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A randomized open-label trial of 2-dose or 3-dose pre-exposure rabies prophylaxis among Thai children

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ABSTRACT

Background: World Health Organization changed the recommendation for pre-exposure rabies prophylaxis from 3-dose to 2-dose regimen in 2018. Given limited data of 2-dose regimens in pediatric population, this study aimed to compare the immunogenicity between 2-dose and 3-dose pre-exposure rabies immunization.

Methods: This study was conducted among healthy children aged 2–12 years. They were randomized to 2-dose vaccination (2D) on days 0 and 28 or 3-dose vaccination (3D) on days 0, 7, and 28. Purified Vero cell rabies vaccine (PVRV-Verorab™) was administered intramuscularly. Rabies virus neutralizing antibody (RVNA) titers were measured at 3 time points: 14-day after complete vaccination, 1-year pre-booster vaccination, and 7-day post-booster dose to mimic scenario of rabies exposure. RVNA titers ≥0.5 IU/ml were considered adequate antibody. T cell specific response to rabies vaccine antigen was measured using the interferon-gamma enzyme linked immunospot assay.

Results: From September to October 2017, 107 participants (51% males), 78 in 2D group and 29 in 3D group were enrolled. Median age was 5.8 years (IQR 4.4–7.3). All participants had RVNA titers ≥0.5 IU/ml after primary vaccination [GMT 2D: 18.6 (95%CI 15.9–21.8) and 3D: 16.3 (95%CI 13.2–20.1 IU/ml), p = 0.35]. At 1-year prior to receiving the booster, only 80% of the children in 2D group maintained RVNA titers ≥0.5 IU/ml compared to 100% of the children in 3D group (p = 0.01). However, all participants in both groups had RVNA ≥0.5 IU/ml at 7-day post booster vaccination [GMT 2D: 20.9 (95%CI 17.4–25.3) and 3D: 22.2 (95%CI 15.8–31.4) IU/ml (P = 0.75)]. The median number of IFN-γ secreting cells at 7-day post-booster dose was 98 and 128 SFCs per 10^6 PBMCs in the 2D and 3D groups, respectively (P = 0.30).

Conclusions: Two-dose primary rabies immunization provided adequate antibody at post primary vaccination and post booster. The results support 2-dose regimen of pre-exposure rabies immunization in the pediatric population.

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1. Introduction

Rabies is a vaccine-preventable zoonotic disease which does cause acute, severely progressive encephalitis in humans. The World Health Organization (WHO) reported that the number of global human rabies death is estimated to be 59,000 annually and occurred predominantly in Asia and Africa [1,2] of which 40% occurred in children aged under 15 years [3]. In Thailand, the reported cases of rabies by Bureau of Epidemiology Ministry of Public Health Thailand has significantly decreased from 370 annual deaths in 1980 to <10 annual deaths in the past 5 years. However, the prevalence of animal bites and administration of post

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exposure rabies prophylaxis are still high in the Thai population. It is estimated that 500,000 of animal bites occur each year of which nearly 35% occurred in children aged 1–15 years [4,5]. Moreover, the compliance, the cost of multiple doses post-exposure rabies prophylaxis and the availability of the rabies immunoglobulin remained to be major problems. Furthermore, the coverage of stray dog vaccination in Thailand is only 62%, below the 70% target set by the WHO [6]. Therefore, the pre-exposure rabies prophylaxis or primary rabies immunization plays an important role to overcome these problems and decreases the need for rabies immunoglobulin use [7].

Pre-exposure rabies immunization is recommended for people who are at high risk of being expose to rabies, for example, laboratory personnel working with rabies, vaccine producers, veterinarians and some travelers visiting rabies endemic areas [8,9]. Although children are not included in the high risk population, they still get rabies and need to receive pre-exposure rabies prophylaxis for many reasons such as they live and play closely with dogs, and their small size makes them that much more vulnerable to get multiple bites [7]. There are some countries such as the Philippines who have implemented pre-exposure rabies vaccination program for school-aged children living in high risk areas [10,11].

