan 1999
211SPbp Samut Prakan 1999
C276BKpv Bangkok 1999
318KJtmk Kanchanaburi 2001
335KJpnt Kanchanaburi 2001
308KJm Kanchanaburi 2001
304NPbl Nakhon Pathom 2001
333KJm Kanchanaburi 2001
303KJtmk Kanchanaburi 2001
326KJtm Kanchanaburi 2001
133SSm Samut Sakhon 1999
62SPbp Samut Prakan 1999
38/43 Chaiyaphum 2000
33/43 Chaiyaphum 2000
39/43 Chaiyaphum 2000
46/43 Chaiyaphum 2000
19/43 Chaiyaphum 2000
Pasteur
Mokola
0.005 substitutions/site

**Figure**
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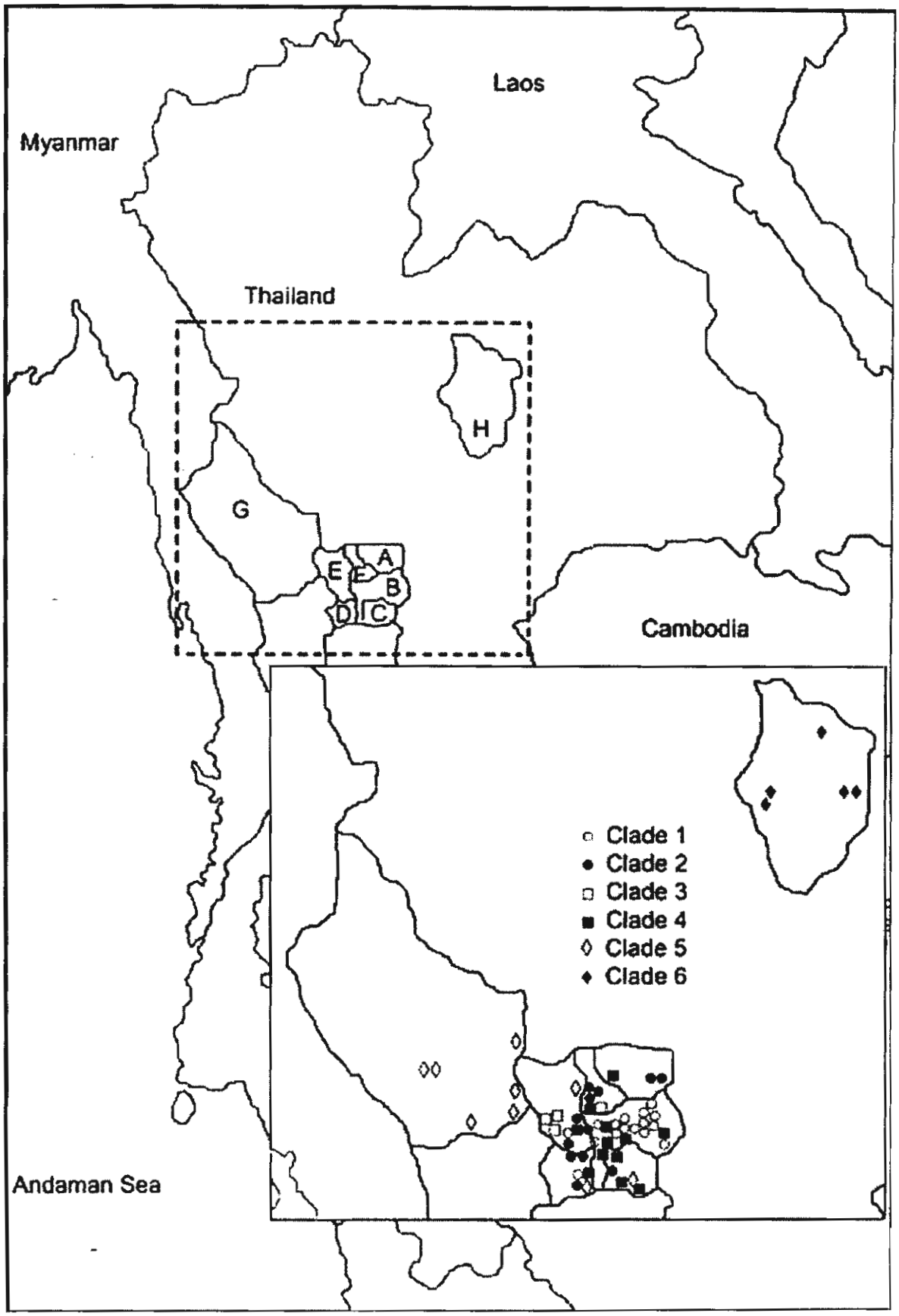

**Figure**
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