

Title: MORE THAN A DECADE AND A HALF OF SERVING THE CAUSE OF CONTROL AND PREVENTION OF RABIES: A REVIEW OF SAFETY AND EFFICACY OF ABHAYRAB - PURIFIED VERO CELL RABIES VACCINE

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Keywords Abhayrab, Anti-Rabies Vaccine (ARV), Post-Exposure Prophylaxis, Pre-exposure Prophylaxis, Purified Vero Cell Rabies Vaccine (PVRV), Rabies, RFFIT

Abstract Prevention of Rabies, a fatal viral disease, depends highly on availability and affordability of potent anti-rabies vaccine. Abhayrab, Human Rabies Vaccine, a modern cell cultured Purified Vero Cell Rabies Vaccine (PVRV) has played a significant role in prevention of Rabies in India and elsewhere since its launch. Manufactured by Human Biologicals Institute, a unit of Indian Immunologicals Limited, a subsidiary under National Dairy Development Board, it provides an affordable alternative to costlier options. Since any vaccine has to be potent and provide desired protection, as well as be affordable. In this review the results of various studies on safety and efficacy of Abhayrab were evaluated and compared with studies of other vaccines. Comparison was done with studies using similar vaccination regimen and route. It was concluded that safety and efficacy profile of Abhayrab is comparable to that of PVRV, PCECV and HDCV anti-rabies vaccines prequalified by WHO

Review Article

MORE THAN A DECADE AND A HALF OF SERVING THE CAUSE OF CONTROL AND PREVENTION OF RABIES: A REVIEW OF SAFETY AND EFFICACY OF ABHAYRAB - PURIFIED VERO CELL RABIES VACCINE

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ABSTRACT:

Prevention of Rabies, a fatal viral disease, depends highly on availability and affordability of potent anti-rabies vaccine. Abhayrab, Human Rabies Vaccine, a modern cell cultured Purified Vero Cell Rabies Vaccine (PVRV) has played a significant role in prevention of Rabies in India and elsewhere since its launch. Manufactured by Human Biologicals Institute, a unit of Indian Immunologicals Limited, a subsidiary under National Dairy Development Board, it provides an affordable alternative to costlier options. Since any vaccine has to be potent and provide desired protection, as well as be affordable. In this review the results of various studies on safety and efficacy of Abhayrab were evaluated and compared with studies of other vaccines. Comparison was done with studies using similar vaccination regimen and route. It was concluded that safety and efficacy profile of Abhayrab is comparable to that of PVRV, PCECV and HDCV anti-rabies vaccines prequalified by WHO.

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INTRODUCTION:

Rabies is a viral disease which is almost always fatal.¹ It is a neglected zoonotic disease which still causes significant mortality, mostly in the developing and third world countries. India and its neighboring countries in Asia and parts of Africa bear the major brunt of the disease with significant loss of life and DALY (disability-adjusted life year) every year.²

The mainstay in prevention of rabies is timely vaccination with a safe and potent anti-rabies vaccine. The Nerve Tissue vaccines used earlier had frequent and significant adverse events, mostly neuritis. With the effort of World Health Organization they were replaced with modern cell/ tissue cultured vaccines. In India, the effort was supported and bolstered by united efforts of some public health experts, especially under APCRI. The efforts culminated in discontinuation of Nerve Tissue vaccines by Government of India. NCDC played a vital role in coordinating and regulating the public health measures as well as in training the staff in government center and bringing out guidelines to ensure best clinical practices in the field. The efforts towards control of Rabies in India started bearing fruits.^{3,4,5}

But, the major stumbling block in control of Rabies was lack of availability and affordability of effective anti-rabies vaccine (ARV). In 1999, the Indian government entrusted Indian Immunologicals Limited to produce a domestic rabies vaccine as part of a larger initiative to domestically develop vaccines against communicable

diseases.¹ The company came out successfully with the world's second and India's first PVRV (Purified Vero Cell cultured Rabies Vaccine), Human Rabies vaccine, I.P., with the brand name 'Abhayrab'.

