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Intradermal post-exposure rabies vaccination with purified Vero cell rabies vaccine: Comparison of a one-week, 4-site regimen versus updated Thai Red Cross regimen in a randomized non-inferiority trial in the Philippines

Beatriz P. Quiambao [a], Cristina Ambas [a], Sherylle Diego [a], Valérie Bosch Castells [b], Joanna Korejwo [b], Céline Petit [b], Guy Houillon [b,*]

[a] Research Institute for Tropical Medicine, Alabang, Muntinlupa City, Philippines
[b] Sanofi Pasteur, 14 Espace Henry Vallée, 69007 Lyon, France

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ABSTRACT

Background: Rabies post-exposure prophylaxis (PEP) via intradermal (ID) administration is standard practice in Asia. Accumulating evidence suggests that PEP shortened to 3 visits in one week does not adversely affect seroconversion rates or immune memory.
Objective: To determine whether the seroconversion rate at Day14 with a 1-week, 4-site (4-4-4-0-0) ID vaccination regimen with or without rabies immunoglobulin (RIG) was non-inferior to the updated Thai Red Cross (TRC) 28-day, 2-site (2-2-2-0-2) ID regimen with RIG during rabies PEP. We also assessed one-year antibody persistence.
Methods: This phase III, mono-center, open-label, randomized-controlled trial assigned participants aged ≤50 years (n = 600) exposed to suspected rabid animals and sustaining WHO Category II injuries (automatic allocation to G1) or Category III injuries (randomized to G2 or G3) to the following groups (1:1:1 ratio): G1 (n = 200), 1-week 4-site ID regimen with the purified Vero cell rabies vaccine (PVRV; Verorab®) without RIG; G2 (n = 201), 1-week 4-site ID regimen with PVRV, and purified equine rabies immunoglobulin (pERIG); G3 (n = 199), TRC 28-day, 2-site ID regimen with PVRV, and pERIG. Non-inferiority tests compared G1 vs. G3 and G2 vs. G3. Seroconversion rate was the proportion (%) of vaccinees with rabies virus neutralizing antibodies (RVNA) titers ≥0.5 IU/mL measured by rapid fluorescent focus inhibition test.
Results: On Day14, after the third vaccine administration, seroconversion rates were non-inferior in both comparisons and were, respectively, 100%, 99.4%, 98.8% in G1, G2, G3 with a decrease to 97.6%, 89%, 79.8% at Year 1. At Day14, RVNA geometric mean titers were 11.3 IU/mL; 9.89 IU/mL; 6.15 IU/mL, respectively, decreasing to 2.96 IU/mL, 1.37 IU/mL, 0.97 IU/mL at Year1. Safety and tolerability were similar between the three groups.
Conclusion: The seroconversion rate at Day 14 with the 1-week 4-site ID regimen, both with and without pERIG, was non-inferior to the reference TRC 28-day 2-site ID regimen with pERIG during rabies PEP with PVRV.
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1. Introduction

Rabies is a viral zoonotic and human infection that occurs following a transdermal bite or scratch from an infected animal (dogs are responsible for transmission in 99% of cases in rabies endemic areas). Clinical presentation is acute encephalitis, either the furious form with hydrophobia or the lesser-known and often misdiagnosed

Abbreviations: AE, adverse event; CI, confidence interval; ID, intradermal; IM, intramuscular; LLOQ, lower limit of quantification; PEP, post-exposure prophylaxis; pERIG, purified equine rabies immunoglobulin; PVRV, purified Vero cell rabies vaccine; RFFIT, rapid fluorescent focus inhibition test; RIG, rabies immunoglobulin; RVNA, rabies virus neutralizing antibodies; SAE, serious adverse event; TRC, Thai Red Cross; WHO, World Health Organization.

* Corresponding author.
E-mail address: Guy.Houillon@sanofi.com (G. Houillon).

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paralytic form. Once established, the disease is almost always fatal [1].

Annual estimates of global rabies mortality are 59,000 [2,3], with 99% of deaths occurring in Africa and Asia and around 40% of deaths occurring among children aged <15 years [1]. In non-endemic areas, the incidence of dog bites is approximately 16 per 1000 per year in the USA and Europe [4,5]. In rural endemic areas, the burden of dog bites is as high as 48 per 1000 per year [6]. The highest incidence is among children aged 6–12 years who commonly sustain injuries to the head [6].

For immunologically naïve individuals with Category II injuries, World Health Organization (WHO)-recommended rabies post-exposure prophylaxis (PEP) requires thorough wound washing and immediate vaccination with one of three regimens: (a) "Institut Pasteur Cambodia 2-2-2-0-0" (2-sites intradermal [ID] on days 0, 3 and 7; total duration 7 days), (b) "Essen 1-1-1-1-0" (1-site intramuscular [IM] on days 0, 3, 7 and between day 14–28; total duration up to 14–28 days), or (c) "Zagreb 2-0-1-0-1" (2-sites IM on days 0 and 1-site IM on days 7, 21; total duration 21 days) [1]. For those with Category III injuries, rabies immunoglobulin is additionally recommended [1].

In 2009, the WHO acknowledged an interest in a 1-week 4-site (4-4-4-0-0) ID regimen and recommended that manufacturers explore this regimen as an alternative to the widely used updated Thai Red Cross (TRC) 28-day 2-site (2-2-2-0-2) ID regimen [7]. The updated TRC regimen was the ID regimen recommended by the WHO until 2018 [1,8]. Although a 1-week 4-site ID PEP regimen requires a total vaccine volume of 1.2 mL, which is higher than that for 2-site ID regimens (0.8 mL for the updated TRC and 0.6 mL for Institut Pasteur Cambodia regimens), compared to the TRC regimen, it reduces both the number of clinic visits (from 4 to 3 visits) and the duration of a complete PEP (from 28 to 7 days). Thus, compared with the updated TRC and IM regimens, a 1-week 4-site ID regimen may reduce clinic costs, vaccine transportation expenses, loss of daily wages, and poor compliance among vaccinees. Based on data from Thailand [9], the WHO also recommend a single-visit, 4-site ID PEP booster vaccination comprising four 0.1 mL injections for previously fully immunized individuals [7]. The 1-week 4-site ID primary PEP has been shown to be immunogenic and well tolerated [9–12].

The current study aimed to identify whether the seroconversion rate at Day 14 with a 1-week 4-site (4-4-4-0-0) ID vaccination regimen is non-inferior to the updated TRC 28-day 2-site (2-2-2-0-2) ID regimen during rabies PEP with purified Vero cell rabies vaccine.

