EVALUATION OF SAFETY AND IMMUNOGENICITY OF ABHAYRAB™ ID REGIMEN ON HEALTHY VIETNAMESE

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ABSTRACT

Rabies is a fatal, acute, progressive viral encephalitis which is only preventable by vaccine. However, multiple times injection schedule and high pricehave limited the usage the rabies vaccine. Compared to intramuscular (IM) regimen, intradermal (ID) regimen has advantage of low price buthas disadvantages of injection technique, vaccine remaining, cost-benefit of provider and limitation of safety and immunigenicity profile, especially in Vietnamese population.

Objectives

We aimed to assess the safety and immunogenicity of a vero cell rabies vaccine named Abhayrab™ by ID regimen.

Methods

We under took an open-labeled in one group phase 4 study between October, 2013 and April, 2014 at Long Xuyen city, An Giang province, Vietnam. The participants (aged 18 - 66) were administered intradermally two doses of vaccine at deltoid regions of two arms on the day 0, 3, 7 and 28. Three blood samples were collected on the day 0, 28 before vaccination and the day 180. Solicited and unsolicited adverse events would be followed for 3 and 30 days post-vaccination, respectively where as serious adverse events were assessed during the trial.

Results

Immunogenicity based on per-protocol population showed that Geometric Mean Titre on Day 28 (GMT_{28}) and GMT_{180} were 2.62 IU/mL (95% CI: 2.44 - 2.80) and 0.70 IU/mL (95% CI: 0.47 - 0.92) respectively. The most common solicited local and systemic adverse events (AEs) are itching (17.0%), redness (10.0%) and headache (13.0%), fatigue (12.0%), respectively. Most of these AEs were mild and occurred within first three days post-vaccination. No unsolicited AEs and serious adverse events (SAEs) were reported.

Conclusion

Abhayrab™ vaccine ID regimen is well tolerated, safe and immunogenic on healthy Vietnamese adults.

Keywords

Abhayrab™, clinical trial, vaccine

BACKGROUND

Rabies is an acute, progressive viral encephalitis transmitted from animal to animal or from animal to humans by exposure to saliva or other sources of infection virus [1]. The annual number of human rabies deaths estimated in the world in 2010 was from 26,400 (95% CI: 15,200 to 45,200) to 61,000 (95% CI: 37,000 to 86,000), the vast majority of deaths occurred in the rural area (84.0%) in developing countries in Asia, Africa and Latin America [2,3]. In Viet Nam, the rabies is an important medical issue which greatly affect to economy and human health in many years [4,5]. Before 1996, Vietnam had 350,000 - 450,000 people exposed to rabies virus and approximately 500 deaths each year because they had not received full schedule of vaccine and immunoglobulin on time [4,6]. Rabies also cause highest mortality rate per 100,000 people in 10 diseases which had highest mortality rate [4,6]. Untill now, there had still been no successful therapy of rabies, therefore preventive treatment for exposure people by rabies vaccine and rabies anti-immunoglobulin is unique therapy to prevent rabies. In Viet nam, rabies vaccines used before 1974 were produced from brain of sheep and calf (Femi, semple). These kind of vaccines had low immunogenicity which required multiple injection schedule (18 - 21 injections) and large dose volume (1.5 - 2.5 mL). Moreover, the presence of myelin originated from brain tissue in vaccine caused encephalomyelitis which led to paralysis and death [1,5].

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In 1974, Pasteur Institute in Paris transferred rabies vaccine producing technology from mice brain according to Fuenzalida - Palacoise method for National Institute of Hygiene Epidemiology (NIHE), therefore, from 1974 to 2007, Vietnam mainly used Fuenzalida vaccine with approximately 4 milion singe doses per year. However, Fuenzalida vaccine usually caused neurologic catactrope such as encephalitis and allergy neurologic inflammation due to presence of myelin from brain tissue and incompletely inactivated virus [1,7]. In addition, sine 1992 there were approximately 30,000 dose of Verorab in the Viet Nam market per year, however, the usage of Verorab was limited due to high price (10 USD/dose). In December 2002, Vietnam Ministry of Health (MOH) approved study results of NIHE to intradermally injection schedule of Veorab on healthy Vietnamese [8]. In 2004, Verorab intradermal injection schedule was licensed in Vietnam. This schedule had decreased 2/3 price for a fully injection schedule of Verorab.