The manufacturing process used Vero Cell Culture, (Purification and Inactivation with beta propiolactone) had a long and successful history of being used for production of Rabies and Polio vaccines worldwide. PVRV was already endorsed by WHO and established to be quite safe for human use.⁶ This third generation ARV was affordable for both government and public purchase. With an initial manufacturing and distribution of 4 million doses per year⁷, it filled a vital gap in the requirement of ARV in India. This made replacement of Nerve tissue vaccine in India possible which was eventually phased out in 2005. Over time, Abhayrab was exported to different countries across the globe and started playing an important role in prevention of Rabies beyond boundaries.

The safety and immunogenicity of Abhayrab has been evaluated by many clinical trials in India and other countries. More than 60 million doses have been sold since its market launch. In this article we discuss and summarize the different studies using Abhayrab in pre-exposure and post-exposure regimens and administration by intramuscular as well as intradermal routes. They encompass studies involving intramuscular dose (>2.5 IU) reconstituted to 0.5 ml as well some studies with reconstitution of the dose to 1 ml.

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The studies discussed adhered to Good Clinical Practice, ICMR guideline and Schedule Y of Drugs & Cosmetic Rules 1945. All these studies were carried out by experts at medical centers regularly carrying out anti-rabies vaccination. Some are randomized clinical trials and some prospective interventional clinical studies. A study on pregnant women was done by a team led by Dr. M. K. Sudarshan, analyzing retrospective data from medical records and prospective follow up of the subjects by visit or phone call.⁸ All the interventional studies followed regimens for pre-exposure or post-exposure prophylaxis recommended by WHO, as applicable. In case of use by intradermal route, vaccination was carried out by trained and experienced staff. For estimation of anti-rabies antibody (IgG) titers rapid fluorescent focus inhibition test (RFFIT) was carried out in laboratories with proper set up and by trained and experienced staff.

These studies include clinical trials performed by Human Biologics Institute to evaluate its safety and efficacy as well as studies done by independent experts/institutes. They are summarized below in different categories as per indication, route of use and use in special population.

Pre-Exposure Prophylaxis (Pr-EP):

Pre-exposure prophylaxis is recommended in populations with a high risk of rabies exposure. That includes anyone who is at continual, frequent or increased risk for exposure to the rabies virus, as a result of their place of stay or occupation. For pre-exposure prophylaxis, one intramuscular dose or one intradermal injection of 0.1 ml is administered on each of days 0, 7 and 21 or 28. A titer above 0.5 IU/ml post vaccination is considered adequate.⁹ For studies in healthy volunteers, administration of ARV as per Pre-Exposure prophylaxis is a good option and the same was adopted in the initial studies of Abhayrab.

The initial trial of Abhayrab was carried out on healthy volunteers, who received 3 intramuscular doses, on days 0, 7 and 28 as pre-exposure regimen. In total, 27 subjects were recruited in three groups. Group-1 (17 numbers) subjects were vaccinated with Abhayrab (reconstituted to 0.5 ml), Group-2 (5 numbers) subjects with Rabipur and Group-3 (5 numbers) subjects with Verorab. The volunteers were followed up till day 35. A blood sample was collected on day 0, 14 and 35 and sent to Pasteur Institute, Coonoor for antibody assay (by RFFIT).

No serious adverse event was noticed in any of the volunteers during the course of the clinical trial. The most common observation was mild pain at the injection site in a few volunteers in each group which subsided within 24-48 hours. No treatment was required in any of the volunteers. No Systemic reaction was noted.

The geometric mean serum antibody titers of <math><0.25</math> was noted on day 0 in volunteers of all three groups. The Group-1 (Abhayrab group) volunteers showed geometric mean titers of 17.29 IU/ml (n=17) and 19.25 IU/ml (n=16) on 14th and 35th day post vaccination respectively. Geometric mean (n=5) serum antibody titers of 12.80 IU/ml and 10.40 IU/ml were observed on 14th and 35th day post vaccination respectively in Group 2 (Rabipur group) volunteers. Similarly, geometric mean (n=5) serum antibody titers of 24.00 IU/ml and 7.00 IU/ml were observed on 14th and 35th day post vaccination respectively in Group 3 (Verorab group) volunteers. All examined titers in all the groups were above the protective level of 0.5 IU/ml (one subject in Abhayrab group was lost to follow up) both on 14th and 35th day.