The recent schedule of pre-exposure rabies prophylaxis recommended by WHO in 2018 was changed from 3-dose to 2-dose regimen [2]. The 2-dose regimen includes cell culture rabies vaccines given 0.5 ml intramuscularly or 0.1 ml intradermally on days 0, and 7. An adequate immunity against rabies is defined by WHO as having rabies virus neutralizing antibody (RVNA) titers of $\geq$0.5 IU/ml by using the rapid fluorescent focus inhibition test (RFFIT) [2,12,13]. For children, pre-exposure rabies prophylaxis is aimed to produce immune memory. When they are bitten by an animal, the booster vaccination can rapidly increase the presence of memory T cells.

There are several research studies among healthy adults which support the 2-dose regimen for pre-exposure rabies prophylaxis recommended by WHO [14–17]. Nonetheless, the interval of schedule for second dose varies between 7 and 28 days [15–21]. There are very limited data of 2-dose pre-exposure rabies prophylaxis in the pediatric population [18,19]. This study aimed to compare the immunogenicity, in terms of humoral immunity and cell-mediated immunity, of the 2-dose and 3-dose primary rabies immunization among healthy children.

2. Material and methods

2.1. Study design

The study was a randomized, open-label clinical trial conducted in children aged 2–12 years at the King Chulalongkorn Memorial Hospital in Thailand. The study protocol was approved by the Institutional Review Board of Chulalongkorn University. Written informed consent was obtained from the parents and informed assent was obtained from the participants aged 7–12 years before enrollment. The study was registered at Thai Clinical trials Registry (TCTR), study number TCTR20171101001.

2.2. Participants

We enrolled children aged 2–12 years, in good general health, who had not previously been vaccinated with rabies vaccine prior to enrollment. Children were excluded if they had either received blood or blood products in the past 3 months or had fever (body temperature $\geq$38 Celsius) on the day of enrollment or baseline RVNA titers of $\geq$0.5 IU/ml.

2.3. Procedures

The children were allocated by block randomization ratio 3:1 into 2 groups. 2-dose group (n = 80) received purified Vero cell rabies vaccine (PVRV) on days 0 and 28. 3-dose group (n = 30) received PVRV on days 0, 7 and 28 as conventional primary rabies immunization. PVRV was administered intramuscularly at the left deltoid. Blood samples were obtained from all participants to evaluate baseline RVNA titers and additional blood was collected from 50 participants to evaluate T cell specific response to rabies vaccine antigen using the interferon-gamma enzyme linked immunospot (IFN-γ ELISpot) assay prior to immunization with PVRV (day 0) and at day 14 after primary rabies immunization (day 42).

One year after primary rabies immunization (day 365), the participants received another dose of PVRV which simulated post-exposure booster vaccination. Blood samples were collected for RVNA titers and T cell specific response to rabies vaccine antigen before booster rabies vaccination (day 365) and at 7 days after booster rabies vaccination (day 372).

One participant was bitten by an animal during the 1-year follow-up period of the study. The participant's blood was drawn to measure RVNA titers, and immediately received one booster rabies vaccination. The follow-up for this participant was censored at this time point.

2.4. Rabies vaccines

The rabies vaccine used in this study was PVRV manufactured by Sanofi Pasteur, France. Each dose, 0.5 ml, contained potency of 8.3 IU/dose for primary rabies vaccination (vaccine lot number M15801M) and 7.7 IU/dose for the booster dose (vaccine lot number P1A861M).

