November 2004

Rabies deaths in Pakistan: results of ineffective post-exposure treatment

Shehzad Parviz
Aga Khan University

Rashid Chotani

Joseph McCormick
Aga Khan University

Sue Fisher-Hoch
Aga Khan University

Stephen Luby
Aga Khan University

International Journal of Infectious Diseases (2004) **8**, 346—352

http://intl.elsevierhealth.com/journals/ijid

Rabies deaths in Pakistan: results of ineffective post-exposure treatment

Shehzad Parviz[a,*], Rashid Chotani[b,c,d], Joseph McCormick[a], Sue Fisher-Hoch[e], Stephen Luby[a]

[a]*Department of Community Health Sciences, The Aga Khan University, Karachi, Pakistan*
[b]*Department of International Health, The Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland, USA*
[c]*Department of Emergency Medicine, School of Medicine, The Johns Hopkins University, Baltimore, Maryland, USA*
[d]*The Global Infectious Disease Surveillance and Alert System, The Johns Hopkins University, Baltimore, Maryland, USA*
[e]*Department of Pathology, The Aga Khan University, Karachi, Pakistan*

Received 24 July 2002; received in revised form 3 February 2004; accepted 18 February 2004
Corresponding Editor: Jane Zuckerman, London, UK

KEYWORDS
Rabies;
Sheep brain vaccine;
Vaccine efficacy;
Incidence;
Pakistan;
Developing country;
Cold chain

Summary

Objectives: To estimate the incidence of rabies and the effectiveness of post-exposure treatment (PET) in Pakistan.
Methods: Rabies cases admitted from July 1993 to December 1994 to a public rabies isolation hospital were analyzed. Two samples (one sample each from a separate peripheral site) of a single batch of sheep brain vaccine (SBV) were also tested for potency by the National Institute of Health (NIH) test in May 1997.
Results: Forty patients were admitted with a history of clinical rabies. The median age was 22 years and 55% were under 15. Thirteen (23%) victims did not receive any vaccine; the remaining 27 (67%) received SBV only, and of these, 16 (40%) received a full course of SBV. No rabies immunoglobulins (RIG) or cell culture vaccines were administered. There were frequent power blackouts and no back-up supply at the public hospital. In-house potency testing of the vaccine batch by the manufacturer was adequate, athough it was not tested by the World Health Organization (WHO) recommended NIH test. Samples of SBV of the same batch collected at the peripheral sites showed no potency. Rabies incidence was estimated to range between 7.0 to 9.8 cases per million annually.

* Corresponding author. Present address: 75 Ave B, Riverside Addition, Richwood Area Community Hospital, Richwood, WV 26261, USA. Tel.: +1 304 846 2573; fax: +1 304 846 9562.
E-mail address: sparviz19@hotmail.com (S. Parviz).

1201-9712/$30.00
doi:10.1016/j.ijid.2004.02.008

Conclusion: A multi-sectorial approach is needed to decrease rabies incidence in Pakistan. Public and healthcare practitioner education on prompt and appropriate PET, especially the use of cost-effective cell culture intradermal regimens, is needed urgently. The NIH test should be employed for vaccine potency testing. An independent agency is needed for monitoring vaccine quality and strategies are needed for maintaining cold chain. SBV should be replaced by locally manufactured second-generation cell culture rabies vaccine. Purified equine rabies immunoglobulin (ERIG) should be manufactured locally to meet national needs. Furthermore, effective dog control strategies should be implemented to decrease the rabies reservoir.

Introduction

Rabies continues to be a public health hazard in at least 87 countries.[1] Despite its preventability, rabies accounts for an estimated 50,000 human deaths each year, mostly in developing countries.[2] Bogel and Motschwiller (1986),[1] using 1979 data, estimated that India (28.8 per million), Ethiopia (12.6 per million), Sri Lanka (10.2 per million), Argentina (8.2 per million) and Thailand (7.2 per million) had the highest incidence rates.

