Comparative immunogenicity and safety trial of 2 different schedules of single-visit intradermal rabies postexposure vaccination

A COMPARATIVE IMMUNOGENICITY AND SAFETY TRIAL OF TWO DIFFERENT SCHEDULES OF SINGLE-VISIT INTRADERMAL RABIES POST-EXPOSURE VACCINATION FOLLOWING A SINGLE-VISIT PRE-EXPOSURE VACCINATION.

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Summary:

In healthy adults, post-exposure prophylaxis (PEP) consisting of a two-dose single-visit intradermal (ID) rabies vaccination was as immunologically adequate and safe as a four-dose single-visit vaccination, following a two-dose single-visit pre-exposure prophylaxis regimen 7 to 28 months earlier.

ABSTRACT

BACKGROUND

Effective and safe single-visit rabies vaccination for pre- and post-exposure prophylaxis (PrEP and PEP) could substantially simplify rabies prevention and therefore increase compliance.

METHODS

In a comparative trial, 303 healthy adults received a primary vaccination consisting of two intradermal (ID) doses of 0.1mL of the purified chicken embryo cell vaccine (PCEV) during a single visit. One year later, subjects were randomly assigned to receive either four or two ID PEP booster doses of 0.1mL of PCEV during a single visit.

The primary endpoint for immunogenicity was the percentage of subjects with an adequate antibody level (>0.5 IU/mL) seven days after the booster doses. The safety endpoint was the proportion of participants developing adverse events (AE) following primary and/or booster vaccination.

RESULTS

All subjects, except one (99.3%) in each study group, had a rabies antibody titer >0.5 IU/mL on day 7 following the booster schedules.

Subjects exposed to the four-dose PEP schedule had a geometric mean titer of 20 IU/mL versus 14 IU/mL for the two-dose PEP schedule (p=0.0228).

Local reactions at the injection site following PrEP and PEP were mild and transient and only seen in 14.9% and 49.6 to 53% of the participants respectively. No serious AE were reported.

CONCLUSION

In healthy adults, a two-dose (2x 0.1mL) single-visit intradermal post-exposure prophylaxis schedule was as immunologically adequate and safe as a four-dose (4x 0.1mL) single-visit PEP schedule, seven to twenty-eight months following a two-dose (2x 0.1mL) single-visit intradermal pre-exposure prophylaxis.

This clinical trial was registered in EudraCT 2014-00183612.

INTRODUCTION

Rabies is a preventable neglected tropical disease with a very high case-fatality rate (1). The annual death toll is approximately 61,000 cases, 40% of them occurring in children, with higher prevalence in Asia and Africa (2-3). In 2015, the World Health Organization (WHO) called for action by setting a goal of zero dog-mediated rabies death in humans by 2030, worldwide (4).

WHO has recently recommended rabies two-visit pre-exposure prophylaxis (PrEP) schedules instead of three-visit schedules, with the main aim to be cost-, dose- and time-sparing, while still assuring the safety and clinical effectiveness of these preventive interventions (3). WHO recommends as first-line two-visit PrEP: a two-dose (0.1mL in two different anatomic sites) intradermal (ID) schedule (2^2ID) (2: two visits, ^{2}: two-dose on each visit) or a single-dose (1mL) intramuscular (IM) schedule (2^1IM) (2: two visits, ^{2}: one-dose on each visit), each administered on days 0 and 7 (3). This new rabies PrEP schedules has been recently implemented in Belgium (5). A recent meta-analysis has confirmed that all PrEP regimens given ID or IM within two-visit or three-visit schedules according to WHO recommendations provide adequate rabies antibody level of >0.5 IU/mL after booster injection(s) (6).

The advantages of priming and "training" the immune system before the risk (through PrEP) (7, 8), the concept of "lifelong boostability" after priming (adequate anamnestic serological response >0.5 IU/mL 7 days after booster doses following an initial primary vaccination administered once before) (9-14), and the need for additional booster doses through post-exposure prophylaxis (PEP) after the risk (3), are all key in rabies prevention. The two-visit ID or IM PrEP regimens, which are safe, sufficiently immunogenic and convenient are in line with these concepts (7,15-23).

There is however some growing evidence that a single-visit PrEP, as well as a single-visit PEP, may constitute a valid and cheaper alternative to this recently recommended two-visit schedules or to the widely used three-visit schedules (ID or IM) (24-27). If proven to be safe and effective, such single-visit PrEP and PEP schedules would be much more convenient for international travelers as well as for children at high risk in endemic low-income countries (LIC) (7-28).

The study hypothesis is that a single-visit administration of 0.1mL ID in two different anatomic sites (hereafter referred as 1^2ID according to our convention) as primary vaccination (PrEP) is sufficient to prime the immune system in such a way that it will result in a fast and adequate anamnestic response following a single-visit booster vaccination (mimicking an immunological response following PEP). The primary objective of this study was to evaluate the immunogenicity of two different PEP schedules (two-dose of 0.1ml (1^2ID) versus four-dose of 0.1mL (1^4ID) of the purified chicken embryo cell vaccine (PCEV) during a single visit planned approximately one year following a single-visit PrEP (1^2ID). Secondary objectives were to determine the "intermediate" immunogenicity of the PrEP schedule and to evaluate the safety of both the PrEP and PEP schedules.