Since 2007, Viet Nam stopped producing and using Fuenzalida vaccine and replacing by high safety and effective rabies vaccine. Currently, there are 4 rabies vaccines licensed in the Vietnam market including Verorab, Abhayrab, Rabipur and Lyssavac N but only Verorab had data of intradermal regimen on the Vietnamese [8]. Although Abhayrab^{TM} was marketed in Vietnam since 2009 with two regimens IM and ID. However, the ID regimen was rarely used due to lack of clinical data about efficacy and safety as well as difficulties of ID injection skill in spite of its low price. Therefore, we conducted this trial to provide immunogenic and safety data on Vietnamese, which then encourages widely application of the ID regimen.

METHODS

Participants

We enrolled 100 healthy men and women who satisfied *the following criteria*: aged from 18

years; female participants showed negative urine pregnancy test at screening day, for females of childbearing potential who are sexually active agreed to use an acceptable contraceptive method during the study; no receipt of any previous rabies vaccine; understanding and compliance with trial procedure; signing and dating a written informed consent prior to the initiation of any trial procedures after the nature of the trial had been explained. *Participants were excluded* if they were using IV administration immunoglobulin or immunosuppressive drugs such as systemic corticosteroids, anticancer drugs, chloroquine…; had autoimmune diseases or immunodeficiency; known to be allergy to any components of investigational vaccine; were pregnant or breastfeeding; intellectual deficiency.

Methods

Study design

We undertook an open-labeled, phase 4 study, compared immunogenicity pre-vaccination and post-vaccination in the same group of participants in Long Xuyen city, An Giang province where there was high ratio of rabies vaccine ID regimen injection since last two year (2012-2013).

Sample

Sample size is calculated based on the following formula:

$$n = Z^2_{1-\frac{\alpha}{2}} \frac{p(1-p)}{d^2}$$

In which: d = 0.05 (estimate error); $Z_{1-\alpha 2}$ = 1.96 (confidence coefficient with α=0.05, confidence interval 95%); p = 0.96 (percentage of subjects had antibody over 0.5 IU/mL based on the result from an IM study of Abhayrab [9]; From the formular, we estimated n ≥ 59 subjects. With the drop out ratio was approximately 10.0%, we decided toenroll 100 healthy men and women in Long Xuyen city, An Giang province.

Based on recruitment data of a dengue vaccine study conducted in Long Xuyen city since 2011 [10], we invited 236 subjects to providegeneral information about study. 117/236 subjects came back to site on screening day to be explained more detail about the nature of study in which 116 participants signed informed consent form and to be examined physically. The remaining subject had previously been injected with rabies vaccine so did not sign ICF. However, there were only 100 participants comingback and were vaccinated on the day 0.

Outcomes

Our primary endpoints were evaluation of safety and immunogenicity of Abhayrab™ ID regimen on healthy Vietnamese adults. In detail, we assessed immunogenicity of Abhayrab including GMT, ratio of seroconversion on day 28 before two last doses and on day 180. For safety, we analyzed incidence and ratio of solicited AEs within 3 days post vaccination, unsolicited AEs within 30 days post-vaccination after each vaccination, and SAEs during study.

Procedure

Abhayrab™ was manufactured from vero cells by Human Biologicals Institute. Vaccine was transferred and stored at 2-8^{0}C. If subjects agreed to participate into trial by signing and dating on ICF, they would be physically examined and checked criteria for inclusion and exclusion. Enrolled participants would be requested to come back study site next two days to receive two first doses of Abhayrab™ by intradermal administration on day 0 and then on the day 3, 7 and 28. Before vaccination on day 0, day 28 and on day 180, participants were taken 3 mL of blood sample to measure antibody response. Solicited adverse events and unsolicited AEs were recorded in diary cards by participants for three days and 30 days, respectively after each vaccination. The diary cards were collected on day 3, 7, 28, 58 respectively. Solicited systemic adverse events were assessed including fever, headache, myalgia, fatigue, dizziness, joint pain. Solicited local adverse events were monitored including

pain, redness, swelling/induration, itching at injection site. We documented and followed up serious adverse events that happened any time during the study.

We measured titres of anti-rabies virus neutralizing antibody in sera collected on day 0, 28 and 180 by rapid fluorescent focus inhibition test (RFFIT)..