Thus Abhayrab vaccine was found to be protective and the safety and immunogenicity results were found to be comparable to that of the two available Cell cultured vaccines in the market: Rabipur (PCECV) and Verorab (PVRV).

In a subsequent clinical trial, 92 healthy volunteers aged between 20 to 55 years were recruited with 60 of them having no previous history of vaccination (Group-1) and rest 32 having a previous history of vaccination (Group-2). Group-1 volunteers received one intramuscular dose (Abhayrab was reconstituted to 0.5 ml) each on day 0, 7 and 28 while Group-2 volunteers received a single intramuscular dose. They were followed up till day 35.

The mean serum antibody titers of <math><0.25</math> IU/ml and 8.33 IU/ml was noted on day 0 in volunteers of Group 1 and 2 respectively. Post vaccination, the Group-1 volunteers showed geometric mean antibody titers of 12.69 IU/ml and 18.19 IU/ml on 14th and 35th day respectively. In Group-2 volunteers, geometric mean serum antibody titers were 38.85 IU/ml and 22.14 IU/ml on 14th and 35th day post vaccination respectively.

The immunogenicity results matched quite well with the results as described by Poh Lian Lim et al in their study of pre-exposure prophylaxis with PVRV, Verorab¹⁰, Renuka Kulkarni et al using a marketed PCECV¹¹ and Turner et al using HDC(S)V manufactured by Merieux Institute¹² for Pre-Exposure Prophylaxis.

In this study, all the subjects were included in safety analysis together. No serious adverse reaction was noticed in any of the volunteers during the course of clinical trial. The most common observation was mild pain at the injection site in a few volunteers which subsided within 24-48 hours. No treatment was required in any of the volunteers. No Systemic reactions were noted.

The results on safety of Abhayrab administered as per pre-exposure prophylaxis regimen by intramuscular

route compared well with the results and experience with PVRV, PCEC or HDCV vaccines approved and marketed in the world as summarized in WHO Information Sheet on Rabies Vaccine.¹⁷

Studies were also carried out with Abhayrab administered intradermally as per Pre-exposure Prophylaxis regimen. In Philippines, where intradermal route was already approved and in practice, a group of 73 subjects were recruited in a randomized, single blind, unicentric trial who received the standard Pre-Exposure Prophylaxis - ID regimen.¹⁸ Each subject received a dose of 0.1 ml Abhayrab (reconstituted to 0.5 ml) given intradermally over either deltoid on days 0, 7 and 28. A blood sample from each subject was drawn on days 0, 14, and 28 for estimation of anti-rabies antibody titres. Safety and immunogenicity were assessed through follow-up of adverse events and anti-rabies antibody response, respectively. Sixty subjects completed the day 28 ID immunization. All 60 subjects demonstrated sero conversions with antibody titers greater than the WHO recommended cut-off level of 0.5 IU/ml on days 14 and 28. The GMC values were 3.30 IU/ml and 4.37 IU/ml on day 14 and 28 respectively. Only a few mild adverse events (local redness, itchiness, pain as local and fever as systemic AE) were observed with no report of moderate or severe events.

The results of immunogenicity and safety of Abhayrab administered as per pre-exposure prophylaxis regimen by intradermal route compared well with the results as summarized in WHO Information Sheet on Rabies Vaccine.¹⁷

Post-Exposure Prophylaxis (PEP):

Post-Exposure prophylaxis is indicated in case of possible exposure to rabies virus from contact with confirmed / suspect rabid animals or in case of laboratory exposures. Depending on the risk, they are classified into three categories. Treatment of Category II and III exposure cases to prevent rabies includes immunization with anti-rabies vaccine as per different regimens approved by WHO.^{17 (Para 7a-7b, 7c)} Among these, ESSEN regimen for intramuscular route and modified Thai Red Cross Regimen for intradermal route are the most commonly followed regimens in India.^{18 (Para 7a, 18.1)}

Many studies were carried out with Abhayrab administered as per post-exposure prophylaxis regimen in both healthy volunteers as well as patients with category II or III animal exposure. In some, Abhayrab was administered by intramuscular route and in others by intradermal route. The study details and the results are discussed in brief below.