METHODS

Study design and endpoints

This single-center, randomized, open-label, clinical trial aimed to compare the immunogenicity and safety of two different single-visit ID rabies PEP schedules planned approximately one year after all participants had received a two-dose single-visit ID primary vaccination (PrEP) (1²ID): 0.1mL of the PCEV vaccine was injected by ID route in each forearm for a total dose of 2x 0.1mL ID on day 0.

The participants were randomized to one of the following two PEP vaccination schedules:

- Group 1 (1^4ID): 0.1mL of the PCEV vaccine was injected by ID route in each forearm and in each M. deltoid for a total dose of 4x 0.1mL ID on day 0 of the booster vaccination;
- Group 2 (1²ID): 0.1mL of the PCEV vaccine was injected by ID route in each forearm for a total dose of 2x 0.1mL ID on day 0 of the booster vaccination.

The primary study endpoints were the proportion of participants with antibody titers >0.5 IU/mL, as measured by rabies fluorescent focus inhibition test (RFFIT), 7 days following one of these two ID booster regimens (1^4ID versus 1²ID) and the difference of those proportions.

Secondary endpoints were to determine and compare (1) the percentage of subjects with RFFIT levels >10.0 IU/mL in the two arms, (2) the geometric mean titer (GMT) of rabies antibody and (3) its fold increases at day 7 compared to the day 0 of the booster vaccination).

Other secondary endpoints were to determine the percentage of subjects with RFFIT levels >0.5 IU/mL, the GMT, and the fold increases on day 14 (compared to day 0) of the single-visit primary vaccination, in order to evaluate the intermediate immunogenicity.

Safety endpoints included the proportion and pattern of adverse events and of serious adverse events within 7 days and 14 days after initial and booster vaccinations respectively.

Study site and subjects

Study participants were recruited from the Belgian Armed Forces. Inclusion criteria were age between 18 - 54 years, being in preparation for overseas deployment, and willingness to provide informed consent. Subjects who had previously received rabies vaccines or had positive rabies serology, and pregnant or breast-feeding women were excluded, as well as subjects with known or suspected immunodeficiency, chronic disease, mefloquine prophylaxis or known allergy to one of the vaccine components. Subjects with planned overseas deployment within the next 28 days (to rabies non-endemic regions) or within 2 years (to rabies endemic regions) were also excluded. No other vaccinations were given simultaneously with the rabies vaccination. Approximately one year following PrEP, subjects were randomized to one of the two ID PEP schedules using block randomization.

The target sample size for the primary analysis was 300. Participation in this study was entirely voluntary and free of any type of coercion or undue influence by superiors.

Ethics and Registration

The trial was conducted in compliance with the Declaration of Helsinki and national regulations (29-30). This clinical trial is registered as EudraCT 2014-00183612.

Vaccination procedure

The 1.0mL purified chicken embryo cell vaccine (PCEV) for rabies (GlaxoSmithKline Biologicals), registered in Belgium, was used. It contains no adjuvants. The rabies vaccine was stored between +2 and +8°C as recommended by the manufacturer. Different lots were used for primary and booster vaccinations (546011G, 555011C, 529011C, 610011A, 533011C).

Preparation of the injecting solution of 0.1mL (from an ampoule of 1.0mL) was performed using a separate Gauche 29 fixed needle for insulin injection for each dose. After intradermal injection (using the Mantoux technique) the papule was measured, and had to be at least 4mm.

Immunogenicity

Antibody titers were measured by RFFIT on day 0 (prior to the primary vaccination), on day 14, on the day of the booster vaccination (planned approximately one year after day 0), and 7 days after the booster vaccination.

Safety

Adverse events (AE's) and serious adverse events (SAE's) were recorded until 7 and 14 days respectively following the completion of the primary and booster vaccination.

Study information

This clinical trial was sponsored by the Institute of Tropical Medicine, Antwerp (ITM). Clinical activities were performed at the Military Hospital Queen Astrid in Brussels. The recruitment began in October 2014, and the study was completed in March 2017.

Statistical analysis

For the immunogenicity component, participants who were seropositive on day 0 and participants who did not fully comply with the protocol were excluded from the statistical analysis. For the safety analysis, all subjects who had received at least one dose were included.

Baseline characteristics were summarized in terms of medians and interquartile ranges and categorical characteristics were described as frequency counts and percentages. Serology measurements are presented as percentages of subjects above different cut-off levels and one-sided 95% Wilson confidence intervals (95% CI), and GMT are presented with two-sided 95% CI.

Two-sided 95% Wilson confidence intervals for the difference (Diff) in proportions between the two groups were used to assess immunogenicity outcomes. The comparison of antibody levels between the two groups was assessed by GMT ratios and their respective t-test p-

values. Mixed models were used to explain the changes in serology over time. Differences in safety results between the two groups were assessed using Fisher's exact test.

RESULTS

Subject accounting and characteristics

Of the 524 screened subjects, a total of 303 subjects were included (57.8%). Reasons for exclusion are described in Table 1. From the 303 subjects completing the primary vaccination schedule, 271 (89.4%) were randomized, completed the booster vaccination (including the 7 day follow-up) and were included in the analyses (Table 2). Among those, 134 subjects (49.4%) received the four-dose booster and 137 subjects (50.6%) received the two-dose booster.