Although rabies mortality approaches 100% once clinical signs set in, prompt debridement of the wound, infiltration of the wound site with rabies immune globulin (RIG) and administration of a potent vaccine (World Health Organization (WHO) expert committee 1992) will serve as a successful antidote against a severe exposure.[3] Vaccinating dogs against rabies, applying leash and dog ownership laws and removing stray dogs, can also substantially reduce the risk to humans.[4] The success of rabies control in Europe and North America has been due to the control of stray dogs, vaccinating susceptible wildlife, and wide use of post-exposure treatment (PET).[4] Lack of resources and technical expertise and the inability to control an increasing stray dog population has resulted in less effective control of rabies in Pakistan. In addition, many low-income countries rely on the inexpensive, sometimes locally produced, less efficacious nerve tissue vaccines, such as sheep brain vaccine (SBV) rather than the more effective tissue culture vaccine.[3] Regrettably, in these circumstances RIG is only rarely administered.[3]

Sheep brain vaccine (SBV), in addition to having poorer efficacy compared to cell culture rabies vaccines, is associated with neuroparalytic reactions reported at a rate of at least one in 200 vaccinees.[5] These range from peripheral neuropathy, myelitis, meningoradiculitis, paraplegia and encephalitis to Guillian Barré syndrome and are due to the immunological reaction to myelin basic protein in SBV.[5–8] There is also a high dropout rate (33%) since giving SBV entails multiple (at least 14) painful injections in the abdominal wall.[9]

The National Institute of Health (NIH) in Pakistan produces all of the anti-rabies SBV in Pakistan. This is purchased by the provincial governments and provided free of charge to the general public. The recommended regimen is to give 2 ml of the 5% SBV suspension subcutaneously in adults and 1.5 ml in children under 12 years of age consecutively for 14 days and then a booster on days 24, 34 and 104. Pakistan's NIH also produces unpurified equine RIG in small quantities. Various imported rabies biologicals available[10] at the time of the study included purified Vero cell vaccine (PVRV; Verorab™, Pasteur Merieux Connaught); purified duck embryo vaccine (PDEV; Lyssavac-N™, Berna Swiss and Serum Institute, Switzerland); purified equine rabies immunoglobulin (ERIG; Swiss Serum and Vaccine, Institute Berne, Switzerland and bioMerieux, Marcy L'Etiole, France) and human rabies immunoglobulin (HRIG; Bayer, West Haven, CT, USA). These can be purchased commercially by those who can afford them. Note that both the PDEV and the ERIG above are no longer produced.

Although Pakistan's neighbour, India, accounts for 60% of all reported rabies mortality worldwide, there are no incidence estimates from Pakistan.[2] Thus, a study was conducted to estimate the incidence of rabies in Karachi and evaluate what proportion of rabies victims had been vaccinated with full or partial courses of SBV.

Methods

Karachi is the largest city in Pakistan with a population of 9.3 million (census of 1998).[11] Civil Hospital (CHK), a government referral hospital, has the only rabies isolation ward in Karachi. The medical records of all the patients admitted with clinical rabies from July 1993 to December 1994 were reviewed. Rabies was clinically diagnosed in

patients with agitation, hydrophobia and a history of dog bite. Laboratory confirmation was not available. Data were extracted on the demographic features of the patients, clinical signs and symptoms, history of dog bite and post-exposure treatment. PET was compared with WHO standards, which consist of the immediate scrubbing of the wound with water and soap, irrigating it with a viricidal agent (20% soap solution, povidine-iodine, 0.1% aqueous iodine or 40—70% alcohol), potent cell culture vaccine [given in five intramuscular (IM) shots (Essen regimen), four IM shots (Zagreb regimen 211), or reduced dose intradermal (ID) regimens (TRC 222011 and Warrell 804011)] and RIG for transdermal bites and mucosal exposures, and vaccine only for minor scratches and abrasions.[3] TRC 222011 consists of id injections at two different sites on day 0, 3, 7 and one site on day 28 and 90 of exposure while the Warrell 804011 consists of id injections at 8 different sites on day 0, four sites on day 7 and one site on day 28 and 90 of exposure. Current vaccines approved for TRC 222011 regimen by WHO are HDCV (Rabivac™) and purified chick embryo cell vaccine (PCEV) (Rabipur™). Current vaccines approved for the Warell 804011 regimen by WHO are PVRV (Verorab™, Imovax™, Rabies vero™, TRC Verorab™) and PCEV (Rabipur™).