2.5. Rabies virus neutralizing antibody measurement [by rapid fluorescent focus inhibition test (RFFIT)]

The RVNA titer was measured by RFFIT. The test was performed at Queen Saovabha Memorial Institute, the Thai Red Cross Society, Bangkok, Thailand, which is a WHO reference laboratory. Baby Hamster kidney cell (BHK-21) and challenge virus standard (CVS-11) were used in the study. Serum samples were inactivated by exposure to 56 Celsius for 30 min. The sera then were diluted at 2-fold in the 96 well plates. Diluted sera were mixed with a constant dose of CVS-11 and incubated at 37 Celsius for 90 min. Then 50 μl of cell suspension 10^5 cells/ml were added into 96 well plates, and incubated in the CO_2 incubator at 37 Celsius. After 21 h in the incubator, the monolayers of the cells were fixed with acetone and stained with a fluorescent antibody in order to detect the presence of non-neutralized virus. RVNA titers were calculated by Spearman-Karber. The limit of detection of the assay for RVNA titer was 0.03 IU/ml [12,20–22].

2.6. T cell specific response to rabies vaccine antigen [using interferon-gamma enzyme-linked immunospot assay]

T cell specific response to rabies vaccine antigen was measured in peripheral blood mononuclear cells (PBMCs) using the interferon-gamma enzyme linked immunospot (IFN-γ ELISpot) assay. Ninety-six-well nitrocellulose membrane plates (MAIP S45; Millipore, Bedford, MA, U.S.A.) were coated with 100 μl/well of 5 μg/mL of anti-human IFN-γ (1-D1K) monoclonal antibody (mAb) (Mabtech, Stockholm, Sweden) in phosphate buffer saline (PBS) and left at 4 Celsius overnight. 2.5×10^5 PBMCs/well were cultured with PVRV at a final concentration of 0.1 and 0.25 IU/ml and incubated at 37 °C with 5% CO_2 for 40 h. Culture medium alone served as a negative control and phytohemagglutinin (PHA) as a

positive control. After incubation, the plates were washed and incubated with 1 μg/mL anti-human IFN-γ-biotinylated mAb (7-B6-1 biotin; Mabtech, Stockholm, Sweden) in PBS for 3 h at RT. After wash, the plates were incubated with 100 μl/well of streptavidin-alkaline phosphatase (ALP; Mabtech, Stockholm, Sweden) diluted 1:1000 in PBS for one hour at RT. 100 μl of the substrate solution (5-bromo-4-chloro-3-indolyl-phosphate/nitro blue tetrazolium; BCIP/NBT) were added into each well after the plates were washed. When the spots became visible, the mean numbers of cytokine-producing and spot-forming cells (SFCs) were calculated from duplicate assays. PVRV-specific responses were calculated by subtracting the negative control value and expressed as SFCs per 10^6 input cells. The validation assay was performed with the standard ELISpot protocol testing with frozen PBMCs that had a response to the PVRV. In addition, for the IFN-γ ELISpot assay, each plate had one well that was negative control and one well that was the positive control. The culture medium alone was used as the negative control and PHA as the positive control. By having these controls, we were able to check for the quality and quantity of the PBMCs [23,24].

The number of IFN-γ secreting cells of 73 SFCs per 10^6 PBMCs was used as a cut off titer for statistical analysis. This number was calculated from the mean + 1.5SD at baseline before primary rabies immunization. The sensitivity analysis was performed using mean and standard deviation (SD) as a cutoff titer of 23 and 33 SFCs per 10^6 PMBCs, respectively.

2.7. Statistical methods

The sample size of this study was calculated based on the assumption that children in 2-dose group and 3-dose group will have RVNA titer ≥0.5 IU/ml after the booster rabies vaccination of 77% and 99%, respectively, with 80% power of test, alpha 0.05, and the ratio of participants in 2-dose and 3-dose regimen 3:1 using two independent proportion without continuity correction formula, which indicated that at least 100 children were needed for the study. When 10% of the children who may be lost to follow-up were accounted, this then indicated that 110 children were needed for the study.

2.8. Outcome measures

The primary endpoint was to compare the proportion of children who had RVNA titer ≥0.5 IU/ml between 2-dose and 3-dose primary rabies vaccination at day 7 (day 372) post booster rabies vaccination. Adequate antibody titer was defined as having RVNA titer ≥0.5 IU/ml by using the rapid fluorescent focus inhibition test [2].