Day 7 results following booster doses

The planned timing of the booster vaccination approximately one year following primary vaccination needed to be adapted because most soldiers had to comply with unexpected security tasks related to the 2016 terrorist attacks in Belgium. As a result, the booster doses (the 1^4ID compared to the 1^2ID booster schedule respectively) were finally given in different timeframes following the primary vaccination (between 7 and 28 months): in 13.4% versus 12.4% in the first 12 months (pooled: 13%), and in 71.6% versus 70.1% between 12 and 24 months (pooled: 71%), and in 14.9% and 17.5% (pooled:16%) after 24 months (Table 1).

All subjects (except one in each group: 99.3%) displayed rabies antibody titers >0.5 IU/mL on day 7 after the booster vaccination, unrelated to the timing of the booster regimen (Table 3). The 95% confidence intervals indicated that the success rate of RFFIT >0.5 IU/mL was at least 96.7% and that the difference in success rate between the two booster schedules did not exceed 2%. Regarding the RFFIT results >10 IU/mL 7 days after booster doses, the proportion of participants reaching this level following the 1^4ID booster tended to be higher than in the 1^2ID group (79.9% versus 69.3%): a difference (95% CI) of 10.6 % (0.23 - 20.8) was demonstrated (p= 0.052) (Table 3).
Of note, the two "slow-responsive" cases post-boosting (a 51-year old male and a 49-year old female, in the 1^4ID and 1^2ID booster group respectively) were followed-up serologically according to protocol, and both had an adequate antibody response (without additional booster doses) at a later timepoint (2.02 IU/ml at 3 months for the first participant and 0.67 IU/ml at 6 months for the second one.

Subjects after the 1^4ID booster had a GMT (95% CI) of 20 IU/mL (16 - 25), as compared with a GMT of 14 IU/mL (12 - 18) for the 1^2ID booster group (p= 0.0228) (Table 4 and Figure 1). Moreover, female subjects after 1^4ID had significant higher GMT levels (95% CI), than male after 1^4ID boosters (42 IU/mL (28 - 62) versus 18 IU/mL (15 - 23) (p< 0.001).

In addition, RFFIT results seem to increase with interval since administration of the PrEP: after 24 months or more in a subgroup of 44 participants GMT levels (95% CI) were 41 IU/mL (29 - 59) and 35 IU/mL (25 - 49) for 1^4ID and 1^2ID PEP schedules respectively. All these 44 participants were male, and were significantly younger (a median age of 25.5 years compared to 42 and 39 years old for the time-group <12 months and 12 to 24 months respectively.

Changes in serology over time are presented in Figure 2. The 1^4ID booster schedule showed a slightly steeper increase (95% CI) after the booster vaccination (35.39; 27.24 - 43.54), compared to the 1²ID booster schedule (26.09; 19.92 - 32.25) (Figure 2).

Day 14 results following primary vaccination

53 of 303 subjects (17,5%) didn't develop adequate antibody responses on day 14 following 1²ID primary vaccination. Fourteen days after completing primary vaccination 82.5% (CI: 78.6 – 85.8) of all subjects in the pooled analysis set attained rabies serology results >0.5 IU/mL: 81.3% (CI: 75.2 – 86.2) and 81.8% (75.7 – 86.2) receiving later after randomization a 1^4ID compared to a 1²ID booster schedule respectively.

Safety

A summary of the safety data for the primary vaccination period and for the booster period is shown in Table 5 – 6. No serious AEs were reported during the study; 14.9% showed local irritation of injection site (mild and transient) after primary vaccination. After the booster vaccination, local irritation was slightly more often observed after the 1^4ID booster regimen compared to the 1²ID schedule 53% (44.6 - 61.2) vs 49.6% (41.4 - 57.9) (p=0.63).

DISCUSSION

In this trial, adequate antibody responses were achieved in 99.3% of healthy adults 7 days after a 1^4ID- or a 1²ID single-visit PEP following a single-visit 1²ID PrEP administered 7 to 28 months earlier. The GMT and the proportion of antibody levels >10 IU/mL were significantly higher in the 1^4ID booster regimen, compared to the 1²ID booster regimen.

This non-commercial clinical trial has several strengths including the randomized controlled design, good follow-up rates, blinding of laboratory study staff, as well as the use of the golden standard for serology (RFFIT) in a laboratory with proficiency in testing, and substantial expertise of nurses in performing appropriate intradermal injections and conducting vaccine trials. Moreover different batches of the PCEV vaccine were used in this trial over three years, reflecting a real-life situation. Study limitations include most participants being healthy young adult males, and the follow-up with booster vaccination not exceeding a three-year period.

The GMT results obtained 7 days post-booster (with cumulative total ID PrEP and ID PEP doses between 0.6 and 0.4 mL) were similar and/or higher compared with some pilot studies conducted in Thailand and evaluating ID single visit priming with ID or IM PEP (total dose between 0.6 and 2.2 mL) (25). They were also similar to results observed in 15 recently published cases of ID PrEP and IM PEP (total dose between 2.2 and 2.6 mL) (27). In contrast, GMT results of IM single-visit priming studies with additional IM PEP (total doses of 1.4 ml to 3 ml) were rather different, with lower and higher GMT results in 33 and 10 cases respectively (25,27). It must however be reminded, that no consensus exists for the optimal GMT levels after booster vaccination (7). In addition, male gender and young age can explain the high GMT levels when booster doses were given more than 24 months following the primary vaccination. Notably, as seen with other vaccines female participants had in general higher antibody responses than males (31).