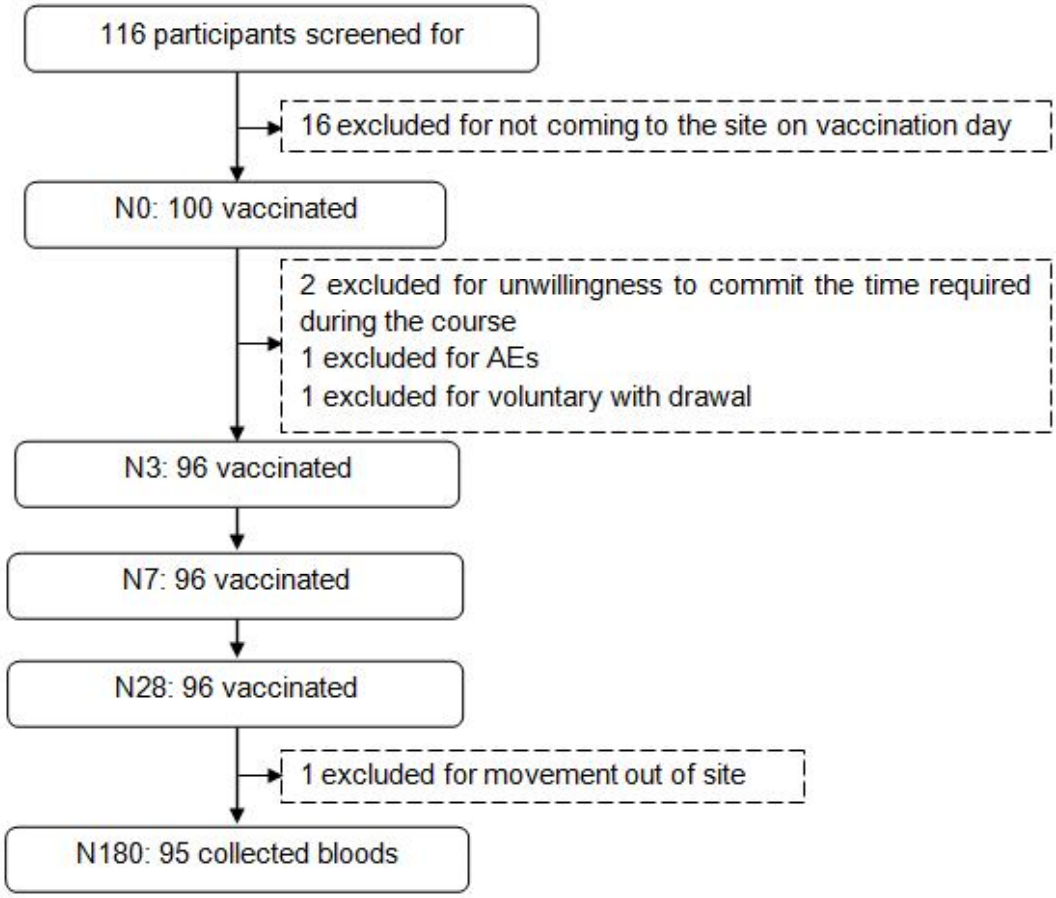

Figure 1. Trial profile

Statistical analysis

We summarized antibody responses with geometric mean titres (GMT) and ratio of seroconversion population at 95% CI. Participants having antibody titres above 0.1 IU/mL on day 0 would be considered to be vaccinated with rabies vaccine in the past and not be incorporated into immunogenicity analyzed population. On day 28 and 180, Abhayrab^{TM} ID regimen shows protective effect against rabies if antibody titres ≥ 0.5 IU/mL and by contrast. We summarized AEs with ratio. Statistical analyses were by intention to treat for safety and per protocol for immunogenicity.

Ethical considerations

The clinical trial protocol was approved by the Ethics Committee of Vietnam Ministry of Health according to the Approval No. 95/CN-BDGDD, dated October 07, 2013 and Decision No. 4169/QD-BYT, dated October 18, 2013 by Ministry of Health. All protocol amendments, Informed consent form (ICF) and subject's documents were approved by Ethics Committee of Vietnam Ministry of Health before conducting study. Subjects were supported with 200,000 VND the reimbursement fee for each visit at site. If the subject appeared AEs or SAEs during study period, they could contact with investigator to be advised or came to An Giang Preventive Medicine Centerand/or An Giang General hospital to be physical examined and treated. Treatment fee for AEs/SAEs which were concluded to be related to vaccine and/or study procedure would be paid by sponsor. The subject's information was kept confidencially. Only investigators who had been delegated had

right to approach the study document. The result studywas approved by the Ethics Committee of Vietnam Ministry of Health according to the Approval No. 07/CN-K2DT, March 25, 2015.