The secondary endpoints were to compare the proportion of children who had RVNA titer ≥0.5 IU/ml between 2-dose and 3-dose primary rabies vaccination at day 14 (day 42) and 1 year (day 365) post primary rabies vaccination. We also compared the geometric mean titer (GMT) of RVNA between 2-dose and 3-dose primary rabies immunization at day 14 (day 42), 1 year (day 365) post primary rabies vaccination and day 7 (day 372) post booster vaccination. Moreover, we compared the number of IFN-γ secreting cells at day 14, 1 year post primary rabies vaccination and day 7 post booster rabies vaccination.

All analyses were conducted using Stata version 15.1 (Stata Corp., College Station, Texas). The per-protocol analysis was used for the immunogenicity results. Participants who had baseline RVNA ≥0.5 IU/mL were excluded from the analysis. We performed descriptive analyses for the demographic and clinical characteristics of the children. Continuous values were presented as median and interquartile range (IQR). Categorical data was presented as number and percentage. The GMT and 95% confidence intervals (95%CI) were calculated. Independent two sample *t*-test was used to compare GMT between 2-dose group and 3-dose group. The Fisher-Exact test was used to compare the proportion of children who had RVNA ≥0.5 IU/ml between 2-dose and 3-dose groups. P values of <0.05 were considered statistically significant.

3. Results

3.1. Study populations

From September to October 2017, 110 Thai children were assessed for eligibility and enrolled in to the study. However, 3 children were subsequently excluded because they had ≥0.5 IU/ml RVNA titers at baseline. All of them had low positive rabies antibody titer (range from 1.0 to 2.4), that possibly from ever exposed to rabies vaccine. Therefore, 107 children received the vaccine according to the study protocol. 78 children in the 2-dose group and 29 children in 3-dose group were vaccinated. 51% of them were males with median age of 5.8 years (IQR 4.4–7.3). Baseline characteristics between 2-dose and 3-dose groups were not statistically significant (Table 1).

There were 5 participants who completed 2 or 3 doses of the rabies vaccination but prematurely discontinued from the study. Four participants who were lost to follow-up: 3 children in 2-dose group and 1 child in 3-dose group. 1 participant from the 2-dose regimen was bitten by the rat on day 32 of the follow-up. She received 1 PVRV booster dose immediately, however the laboratory result subsequently showed that she had high titers of RVNA (29.5 IU/ml). Therefore, 102 participants (74 children in 2-dose group and 28 children in 3-dose group) were included in per-protocol analysis (Fig. 1).

3.2. Rabies virus neutralizing antibody

The proportion of participants who had RVNA titer ≥0.5 IU/ml in 3-dose rabies vaccine regimen and 2-dose rabies vaccine regimen were 100% at day 14 post primary rabies immunization and at day 7 post booster rabies vaccination (Fig. 2).

However, only 59 children (80%) who received 2 doses of the rabies vaccine regimen maintained RVNA ≥0.5 IU/ml at 1 year (pre-booster) compared to 100% among children who received 3-dose regimen (p = 0.01). 15 children who received 2 doses of the rabies vaccine had RVNA titers <0.5 IU/ml at 1 year pre-booster vaccination; 60% were males and 60% were children aged >6 years.

Table 1
Demographic characteristics of the eligible participants at enrollment.