The nursing personnel and staff physicians at CHK were interviewed to obtain the proportion of victims clinically diagnosed with rabies but who did not request admission after knowing about the grave prognosis. The annual number of rabies cases was estimated by dividing the number of observed cases with the proportion of the victims seeking admission. Karachi's population was calculated for 1994 by using the census figures and compound annual growth rate (3.49%) between 1981 (pop. 5,208,132) and 1998 (pop. 9,339,023).[11] The projected population was calculated by using the formula[12]: projected population = initial population X (1 + growth rate)$^{\text{number of years}}$.

Information from Pakistan's NIH was obtained regarding its vaccine production. After noticing a very high proportion of rabies victims failing with SBV, we collected two vials of SBV of the same batch (#420) in May 1997 from the two largest public hospitals in Karachi: Jinnah Post-graduate Medical Center and CHK. These vials were obtained from a storage refrigerator kept at 4 °C in the respective hospitals; the batch was being used for PET. The samples were placed in a cooling container packed with insulating material and ice, and transported by air to Aventis-Pasteur, Lyon, France (an international manufacturer of rabies vaccine) for potency testing by the National Institute of Health (NIH) test.[13]

Results

In the 18 months between July 1993 and December 1994, 40 patients clinically diagnosed with rabies were admitted to the isolation ward of CHK. Thirty-four (85%) were males. Their median age was 22 years; 22 (55%) were under 15 years of age. All 40 patients had symptoms of agitation and hydrophobia. In addition, 28 patients (70%) had at least two other neurological symptoms, most commonly anxiety and dysphagia. All had a history of dog bite in the year prior to the onset of illness, 20 (50%) within the preceding 30 days. Fifteen (37.5%) were from Karachi's East district, 11 (27.5%) were from West district, three (7.5%) were from Central district, and one (2.5%) from South district. In five cases (12.5%) residence could not be determined and five (12.5%) were outside Karachi limits.

Of the 14 individuals who had documentation in their medical records of where on the body they had been bitten, seven (50%) had been bitten on the face, three (21%) on the leg, three (21%) on the hands, and one on both head and arm. Of the seven individuals who had been bitten on the face, five (71%) presented within one month, which is consistent with the shorter incubation period from bites in close proximity to the central nervous system.[14] In these records there was no documentation of wound care after the bite.

Twenty-seven rabies victims (67%) had received PETwith a rabies vaccine, which was SBV in all cases. None had received the rabies cell culture vaccine. Sixteen (40%) completed a full course of SBV, consisting of 17 injections. Among the 11 victims who received incomplete vaccination, one person received 12 doses, while the rest received five doses or less. None of the 27 received RIG after the dog bite. Sixteen (40%) of the victims died of rabies while in the isolation unit; the other 24 were taken home by their families after learning the poor prognosis.

Among the 40 rabies cases, 30 were confirmed as having lived within Karachi city limits. Based on interviews at the CHK's emergency room and discussion with medical staff at the other large government hospital in the city that dispenses rabies vaccine, it is estimated that 25—35% of all children presenting with a syndrome and history consistent with classical rabies were admitted to the CHK's rabies isolation ward. Thus these 30 deaths would represent 86 to 120 deaths in Karachi during the 18 months of this study (30/0.25—0.35) and would equate to 57 to 80 deaths in a year. Using the formula for the projected population (see Methods section), the estimated population of Karachi in 1994 was 8.1 million inhabitants. The estimated

incidence of human rabies deaths in Karachi ranged from 7.0 to 9.8 cases per million per year.