Intramuscular Route

In the same second trial as described above under pre-exposure prophylaxis, another 28 patients, aged between 6 to 40 years, 12 with Category II (Group A) and 16 with Category III (Group B) exposure, were also recruited who received post-exposure prophylaxis as per WHO approved (one dose each on Day 0, 3, 7, 14, 30 and 90) regimen. The class III exposure subjects also received rabies immunoglobulin as per WHO recommendation. The mean serum antibody titers of Group A on day 0, 14, 30, and 90 post vaccination were <0.25 IU/ml, 10.83 IU/ml, 14.16 IU/ml, and 5.81 IU/ml respectively. The mean serum antibody titers in Group B on day 0, 14, 30, and 90 post vaccination were <0.25 IU/ml, 8.37 IU/ml, 13.62 IU/ml and 4.84 IU/ml respectively.

These titer levels were well above the protective level of 0.5 IU/ml. The results were comparable to the results observed in the study done by Suntharasamai & Warrell et al¹⁹ using both PVRV and HDCV vaccines and another study done by Phampak et al where Verorab was used in one group intramuscularly (reconstituted to 0.5 ml) by ESSEN regimen.²⁰

After initial evaluation and finding Abhayrab to be safe and protective, a Phase III Clinical Trial of Abhayrab was carried out in which 230 patients (75 with Category II exposure and 155 with Category III exposure) of both sexes in age group between 3 to 60 years were recruited. All were administered post exposure treatment as per WHO recommendation including intramuscular vaccination with Abhayrab reconstituted to 0.5 ml. The subjects with category III also received rabies immunoglobulin. A blood sample was collected from each volunteer who came for follow up on day 0, 14 and 90 and tested for anti-rabies antibody levels using Rapid Fluorescent Focus Inhibition Test (RFFIT) at Pasteur Institute, Coonoor.

GMT values were < 0.5 IU/ml (n=75), 13.58 IU/ml (n=72) and 7.04 IU/ml (n=48) on day 0, 14 and 90 respectively in subjects of Category II exposure. GMT values were: 0.5 IU/ml (n=155), 12.76 IU/ml (n=147) and 8.84 IU/ml (n=98) on day 0, 14 & 90 respectively, in subjects of Category III exposure. Thus, the immunogenic response elicited by Abhayrab in all patients as indicated by sero-conversion studies was found to be good. No serious adverse reaction was noted in any patient during the course of the clinical trial. Twelve patients showed mild fever and two patients reported itching at the site of administration. The most common observation was mild pain at the injection site which subsided within 24-48 hours. No treatment was

required in any of the patients. The investigation indicated that Abhayrab vaccine is safe, immunogenic and potent.

These results were similar to the results recorded in earlier studies of Abhayrab, the study by Phanupak et al where a PVRV was used in one group intramuscularly (reconstituted to 0.5 ml) by ESSEN regimen.¹⁷ The results were also comparable to the results found with administration of PCECV (reconstituted to 0.5 ml or 1 ml) when administered by ESSEN regimen as in a study carried out by Renuka Kul karini et al.¹⁸

Intradermal Route

(A) A prospective unicentric randomized single blind study was taken up to compare the safety and immunogenicity of Abhayrab vaccine reconstituted to 1 ml, with commercially available Rabipur vaccine both administered through intradermal route as per updated Thai Red Cross regimen (2-2-2-0-2) in healthy volunteers as a simulated post-exposure study.

In total, 106 subjects were recruited in a single center. Subjects were randomized in 1:1 ratio to Abhayrab and Rabipur vaccine groups. Subjects were administered two doses of 0.1 ml Abhayrab vaccine (53 subjects) reconstituted to 1 ml or commercially available Rabipur vaccine (53 subjects), intradermally, one on each deltoid, according to randomization, on days 0, 3, 7 and 28. A venous blood sample was collected from the subjects on days 0, 3, 7, 28 and 38 for anti-rabies antibody titer estimation. Ninety two subjects (42 in Abhayrab group and 50 in Rabipur group) completed the study and were included in immunogenicity analysis.