	Total (N = 107)	Rabies vaccine 2-dose regimen (N = 78)	Rabies vaccine 3-dose regimen (N = 29)
Age, median (IQR), year	5.8 (4.4–7.3)	6 0.0 (4.4–7.3)	5.8 (4.4–7.8)
Gender male, n (%)	55 (51)	37 (47)	18 (62)
Weight, median (IQR), kilograms	19.2 (15.9–24.4)	18.9 (15.8–24.2)	19.6 (17.1–24.4)
Height, median (IQR), centimeters	113 (105–126)	113 (104–126)	115 (105–127)

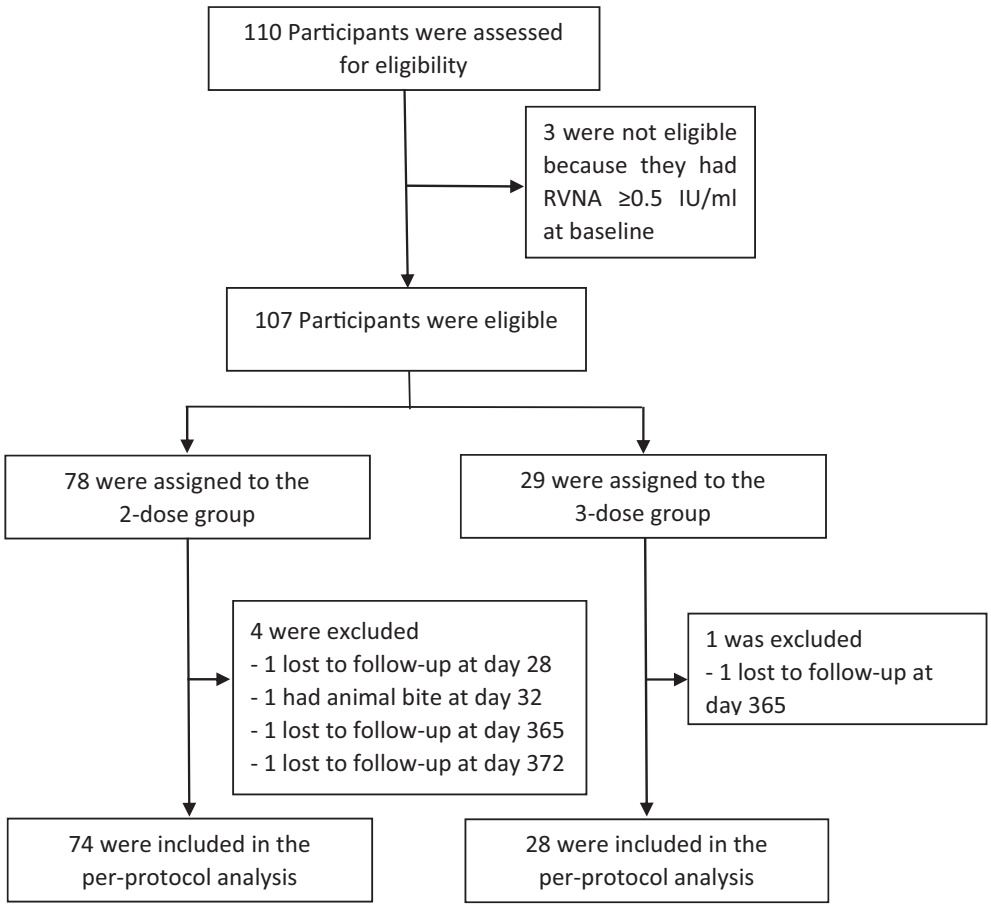

Fig. 1. Enrollment, randomization, and follow-up.

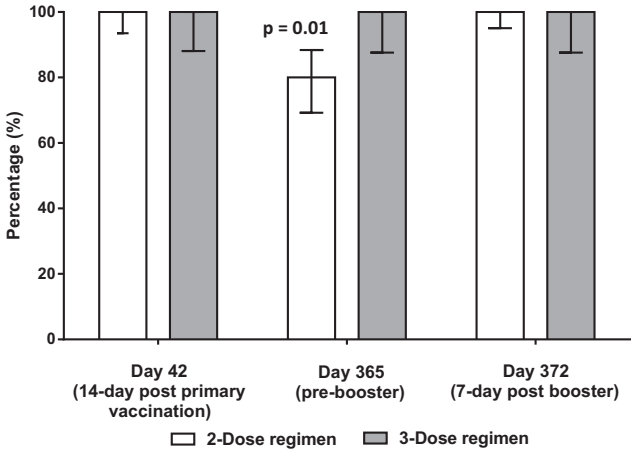

Fig. 2. The proportion of participants who had RVNA titer ≥0.5 IU/ml compared between 2-dose regimen and 3-dose regimen at day 42, day 365, and day 372.