RESULTS

Demographic analysis showed that the meanage of participants was 36.4 years and ranged from 17 to 66 years old. There was one participant whose age was 17 year 9 months at the time of enrolment, this case was deviated from protocol therefore his immunogenicity data was not included in final analysis (Table 1). 13 participants informed to investigators to be previously bitten by animals and 100% had not been receivedpreviously rabies vaccination.

Table 1. Baseline characteristics

Characteristics (n=100)	No.	%
Age group		
17- 20 years old	14	14.0
21 - 30 years old	31	31.0
31 - 40 years old	17	17.0
41 - 50 years old	19	19.0
51 - 60 years old	11	11.0
61 - 70 years old	8	8.0
Mean: 36.4 (33.6-39.2); Median: 35 years old; Min: 17 years old; Max: 66 years old		
Sex		
Male	40	40.0
Female	60	60.0
Pregnancy test		
Negative	53	53.0
Not applicable	47	47.0
Rabies exposure history		
No	87	87.0
Yes	13	13.0
Rabies vaccination history		
No	120	100.0
Yes	0	0.0

There were no immediately adverse events, serious adverse events and unsolicited adverse eventsoccurred during study period. One participant who was bitten by dog three months after the two last doses vaccination was not indicated with rabies vaccine (Table 1).

Table 2. Percentage of patients who had at least one AEs after each injection

≥ 1 AEs	After N0 n (%)	After N3 n (%)	After N7 n (%)	After N28 n (%)
No.	100	96	96	96
Local	25 (25.0%)	26 (27.0%)	17 (17.7%)	11 (11.5%)
Systemic	27 (27.0%)	14 (14.6%)	9 (9.4%)	7 (7.3%)

Table 2: The frequency of solicited adverse events appeared at reach after injections on day 0 with 25.0% local AEs and 27.0% systemic AE. These ration gradually decreased by following vaccinations (table 2) for both local AEs and systemic AEs. Local AEs decreased from 25.0% after injections on day 0 to 27.0%, 17.7%, 11.5% after injections on day 3, day 7 and day 28, respectively. Compared to local AEs, systemic AEs had more significantly decrease from 27.0% after after injections on day 0 to 14.6%, 9.4%, 7.3% after injections on day 3, day 7 and day 28, respectively.

Table 3. Percentage solicited AEs after each injection

Solicited AEs	After N0 n (%)	After N3 n (%)	After N7 n (%)	After N28 n (%)
No.	100	96	96	96
Local solicited AEs				
Redness	10 (10.0%)	11 (11.5%)	11 (11.5%)	6 (6.3%)
Swelling	3 (3.0%)	1 (1.0%)	0 (0.0%)	3 (3.1%)
Pain	6 (6.0%)	7 (7.3%)	6 (6.3%)	3 (3.1%)
Itching	17 (17.0%)	17 (17.7%)	10 (10.5%)	4 (4.2%)
Systemic solicited AEs				
Fever	5 (5.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Erythema	9 (9.0%)	1 (1.1%)	0 (0.0%)	4 (4.2%)
Fatigue	12 (12.0%)	10 (10.4%)	3 (3.1%)	2 (2.1%)
Headache	13 (13.0%)	9 (9.4%)	5 (5.2%)	2 (2.1%)
Dizziness	6 (6.0%)	2 (2.1%)	0 (0.0%)	1 (1%)
Joint-pain	2 (2.0%)	3 (3.1%)	2 (2%)	0 (0.0%)
Myalgia	6 (6.0%)	3 (3.1%)	3 (3.1%)	3 (3.1%)

Itching and redness were the most commonly solicited local AEs reported with 17.0% and 11.0% participants appeared itching and redness after injections on day 0 and slightly changed after injections on day 3, 7 and dramatically reduced after injections on day 28 with 4.2% and 6.3%, respectively (table 3). For solicited systemic adverse events, headache and fatigue were the most commonly reported systemic AEs. 13.0% and 12.0% participants had headache and fatigue, respectively after the injections on day 0 and declined dramatically after vaccination on day 7 and day 28 with 5.2% of headache, 3.1% of fatigue on after day 7 and 2.1% of headache, 2.1% of fatigue after day 28, respectively (table 3).

Most AEs were mild (13.5% - 37.5% local AE and 12.6% - 43.3% systemic AE). The moderate AEs changed from 1.0% - 3.0% for local AEs and 3.0% - 8.0% for systemic AE. There were 3.0% solicited systemic AEs and occurred after injections on day 0. Most AEs appeared within 3 days post-vaccination, only one case of systemic AE occurred after three days post-vaccination.