2. Methods

2.1. Study setting, design and participants

The study is an ongoing 5-year Phase III, open-label, randomized controlled trial that began on 29 June 2012 at a single center, the Animal Bite Treatment Center at the Research Institute for Tropical Medicine, Muntinlupa City, The Philippines. The study is expected to complete on 30 October 2018.

Participants (aged ≤50 years) exposed to a suspected rabid animal within 48 h of presentation to the study site were allocated in a 1:1:1 ratio to one of the following groups. Group 1: participants with WHO Category II exposure received PEP with purified Vero cell rabies vaccine (PVRV) (Verorab®), using a 1-week, 4-site (4-4-4-0-0) ID vaccination regimen. Group 2: participants with WHO Category III exposure received PEP with PVRV, using a 1-week, 4-site (4-4-4-0-0) ID vaccination regimen, and purified Equine Rabies Immunoglobulin (pERIG; Favirab®) at Day 0. Group 3: participants with WHO Category III exposure received PEP with PVRV, using the updated 2-site TRC (2-2-2-0-2) ID vaccination regimen, and pERIG at Day 0. All participants received a single-visit, 4-site ID booster vaccination five years later.

Participants with WHO Category III exposure were randomly assigned to Groups 2 or 3. Randomization was prepared by the Biostatistics platform of the sponsor and the investigator was given a scratchable list. Once opened, the randomization list was dated and signed for the corresponding patient number by the investigator. Participants with WHO Category II exposure were automatically assigned to Group 1.

Key exclusion criteria were receipt of chloroquine or other medications used for malaria chemoprophylaxis, prior mammalian bite within the past 5 years, previous rabies immunization, self-reported seropositivity for Human Immunodeficiency Virus, Hepatitis B virus, or Hepatitis C virus, and participation or planned participation in another clinical trial investigating a vaccine, drug or medical device.

All participants provided blood samples for immunogenicity assessment at Day 0 for baseline (pre-vaccination), Day 14 and Day 90 after initiation of vaccination, and at Years 1, 2, 3, and 4 after the last dose of primary vaccination. At Year 5 after the last dose of primary vaccination, one sample was collected before booster vaccination and one at Day 11 ± 3 post-booster. Here, we present results up to 1 year of follow-up.

The study is being conducted per the Declaration of Helsinki and the International Council on Harmonisation guidelines for Good Clinical Practice. The Institutional Review Board of the Research Institute for Tropical Medicine approved the trial protocol with Level 3 Accreditation from the Philippines Health Research Ethics Board. Adult and adolescent (aged 12 to <18 years) participants or parents or a legally acceptable representative of minors provided a signed informed consent/assent form.

2.2. Vaccine

The PVRV Verorab®, manufactured by Sanofi Pasteur, is a freeze-dried inactivated rabies virus reconstituted with diluent. PVRV (lot numbers G1087F01, potency 7.7 IU/dose, and G1553F01, potency 9.1 IU/dose) was administered by intradermal injection at a dose of 0.1 mL per injection site using insulin syringes and needles provided by Sanofi Pasteur. For Groups 1 and 2, injections were administered at both deltoids and both anterior thighs on Days 0, 3 and 7. For Group 3, injections were administered at both deltoids on Days 0, 3, 7 and 28. For Groups 2 and 3 (Category III exposures), pERIG Favirab® (Sanofi Pasteur, lot numbers G10G9709; 2/H-8058–187; G1553) was administered on Day 0 at a dose of 40 IU/kg of body weight and infiltrated around and into the wound as much as anatomically feasible with the remainder administered intramuscularly (gluteal region for Group 2 and thigh for Group 3).

2.3. Immunogenicity

The primary immunogenicity objective was to show that PEP using the 1-week, 4-site (4-4-4-0-0) ID vaccination regimen was non-inferior to PEP using the updated TRC 28-day 2-site (2-2-2-0-2) ID vaccination regimen by comparing seroconversion rates at Day 14 after the start of vaccination between Group 1 versus Group 3, and Group 2 versus Group 3. Seroconversion rate was defined as the proportion of vaccinees achieving rabies virus neutralizing antibody (RVNA) titers ≥0.5 IU/mL.

RVNA was measured using the rapid fluorescent focus inhibition test (RFFIT) method, which is based on the ability of specific anti-rabies antibodies present in test serum to inhibit virus infectivity of Baby Hamster Kidney (BHK)-21 cells in culture [13]. The RFFIT was performed in 8-well LabTek chamber slides. Two-fold dilutions of test serum samples and controls were incubated with

a fixed predetermined amount of rabies Challenge Virus Standard-11 before adding diethylaminoethyl treated BHK-21 cells. After incubation, the un-neutralized rabies virus was detected using a fluorescein isothiocyanate conjugated anti-rabies nucleocapsid monoclonal antibody. An automated cell imaging reader was used to scan the slides that were then manually scored for viral-infected cells (foci) by counting the number of positive fields. The RVNA titer at the Effective Dose 50% was mathematically interpolated using the Reed & Muench method [14]. The endpoint neutralizing titer of the test serum was reported in IU/mL after calibration with the endpoint neutralizing titer of the WHO standard rabies immune globulin with an assigned value of 2.0 IU/mL and tested in the same assay run. The lower limit of quantification (LLOQ) of the assay was 0.2 IU/mL. Each serum sample was tested in two independent assay runs and each value was reported. The geometric mean of the two values was then calculated for immunogenicity analysis according to rules governing the handling of extreme values: if a value was <LLOQ, then the computed value LLOQ/2 was used; if a value was between ≥LLOQ, then the value itself was used. The RFFIT testing was conducted by Global Clinical Immunology, Sanofi Pasteur, USA [15,16].

The rabies RFFIT method was validated per the International Council on Harmonisation guidelines and shown to be precise, accurate, linear, specific, and robust to quantitate specific RVNA levels in human serum samples [16,17]. RVNA titers were considered available for analysis if each of the two independent tests gave results within pre-defined acceptance criteria (±30%), the sample was not hemolyzed, and if the sample was drawn within the relevant visit window (±0 days at Day 14, ±3 days at Day 90, and ±15 days at Year 1).

Secondary immunogenicity objectives included: seroconversion rates at Day 90; geometric mean titers (GMTs) at Day 14 and Day 90; geometric mean titer post-/pre-primary immunization ratios (GMTRs) at Day 14/Day 0 and Day 90/Day 0; and antibody persistence evaluated as seroconversion rate at Year 1, GMTs at Year 1, and GMTRs at Year 1/Day 90.