The GMT of RVNA at day 14 post primary rabies immunization in 15 seronegative children was significantly lower than 59 children who had adequate antibody titer; 9.4 IU/mL (95% CI 6.6–13.3) versus 22.5 IU/ml (95% CI 19.3–26.3), respectively ($p < 0.01$).

The GMT of RVNA at day 14, 1 year post primary rabies vaccination and day 7 post booster rabies vaccination are also shown in Table 2. At day 7 post booster rabies vaccination, the GMT of RVNA in 2-dose group significantly increased to 20.9 IU/ml (95% CI 17.4–25.3) and for those in the 3-dose group, it increased to 22.2 IU/ml (95% CI 15.8–31.4) ($p = 0.75$).

Exploratory statistical analysis was performed to find whether there was any association between age and sex to RVNA. Children were divided according to their age. One group had children aged 2–6 years and the other group had children aged >6–12 years. The GMT of RVNA between 2-dose and 3-dose group were compared. There were no significant differences between the younger and older participants at all 3 time points. Also, the sex of the participants was not associated with GMT of RVNA level.

3.3. T cell specific response to rabies vaccine antigen

T cell specific response to rabies vaccine antigen at day 14, 1 year post primary rabies vaccination and day 7 post booster rabies vaccination are shown in Table 3. The median number of IFN-γ secreting cells at day 14 post primary rabies immunization between 2-dose and 3-dose groups were 92 SFCs per 10^6 PBMCs (IQR 40–152) and 121 SFCs per 10^6 PBMCs (IQR 12–236), respectively ($p = 0.86$). At day 7 post booster immunization, the median number of IFN-γ secreting cells in 2-dose and 3-dose groups increased to 98 SFCs per 10^6 PBMCs (IQR 72–138) and 128 SFCs per 10^6 PBMCs (IQR 90–188), respectively ($p = 0.30$).

The proportion of participants who had IFN-γ secreting cells ≥73 SFCs/10^6 PBMCs (mean + 1.5SD) at day 14 post primary rabies vaccination between 2-dose group and 3-dose group were 62% and 67%, respectively ($p = 0.76$). At day 7 post booster rabies immunization, the proportion of children who had IFN-γ secreting cells ≥73 SFCs/10^6 PBMCs were increased in both 2-dose and 3-dose groups to 69% and 90%, respectively ($p = 0.40$). This increase may be due to the booster effect of rabies vaccine.

Table 2
GMT of RVNA compared between 2-dose regimen and 3-dose regimen at day 42, day 365, and day 372.

	Rabies vaccine 2-dose regimen	Rabies vaccine 3-dose regimen	P-value
Day 42 (14-day post primary)	N = 76	N = 29	
GMT (95% CI), IU/ml	18.6 (15.9–21.8)	16.3 (13.2–20.1)	0.35
Min-Max	4.6–100.0	5.2–32.2	
Day 365 (Pre-booster)	N = 75	N = 28	
GMT (95% CI), IU/ml	0.8 (0.71–0.97)	1.7 (1.28–2.25)	<0.01
Min-Max	0.2–4.6	0.5–8.1	
Day 372 (7-day post booster)	N = 74	N = 28	
GMT (95% CI), IU/ml	20.9 (17.4–25.3)	22.2 (15.8–31.4)	0.75
Min-Max	1.7–87.8	0.9–77.7	

GMT = Geometric mean titer

Table 3
T cell specific response to rabies vaccine antigen using Interferon-gamma enzyme linked immunospot assay (IFN-γ ELISpot) compared between 2-dose regimen and 3-dose regimen at day 42, day 365, and day 372.