After contacting NIH, it was found out that SBV is a killed vaccine prepared from sheep brain tissue infected with fixed rabies virus and inactivated with phenol at 37 °C. The final vaccine is 5% brain tissue suspension with 0.5% phenol and hence does not require reconstitution. After it is manufactured, it is kept in cold rooms at 4 °C and is transported by air on ice packs maintaining temperature for 24 hours. It was noticed that there were frequent power cuts at CHK, without backup for maintaining the cold chain. NIH has a National Quality Control Laboratory which tests each batch for sterility, safety, potency, phenol content and toxicity before it is released. The two vials of batch #420 of SBV that had been collected during May 1997 from peripheral sites did not show any protection when tested with the NIH test for potency. At the NIH, 45 of 1580 vials of the batch were tested for potency by the Habel test[13] before release and the vaccine titer was 3.181 IU/ml (the minimum potency requirement set by NIH is 3.1 IU/ml).

Discussion

Our estimated incidence of human rabies in Karachi, Pakistan, is consistent with the high incidence of rabies in South-East Asia. The sheep brain vaccine (SBV), which is the cornerstone of rabies prevention in Karachi, is ineffective. There is a lack of controlled trials looking at the efficacy of SBV. Indian-produced SBV of the 1950s had an estimated efficacy of 84% in completely treated patients, which dropped to 58% in incompletely treated patients (the data were based on a retrospective analysis of infected dog bites).[15]

Although SBV has some efficacy against rabies exposures and has saved many lives, it becomes useless in severe exposures when protection is most needed.[16] The figure found here, of 40% of rabies victims acquiring rabies, despite complete vaccine regimen, is higher than Burney et al.'s previous estimate in Karachi in the mid 1960s of 23% and from previous studies in Thailand and India of 11% and 6.5% respectively.[15,17,18] In India, an increasing SBV dosage is given depending upon the severity of exposure (four times the adult dose of phenol-inactivated Indian SBV is recommended for severe exposures compared to minor exposures and Pakistan's SBV recommended adult dose).[19] Burney et al reported in the 1960s that after a full course of Pakistan's SBV, only 50% of the vaccinated patients achieved neutralizing antibodies.[17] Similarly in Thailand, in a randomized trial by Warrell et al of Thailand's SBV vs. human diploid cell vaccine (HDCV) with RIG, given when indicated, protective antibody titers were present in 42% vs. 100% at day 14, and 6% vs. 100% at one year.[20] Furthermore, the immune response after SBV is not long lasting.[21] WHO recommends all countries producing rabies nerve tissue vaccine to shift to the cell culture vaccine.[3] Yet SBV is still the most common rabies vaccine used in developing countries, where better vaccines are most needed (in India 700,000 people received SBV in 1997).[22]

The other deficiencies in PET in our study limiting rabies prevention in Karachi were (1) no post-exposure vaccination as nearly a quarter did not receive any vaccine (2) low compliance with the SBV vaccination schedule with more than half receiving an incomplete course (3) no use of RIG and (4) inability to maintain the cold chain, which was also seen in the dog bite study.[10] Factors that could not be assessed from the study but that might have played a role include (5) inadequate and inappropriate wound care as seen in the previous dog bite study[10] in which wounds of 69% of 143 dog bite victims were not cleansed with soap, iodine or alcohol, thus failing WHO standards[3] and (6) possible delays in initiating PET.[23] The last factor was corroborated with a randomized community survey of households in a squatter settlement in Karachi during the same period as the study, in which 23% of respondents with a dog bite in the household did not go to a hospital for care. Among those who went to hospital, 31% did not complete the full course of vaccination (Noorudeen Punjwani, The Aga Khan University; personal communication).

The WHO recommends, for rabies-endemic developing countries, the use of second-generation tissue culture vaccines like purified chick embryo (PCEV) and purified vero cell (PVRV) in cost-effective ID regimens.[3] Dog bite victims can be vaccinated intradermally (ID) with these vaccines for as little as US$ 30 at commercial prices in cost-effective regimens of ID TRC 222011 (PVRV and PCEV) and ID Warrell 804011 (PCEV).[20,24]