Table 4. Comparison of antibody level before vaccination, before two last doses and after 6 months since the first doses (n=80)

Antibody concentration		N0 n (%)	N28 n (%)	N180 n (%)
No (< 0.1 IU/mL)		80 (100.0%)	0 (0.0%)	0 (0.0%)
Non-protective (< 0.5 IU/mL)		0 (0.0%)	0 (0.0%)	5 (6.25%)
Protective	0.5 - 5 IU/mL	0 (0.0%)	9 (11.25%)	63 (78.75%)
	≥ 5 - 10 IU/mL	0 (0.0%)	12 (15.0%)	5 (6.25%)
	≥ 10 - 20 IU/mL	0 (0.0%)	42 (52.5%)	4 (5.0%)
	≥ 20 - 30 IU/mL	0 (0.0%)	6 (7.5%)	0 (0.0%)
	> 30 IU/mL	0 (0.0%)	11 (13.75%)	3 (3.75%)

Immunogenic analysis based on per protocol population including 80 participants. twenty participants were excluded because of protocol deviation: fourteen participants had anti-rabies antibody titres ≥ 0.1 IU/mL pre-vaccination; four participants did not complete the injection schedule; one participant aged 17 years at enrolled time; one participant from whom blood sample was not collected on day 180. Before last two doses on day 28, 100.0% per protocol population reached antibody titer above 0.5 IU/mL, in which 88.75% population reached over 5 IU/mL. The antibody response gradually decreased in a time dependent manner. Six months later, 93.75% participants still maintained antibody titer above protective antibody threshold with 15.0% participants showing antibody titer above5 IU/mL (table 4). The sero-conversion ratio reached 100.0% from non-protective to protective antibody level on day 28. 5 (6.25%) participants had sero-conversion from protective (on day 28) to non-protective antibody level on day 180. Among 5 participants who had under-protective antibody titer on day 180, we could notmake a relationship between antibody titer decrease with baseline as well as geographic characteristics (data not shown).

Table 5. GMT_{28} and GMT_{180} of unvaccinated group (per protocol analysis) and vaccinated group

Day	n	GMT	95%CI	SD	Min; Max
Unvaccinated group					
N28	80	2.62	2.44 -2.79	0.09	1.08; 4.43
N180	80	0.70	0.47 -0.92	0.11	0.92; 3.96
Vaccinated group					
N28	14	3.85	3.29 -4.41	0.98	2.51; 5.87
N180	14	2.20	1.60 -2.79	1.03	0.43; 4.67

GMT values on day 28 and on day 180 were 2.62 (95% CI: 2.44-2.79) and 0.70 (95% CI: 0.47-0.92), respectively. GMT_{180} declined significantly compared to GMT_{28} although it was still higher than protective threshold (table 5).We performed extra analysis for 14 participants who had antibody titer above 0.1 IU/mL on the day 0 which ranged from 0.11 to 2.0 IU/mL,the results showed GMT_{28} and GMT_{180} in this group were 3.85 (95% CI: 3.29 - 4.41) and 2.20 (95% CI: 1.60 - 2.79), respectively and were significantly higher than previously unvaccinated group (table 5).

DISCUSSION

We did not observed any immediately adverse events, unsolicited adverse event as well as serious adverse events after all injections during study period. Solicited AEs appeared mainly after injections on day 0 with 25.0% local AE and 27.0% systemic AE. These ratio slightly changedor decreased after injections on

day 3 (27.0% local AE and 14.6% systemic AE), day 7 (17.71% local AE and 9.4% systemic AE) and day 28 (11.5% local AE and 7.3% systemic AE).

Itching and redness at injection site were most commonly local AEs reported after all injections which changed from 4.2% - 17.0% and 6.3% - 11.5%, respectively. These ratio of itching and redness observed in this study were lower than the ratio observed in a study conducted in Philippine [11] in which itching changed from 5.0% - 33.3% and redness changed from 3.9% - 63.5%. Fatigue (2.1% - 12.0%) and headache (2.1% - 13.0%) were the most commonly systemic AEs reported especially after injections on day 0, 3 and 7. In addition, fever, erythema, dizziness and myalgia also reported but at lower frequency. The frequency of systemic AEs in our study occurred at higher rate compared to study conducted in Philippine (fever: 2.6%) [11]. However, the frequency of solicited AEs in our study were still in the range reported by WHO with 35 - 40% local AEs and 5% - 15% systemic AE [2].