	Rabies vaccine 2-dose regimen	Rabies vaccine 3-dose regimen	P-value
Day 42 (14-day post primary)	N = 26	N = 12	
IFN-γ secreting cells (IQR), SFCs/10^6 PBMCs	92 (40–152)	121 (12–236)	0.86
Proportion of participants*, n (%)	16 (62)	8 (67)	0.76
Day 365 (Pre-booster)	N = 16	N = 3	
IFN-γ secreting cells (IQR), SFCs/10^6 PBMCs	21 (10–27)	8 (8–10)	0.18
Proportion of participants*, n (%)	0	0	NA
Day 372 (7-day post booster)	N = 29	N = 10	
IFN-γ secreating cells (IQR), SFCs/10^6 PBMCs	98 (72–138)	128 (90–188)	0.30
Proportion of participants*, n (%)	20 (69)	9 (90)	0.40

SFCs = Spot forming cells, PBMCs = Peripheral blood monoclonal cells
* Proportion of participants who had IFN-γ secreting cells ≥73 SFCs/10^6PBMCs.

The sensitivity analysis to determine the cut off point for IFN-γ secreting cells was evaluated by using mean plus different SD. The result showed that the proportion of participants who had IFN-γ secreting cells ≥56 SFCs/10^6 PBMCs (mean + 1SD) at post primary vaccination in 2-dose group was 65% and 3-dose group was 67% (p = 0.94). After the booster vaccination, the proportion of participants who had IFN-γ secreting cells ≥56 SFCs/10^6 PBMCs in 2-dose group and 3-dose group increased to 86% to 90%, respectively (p = 0.76).

4. Discussion

This study showed that all children who received 2 doses or 3 doses of purified Vero cell rabies vaccine achieved RVNA titers ≥0.5 IU/ml at day 14 post primary rabies vaccination and at day 7 post booster dose in the subsequent year. Only 80% of the children who received 2-dose regimen maintained RVNA titers ≥0.5 IU/ml at 1 year post primary vaccination. However, after 7 days post booster vaccination, all of the participants had RVNA titers ≥0.5 IU/ml. This mimics the real life situation that children do not need to maintain high RVNA all the time like veterinarians or rabies laboratory workers [8,9]. They just need a booster rabies vaccination after an animal bite to increase the immune response.

A study conducted in Thailand among children aged 5–8 years who received 2 doses of the regimen were compared to those who received 3 doses of the regimen; they used purified chick embryo cell rabies vaccine which was administered intradermally. Only 7% and 35% of the children who received 2-dose and 3-dose regimens achieved RVNA titer ≥0.5 IU/ml at 1 year post primary immunization and this increased to 100% post booster immunization [19]. The proportion of children who maintained RVNA titer ≥0.5 IU/ml at 1 year after primary vaccination was quite low compared to our study. This discrepancy may be due to the different dose of the vaccine that was used only 0.1 ml and the site of vaccination. In another study conducted in France, adults aged 15–65 years who received 2 doses of the regimen and 3 doses of the regimen of PVRV intramuscularly showed that the proportion of participants who achieved RVNA titer ≥0.5 IU/ml at 1 year post primary vaccination were 46.6% and 87.9%, respectively, and this increased to 100% post booster immunization in both groups [14].

Moreover, our study showed that the GMT of RVNA after booster rabies immunization were anamnestic response in both 2-dose and 3-dose groups. The study among adult participants in France who received PVRV intramuscularly of 2-dose and 3-dose regimen showed that the GMT of RVNA pre-booster vaccination of 1.3 IU/ml and 1.9 IU/ml and increased at post booster vaccination to titers of 24.0 IU/ml and 50.6 IU/ml, respectively. Furthermore, our study found no association between younger and older age groups; the GMT of RVNA level was consistent with the result from another large study in adult participants that showed there were no association between age groups and RVNA level [14].