In an unpublished study in Karachi (Khalid et al. The Aga Khan University, 1997) the direct (at market price) and indirect costs (loss of wages and transportation costs, excluding failure rates with vaccine and life lost) of ID vaccine regimens with PVRV was cheaper than SBV. Thailand in the 1980's, by adopting an ID cell culture vaccine regimen with RIG strategy, decreased rabies incidence from 7.6 per million in 1979 to 0.9 per million in 1997.[1] Two other developing countries Philippines and Sri-Lanka have stopped nerve tissue vaccine production and in 1997 adopted ID regimens using imported cell culture vaccine. Similarly to Thailand, China has

considerably reduced rabies incidence with the availability of improved PET, namely by using ERIG and primary hamster kidney cell vaccine (PHK) that can be given in five shots intramuscularly.[25,26] Pakistan's neighbour, India is also gradually phasing out its SBV production to cell culture vaccine. Three out of 25 states have moved to using solely cell culture vaccine, consisting of PVRV and purified chick embryo culture (PCEC) manufactured by a government-funded company and a private company respectively.[27] Although ID regimens have not been officially approved, studies have shown that ID regimens are effective in the Indian population.[28,29]

The NIH in Pakistan was the first among developing countries to locally produce expensive freeze-dried HDCV in 1988 but stopped production after five years for political and technical reasons, though it still has equipment for cell culture vaccine production.[30,31] Restarting production of one of the second generation tissue culture vaccines like PVRV, which is much cheaper than HDCV, would be within NIH's capacity.[32] Local production will make the vaccine cheaper and even more economical when given in the ID regimens.

The fact that two randomly selected vials of SBV from the NIH in Pakistan had no potency suggests problems with either vaccine quality control/production or cold chain at the peripheral sites. There has been a report of a vaccine with no antigenic value from Nigeria but that was a rabies vaccine of unknown origin, purchased at an open market during a vaccine shortage period.[33] WHO recommends the NIH test instead of the Habel test (which was used by NIH Pakistan) for potency testing and calibration of rabies vaccines.[3] India and Bangladesh also use the NIH test for SBV potency testing (personal communication: S.N. Madhusudana and Z. Ahmed respectively). Pakistan does not have an independent vaccine monitoring body. This raises the question as to how other developing countries maintain quality control of their own biological products. An external monitoring agency, such as the Food and Drug Administration (FDA) in the USA, is essential for vaccines, drugs and other biologicals.[34] An international agency for monitoring vaccines for developing countries is another option. This would require international prioritization, funding and cooperation from the countries involved. Maintenance of the cold chain is a major hurdle in developing countries, especially at peripheral sites with heat-intolerant SBV.[35] Freeze dried HDCV tested in Pakistani medical staff was stable for 11 weeks at 26—37 °C.[36] PCEV (Chiron Institute) is stable for three months at 37 °C.[37] Similarly, PHK, PDEV, PVRV are likely to be more thermostable than SBV.[35] This is why replacing SBV with a freeze dried cell culture vaccine would be a solution to the problem. Nevertheless, improved strategies to monitor and maintain cold chain are needed. These are major critical public health challenges.

Although it has been known for over half a century that RIG is critical in severe rabies wounds, none of the patients received rabies immune globulin (RIG) because both commercially available RIGs were expensive.[38] HRIG was eight times more expensive than ERIG (US$ 293 vs. 35 for an equivalent dose for a 60 kg adult). Currently, there is a shortage of ERIG worldwide as international manufacturers have stopped production because of complex regulations, increasing production costs and pressure from animal rights activists.[39] In Thailand, purified ERIG as well as HRIG (via plasmapharesis from an adequate number of human donors) is produced locally.[40] Production of purified ERIG has a substantial low cost yield that is effective for human PET with very low risk of anaphylaxis and hence should be carefully considered by NIH.[41] In India, locally produced ERIG costs US$ 15 for a 60 kg adult. In the absence of RIG the ID Warrell regimen is preferred as it produces an earlier neutralizing antibody response on day seven compared to IM Essen and ID TRC-222011 regimens.[20,24]

Furthermore, failure of the simple but crucial measure of proper wound care to reduce the viral inoculum is not routinely observed in Karachi, as noted previously.[3,10] In a report detailing PET failures, the PET apparently failed, despite patients receiving a proper tissue culture vaccine and RIG, because none of their wounds were locally cleansed.[26] Public and health practitioner education is essential regarding the dangers of dog bites and the importance of prompt irrigation and cleansing of wounds and applying antiviral agents like povidine or alcohol as recommended by WHO.[3]