Although only 82.5% (N = 250) of subjects after primary vaccination attained rabies seroconversion results >0.5 IU/mL at day 14, this initial priming was sufficient in almost all participants to induce an adequate anamnestic response within 7 days after additional boosters around 1 year later. This time interval of 14 days between PrEP priming and serological testing is likely too short to evaluate the total amount of seroconverters following low dose ID PrEP: 53 of subjects didn't respond adequately.

The 1²ID single-visit ID PrEP regimen gives less side effects in total than two- or three-visit ID regimens (7). Only some minor local irritation, was seen in 14.9% of the participants. In our previous trial comparing a two-dose two-visit PrEP 2²ID regimen with a single-dose three-visit PrEP 3^1ID regimen, the proportions of participants with general discomfort were similar, while local site irritations were more frequent, directly related to the cumulation of AE, higher total vaccine doses and more ID injection-related immunological triggers during each visit (7). In this trial, more local adverse events were reported during the post-PEP period compared to the PrEP vaccination, which might be related to a "trigger" effect in the PEP regimen group following the earlier primary vaccination.

Although a single-visit 1²ID PrEP course confers adequate immune responses in at least 78.6% and 97.8% of the subjects after PrEP and PEP respectively, it is not recommended by the WHO as first-line regimen at this stage (4). Still, the WHO recommends in their new guideline, to give to last-minute travelers, at least one ID or IM PrEP course (of the complete two-visit regimen), instead of no injections at all due to late presentation. Moreover, in such cases, a second PrEP visit has to be scheduled after travel to complete the full PrEP regimen. In case of exposure during travel, a full PEP (4 or 5 injections of vaccine and immunoglobulins) is required. This new guideline is changing slowly towards a new paradigm of rabies PrEP regimen: from a strict three-visit 3^1ID or 3^1IM regimen to a more convenient two-visit 2²ID or 2^1IM, and - but only as second choice, if there is not enough time - to a single-visit 1²ID PrEP (3). In individuals, that received PrEP (two-visit regimen), additional PEP injections are always promptly needed after a risk exposure (3).

This trial, with 271 subjects, adds further evidence to the previous 74 cases in the literature (345 subjects in total), that a single-visit 1²ID PrEP can induce robust anamnestic responses 7 days after additional booster doses (25, 27). In contrast, only 43 subjects were evaluated so far with single-visit 1^1IM PrEP (25, 27).

A 1²ID PrEP schedule, given only once or possibly repeated without a specific time window, may be appropriate for any type of healthy traveler. Further research is required to assess whether it would also be immunogenic in more vulnerable travelers or groups of populations (children, elderly, and immunosuppressed), particularly in low-income countries (28).

Additional randomized controlled trials are currently evaluating single-visit PrEP and boostability after PEP with PCEV vaccine. The first study in a Dutch population aims to compare a single-visit 1^1IM (N= 70) and 1²ID (N= 70), with a two-visit IM (2^2IM) and three-visit IM (3^1IM) PrEP schedule, and their respective B-cell and T-cell responses (EudraCT 2017-000089-31). The second trial is conducted by our research group and evaluates the immunological added-value of topical imiquimod and the use of an ID device (VAX-ID™) in Belgian soldiers (N = 268) subjected to single-visit 1²ID PrEP (EudraCT 2017-002953-12 / MedDev 80M0688).

CONCLUSION

In our cohort of healthy adults, a 1²ID PEP schedule was immunological adequate to, and as safe as, a 1^4ID PEP schedule, following a 1²ID PrEP regimen approximately 7 to 28 months earlier.

Single-visit two-dose pre- and post-exposure prophylaxis appear to be adequate, give minor local side effects, and are more convenient for travelers.

Author contributions

PS conceived the research project, researched, designed the trail, PS and KD executed the trial, HVL created the database (eCRFs) and coordinated data management, AT analyzed the data and PS, EB, YVH, KD, NH, ST, SVG, DVB, AT, PV wrote the paper. ST and SVG were responsible for laboratory analyses.

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Disclosures

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REFERENCES

1. Hemachudha T, Ugolini G, Wacharapluesadee S, Sungkarat W, Shuangshoti S, Laothamatas J. Human rabies: neuropathogenesis, diagnosis, and management. Lancet Neurol, 2013: 12: 498-513.

2. Hampson K, Coudeville L, Lembo T, Sambo M, Kieffer A, Attlan M, Barrat J, Blanton JD, Briggs DJ, Cleaveland S, Costa P, Freuling CM, Hiby E, Knopf L, Leanes F, Meslin FX, Metlin A, Miranda ME, Müller T, Nel LH, Recuenco S, Rupprecht CE, Schumacher C, Taylor L, Vigilato MA, Zinsstag J, Dushoff J. Estimating the global burden of endemic canine rabies. PloS Negl Trop Dis, 2015: 11: 9(5): e0003786.

3. WHO Expert Consultation on Rabies: third report: WHO Technical Report Series N°1012. Apr 2018. http://apps.who.int/iris/bitstream/handle/10665/272364/9789241210218-eng.pdf?ua=1. Accessed 19 Apr 2018.

4. United Against Rabies launches global plan to achieve zero rabies human deaths. http://www.who.int/rabies/news/RUA-Rabies-launch-plan-achieve-zero-rabies-human-deaths-2030/en/. Accessed 29 Jun 2018.

5. Summary of Belgian Consensus meeting, Scientific Study Group on Travel Medicine, Brussels, 25th January 2018: page 3-4. https://www.itg.be/Files/docs/Reisgeneeskunde/summaryconsensus2018.pdf. Accessed 29 Jun 2018.