2.4. Safety

The following safety outcomes were recorded: unsolicited immediate systemic adverse events (AE) occurring within 30 min after each vaccination; solicited (prelisted in participants' diaries and the case report form) and unsolicited injection-site reactions occurring within seven and 21 days, respectively, after each vaccination. Solicited and unsolicited systemic reactions occurring between the first and second vaccination, the second and third vaccination, and the third and fourth vaccination (unsolicited only), and up to 7 days (solicited) or 28 days (unsolicited) after the remaining vaccinations. The occurrence of any serious adverse event (SAE) up to 28 days post-primary vaccination (only related or fatal SAEs were reported from 28 days post-primary vaccination to Year 1). Pregnancies and pregnancy-related or perinatal AEs/SAEs in both mother and newborn were also recorded.

2.5. Statistical methods

Sample size calculations proceeded as follows. An alpha level of 2.5% (one-sided hypothesis), a minimum clinically relevant non-inferiority margin of 5% for the seroconversion rate, and a single hypothesis power of at least 90% were chosen to calculate the sample size for each hypothesis tested. Assuming a seroconversion rate of 99% for both regimens, and a balanced 1:1 randomization, 170 participants in each group were necessary to test the null hypotheses, using the Farrington and Manning method. Assuming that almost 15% of participants were not evaluable, 200 participants in each group were necessary to be enrolled to provide an overall power of at least 80%.

For the primary immunogenicity objective, if the non-inferiority criterion was met among participants complying with the study protocol (per-protocol analysis set), the same analysis was done among participants who had received at least one dose of study vaccine and had at least one post-baseline blood sample at Day 14 or Day 90, irrespective of protocol compliance (full analysis set). For secondary immunogenicity objectives, immune responses were analyzed in the full analysis set. Missing data were not replaced.

Non-inferiority was shown if the lower bound of the two-sided 95% confidence interval (CI) for the inter-group differences in seroconversion rate was higher than the non-inferiority margin, defined as −5%. The CI of the difference in proportions was calculated using the Wilson score method without continuity correction [18]. CIs for single proportions were calculated using the exact binomial method (Clopper-Pearson [19]). Antibody titers were first $\log_{10}$-transformed to calculate 95% CIs assuming a normal distribution and were then back-transformed to obtain GMTs and their associated 95% CIs. Other than testing non-inferiority, no between group statistical comparisons were undertaken.

3. Results

3.1. Study population

Of the 600 participants enrolled, 200 were assigned to Group 1, 201 to Group 2 and 199 to Group 3. All participants except for two in Group 3 received the full primary vaccination (Fig. 1). One participant in Group 1 (inclusion criterion: WHO category II bite) with a WHO Category III bite was allocated to that group in error. Therefore, this participant was excluded from all analyses.

During the primary vaccination phase up to Day 90, 25 participants (4.2%) discontinued the study; 15 (2.5%) because of repeat animal bites requiring booster vaccination, 9 (1.5%) due to missed visits, relocation, or refusing follow-up, and 1 (0.2%) due to non-compliance with the protocol. Five participants who discontinued due to missed visits during the primary vaccination phase re-entered the study. From Day 90 to Year 1, a further 44 participants discontinued; 23 (3.8%) due to repeat animal bites requiring booster vaccination and 21 (3.5%) due to missed visits, relocation, or lost to follow-up. Of 66/600 participants (11.0%) who discontinued over the entire one-year period, 38/66 (57.6%) discontinued due to repeat animal bites requiring booster vaccination (Fig. 1). No participants died or discontinued the study due to an AE or SAE.

Baseline characteristics were balanced across all three groups despite random allocation occurring only to Groups 2 and 3 (Table 1). Participants comprising the full and per-protocol analysis sets are described in Table 1. For both baseline and repeat exposure to animal bites, most participants were bitten by dogs on the lower limb (Table 2; data obtained from on-site clinical investigators).

3.2. Immunogenicity

3.2.1. Seroconversion

The primary objective of non-inferiority of the investigational regimens versus the reference regimen was met in the per-protocol analysis set (Table 3). All participants in Group 1, 99.4% of participants in Group 2, and 98.8% of participants in Group 3 seroconverted at Day 14 (7 days after the 3rd vaccine injection). The lower limit of the two-sided 95% confidence intervals of the differences between seroconversion rates were above delta (−5%) for both comparisons, demonstrating non-inferiority of the 1-