There are important limitations to this study. The estimate of rabies incidence is imprecise; victims might seek care at other hospitals and rabies might be under-diagnosed because of the unknown number of elusive dumb or paralytic clinical presentations, which may account for up to 30% of rabies cases.[42] Some patients may have been misdiagnosed, because there was no access to laboratory confirmation. There was also no follow-up record of the 24 rabies victims who left the hospital after learning the poor prognosis.

Rabies is, however, a clinically distinctive disease, usually easily and accurately recognized by physicians familiar with the condition and the prognosis is virtually hopeless with only one fully documented human survivor who remained severely impaired neurologically.[2,42] Overall, the estimates reported here are consistent with reported

incidences from neighboring countries in South Asia. It could not be ascertained that the lack of potency of the SBV vials tested was due to the failure of maintaining cold chain or improper vaccine manufacturing/quality control, although the vaccine was collected from two different hospitals. The exact time from exposure to vaccination from patients' records could not be determined due to lack of documentation. The result of 40% of rabies victims developing rabies despite a full course of SBV should not be equated with vaccine efficacy or failure rate as such an analysis requires knowledge of the incidence of rabies in both vaccinated and unvaccinated groups.

Karachi has a high incidence of rabies deaths. Ideally there needs to be a paradigm shift in canine rabies-endemic countries towards an effective dog control program which will decrease the virus reservoir, the demand for expensive PET, the number of rabies deaths, and would be economical in the long run.[43,44] Unfortunately, canine control programs have only been effective in a few countries in Asia that have closed borders i.e. peninsular Malaysia and Singapore.[2,45] Canine control programs also require multi-sectorial coordination, years of implementation to accrue economic benefits and are costly at inception.[43,44] Still, an ineffective and obsolete vaccine wastes scarce resources, undercuts professional motivation, erodes public confidence and costs lives, especially when cost-effective alternatives are available.

Indeed many things need to be done but because of other priority infectious diseases, low health expenditure, particularly on public health and external debt, resources need to be tapped and strategies prioritized. To save human lives the most cost-effective and foremost step would be educating the general public and healthcare physicians regarding prompt, appropriate wound care and rabies PET. We should educate and train health care physicians, especially those at government-approved rabies PET centers, about the option of cheaper cell culture intradermal regimens. This would increase awareness and use of these regimens as well as exerting pressure on national authorities to eventually change to cell culture vaccine production. Vaccine potency testing by the manufacturer has to be undertaken with the WHO-approved NIH test. An independent agency for monitoring locally produced and imported vaccines would be essential for ensuring safe and effective vaccines. Strategies are needed for monitoring compliance of cold chain. SBV needs to be phased out and replaced by second generation rabies cell culture vaccine production and the existing RIG facility should be upgraded to produce sufficient amounts of purified ERIG. Availability of better PET will still not obviate the need for effective and coordinated strategies to control canine rabies. These need to be planned and implemented to eliminate rabies in the long term.

Acknowledgments

We appreciate the technical support and assistance of Tariq Ayubi, Senior Casualty Officer and Abbas Khan, Medical Superintendent, CHK, Khaulda Nauman, Senior Medical Officer, CHK, Rashid Juma at JPMC, Shahid Akhtar and Abdul Samad Khan at the NIH, Islamabad. We also thank Adnan Hashmi, Syeda Batool, Aliya Rehman, Aon Ali Mohib, Amina Patel, Rose Paul and Lowell Stoltzfus who assisted in the initial design of the study and in data collection. We thank Paul Arguin and Melanie Fischer for reviewing a draft of this manuscript. We would like to thank the NIH, Pakistan for their cooperation in sharing information. We would like to thank Jean Francois Saluzzo at Aventis-Pasteur, Lyon, France for performing the analysis of the vaccine samples.

Conflict of interest: The Department of Community Health Science, The Aga Khan University, Karachi funded the project.