6. Langedijk AC, de Pijper CA, Spijker R, Holman R, Grobusch MP, Stijnis C. Rabies Antibody Response after Booster Immunization: A Systematic Review and Meta-Analysis. Clin Infect Dis. 2018. doi: 10.1093/cid/ciy420.

7. Soentjens P, Andries A, Aerssens A, Tsoumanis A, Ravinetto R, Heuninckx W, van Loen H, Brochier B, Van Gucht S, Van Damme P, Van Herrewege Y, Bottieau E. Simplifying the rabies pre-exposure vaccination schedule from 28 days to 7 days: a randomized clinical trial in healthy adults.' Clin Infect Dis 2018. doi: 10.1093/cid/ciy513.

8. Blok BA, Arts RJ, van Crevel R, Benn CS, Netea MG. Trained innate immunity as underlying mechanism for the long-term, nonspecific effects of vaccines. J Leukoc Biol, 2015; 98(3): 347-56.

9. Ruprecht C, Plotkin S. Rabies vaccines. In: Plotkin S, Orenstein W, Offit P, eds. Vaccines, 6th edition. Saunders Elsevier, 2013: 646-662.

10. Shantavasinkul P, Tantawichien T, Jaijaroensup W, Lertjarutorn S, Banjongkasaena A, Wilde H, Sitprija V. A 4-site, single-visit intradermal postexposure prophylaxis regimen for previously vaccinated patients: experiences with >5000 patients. Clin Infect Dis, 2010; 51(9): 1070-2.

11. Suwansrinon K, Wilde H, Benjavongkulchai M, Banjongkasaena U, Lertjarutorn S, Boonchang S, Suttisri R, Khowplod P, Daviratanasilpa S, Sitprija V. Survival of neutralizing antibody in previously rabies vaccinated subjects: a prospective study showing long lasting immunity. Vaccine. 2006; 24: 3878-3880.

12. Brown D, Featherstone JJ, Fooks AR, Gettner S, Llyod E, Schweiger M. Intradermal pre-exposure rabies vaccine elicits long lasting immunity. Vaccine, 2008; 26: 3909–3912.

13. Naraporn N,Khawplod P, Limsuwan K, Thipkong P, Herzog C, Glueck R, Tantawichien T, Wilde H.. Immune response to rabies booster vaccination in subjects who had postexposure treatment more than 5 years previously. J Trav Med, 1999; 6: 134-136.

14. Strady A, Lang J, Lienard M, Blondeau C, Jaussaud R, Plotkin SA. . Antibody persistence following preexposure regimens of cell-culture rabies vaccines: 10-year follow-up and proposal for a new booster policy. J Infec Dis, 1998;177: 1290-1295.

15. Recuenco S, Warnock E, Osinubi MOV, Rupprecht CE. A single center, open label study of intradermal administration of an inactivated purified chick embryo cell culture rabies virus vaccine in adults. Vaccine, 2017; 35(34): 4315-4320.

16. Kamoltham T, Thinyounyong W, Khawplod P, Phraisuwan P, Phongchamnaphai P, Anders G, Malerczyk C. Immunogenicity of Simulated PCECV Postexposure Booster Doses 1, 3, and 5 Years after 2-Dose and 3-Dose Primary Rabies Vaccination in Schoolchildren. Adv Prev Med, 2011: 403201.

17. Pengsaa K, Limkittikul K, Sabchareon A, Ariyasriwatana C, Chanthavanich P, Attanath P, Malerczyk C. A three-year clinical study on immunogenicity, safety, and booster response of purified chick embryo cell rabies vaccine administered intramuscularly or intradermally to 12- to 18-month-old Thai children, concomitantly with Japanese encephalitis vaccine. Pediatr Infect Dis J, 2009: 28: 335-7.

18. Wongsaroj P, Udomchaisakul P, Tepsumethanon S, Khawplod P, Tantawichien T. Rabies neutralizing antibody after 2 intradermal doses on days 0 and 21 for pre-exposure prophylaxis. Vaccine, 2013; 31(13): 1748-51.

19. Vien NC, Feroldi E, Lang J. Long-term anti-rabies antibody persistence following intramuscular or low-dose intradermal vaccination of young Vietnamese children. Trans R Soc Trop Med Hyg, 2008: 102(3): 294-6.

20. Strady C, Andreoletti L, Baumard S, Servettaz A, Jaussaud R, Strady A. Immunogenicity and booster efficacy of pre-exposure rabies vaccination. Trans R Soc Trop Med Hyg, 2009; 103(11): 1159-64.

21. Khawplod P, Wilde H, Benjavongkulchai M, Sriaroon C, Chomchey P. Immunogenicity study of abbreviated rabies preexposure vaccination schedules. J Travel Med, 2007; 14: 173-6.

22. Mills D, Lau C, Fearnley E, Weinstein P. The immunogenicity of a modified intradermal pre-exposure rabies vaccination schedule - A case series of 420 travellers. J Travel Med, 2012; 18: 327-32.

23. Lau CL, Hohl N. Immunogenicity of a modified intradermal pre-exposure rabies vaccination schedule using a purified chick embryo cell vaccine: an observational study. Travel Med Infect Dis, 2013; 11(6): 427-30.

24. Tantawichien T, Benjavongkulchai M, Limsuwan K, Khawplod P, Kaewchompoo W, Chomchey P, Sitprija V. Antibody response after a four-site intradermal booster vaccination with cell-culture rabies vaccine. Clin Infect Dis, 1999; 28(5): 1100-3.