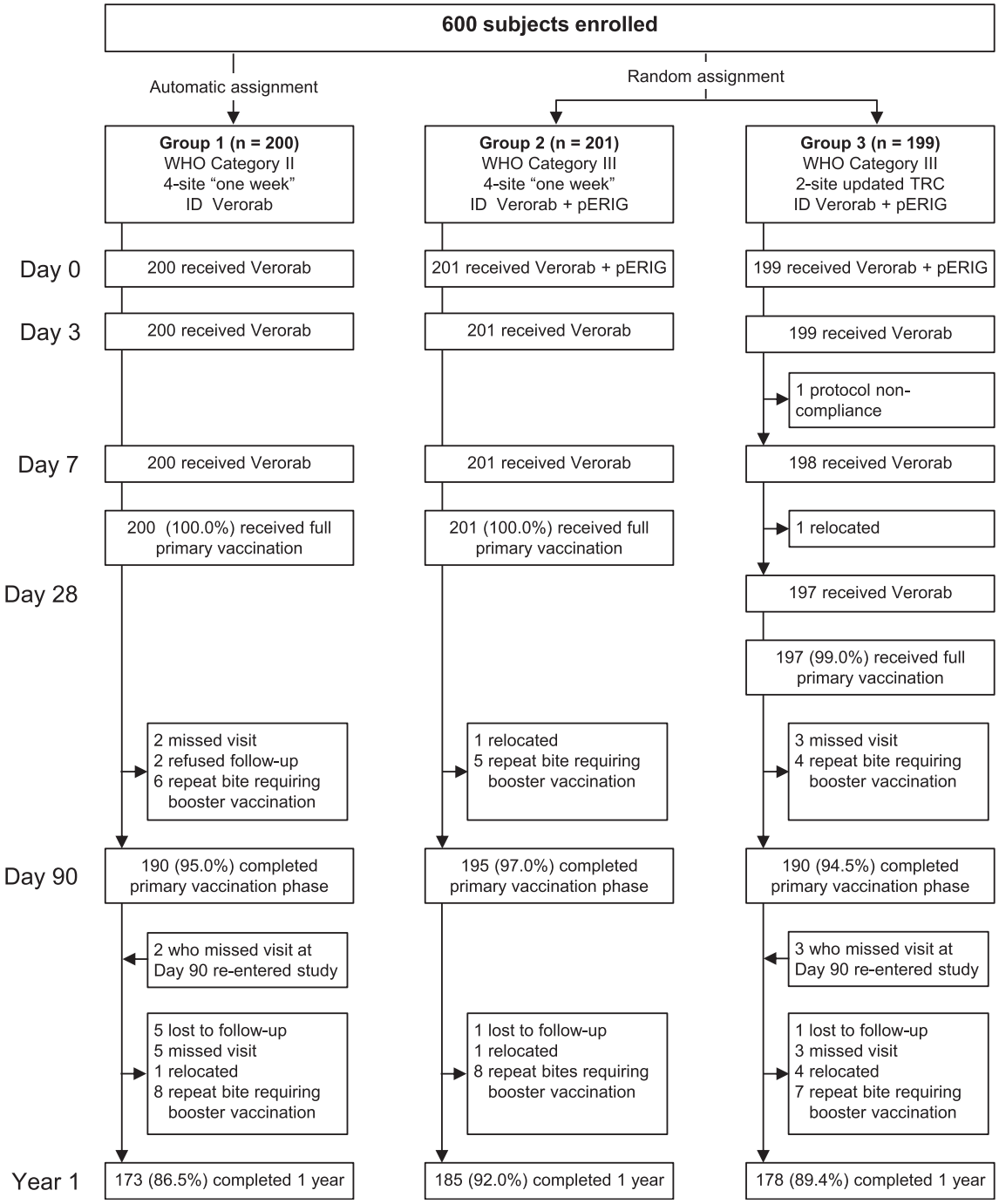

Fig. 1. CONSORT flow diagram. ID, intradermal; pERIG, purified Equine Rabies Immunoglobulin; WHO, World Health Organization. *Received full primary vaccination.

week 4-site ID regimen (after Category II or Category III exposure) versus the updated 2-site TRC regimen (after Category III exposure) (Table 3). Analysis in the full analysis set confirmed non-inferiority of the 1-week 4-site ID regimen (Table 3).

At Day 90, seroconversion rates remained high in all groups (>94%) (Fig. 2). At Year 1 (Fig. 2), the percentage of participants that showed anti-RVNA titers remaining ≥0.5 IU/mL was highest in Group 1 (97.6%), intermediate in Group 2 (89.0%), and lowest in Group 3 (79.8%).

Immunogenicity results by age groups are presented in Supplementary Figs. S1–4. All pediatric age groups (<2 years, 2–11 years, and 12–17 years) that received the 1-week 4-site regimen (Groups 1 and 2) achieved 100% seroconversion rates at Day 14, Day 90 and Year 1, except for children aged 2–11 years in Group 2, for whom the seroconversion rate at Year 1 was 98.6%. All participants in the pediatric age groups who received the updated 28-day 2-site TRC regimen (Group 3) achieved 100% seroconversion at each time point except at Year 1 for the group aged 2–11 years (98.5%) and the group aged 12–17 years (81.0%).

3.2.2. Geometric mean titers

At Day 14, GMTs had increased from baseline in all groups and appeared slightly higher in Groups 1 and 2 versus Group 3 (Fig. 3, Table 4). From Day 14–90, GMTs decreased in all three groups and remained highest in Group 1 (Fig. 3, Table 4). At Year 1, GMTs had decreased compared to D90 and remained highest in Group 1 at

Table 1
Baseline characteristics of all enrolled participants and analysis sets.

	Group 1 (N = 200)	Group 2 (N = 201)	Group 3 (N = 199)	All (N = 600)
Enrolled participants				
Male sex, n (%)	98 (49.0)	106 (52.7)	103 (51.8)	307 (51.2)
Age, years*				
Mean (SD)	17.5 (14.0)	19.2 (13.8)	19.5 (14.1)	18.7 (14.0)
Min, Max	1.0, 47.0	1.0, 48.0	0.0, 49.0	0.0, 49.0
Median	13.0	16.0	17.0	15.5
Age groups, n (%)*				
<2 years	11 (5.5)	3 (1.5)	3 (1.5)	17 (2.8)
2–11 years	84 (42.0)	79 (39.3)	74 (37.2)	237 (39.5)
12–17 years	18 (9.0)	23 (11.4)	24 (12.1)	65 (10.8)
18–50 years	87 (43.5)	96 (47.8)	98 (49.2)	281 (46.8)
Weight, kg, mean (SD)*	38.9 (20.8)	42.5 (21.4)	42.7 (20.9)	41.3 (21.1)
Concomitant medication† up to Day 56				
Antipyretic/anti-inflammatory drugs‡	69 (34.5)	50 (24.9)	68 (34.2)	187 (31.2)
Prophylactic use	1 (0.5)	1 (0.5)	1 (0.5)	3 (0.5)
Other	1 (0.5)			
Full analysis set	199 (99.5)	201 (100.0)	198 (99.5)	598 (99.7)
Group allocation error	1 (0.5)	0 (0.0)	0 (0.0)	1 (0.2)
Samples not done at Day 14 and Day 90	0 (0.0)	0 (0.0)	1 (0.5)	1 (0.2)
Full analysis set participants with available RVNA titers				
Day 0	199 (99.5)	201 (100.0)	198 (99.5)	598 (99.7)
Day 14	182 (91.0)	170 (84.6)	177 (88.9)	529 (88.2)
Day 90	188 (94.0)	193 (96.0)	189 (95.0)	570 (95.0)
1 Year	169 (84.5)	182 (90.5)	178 (89.4)	529 (88.2)
Per-protocol analysis set	175 (87.5)	162 (80.6)	172 (86.4)	509 (84.8)
Participants with at least one deviation	24 (12.0)	39 (19.4)	27 (13.6)	90 (15.0)
Participant did not complete vaccination schedule at Day 0, Day 3 and Day 7	0 (0.0)	0 (0.0)	1 (0.5)	1 (0.2)
Preparation and/or administration of vaccine was not done as per protocol at Day 0, Day 3 and Day 7	0 (0.0)	0 (0.0)	1 (0.5)	1 (0.2)
Participant did not receive injection 3 at Day 7	1 (0.5)	5 (2.5)	1 (0.5)	7 (1.2)
Participant with rabies virus neutralizing antibodies ≥0.2 IU/mL or missing value at baseline	4 (2.0)	4 (2.0)	3 (1.5)	11 (1.8)
Sample not done at Day 14	3 (1.5)	1 (0.5)	2 (1.0)	6 (1.0)
Sample at Day 14 did not produce a valid test result	17 (8.5)	31 (15.4)	21 (10.6)	69 (11.5)

SD, standard deviation. Similar baseline characteristics were observed in the per-protocol and full analysis sets.
* At Day 0.
† Up to Day 35 for Groups 1 and 2, and up to Day 56 for Group 3.
‡ Antipyretics, analgesics, non-steroidal anti-inflammatory drugs, corticosteroids and other immune modulators.

Year 1. This decrease at Year 1 appeared greater in Group 3 than in the other groups. (Fig. 3, Table 4).