On day 0, all the 42 and 50 subjects in Abhayrab and Rabipur vaccine groups respectively were having < 0.5 IU of antibody titer. On day 7, thirty eight (90.48 %) subjects in Abhayrab and forty seven (94 %) subjects in Rabipur vaccine groups were seroconverted. There was no statistically significant difference between the vaccine groups (p-value=0.6982). On day 14, day 28 and day 38, all the subjects in both Abhayrab (42 out of 42) and Rabipur (50 out of 50) vaccine group were seroconverted.

The GMT (n=42) in Abhayrab group on day 0 and day 38 were 0.12 IU/ml and 60.39 IU/ml respectively. Similarly GMT (n=50) in Rabipur group on day 0 and day 38 were 0.10 IU/ml and 56.55 IU/ml respectively. The statistical difference between the two groups was insignificant (p=0.7266).

All 106 subjects were included in safety analysis. No Serious Adverse Events (SAEs) were recorded in the study. Among the local adverse events, one subject had pain and two subjects had rashes at the site of injection

in Abhayrab group. Similarly, in Rabipur group, one subject had itching and two subjects had rashes at the site of injection. Among systemic adverse events, in Abhayrab group, three (5.66%) had fever, two (3.77%) had headache, two (3.77%) had generalized weakness and one (1.89%) subject had body pain. Drowsiness, giddiness, neck pain, right upper limb pain were also reported. In Rabipur group five (9.43%) had body pain and four (7.55%) had generalized weakness. Headache, malaise, itching, drowsiness and cold & cough were also reported. The reported adverse events were considered comparable.

It was concluded that Abhayrab vaccine is equally immunogenic and safe as Rabipur vaccine when administered intradermally (reconstituted to 1 ml) as per Updated Thai Red Cross regimen.

(B) Comparative Study by ICMR with different brands¹⁹: the Government of India had asked the Indian Council of Medical Research to undertake a study to assess feasibility, safety and immunogenicity of indigenously manufactured TCARVs (Tissue Cultured Anti Rabies Vaccines) to be administered intra-dermally in healthy human volunteers. In order to assess the feasibility of introducing intra-dermal anti-rabies vaccination (IDRV) in government institutions, a survey was carried out to assess:

- Availability of different facilities (physical, cold chain, manpower and injection supplies) at the anti-rabies vaccination clinics at the district hospitals
- Animal bite load
- Skill for Intra-dermal injection and
- Acceptability of IDRV among the patients receiving nerve tissue vaccine

The TCARVs used for intradermal administration were Purified Vero cell Rabies vaccine (PVRV: Abhayrab and Coonoor), Purified Chicken Embryo Cell vaccine (PCEC: Rabipur) and Purified Duck Embryo vaccine (PDEV: Vaxirab) with a 2-2-2-0-1-1 regimen. Responses to intradermal TCARVs were compared with that of French PVRV (Aventis) administered intramuscularly on 0, 3, 7, 14 and 28 days. Ten volunteers were recruited for each of the TCARV arm in each center as well as for control group receiving French PVRV. Vaccinated individuals were observed for immediate hypersensitivity reactions and their follow-up blood samples were collected on days 14, 28, 90 and tested for anti-rabies antibody levels using Rapid Fluorescent Focus Inhibition Test (RFFIT) at Pasteur Institute, Coonoor.

A total of 257 volunteers from five centres were recruited in the study after obtaining their consent. On scrutiny of the data, it was observed that two centres in

Delhi experienced several operational problems. Hence the data from 104 individuals from these 2 centres were not considered for the purpose of analysis. From the remaining 3 centres, day zero blood samples were available from 153 individuals. Twenty seven individuals were excluded from the analysis as their sera samples had a pre-vaccination antibody concentration of > 0.5 IU/ml. Thus the primary analysis was restricted to 126 sero-negative individuals. Six individuals were withdrawn from the trial for various reasons at different times during follow-up, four by day 14 and two by day 90.