Although, the role of cellular mediated immunity in rabies infection and vaccination are not clearly understood unlike RVNA [25], some studies showed that cellular immune response may play an important role in clearing the rabies virus from the central nervous system [26,27]. Our study also measured the cellular mediated response after rabies vaccination. IFN-γ, representative of type 1 cytokine from Th1 cell, increased rapidly after the booster rabies vaccination in both 2-dose and 3-dose groups. This finding was the same as the study that was conducted in adults which showed that both type 1 and type 2 cytokines were highly present after rabies vaccination [23]. However, there is no exact criteria to determine the positive CMI response for the rabies specific IFN-γ secreting cells in contrast to the pertussis specific IFN-γ secreting cells using stimulation index criteria [28]. Sensitivity analysis was performed to determine the positive CMI response and the booster effect of the IFN-γ secreting cells. The result showed that

the booster effect of the IFN-γ secreting cells were not dependent on the cut off point value.

According to WHO recommendation for the route of rabies vaccine injection, both intradermal and intramuscular routes were equally recommended. In our study, intramuscular injection was preferred for the reason that this regimen was more practical to use in real community setting and some studies showed that the GMT of RVNA of intramuscular injection group was higher than the intradermal injection group [18,29]. The study among Thai children aged 5–8 years who received 2 doses or 3doses of the purified chick embryo cell rabies vaccine intradermally showed that the GMT of RVNA at day 7 post booster immunization were lower (4.7 IU/ml and 11.0 IU/ml, respectively) [19] compared to our study. For our study, the GMT of RVNA at post booster vaccination for 2 doses and 3 doses of the regimen were 20.9 IU/ml and 22.2 IU/ml, respectively. Although, intradermal regimen was more economical compared to intramuscular regimen, however intradermal regimen was only suitable for a large center that had many patients per day, otherwise the cost of the vaccine was similar to the intramuscular regimen that used 1 vial per visit.

The strength of this study was its coverage of a large range of ages compared to other 2-dose studies which had a much narrower age range [18,19]. We included the children from toddler aged 2 years until adolescent aged 12 years. Moreover, to our knowledge this study was the first study to access the cellular immunity in children after rabies vaccination. This finding can be used for reference and further studies.

The limitation of this study were the schedule date of pre-exposure prophylaxis. WHO recently recommended the 2 doses of pre-exposure rabies prophylaxis be administered on days 0 and 7. 2-dose pre-exposure rabies prophylaxis studies conducted in adults were done at days 0 and 7 [15–17]. However, most of the studies done in children were vaccinated on days 0 and 28 which was the same as our study [18,19]. The 2-dose regimen administered on days 0 and 7 are more appropriate for travelers or other people who are exposed to animal for a short period of time. Regimen administered on days 0 and 28 are easier to be included in the Expanded Programme on Immunization (EPI) because it can be integrated with other vaccine schedule or clinic visit for the child (every 1–3 months) [18,30,31]. Although, pre-exposure rabies vaccination is recommended by Pediatric Infectious Disease Society of Thailand (PIDST) as an optional vaccine [32], this vaccine is in the pipeline to be included in the EPI.

Another limitation of our study were the target population included only the healthy children. We could not suggest to apply our 2-dose regimen of pre-exposure rabies vaccination to non-healthy or immunocompromised children.

Other schedule for pre-exposure rabies prophylaxis such as a single dose of rabies vaccine was previously studied in an adult population and the result showed that all participants had RVNA titer $\geq$0.5 IU/ml after booster rabies vaccination [29,33,34]. However, there are no studies being conducted in children using a single dose of the rabies vaccine. Therefore, other regimens, including single dose regimen, should be further studied in the pediatric population.

In conclusion, primary rabies immunization using 2-dose of intramuscular regimen on days 0 and 28 can provide adequate antibody titer at post primary vaccination, and especially post booster. The results support WHO's recommendation of 2-dose regimen of pre-exposure rabies immunization.

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Declaration of Competing Interest

None.

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