Similar findings were shown for GMTRs (Fig. 4, Table 4).

3.3. Safety

Solicited injection-site reactions of tenderness/pain and swelling were similar across groups (Table 5). Erythema was reported more frequently in Group 1 and Group 2 versus Group 3 (percentage point differences 14.6% and 10.7%, respectively).

Overall, solicited systemic reactions of fever, headache, malaise, and myalgia were similar across groups (Table 5). For those systemic reactions evaluated only in children <2 years of age (vomiting, abnormal crying, drowsiness, lost appetite, and irritability), the number of children in this age group (11, 3, and 3 in Groups 1, 2, and 3, respectively) were too small to make meaningful comparisons.

Seventeen participants experienced 19 SAEs, all occurred within 28 days of the last injection and all resolved. Five SAEs among four participants were most likely reactions after pERIG (Favirab) administration, rather than being related to the vaccine. Three participants most likely had hypersensitivity after pERIG, and 1 case was most likely a procedure-related vaso-vagal reaction. The first participant developed an immediate hypersensitivity reaction with generalized urticaria, dry cough, and erythema at pERIG sites 10 min post-vaccination which resolved on Visit 1 after treatment. A second episode of itching occurred three days later after the second vaccination, but this occurred at the original pERIG injection site, not the vaccination site. This was considered by the Investigators as 1 continuous episode of hypersensitivity to pERIG. The second participant developed an immediate hypersensitivity reaction with itching and erythema at the pERIG injection site with a maculopapular rash on the chin. The third participant experienced syncope, probably of vasovagal origin (with possible seizure disorder), characterized by jerking, tonic-clonic contractions of both upper and lower extremities with upward rolling eyeballs. The patient had a medical history of several instances of loss of consciousness, with or without convulsive disorder, triggered by severe pain, fear, or emotion. The fourth participant developed erythema and swelling at the pERIG site one day after injection. Considering that in the 3 SAE cases occurring on the day of PEP initiation and accompanied with systemic symptoms, the role of each element of PEP (rabies vaccine, pERIG, and other associated treatments such as tetanus vaccine, tetanus immunoglobulins and antibiotics) cannot be assessed separately, all these treatments were considered as suspect. However, none of the subjects discontinued due to adverse event, and none experienced recurrence of symptoms after subsequent doses of Verorab. Other SAEs were: pneumonia (n = 11); urinary tract infection (n = 1); viral exanthema (n = 1); and fractured radius/ulna (n = 1), all considered unrelated to vaccination.

Pregnancy is not a contraindication for PEP, and 7 subjects received the vaccinations during pregnancy. In addition, 10 subjects reported pregnancy between 1 month after having received the last primary vaccination and up to the Y1 visit (considered unexposed pregnancies). Two women exposed during pregnancy developed AEs considered unrelated to the vaccine: one developed transient abdominal pain/headache with later onset of pregnancy-

Table 2
Characteristics of exposures to potential rabid animals among enrolled participants at study entry and on repeat exposure.*

Exposure at study entry	Group 1 (N = 200)	Group 2 (N = 201)	Group 3 (N = 199)	All (N = 600)
Biting animal				
Dog	143 (71.5)	151 (75.1)	140 (70.4)	434 (72.3)
Cat	57 (28.5)	50 (24.9)	59 (29.6)	166 (27.7)
Relationship to victim				
Family pet	88 (44.0)	103 (51.2)	97 (48.7)	288 (48.0)
Neighbor's pet	77 (38.5)	67 (33.3)	69 (34.7)	213 (35.5)
Stray	28 (14.0)	24 (11.9)	31 (15.6)	83 (13.8)
Other	7 (3.5)	7 (3.5)	2 (1.0)	16 (2.7)
Wound location				
Head and neck	0 (0.0)	5 (2.5)	14 (7.0)	19 (3.2)
Trunk	13 (6.5)	8 (4.0)	3 (1.5)	24 (4.0)
Upper limb	70 (35.0)	57 (28.4)	52 (26.1)	179 (29.8)
Lower limb	113 (56.5)	124 (61.7)	120 (60.3)	357 (59.5)
Multiple areas	4 (2.0)	7 (3.5)	10 (5.0)	21 (3.5)
Repeat exposure	Group 1 (N = 14)	Group 2 (N = 15)	Group 3 (N = 12)	All (N = 41)
Biting animal				
Dog	9 (64.3)	9 (60.0)	7 (58.3)	25 (61.0)
Cat	5 (35.7)	6 (40.0)	5 (41.7)	16 (39.0)
Relationship to victim				
Family pet	9 (64.3)	10 (66.7)	7 (58.3)	26 (63.4)
Neighbor's pet	4 (28.6)	3 (20.0)	2 (16.7)	9 (22.0)
Stray	1 (7.1)	2 (13.3)	3 (25.0)	6 (14.6)
Different animal to original bite	9 (64.3)	13 (86.7)	12 (100.0)	34 (82.9)
WHO Category				
II	9 (64.3)	4 (26.7)	5 (41.7)	18 (43.9)
III	5 (35.7)	11 (73.3)	7 (58.3)	23 (56.1)
Wound location				
Head and neck	0	1 (6.7)	0	1 (2.4)
Trunk	0	0	1 (8.3)	1 (2.4)
Upper limb	7 (50.0)	9 (60.0)	5 (41.7)	21 (51.2)
Lower limb	7 (50.0)	5 (33.3)	6 (50.0)	18 (43.9)
Days after original bite, mean (SD)	122 (1 0 3)	121 (1 2 0)	153 (1 2 0)	131 (1 1 5)
Discontinued study to receive booster	14 (100.0)	13 (86.7)	11 (91.7)	38 (92.7)
Continued in study†	0 (0.0)	2 (13.3)	1 (8.3)	3 (7.3)

Data are n (%) unless otherwise specified.
* Data obtained from on-site clinical investigators.
† Because re-exposure occurred during primary vaccination.

Table 3
Immunogenicity – Non-inferiority tests for proportions of participants with RVNA titer ≥0.5 IU/mL (RFFIT method) at Day 14 by Group.

	n/N	% (95% CI)	Non-inferiority*
Per-protocol analysis set			
Group 1	175/175	100 (97.9, 100.0)	
Group 2	161/162	99.4 (96.6, 100.0)	
Group 3	170/172	98.8 (95.9, 99.9)	
Group 1 vs Group 3		1.16 (−1.145, 4.140)	Yes
Group 2 vs Group 3		0.55 (−2.375, 3.566)	Yes
Global conclusion			Yes
Full analysis set†			
Group 1	182/182	100 (98.0, 100.0)	
Group 2	169/170	99.4 (96.8, 100.0)	
Group 3	175/177	98.9 (96.0, 99.9)	
Group 1 vs Group 3		1.13 (−1.094, 4.026)	Yes
Group 2 vs Group 3		0.54 (−2.249, 3.478)	Yes
Global conclusion			Yes

n, number of participants experiencing the endpoint; N, number of participants with available data for the relevant endpoint.
* Non-inferiority concluded if the lower limit of the two-sided 95% CI of the difference was above delta (−5%).
† includes only participants with available RVNA titers.