All the vaccines administered intradermally were well tolerated. No adverse drug reactions were reported from any of the vaccinated volunteers from the three participating centers and none of the volunteers was withdrawn from the trial on account of vaccine related reasons.

Proportions of individuals sero-protected on day 14, 28 and 90 for the French PVRV vaccine (standard) were 100%, 100% and 95.7% respectively with the GMT of 6.73 IU/ml, 10.08 IU/ml and 4.65 IU/ml. The sero-protection rate and GMT dropped to 40.8% and 0.80 IU/ml by day 180. All the volunteers who received Abhayrab PVRV were sero-protected on day 14, 28 and 90. Results were similar for the PVRV from Coonoor. For volunteers receiving Rabipur PCECV, the responses were on the lower level but the sero-conversions achieved was 100%. For the volunteers who received Vaxirab PDEV the responses were very much on the lower side, both in terms of GMT values and the sero-conversions over all the three periods of observation.

The results of the study indicated that three TCARVs (Abhayrab PVRV, Coonoor PVRV and Rabipur PCECV) could be used for intradermal administration in India.

(C) A study was carried out at Anti Rabies Vaccination Clinic of VSS Medical College Hospital, Orissa, India to assess the safety and immunogenicity of PVRV (Abhayrab) when administered intradermally as per updated Thai Red Cross regimen.

In all, 218 subjects were screened to eventually have 100 subjects between 18 to 70 years of age presenting within 24 hours of WHO category II or III exposure to known or potentially rabid animals. The subjects were followed up for the 365 day long study period with a grace period of 15 days. Seventy seven patients had exposure of category III and 23 patients of category II. All the category III patients were co-administered ERIG (Equine rabies immunoglobulin). Each patient received two 0.1 ml injection of Abhayrab PVRV intradermally over the deltoid area on days 0, 3, 7 & 28 according to the Updated-TRC Regimen. Venous blood sample was

collected from each patient on days 0, 7, 14, 28, 90 and 365. Anti-rabies antibody titres were measured at day 0, 7, 28, 90 and 365 by Rapid Fluorescent Focus Inhibition Test (RFFIT).

The seroconversion rates were 0% on day 0, 62.9% on day 7 and 100% on the days 14, 28, 90 and 365. The GMT of anti-rabies antibody titres were 0.35 IU/ml, 5.01 IU/ml, 7.81 IU/ml, 4.12 IU/ml and 2.15 IU/ml on days 7, 14, 28, 90 and 365 respectively. All adverse events reported were minor and transient, mostly in the form of local effects. No serious adverse reactions were identified and reported during the one year follow up period.

It was concluded that PVRV (Abhayrab) is a highly immunogenic vaccine when administered in 0.1 ml intradermal doses (after reconstitution to 0.5 ml) in post exposure treatment of the WHO category-II and category-III patients, with immunogenicity reaching its acceptable levels by day 14.

(D) In a randomized, single blind, unicentric trial done in Philippines, a group of 76 subjects (healthy volunteers) were administered with the modified Thai Red Cross ID (simulated) regimen (2-2-2-0-2) using a dose of 0.1 ml (after reconstitution to 0.5 ml) of PVRV (Abhayrab) each given intradermally on both deltoids on days 0, 3, 7, and 28. Following administration of the vaccine, subjects were observed closely for 30 minutes at the site for adverse reactions. A blood sample from each subject was drawn on days 0, 14, and 28 for anti-rabies antibody titres. Safety and immunogenicity were assessed through follow-up of adverse events and anti-rabies antibody response, respectively.

Eventually, 60 subjects completed the day 28 ID immunization. All 60 of them demonstrated seroconversions with antibody titers greater than the WHO recommended cut-off level of 0.5 IU/ml on days 14 and 28. The GMC values were 3.73 IU/ml and 4.82 IU/ml, respectively. Only a few mild adverse events (local redness, itchiness, pain as local AE, and fever as systemic AE) were observed with no incidences of moderate or severe events. It was concluded that Abhayrab is a safe and immunogenic rabies vaccine when administered intradermally.