References

1. Bogel K, Motschwiller E. Incidence of rabies and post-exposure treatment in developing countries. *Bull World Health Organ* 1986;**64**:883—7.
2. Wilde H. Rabies. *Int J Infect Dis* 1996;**1**:135—42.
3. WHO expert committee on rabies. World Health Organ Tech Rep Ser 1992; Report No. 824.
4. Fishbein DB, Robinson LE. Rabies. *N Engl J Med* 1993;**25**: 1632—8.
5. Arya SC. Acquisition of spongiform encephalopathies in India through sheep-brain rabies vaccination. *Indian J Pediatr* 1991;**58**:563—5.
6. Ahasan HA, Chowdhury MA, Azhar MA, Rafiqueuddin AK. Neuroparalytic complications after anti—rabies vaccine (inactivated nervous tissue vaccine). *Trop Doct* 1995;**25**:94.
7. Swaddiwuthipong W, Weniger BG, Wattanasri S, Warrell MJ. A high rate of neurological complications following Semple anti-rabies vaccine. *Trans R Soc Trop Med Hyg* 1988;**82**: 472—5.
8. 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.
9. Hemachudha T. Rabies. In: McKendall RR., editor. *Handbook of Clinical Neurology*. Amsterdam: Elsevier; 1989. p. 383—404.
10. Parviz S, Luby S, Wilde H. Postexposure treatment of rabies in Pakistan. *Clin Infect Dis* 1998;**27**:751—6.
11. Government of Pakistan. Statistic Division, http://www.statpak.gov.pk/.