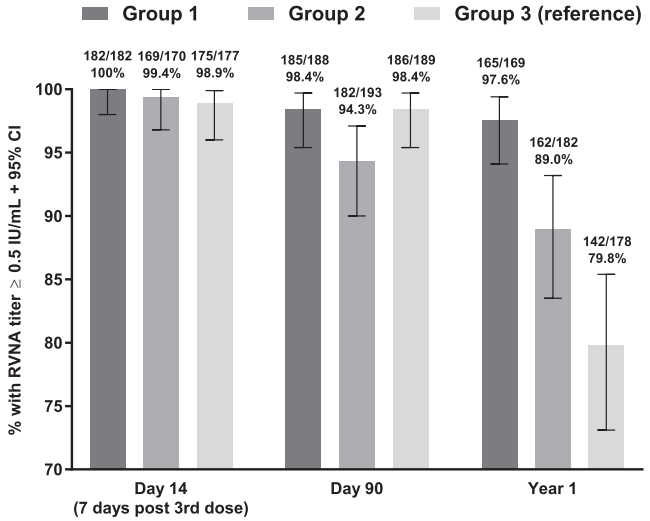

Fig. 2. Seroconversion rate (RVNA titer ≥0.5 IU/mL) (full analysis set; participants with available RVNA titers). RVNA, rabies virus neutralizing antibody.

induced hypertension/eclampsia, and the second one developed transient subchorionic bleeding. Among unexposed pregnancies all reported AEs/SAEs were considered unrelated to the vaccine: there was one case of premature delivery at 30 weeks of gestation with neonatal death due to respiratory distress syndrome, one case in which the baby was diagnosed with glucose-6-phosphate dehydrogenase deficiency, and one case of non-serious, transient subchorionic bleeding. The remaining 12 women underwent normal pregnancies without feto-maternal complications.

No PEP failures were documented up to 1 year of follow-up, despite the fact that of 5 animals that were tested, three had laboratory confirmed rabies (data from on-site clinical investigators).

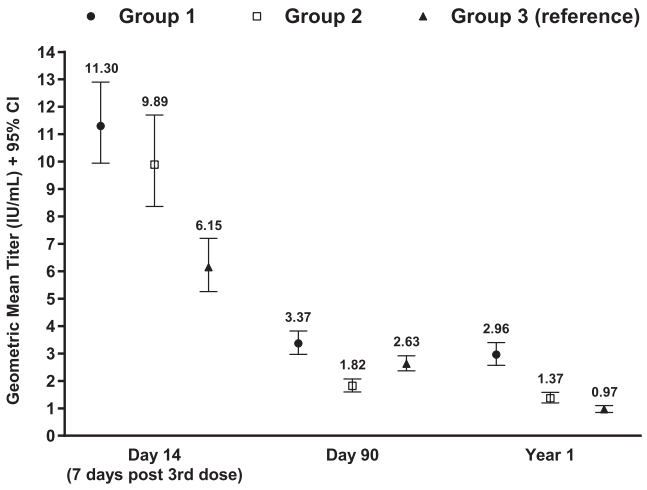

Fig. 3. Geometric Mean Titers (full analysis set; participants with available RVNA titers).

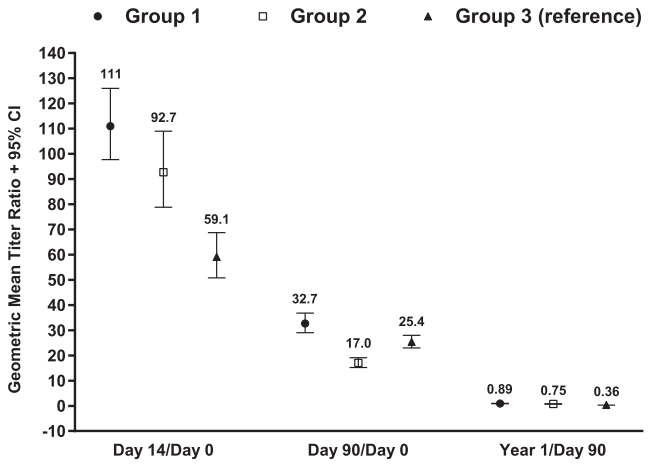

Fig. 4. Geometric Mean Titer Ratios (full analysis set; participants with available RVNA titers).

4. Discussion

The shortened 1-week 4-site ID PEP regimen showed seroprotection at Day 14 that was non-inferior to the 28-day 2-site ID TRC regimen. Although the study was not designed to test for superiority, immunogenicity at Day 14 and antibody persistence at Year 1 appeared higher with the 1-week 4-site regimen than the 28-day 2-site regimen. Other advantages of the 1-week 4-site regimen compared with the 28-day 2-site regimen included completion of primary vaccination within 1 week versus 28 days, no increase in tenderness/pain adverse reactions (Group 1 or Group 2 versus Group 3), robust immunogenicity results up to Year 1 among pediatric patients who are most at risk from animal bites, and no influence of pERIG administration on immunogenicity at Day 14 (Group 1 versus Group 2) in terms of both seroconversion rate and rabies antibody level.

Of interest, the immunogenicity results at Day 14 for Group 3 (before the 4th injection at Day 28) in the current study can serve to document the use of PVRV with the 2-2-2-0-0 Institut Pasteur Cambodia abbreviated regimen that was recommended by the WHO in their April 2018 position paper [1]. At Day 14, seroconversion rates were slightly higher, and GMTs were considerably higher in both groups receiving the 4-site regimens compared with the group receiving the 2-site regimen.

Table 4
Geometric mean RVNA titers.