(E) In a Phase IV study carried out in Vietnam, 100 volunteers aged 18 to 66 years were recruited and they were intradermally administered two 0.1 ml doses of vaccine (after reconstitution to 0.5 ml) at deltoid regions of both arms on day 0, 3, 7 and 28. Blood samples were collected on day 0 (before vaccination), at day 28 and day 180. Solicited and unsolicited adverse events were followed up for 3 days and 30 days post-vaccination respectively, whereas serious adverse events were

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assessed throughout the trial.

Immunogenicity : GMT values on 28th and 180th day was 2.618 IU/ml and 0.695 IU/ml respectively. The solicited local adverse events were itching (17%) and redness (10%). Similarly the systemic adverse events were headache (13%), fatigue (12%), generalized itching (9%) and dizziness (6%). Most of these AEs were mild and occurred within first three days post-vaccination. No unsolicited AEs or SAEs were reported.

It was concluded that Abhayrab vaccine administered ID by TRC regimen is well tolerated, safe and immunogenic on healthy Vietnamese people.

(F) A PMS study for safety evaluation was carried out for Abhayrab vaccine reconstituted to 1 ml, when administered by either intramuscular route or intradermal route in category II animal exposure subjects in India. In this prospective, open label, two arm, single center study, a total of 120 subjects with Category II exposure were enrolled into the study. Out of them, 111 subjects completed the study as per the protocol as nine subjects were lost-to follow up. Subjects recruited in the first arm received one dose of Abhayrab reconstituted to 1 ml by intramuscular route on days 0, 3, 7, 14 and 28 as per EESEN regimen. In the second arm, the subjects received two doses of 0.1 ml of Abhayrab reconstituted to 1 ml administered intradermally, one on each deltoid region on days 0, 3, 7 and 28 as per Updated Thai Red cross regimen. The subjects were followed up for 7 days post last dose of vaccination.

At the end of the study, a total of 87 mild or moderate local and systemic adverse events were reported (33 in intramuscular route group and 54 in intradermal route group) in the study. No serious adverse event was reported during the study period. Among the local adverse events, in intramuscular route group, 18 (30%) subjects were having pain at the injection site and it was the only local adverse event observed. In intradermal route group, pain at the injection site was the most common local adverse event and was found in 13 (21.7%) subjects followed by local redness in 12 (20%) and local itching in 8 (13.3%) subjects. Among the systemic adverse events, in intramuscular route group, fever was the most common and was found in 7 (11.7%) of the subjects followed by body pains in 3 (5%), headache in 3 (5%), backache in 1 (1.7%) and tingling sensation in the lower limbs in 1 (1.7%) of the subjects. In intradermal route group also fever was the most common systemic AE and was found in 7 (11.7%) of the subjects followed by body pains in 6 (10%), headache in

6 (10%), joint pain in 1 (1.7%) and dizziness in 1 (1.7%) of the subjects. The severity assessment of all the local and systemic adverse events showed that they were either mild or moderate only.

Thus, adverse events were found to be less when Abhayrab is administered by intramuscular route after reconstitution to 1 ml in comparison to administration by intradermal route.

Summarizing the results of the above studies, it can be concluded that Abhayrab vaccine was found to be safe and protective when used either by intramuscular route or intradermal route and whether reconstituted to 0.5 ml or 1 ml. There were only few mild and moderate transient and reversible local as well as systemic adverse events. The immunogenicity was timely and adequate when administered by either route and with reconstitution volume of either 0.5 ml or 1 ml. These results are comparable to the results as in published data of different studies of anti-rabies vaccines (D. J. Briggs et. al.¹⁹; Yuan Fang et. al.²⁰; M. K. Sudarshan et. al.²¹; M. Chhabra et. al.²²) including that by Phanupak et al.²³.

Post Exposure Use in Specific Groups:

Some independent clinical investigators studied the efficacy and safety of anti-rabies vaccine in some specific group of subjects where Abhayrab was used. The studies and the results are discussed below.