25. Khawplod P, Jaijaroensup W, Sawangvaree A, Prakongsri S, Wilde H. One clinic visit for pre-exposure rabies vaccination (a preliminary one year study). Vaccine, 2012; 30: 2918-20.

26. Wieten RW, Leenstra T, van Thiel PPA, van Vugt M, Stijnis C, Goorhuis A, Grobusch MP. Rabies Vaccinations: are abbreviated intradermal schedules the future? Clin Infect Dis, 2013; 56(3): 414-9.

27. Jonker EFF, Visser LG. Single visit rabies pre-exposure priming induces a robust anamnestic antibody response after simulated post-exposure vaccination: results of a dose-finding study. J Travel Med, 2017; 24(5): 1-8.

28. Soentjens P, Aerssens A, Van Gucht S, Ravinetto R, Van Gompel A. 'Low-cost intradermal rabies vaccination is indeed very promising.' Clin Infect Dis, 2013; 56(10): 1509-10.

29. Declaration of Helsinki version 2013: World Medical Association, Inc. All Rights Reserved. All intellectual property rights in the Declaration of Helsinki are vested in the World Medical Association. https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects/ Accessed 29 Jun 2018.

30. International Conference of Harmonization (ICH). ICH Tripartite Guideline for Good Clinical Practices E6 (R1), June 10, 1996. http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Efficacy/E6/E6_R1_Guideline.pdf. Accessed 29 Jun 2018.

31. Flanagan KL, Fink AL, Plebanski M, Klein SL. Sex and Gender Differences in the Outcomes of Vaccination over the Life Course. Annu Rev Cell Dev Biol, 2017; 33: 577-599.

Figure Legends:

Figure 1: Serology results (IU/mL; GMT and 95% CI) before and 7 days after booster vaccination.

GMT: geometric mean titers; PP: Per-Protocol; PEP: single-visit post-exposure prophylaxis schedule of either 4 intradermal doses of 0.1 mL (14ID) or 2 intradermal doses of 0.1 mL (1²ID); CI: confidence interval.

Figure 2: Segmented mixed-models of respective serology slopes (PP-analysis).

GMT: geometric mean titers; PEP: single-visit post-exposure prophylaxis schedule of either 4 intradermal doses of 0.1 mL (14ID) (blue lines) or 2 intradermal doses of 0.1 mL (1²ID) (red lines). D0: serology check at day 0 of start primary vaccination; D14: serology check at day 14 after start of primary vaccination; B0: serology check before booster dose; B7: serology check 7 days after booster dose.

14ID PEP: model predictions on population (thick blue line) and on individual base (thin blue line). 1²ID PEP model predictions on population (thick red line) and on individual base (thin red line).

The changes in serology over time in the two groups were evaluated using segmented mixed-models with random intercept and random slopes fitted separately in the subsets of each vaccination schedule. Time and indicator variables before and after booster were used as fixed effects.