Immunogenicity analysis on day 0 showed that 86.0% participants did not have anti-rabies antibody and 14.0% participants have had anti-rabies antibody from whom reported administration of rabies vaccine previously. Antibody response increased considerably on day 28 with GMT_{28} reached 2.62 IU/mL (95% CI: 2.44-2.80), 5 times higher than protective threshold antibody titer but is still lower than GMT_{28} in a study conducted in Philippine, in which GMT_{28} is 4.82 IU/mL (95% CI: 3.90-5.97) [11]. The difference in GMT_{28} between two studies might be attributable to investigational population's age. The study in Philippine enrolled subjects aged from 5 to 50 whereas participants in our study aged from 17 to 60 years.

Our study also observed the substantial decline of antibody titerafter 6 months since the first doses. Certainly, 15.0% participants remained the antibody concentration titer over 5 IU/mL, GMT_{180} declined to 0.70 IU/mL (95% CI: 0.47 - 0.92). In a study conducted in India, GMT_{28} and GMT_{180} of Abhayrab were 10.08 (95% CI: 5.27 - 17.62) and 3.31 (95% CI: 2.01 - 5.45), respectively which was much higher than results observed from our study [9]. It was possible that small sample size and a booster dose on day 90 in the study in India were attributable for this difference. However, the decline pattern of antibody titer from day 28 to 180 was not significantly different between two studies. However, although $GMT_{28}$of Verorab in this study was high with 11.04 IU/mL (CI 95%: 7.24 - 16.83) but and GMT_{180} was equal to our result with 0.8 IU/mL (CI 95%: 0.47 - 1.37).

Although GMT_{180} in our study was lower than two other studies as described above but it was still higher than threshold indicated for ID regimen (approximately 0.5 IU/mL) and much lower than level of IM regimen (above 1 IU/mL) in a review study [1]. This result supports for the recommendation of booster doses in which, if subjects are pre-exposure vaccinated with rabies vaccine, they will be boosted with 2 doses when they are exposed to rabies virus.

CONCLUSIONS

Our study showed that Abhayrab™ ID regimen was well tolerated, safe and induced antibody response which should be applied more commonly in Vietnam and then reduced rabies-induced death ratio.

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REFERENCES

1. CE Rupprecht and SA Plotkin. Rabies vaccine, chapter 29. Vaccines 6th edition. 2013.

2. WHO. Weekly epidemiological record 2010; 32(85): 309-320.

3. WHO technical report series 982. WHO Expert Consultation on Rabies, second report 2013.

4. Thuong Van Nguyen. The 10 years report of Vietnam MOH 10/2006.

5. Xuyen Dinh Kim. Some comments about rabies in the North of Viet Nam in 20 years from 1972 to 1991. Journal of Preventive Hygiene 1995; III; 1(9): 34-39.

6. Xuyen Dinh Kim. Some comments about 2,988 people who were bitten by dogs, cats and 110 deaths who were unvaccinated at Gia Lam (Ha Noi), Thuan Thanh (Ha Bac) and Me Linh (Vinh Phu). Journal of Preventive Hygiene 1995; V; 5(25): 572-576.

7. Xuyen Dinh Kim. Immunogenicity of intradermal regimen of Fuenzalida rabies vaccine manufactured in Vietnam. Journal of preventive medicine 2003; XIII; 6(63): 129 - 133.

8. Xuyen Dinh Kim. Immunogenicty and safety of vero cell line rabies vaccine manufactured in France (Verorab) administered intradermally and intramuscularly in volunteered healthy Vietnamese 2002. Journal of preventive medicine 2003; XIII; 6(63): 134-139.

9. Multi-centric study on the use of intradermal administration of tissue culture antirabies vaccines in India. Clinical Trials, National Institute of Epidemiology.

10. Quang Luong Chan, Uyen Doan Y, Hoang Phan Kim et al. The participation in phase III dengue vaccine clinical trial of children from 2 to 14 years old at Long Xuyen and My Tho in 2001. Journal of preventive medicine 2013; XXIII(10): 231-235.

11. Magpantay RL, Bermal N, Medina P, et al. Safety and immunogenicity of rabies pre- and postexposure intradermal regimens using Abhayrab, a purified vero cell rabies vaccine (PVRV) produced in India in healthy volunteers: towards greater affordability of rabies prophylaxis. Asian Biomedicine 2010; 4(1): 61-67.