

# Immunogenicity of Purified Vero Cell Rabies Vaccine Used in the Treatment of Fox-Bite Victims in India

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(See the editorial commentary by Wilde on pages 614–5)

**Purified Vero cell rabies vaccine was used to treat 19 patients who experienced fox bite. Seventeen patients survived, and 2 patients died of rabies. Maximum antibody titers determined using a rapid fluorescent focus inhibition test were 25 IU,  $\leq$ 90 days after the first vaccination and were 30 IU on day 1050 after booster vaccination.**

Rabies, a fatal viral disease, is endemic in India [1, 2]. Rabies in humans is associated with the exposure of individuals to rabid domestic or wild animals. Rabies in developed countries is associated with bat bites and rarely with bites from domestic animals. In contrast, human rabies due to dog and wild animal bites is quite common in developing and less-developed countries. Unavailability of modern tissue-culture vaccine and rabies immunoglobulin in primary health centers in villages complicates the treatment of patients exposed to rabid animals. Approximately 50% of persons bitten by animals receive postexposure prophylaxis (PEP). As many as 40% of the patients receive Semple vaccine, and the other 60% receive tissue culture vaccine [3]. In India, villagers often report for treatment at the district medical facilities when an animal suspected of being rabid bites several persons. Many government hospitals use the Semple vaccine in spite of its disadvantages because the cell culture–derived vaccine is expensive. The use of Semple vaccine for treatment of patients bitten by a rabid wolf has been reported previously [4].

Purified Vero cell rabies vaccine (PVRV; marketed as Abhayrab, Human Biologicals Institute) has been used in a clinical trial to assess its safety and immunogenicity in accordance with

the international guidelines on good clinical practices [2]. This modern vaccine is made available at an affordable cost to doctors in district-level and taluk (i.e., county)–level hospitals, enabling patients to obtain PEP without traveling long distances immediately after exposure to rabid animals. This article reports the use of PVRV in 19 patients bitten by a fox in Karjat taluk, Ahmednagar district, Maharashtra, India.

**Materials and methods.** Nineteen patients aged 6–70 years, of both sexes, from 6 hamlets in Ahmednagar district presented at the outpatient clinic of the Civil Hospital, Ahmednagar, with a history of fox bite. The patients had bite wounds on different parts of the body, such as the head, hand, neck, chest, face, nose, inner lips, and buttocks. All the patients had category 3 bites. The patients were bitten during a period of 7 days, from 27 March to 4 April 2001.

Fifteen bite victims were administered the first dose of vaccine on the day of the bite; 1 patient received the first dose 1 day after the bite; 1 patient received the first dose 3 days after the bite; and 2 patients received their first doses 4 days after the bite. PVRV (Abhayrab batch no. 57/01; potency, 7.26 IU/0.5 mL) was administered to all patients intramuscularly on days 0, 3, 7, 14, and 28 after they received the first dose, in accordance with the World Health Organization's (WHO) recommendations for rabies PEP. Six patients were treated with equine rabies antiserum (Central Research Institute), which was administered as infiltrations around the wounds. Eleven patients were administered a booster dose of PVRV (Abhayrab vaccine batch no. 10/03; potency, 7.56 IU/0.5 mL) on day 1020 after the first dose.

Blood was collected for neutralizing antibody titer estimation on days 30, 90, 870, 1020, and 1050 after the first dose of vaccine. Coded samples were sent to Indian Immunologicals, Hyderabad, for determination of antibody titers by rapid fluorescent focus inhibition test [5]. Results were decoded in the presence of Dr. V. Suhasini Reddy (former Director, Institute of Preventive Medicine, Hyderabad) by Dr. A. M. Ghanekar (former Quarantine Officer, International Crop Research Institute for Semi-Arid Tropics [ICRISAT], Hyderabad).

**Results and discussion.** Rabies acquired from foxes is often of short duration and fatal. According to the patients who reported for PEP, the bites happened over a period of 7 days. This may mean that  $>1$  fox was involved. Further, there is no laboratory confirmation of the rabies in the fox(es), as this episode occurred in distant villages away from the urban centers.

Fifteen patients received treatment on the day of the bite.

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Other patients reported for PEP on different numbers of days after the bite. One female child, aged 7 years, had severe laceration wounds (category 3) on her nose that were inflicted by the animal; she received PEP 4 days after exposure. The child died 11 days after exposure after receiving 3 doses of vaccine. Another patient, aged 70 years, died 18 days after the bite after receiving 4 vaccinations. Both of these patients reported for PEP 4 days after the bite, and equine rabies immune globulin was not administered. These patients reported general malaise, headache, and body-ache, and the 70-year-old patient exhibited aerophobia. The clinical signs were indicative of rabies because of the case-history. In both cases, no laboratory investigations were performed to confirm rabies. The site of bite (hand and/or nose), delay in initiation of PEP, and lack of immunoglobulin treatment might have resulted in the development of symptoms of rabies and eventual death in these 2 patients. Failures of postbite therapy despite adherence to WHO treatment guidelines have been reported [6–9]. An 8-site intradermal vaccination schedule without administration of rabies immune globulin was unsuccessful in PEP [10].

The PVRV has induced good seroconversion, as indicated by the antibody titer values in table 1. The geometric mean antibody titer values were  $>0.50$  IU on days 30 and 90 after the first dose of vaccine in all the patients. Geometric mean antibody titer values were not determined on day 14. None of the patients received a booster dose on day 90, because this practice has been abandoned.