(A) In Pregnant Women:

Fourteen pregnant women who received rabies post-exposure prophylaxis (PEP) at the anti-rabies clinic (ARC) of Kempegowda Institute of Medical Sciences (KIMS) were followed up for assessing the safety of modern rabies vaccines and equine rabies immunoglobulin (ERIG) in pregnancy. The women were in the age range of 18 to 28 years, mostly from urban area (64%) and exposed to suspect rabid dogs (86%). Four of them had received Abhayrab as ARV by ESSEN regimen, intramuscularly in deltoid, one dose each on days 0, 3, 7, 14 and 28. Two of them had received ERIG, while two had not received any RIG. They were pregnant with gestational age of 12 to 24 weeks, 1 in first trimester and 1 in second trimester and 2 in third trimester (28th week of gestation). None of the women reported any adverse event. All had safe vaginal deliveries and in all cases both the mother and the child were found to be healthy and normal. The children did not have any obvious congenital anomalies. In conclusion, PVRV (including Abhayrab) & PCECV and ERIG were found to be safe in pregnancy.²⁴

(B) In Fox bite victims:

Nineteen patients aged 6 to 70 years, of both sexes, from 6 hamlets in Ahmednagar district of Maharashtra, India, 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 III bites.

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 vaccine, reconstituted to 0.5 ml) was administered to all patients intramuscularly on days 0, 3, 7, 14 and 28 in accordance with the World Health Organization's 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 reconstituted to 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 vaccination. The PVRV was found to have induced good sero-conversion. The geometric mean antibody titer values were 3.16 IU/ml (n=12), 25 IU/ml (n=16) and 0.77 IU/ml (n=11) on days 30 and 90 and 870 after the first dose (day 0) of vaccination. The geometric mean antibody titer was 0.48 IU/ml (n=11) on day 1020. After a booster dose on day 1020, the geometric mean antibody titer value was 30.08 IU/ml (n=11) on day 1050.

In conclusion, PVRV (Abhayrab) was found to be effective in preventing death in human beings exposed to rabid animal (fox) 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.

Summary of Study Results:

The immunogenicity results in different studies using Abhayrab administered as pre-exposure prophylaxis or post-exposure prophylaxis, by either intramuscular or intradermal route were well above the desired level. The titers were consistent across the studies with a variation that can be attributed to physiological variation across the subject population. The results were also consistent whether the vaccine was used in the particular study after reconstitution to a volume of 0.5 ml or 1 ml. The results show that Abhayrab elicits a good

immunogenic response with anti-rabies antibody titers much above the required level of 0.5 IU/ml by day 14 and it is sustained above that level till day 90, 180 and 365. There was no report of any subject with history of category II or III of animal exposure developing rabies or experiencing any serious adverse event during the study period in any of the studies. The adverse events reported in clinical trials showed that the vaccine is safe with a few mild to moderate reversible and acceptable undesirable effects. The common local adverse events were pain, itching, redness and swelling at the site of injection. The common systemic adverse events were fever, headache, generalized weakness and body ache. The overall safety profile is found to be comparable to that of other marketed modern cell cultured vaccines.

CONCLUSION:

Abhayrab is a modern cell cultured (Purified Vero Cell Cultured) rabies vaccine which has undergone extensive clinical evaluation over long periods since its inception. The clinical trials discussed here, concluded that it is safe and immunogenic when used by either intradermal or intramuscular route, whether reconstituted to 0.5 ml or 1 ml. There was no serious adverse event reported in any of the clinical trials. The adverse events were mild in nature and reversed within a few days. The immunogenicity was quick and well above sero-protection level by day 14 of vaccination. The patients of animal exposure of both category II and III were found to be protected during the period follow up, with minimum follow up duration being 35 days and maximum 1050 days from the day of first dose (day 0). Abhayrab was found to be comparable in safety and efficacy with brands marketed in India and elsewhere in the world.

More than 60 million doses of the vaccine have been sold till date in India and over 29 countries across the world. The post marketing surveillance data for years together found its benefits significantly outweighing the minor risks of temporary adverse events in a small percentage of people. Currently, Abhayrab is one of the most used Anti Rabies vaccine in India and saves millions of lives from the dreaded and highly fatal infection of Rabies. It was launched to provide affordable anti-rabies vaccination to millions of animal exposure victims in India and elsewhere in the world who could not afford the costly options available. It has done quite well in serving its purpose in decreasing mortality from rabies and is expected to help in its eventual elimination.

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