Figure 1

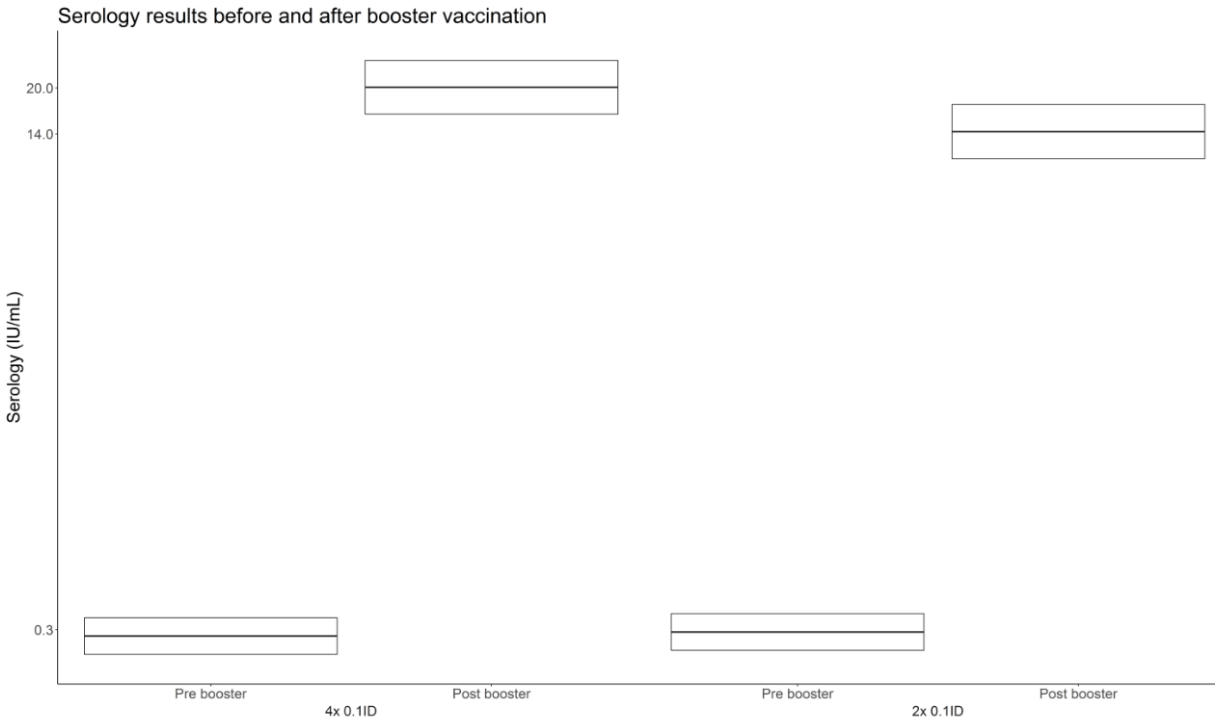
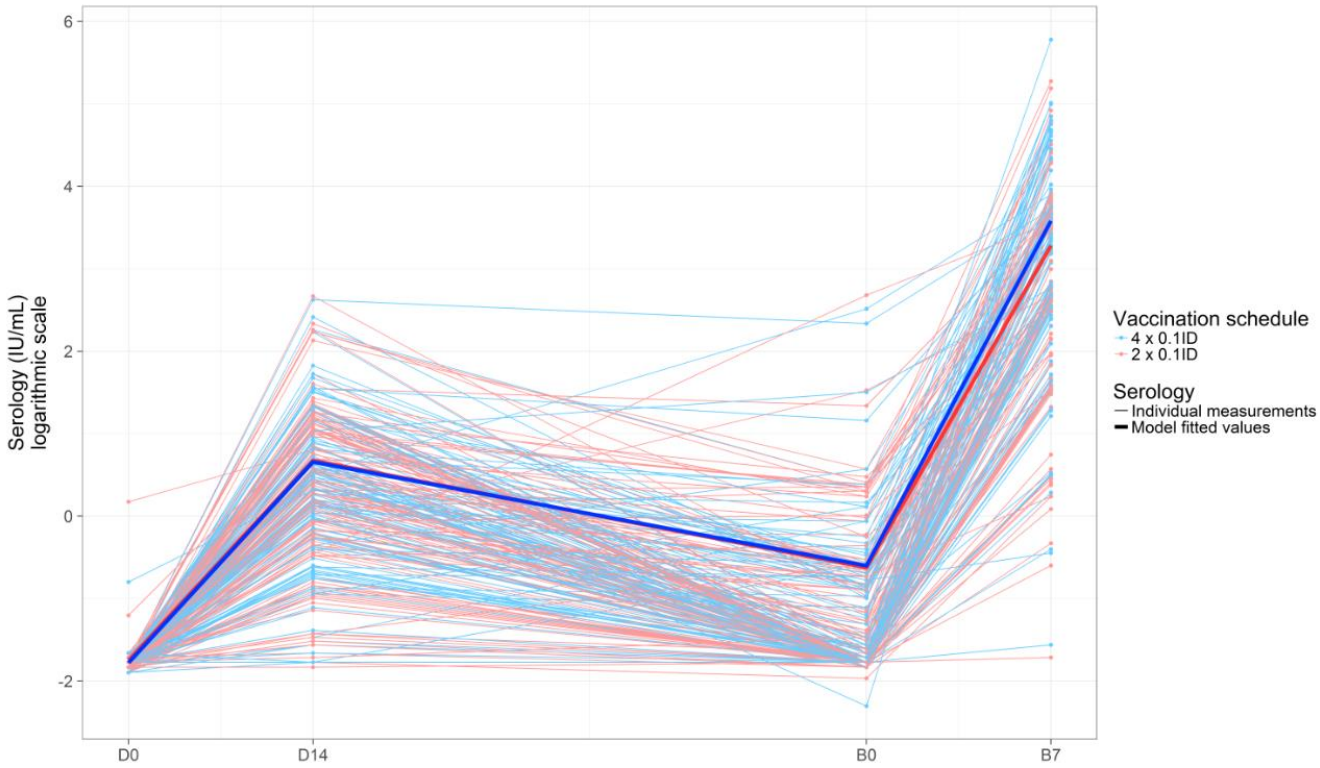


Figure 2



Tables:

Table 1: Study participants accounting on day 7 after booster dose injection

	Initial screening n (%)	
N	**524**	
Screening failures	N = 221	
Not interested - unwilling	116 (52.5%)	
- Unable to respect timelines due to deployment	16 (7.2%)	
- Seropositive at screening for rabies	38 (17.2%)	
- Intake of immunomodulating medication	5 (2.3%)	
- Known allergy to vaccine	2 (0.9%)	
-Not deployable anymore	44 (19.9%)	
	Initial recruitment n (%)	
N	**303**	
Completed primary vaccination period (including day 14 follow-up)	303 (100)	
Did not complete PEP (including day 7 follow-up)	32 (10.6)	
- Lost to Follow Up	14 (43.8)	
- On military mission	1 (3.1)	
- Subjects became out of service	5 (15.6)	
- Subjects discontinued (consent of withdrawal)	12 (37.5)	
Completed PEP (including Day 7 Follow-Up)	271 (89.4)	
	PEP schedule	
	4-doses 1^4ID n/N (%)	**2-doses 1^2ID n/N (%)**
N	134/271 (49.4)	137/271 (50.6)
Time of PEP following primary vaccination		
< 12 months	18/134 (13.4)	17/137 (12.4)
12 - 24 months	96/134 (71.6)	96/137 (70.1)
> 24 months	20/134 (14.9)	24/137 (17.5)

PEP: single-visit post-exposure prophylaxis schedule: 4 doses: 4 x 0.1ID (1^4ID) or 2 doses: 2 x 0.1ID (1^2ID) during a single visit