A follow-up study was conducted to evaluate the immunogenicity of the PVRV. The villagers and/or their children are agricultural laborers, or they migrate to other areas in search of jobs. Hence, it was not possible to obtain serum samples from all the patients at visits. Blood samples were collected again on days 870, 1020, and 1050 after the first dose of vaccine, and the geometric mean antibody titer values were estimated using the rapid fluorescent focus inhibition test. Five of 11 patients and 4 of 10 patients had geometric mean antibody titer values of  $<0.50$  IU on days 870 and 1020, respectively (table 1). A booster dose given on day 1020 elicited a good anamnestic response, as indicated by the increased values in antibody titers for all 10 patients. The mean value for geometric mean antibody titer on day 30 after the booster dose (i.e., day 1050 after the first dose of vaccine) was 26.11 IU. This clearly indicated that the antibody persisted for a long time after the vaccination, and that a booster dose could increase the geometric mean antibody titer to a high level. None of the patients had a titer of  $<0.50$  IU on day 30 after the booster vaccination (day 1050 after the first dose of vaccine). Earlier observations [11] indicated the persistence of satisfactory titers in 56% of patients after day 1100 after the first dose of vaccine. Of the 6 patients administered equine rabies antiserum, only 3 patients

**Table 1. Geometric mean antibody titer (GMAT) values in serum samples from patients who received postexposure prophylaxis against rabies after a fox bite.**

| Days after first dose of vaccine | No. of patients | GMAT value, IU |            | No. of patients with GMAT $<0.50$ IU |
|----------------------------------|-----------------|----------------|------------|--------------------------------------|
|                                  |                 | Mean           | Range      |                                      |
| 30                               | 12              | 3.16           | 1.00–8.00  | 0                                    |
| 90                               | 16              | 25.00          | 8.00–64.00 | 0                                    |
| 870                              | 11              | 0.77           | 0.26–2.10  | 5                                    |
| 1020                             | 11              | 0.48           | 0.11–2.00  | 4                                    |
| 1050                             | 11              | 30.08          | 2.57–69.18 | 0                                    |

**NOTE.** The titers were determined by the rapid fluorescent focus inhibition test (RFFIT). The 11 patients who were included in the follow-up study on day 1020 after the first dose of vaccine was given a booster dose on the same day.

were included in the follow-up study until day 1050. Geometric mean antibody titers in these 2 patients were  $>0.50$  IU, and 1 patient had a geometric mean antibody titer of  $<0.50$  IU on day 1020. Booster vaccination on day 1020 increased the antibody level, as observed by the higher geometric antibody titer (table 1). PVRV was found to be effective in preventing death in human beings exposed to rabid animal bite and elicited a good anamnestic response to a booster vaccination on day 1020 after the first dose of vaccine in patients who had received either vaccine alone or vaccine and equine rabies antiserum.

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## References

1. Jackson AC, Warrell MJ, Rupprecht CE, et al. Management of rabies in humans. *Clin Infect Dis* **2003**; 36:60–3.
2. Sampath G, Reddy SV, Rao ML, Rao YU, Palaniappan C. An immunogenicity study of a newly introduced purified Vero cell rabies vaccine (Abhayrab) manufactured in India. *Vaccine* **2005**; 23: 897–900.
3. Bharadwaj M, Bhattacharya D, Chhabra M, Ichhupujani RL. Immunogenicity of purified chick embryo cell anti-rabies vaccine in post-exposure treatment of animal bite cases. *J Commun Dis* **2000**; 34:40–2.
4. Shah J, Jaswal GS. Victims of a rabid wolf in India—effect of severity and location of bites on development of rabies. *J Infect Dis* **1976**; 134:25–9.
5. Smith JS, Yager PA, Baer GM. A rapid fluorescent focus inhibition test (RFFIT) for determining rabies virus-neutralizing antibody. In: FX Meslin, MM Kaplan, H Koprowski, eds. *Laboratory techniques in rabies*. 4th edition. Geneva: World Health Organization, **1996**: 181–91.
6. Haupt W. Rabies—risk of exposure and current trends in prevention of human cases. *Vaccine* **1999**; 17:1742–9.
7. Rathod NJ, Salunke S, Bawskar VR. Clinical profile of wolf bite cases in Jalgaon district. *J Assoc Physicians India* **1997**; 45:866–7.

8. Satpute PG, Satpute GY. Incidence of rabies in a wolf with severe zoonotic implication. *Indian Vet J* **2000**; 80:1180.
9. Gacouin A, Bourhy H, Renaud JC, Camus C, Suprin E, Thomas R. Human rabies despite postexposure vaccination. *Eur J Clin Microbiol Infect Dis* **1999**; 18:233–5.
10. Sriaroon C, Daviratanasilpa S, Sansomranjai P, et al. Rabies in a Thai child treated with the eight-site post-exposure regimen without rabies immune globulin. *Vaccine* **2003**; 21:3525–6.
11. Vodopija R, Lafont M, Baklaic Z, Ljubicic M, Sujetlicic M, Vodopija I. Persistence of humoral immunity to rabies 1100 days after immunization and effect of a single booster dose of rabies vaccine. *Vaccine* **1997**; 15:571–4.