	Group 1	Group 2	Group 3
Per-protocol analysis set			
Day 14			
Available data	175	162	172
GMT (95% CI)	10.80 (9.53, 12.3)	9.77 (8.31, 11.50)	5.89 (5.08, 6.84)
Min–Max	0.55–136	0.45–129	0.35–65.7
D14/D0 GMTR (95% CI)	108.0 (95.0, 123.0)	97.7 (83.1, 115.0)	58.9 (50.8, 68.4)
Min–Max	5.48–1355	4.47–1293	3.46–657
Day 90			
Available data	166	157	165
GMT (95% CI)	3.16 (2.78, 3.59)	1.76 (1.56, 1.99)	2.53 (2.28, 2.81)
Min–Max	0.10–30.0	0.10–20.6	0.39–12.6
D90/D0 GMTR (95% CI)	31.5 (27.8, 35.8)	17.6 (15.6, 19.9)	25.3 (22.8, 28.1)
Min–Max	1.00–300	1.00–206	3.87–126
Full analysis set			
Day 14			
Available data*	182	170	177
GMT (95% CI)	11.30 (9.94; 12.9))	9.89 (8.36; 11.7)	6.15 (5.26; 7.20)
Min–Max	0.55–246	0.45–282	0.35–461
D14/D0 GMTR (95% CI)	111.0 (97.7, 126.0)	92.7 (78.8, 109.0)	59.1 (50.8, 68.7)
Min–Max	5.48–1355	4.00–1293	3.46–657
Day 90			
Available data*	188	193	189
GMT (95% CI)	3.37 (2.97; 3.82)	1.82 (1.60; 2.07)	2.63 (2.37; 2.92)
Min–Max	0.10–146	0.10–160	0.39–40.7
D90/D0 GMTR (95% CI)	32.7 (29.0; 36.8)	17.0 (15.2; 19.1)	25.4 (23.0; 28.0)
Min–Max	1.00–300	1.00–206	2.75–126
Year 1			
Available data*	169	182	178
GMT (95% CI)	2.96 (2.57, 3.40)	1.37 (1.20, 1.58)	0.97 (0.85, 1.10)
Min–Max	0.10–34.4	0.10–97.2	0.10–24.2
Y1/D90 GMTR (95% CI)	0.89 (0.82, 0.98)	0.75 (0.70, 0.81)	0.36 (0.33, 0.40)
Min–Max	0.22–5.26	0.24–3.07	0.04–2.33

D0, Day 0; D14, Day 14; D90, Day 90; GMT, geometric mean titer; GMTR, GMT ratio; RVNA, rabies virus neutralizing antibody; Y1, Year 1.
* Full analysis set participants with available RVNA titers.

Table 5
Safety overview during the primary vaccination phase.

	Group 1 (N = 199)		Group 2 (N = 201)		Group 3 (N = 199)	
Participants experiencing ≥ 1 event	n/N	% (95% CI)	n/N	% (95% CI)	n/N	% (95% CI)
From Day 0 up to 28 days after last injection*						
Immediate unsolicited AE†	0/199	0 (0.0, 1.8)	2/201	1 (0.1, 3.5)	5/199	2.5 (0.8, 5.8)
Immediate unsolicited AR†	0/199	0 (0.0, 1.8)	1/201	0.5 (0.0, 2.7)	2/199	1.0 (0.1, 3.6)
Solicited reaction	164/199	82.4 (76.4, 87.4)	178/201	88.6 (83.3, 92.6)	172/199	86.4 (80.9, 90.9)
Solicited injection-site reaction	145/199	72.9 (66.1, 78.9)	166/201	82.6 (76.6, 87.6)	148/199	74.4 (67.7, 80.3)
Tenderness/pain	109/199	54.8 (47.6, 61.8)	140/201	69.7 (62.8, 75.9)	126/199	63.3 (56.2, 70.0)
Erythema	85/199	42.7 (35.7, 49.9)	78/201	38.8 (32.0, 45.9)	56/199	28.1 (22.0, 34.9)
Swelling	33/199	16.6 (11.7, 22.5)	37/201	18.4 (13.3, 24.5)	33/199	16.6 (11.7, 22.5)
Solicited systemic reaction	116/199	58.3 (51.1, 65.2)	119/201	59.2 (52.1, 66.1)	133/199	66.8 (59.8, 73.3)
All participants						
Fever	19/199	9.5 (5.8, 14.5)	14/201	7.0 (3.9, 11.4)	15/199	7.5 (4.3, 12.1)
Participants aged ≥2 years						
Headache	76/188	40.4 (33.3, 47.8)	88/198	44.4 (37.4, 51.7)	89/196	45.4 (38.3, 52.7)
Malaise	78/188	41.5 (34.4, 48.9)	83/198	41.9 (35.0, 49.1)	103/196	52.6 (45.3, 59.7)
Myalgia	66/188	35.1 (28.3, 42.4)	83/198	41.9 (35.0, 49.1)	92/196	46.9 (39.8, 54.2)
Participants aged <2 years						
Vomiting	2/11	18.2 (2.3, 51.8)	0/3	0.0 (0.0, 70.8)	0/3	0.0 (0.0, 70.8)
Abnormal crying	3/11	27.3 (6.0, 61.0)	0/3	0.0 (0.0, 70.8)	1/3	33.3 (0.8, 90.6)
Drowsiness	2/11	18.2 (2.3, 51.8)	0/3	0.0 (0.0, 70.8)	1/3	33.3 (0.8, 90.6)
Loss of appetite	5/11	45.5 (16.7, 76.6)	0/3	0.0 (0.0, 70.8)	0/3	0.0 (0.0, 70.8)
Irritability	5/11	45.5 (16.7, 76.6)	0/3	0.0 (0.0, 70.8)	1/3	33.3 (0.8, 90.6)
Unsolicited AE	105/199	52.8 (45.6, 59.9)	90/201	44.8 (37.8, 51.9)	112/199	56.3 (49.1, 63.3)
Unsolicited non-serious AE	104/199	52.3 (45.1, 59.4)	89/201	44.3 (37.3, 51.4)	110/199	55.3 (48.1, 62.3)
Unsolicited non-serious systemic AE	102/199	51.3 (44.1, 58.4)	89/201	44.3 (37.3, 51.4)	110/199	55.3 (48.1, 62.3)
Unsolicited AR	12/199	6 (3.2, 10.3)	11/201	5.5 (2.8, 9.6)	11/199	5.5 (2.8, 9.7)
Unsolicited non-serious AR	12/199	6 (3.2, 10.3)	10/201	5 (2.4, 9.0)	10/199	5 (2.4, 9.0)
Unsolicited non-serious injection-site AR	2/199	1 (0.1, 3.6)	6/201	3 (1.1, 6.4)	2/199	1 (0.1, 3.6)
Unsolicited non-serious systemic AR	10/199	5 (2.4, 9.0)	4/201	2 (0.5, 5.0)	9/199	4.5 (2.1, 8.4)
AE leading to study discontinuation	0/199	0 (0.0, 1.8)	0/201	0 (0.0, 1.8)	0/199	0 (0.0, 1.8)
SAE	5/199	2.5 (0.8, 5.8)	3/201	1.5 (0.3, 4.3)	9/199	4.5 (2.1, 8.4)
Death	0/199	0 (0.0, 1.8)	0/201	0 (0.0, 1.8)	0/199	0 (0.0, 1.8)
From Day 0 up to Day 90‡						
SAE	5/199	2.5 (0.8, 5.8)	3/201	1.5 (0.3, 4.3)	9/199	4.5 (2.1, 8.4)
Death	0/199	0 (0.0, 1.8)	0/201	0 (0.0, 1.8)	0/199	0 (0.0, 1.8)