Table 2: Baseline characteristics of all study participants

	Pooled PrEP ID n (%)	PEP 4-doses 1^4ID n (%)	PEP 2-doses 1^2ID n (%)
N	303	134	137
Age (yr): median (IQR)	36 (26 – 47)	35 (26 – 46)	39 (27 – 47)
Age category: n (%)			
≤20	10 (3.3)	7 (5.2)	3 (2.2)
21-30	93 (30.7)	39 (29.1)	42 (30.7)
31-40	75 (24.8)	40 (29.9)	28 (20.4)
41-50	80 (26.4)	31 (23.1)	45 (32.8)
> 50	45 (14.9)	17 (12.7)	19 (13.9)
Gender: n (%)			
Male	269 (88.8)	119 (88.8)	122 (89.1)
Female	34 (11.2)	15 (11.2)	15 (10.9)

Pooled after inclusion; PrEP ID: single-visit pre-exposure prophylaxis schedule of 2 intradermal doses of 0.1 mL (1^2ID); PEP: single-visit post-exposure prophylaxis schedule of either 4 intradermal doses of 0.1 mL (1^4ID) or 2 intradermal doses of 0.1 mL (1^2ID); yr: year; IQR: interquartile range;

Table 3: Seroprotection rates - day 7 after booster vaccination

	Pooled n/N (%; 95% CI)[1]	4-doses (1^4ID) PEP n/N (%; 95% CI)[1]	2-doses (1^2ID) PEP n/N (%; 95% CI)[1]	Proportion difference % (95% CI)[2]	p-value
Number of subjects with serology > 0.5 IU/mL	269/271 (99.3 ;97.8 – 99.8)	133/134 (99.3; 96.7 – 99.8)	136/137 (99.3; 96.8 – 99.8)	-0.2 (-2.1 – 2.2)	1
Number of subjects with serology > 10 IU/mL	202/271 (74.5; 70.0 – 78.6)	107/134 (79.9; 73.6 – 84.9)	95/137 (69.3; 62.5 – 75.4)	10.5 (0.23 – 20.8)	0.052

Pooled PEP results; PEP: single-visit post-exposure prophylaxis schedule of either 4 intradermal doses of 0.1 mL (1^4ID) or 2 intradermal doses of 0.1 mL (1^2ID); CI: confidence interval.

[1]One-sided 95% confidence interval

[2]Two-sided 95% confidence interval

Table 4: Geometric Mean Titers (GMT) - before and after booster vaccination

	4-doses 1^4ID PEP (N = 134) (GMT; 95% CI)	**2-doses 1^2ID PEP (N = 137)** (GMT; 95% CI)	**Geometrical mean ratio** (95% CI)	**p-value**
GMT overall				
Pre-booster serology (IU/mL)	.29 (.25 – .33)	.30 (.26 – .34)	0.97 (0.79 – 1.18)	0.7542
Post-booster serology (IU/mL)	20 (16 – 25)	14 (12 – 18)	1.41 (1.05 – 1.89)	0.0228
GMT by timing of booster injections				
Pre-booster serology (IU/mL) < 12 months	.21 (.16 – .28)	.24 (.16 – .37)	0.88 (0.55 – 1.42)	0.5946
12 – 24 months	.27 (.23 – .31)	.27 (.23 – .32)	0.97 (0.78 – 1.21)	0.8042
> 24 months	.52 (.31 – .87)	.46 (.31 – .67)	1.14 (0.62 – 2.10)	0.6657
Post-booster serology (IU/mL) < 12 months	12 (7.4 – 21)	8.6 (4.3 – 17)	1.44 (0.63 – 3.32)	0.3780
12 – 24 months	19 (15 – 25)	12 (9.7 – 16)	1.52 (1.07 – 2.17)	0.0211
> 24 months	41 (29 – 59)	35 (25 – 49)	1.18 (0.73 – 1.90)	0.4848

GMT: geometric mean titers; PP: Per-Protocol; PEP: single-visit post-exposure prophylaxis schedule of either 4 intradermal doses of 0.1 mL (1^4ID)) or 2 intradermal doses of 0.1 mL (1^2ID)

Table 5: Safety analyses of all participants for the primary vaccination period

Number of subjects (%) with:	All participants (N = 303)
- any adverse event	54 (17.8)
- any possibly, probably or definitely drug-related adverse event	46 (15)
- any serious adverse event	0 (0.0)
- local irritation of injection site (redness, swelling, rash, itching)	45 (14.9)
- general side effects related to injections	13 (4.3)

Table 6: Safety analyses for the booster vaccination period

Number of subjects (%; 95% CI) with:	4-doses 1^4ID PEP (N=134)	2-doses 1^2ID PEP (N=137)	P-value
- any adverse event	73 (54.5; 46.0 – 62.7)	70 (51.1; 42.8 – 59.3)	0.63
- any possibly, probably or definitely drug-related adverse event	72 (53.7; 45.3 – 62.0)	68 (49.6; 41.4 – 57.9)	0.54
- any serious adverse event	0 (0)	0 (0)	-
- local irritation of injection site (redness, swelling, rash, itching)	71 (53.0; 44.6 – 61.2)	68 (49.6; 41.4 – 57.9)	0.63
- general side effects related to injections	6 (4.5; 2.07 – 9.42)	11 (8.0; 4.54 – 13.8)	0.32

PEP: single-visit post-exposure prophylaxis schedule: 1^4ID versus 1^2ID.