12. Pollard AH, Yusuf F, Pollard GN. *Demographic Techniques*. 3rd edition Sydney: Pergamon Press; 1990 p. 118.
13. Meslin FX, Kaplan MM, Koprowski H, editors. *Laboratory techniques in rabies*. Geneva: World Health Organization; 1996.
14. Hattwick MAW. Human rabies. *Public Health Rev* 1974;**3**: 229—74.
15. Veeraaraghavan N, Subarahmanyan TP. The value of 5% semple vaccine prepared in distilled water in human treatment: comparative mortality among the treated and untreated. *Indian J Med Res* 1958;**46**:518—24.
16. Baltazard M, Ghodsii M. Prevention of human rabies; treatment of persons bitten by rabid wolves in Iran. *Bull World Health Organ* 1954;**10**:797—802.
17. Burney MI, Khan ZH, Lari FZ. The rabies problem in Pakistan. *Trop Doct* 1976;**6**:60—2.
18. Vibulbandhitkii S. Work Report. Data from rabies patients at Bamranradura Hospital between 1971 and 1977. In: Thongcharoen P, editor. *Rabies*. Bangkok: Aksarasamai; 1980. p. 235—51.
19. Tripathi KK, Madhusudana SN, Sahu A. Reduction in the dosage of BPL-inactivated neural tissue vaccine for rabies prophylaxis in man. *Indian J Med Res* 1990;**91**:334—9.
20. Warrell MJ, Nicholson KG, Warrell DA, Suntharasamai P, Chanthavanich P, Viravan C, et al. Economical multiple-site intradermal immunisation with human diploid-cell-strain vaccine is effective for post-exposure rabies prophylaxis. *Lancet* 1985;**1**:1059—62.
21. Khawplod P, Wilde H, Yenmuang W, Benjavongkulchai M, Chomchey P. Immune response to tissue culture vaccine in subjects who had previous postexposure treatment with Semple or suckling mouse brain vaccine. *Vaccine* 1996;**14**: 1549—52.
22. John TJ. An ethical dilemma in rabies immunisation. *Vaccine* 1997;**15**:S12—5.
23. Haupt W. Rabies — risk of exposure and current trends in prevention of human cases. *Vaccine* 1999;**17**:1742—9.
24. Phanuphak P, Khawplod P, Sirivichayakul S, Siriprasomsub W, Ubol S, Thaweepathomwat M. Humoral and cell-mediated responses to various economical regimens of purified Vero cell vaccine. *Asian Pac J Allergy Immunol* 1987;**5**:33—7.
25. Lin FT. The protective effect of the large-scale use of PHKC rabies vaccine in humans in China. *Bull World Health Organ* 1990;**68**:449—54.
26. Fangtao L, Shubeng C, Yinzhon W, Chenzhe S, Fanzhen Z, Guanfu W. Use of serum and vaccine in combination for prophylaxis following exposure to rabies. *Rev Infect Dis* 1988;**10**:S766—70.
27. Strategies for the control and elimination of rabies in Asia. Report of a WHO Interregional Consultation. Geneva: Switzerland; 17—21 July 2001. Report No. WHO/CDS/CSR/EPH/2002.8.
28. Madhusudana SN, Anand NP, Shamsundar R. Evaluation of two intradermal vaccination regimens using purified chick embryo cell vaccine for post-exposure prophylaxis of rabies. *Natl Med J India* 2001;**14**:145—7.
29. Madhusudana SN, Anand NP, Shamsundar R. Economical multi-site intradermal regimen with purified chick embryo cell vaccine (Rabipur) prevents rabies in people bitten by confirmed rabid animals. *Int J Infect Dis* 2002;**6**:210—4.
30. Burney MI. Transferring Manufacturing Technology. Producing essential vaccines in Pakistan. *Int J Technol Assess Health Care* 1993;**9**:397—406.
31. Waheed-uz-Zaman T, Malik IA. *Rabies. Myths and Facts. Armed Forces Institute of Pathology*. Rawalpindi, Pakistan: Al Tirmizi Publications; 1995.
32. Halstead SB. Tissue culture-based rabies vaccines: vaccine production technology transfer. *Rev Infect Dis* 1988;**10**: S764—5.
33. Ogunkoya AB, Macconi F. Emergence of antirabies vaccine of unknown origin for human treatment in Nigeria. *Vaccine* 1986;**4**:77—8.
34. Chen RT, Hibbs B. Vaccine safety: Current and future challenges. *Pediatric Annals* 1998;**27**:445—55.
35. Galazka A, Milstein J, Zaffran M. *Thermostability of vaccines*. Geneva: Switzerland: WHO; 1998 Report No. WHO/GPV/98.07.
36. Nicholson KG, Burney MI, Ali S, Perkins FT. Stability of human diploid-cell-strain rabies vaccine at high ambient temperatures. *Lancet* 1983;**1**:916—8.
37. Barth R, Bijok U, Grushkau H, Smerdel J, Vodopija J. Purified chicken embryo cell rabies vaccine for human use. *Lancet* 1983;**1**:700.
38. Bahmanyar M, Fayaz A, Nour-Salehi S, Mohammadi M, Koprowski H. Successful protection of humans exposed to rabies infection. Postexposure treatment with the new human diploid cell rabies vaccine and antirabies serum. *JAMA* 1976;**236**:2751—4.
39. 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.
40. Chantanakajornfung A, Naraporn N, Khumphai W, Bejavongkulchai M, Mitmoonpitak C, Wilde H. A study of human rabies immune globulin manufactured by the Thai Red Cross. *Vaccine* 1999;**17**:979—81.
41. Wilde H, Chutivongse S. Equine rabies immune globulin: A product with an undeserved poor reputation. *Am J Trop Med Hyg* 1990;**42**:175—8.
42. Warrell MJ, Warrell DA. Rabies and Related Viruses. In: Strickland GT, editor. *Hunters Tropical Medicine and Emerging infectious Disease*. 8th edition Philadelphia: W.B. Saunders; 2000. p. 257—63.
43. Fishbein DB, Miranda NJ, Merrill P, Camba RA, Meltzer M, Carlos ET, et al. Rabies control in the Republic of the Philippines: benefits and costs of elimination. *Vaccine* 1991; **9**:581—7.
44. Meslin FX, Fishbein DB, Matter HC. Rationale and prospects for rabies elimination in developing countries. *Curr Top Microbiol Immunol* 1994;**187**:1—26.
45. Tan YS. Rabies in Malaysia. *Southeast Asian J Trop Med Public Health* 1988;**19**:535—6.