n: number of participants experiencing the endpoint listed in the first column; N: number of participants with available data for the relevant endpoint.
Injection site reactions are collected within 7 days after each injection.
Solicited systemic reactions are collected between the first and the second injections, between the second and the third injections and within 7 days after the remaining injections.
AE, adverse event; AR, adverse reaction.
Table adapted from Clinical Study Report, Tables 6.1 and 6.3.
* From Day 0 to Day 35 (groups 1 and 2) or Day 56 (group 3).
† Occurring within 30 min of any vaccination.
‡ All SAEs up to 28 days after primary vaccination and only related or fatal during Day 35 (for group 1 and 2) or Day 56 (for group 3) to Day 90.

Given that repeat animal bites occurred in 6.3% of the population, the high rate of antibody persistence at Year 1 is encouraging. Subsequent results from this study assessing antibody persistence at Years 2, 3 and 4, and immune response to a booster vaccine at Year 5 will further evaluate the longer-term antibody persistence and immune memory of the 4-site versus the 2-site TRC regimen.

Although comparisons across studies are difficult owing to differing study designs, study populations, and RVNA assays, the current results are broadly consistent with those from other studies investigating the shortened 1-week 4-site ID regimen using Verorab or the purified chick embryo cell culture vaccine Rabipur [9,10,12,20].

Among participants receiving the 4-site regimen in the current study, seroconversion rates, GMTs, and GMTRs at Day 90 and Year 1 were slightly higher in Group 1 (not receiving pERIG) than in Group 2 (receiving pERIG). Lower RVNA titers when rabies vaccines are administered with rabies immunoglobulin (RIG) have been reported with other rabies vaccine regimens [21,22] and other 4-site regimens [9,20]. However, RIG for Category III wounds remains essential to cover the early post-exposure period (<14 days) while an adequate antibody response develops [1,23]. Recent strategies to minimize RIG use by local wound infiltration without systemic intramuscular administration seem to be effective in small-scale studies [24,25], but larger studies using this strategy are needed to evaluate vaccine efficacy and whether this approach produces less immunosuppression of RVNA response.

Overall, the local tolerability of the 4-site regimen (total of 12 injections over one week) appeared similar to that of the 2-site regimen (total of 8 injections over 28 days). Injection-site erythema appeared to be more frequent with the 4-site as opposed to the 2-site regimen, reflecting the greater number of injections; however, injection-site swelling and pain were similar between to the two regimens. Serious hypersensitivity reactions appeared most likely related to the pERIG component rather than to the Verorab component, and the frequency of 4/600 (0.7%) was comparable with a rate of 1.1% in a large series of 72,132 participants receiving either pERIG or human RIG [26].

The strengths of the current study are detailed as follows. First, this is the largest rabies vaccine PEP study to date conducted among participants across the age spectrum who were exposed to bites from potentially rabid animals, reflecting real-life exposures. Second, the study investigators provided detailed information on the frequency of repeat animal bites, which emphasizes the need for PEP regimens with adequate longer-term antibody persistence. Third, the study was designed to administer a booster vaccination at Year 1 and to follow up participants for five years.

Although these longer-term results are not yet available, they should provide valuable information on the durability of immune responses with the 1-week 4-site versus the 28-day 2-site regimen. Fourth, excluding participants discontinued due to repeat bites requiring booster vaccination, the discontinuation rates up to Year 1 were low. Fifth, optimal rabies RFFIT conditions were selected to accurately and precisely measure specific RVNA in human serum samples. These RFFIT conditions included two-fold serial dilutions of serum samples (as opposed to 5-fold serial dilution) and use of a cell imaging reader for scanning slides [15,16] that provided traceability and permanent records of the raw data meeting requirements for Good Clinical Laboratory Practice [17].

The study had limitations. First, random allocation was only undertaken to Groups 2 and 3, which could potentially bias results due to between-group baseline differences among participants. For example, pediatric participants aged ≤11 were over represented in Group 1 (95/200, 47.5%) compared to Group 2 (82/201, 40.8%) and Group 3 (77/199, 38.7%). Given that children are known to have higher RVNA responses than adults [27,28], this could potentially bias immunogenicity results. However, seroconversion at Day 14 was equivalent between the three groups across differing age ranges (Supplementary Fig. S1–4). Second, participants who were bitten again during the study and who required a booster injection discontinued study follow-up, reducing the population available for longer-term assessment of immune responses. Responsibility for administration of the booster injection was not under the control of the study personnel, so it was not possible to verify if these participants received the booster injection, except for those who opted to receive their booster at the study center.

5. Conclusions

The seroconversion rate at Day 14 with the shortened 1-week 4-site ID regimen was non-inferior to the reference TRC 28-day 2-site ID regimen during rabies PEP with PVRV. Immunogenicity at Day 14 and antibody persistence at Year 1 was higher with the 1-week 4-site ID regimen than with the 28-day 2-site regimen. The robust immunogenicity of PVRV given with the 1-week 4-site ID regimen among pediatric patients who are most at risk from animal bites, the similarity in all immunogenicity parameters at Day 14 between the 1-week 4-site ID regimen groups receiving or not receiving pERIG, the shorter primary vaccination completion time of the 1-week 4-site ID regimen, which may reduce clinic costs, vaccine transportation expenses, loss of daily wages, and poor compliance among vaccinees, and the similar frequency of tenderness/pain adverse reactions between the 4-site and 2-site regimens suggest a valuable role for the 1-week 4-site regimen in rabies PEP.

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Role of funding source

This study was sponsored by Sanofi Pasteur. The sponsor participated in operational aspects of the study including data collection and statistical analyses.

Competing interests

BPQ has received research grants from Sanofi for other research studies on rabies and is a member of Sanofi speakers' bureau for rabies. CA and SD have no conflicts of interest to declare. VBC, JK, CP, and GH are employees of Sanofi Pasteur.

Data statement

Qualified researchers may request access to patient level data and related study documents including the clinical study report, study protocol with any amendments, blank case report form, statistical analysis plan, and dataset specifications. Patient level data will be anonymized and study documents will be redacted to protect the privacy of trial participants. Further details on Sanofi's data sharing criteria, eligible studies, and process for requesting access can be found at: https://www.clinicalstudydatarequest.com.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2019.02.083.

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