Vaccination: intradermal administration, duration of protection, and compromised immunity

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Chapter 2

Single visit rabies pre-exposure priming induces a robust anamnestic antibody response after simulated post-exposure vaccination: results of a dose-finding study

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2.1 Abstract

Background

The current standard 3-dose intramuscular rabies PrEP schedule suffers from a number of disadvantages that severely limit accessibility and availability. The cost of is often prohibitive, it requires 3 visits to the clinic, and there are regular vaccine shortages.

There is accumulating evidence that PrEP can be shortened to 2 visits without affecting seroconversion rates or memory formation. The primary objective of this dose finding study is to determine the optimal pre-exposure priming regimen that would require only a single visit to the clinic in order to produce an adequate memory response in all subjects one year later.

Methods

Volunteers (N=30) were randomly assigned to 4 study arms: 1 standard dose intramuscular (IM) dose of PVRV (purified Vero cell rabies vaccine, Verorab), and 1/5th, 2/5th or 3/5th- fractional intradermal (ID) dose of PVRV in a single visit. All subjects received a simulated rabies post-exposure prophylaxis (D0, D3) one year later. Rabies virus neutralizing antibodies (RVNA) were determined by virus neutralization microtest (FAVN) on D0, D7, D28, Y1, and Y1+D7.

Results

28 out of 30 subjects (93%) seroconverted 1 month after primary vaccination; 1 subject in the 1-dose IM arm and 1 in the 1/5th-fractional dose ID arm did not. After 1 year, 22 out of 30 subjects (73%) no longer had RVNA above 0.5 IU/mL, with no discernible difference between study groups. After 1 year, all 30 subjects mounted a booster response within 7 days after simulated PEP, with the highest titers found in the single dose IM group ($p<0.03$).

Conclusions

This dose finding study demonstrates that priming with a single dose of rabies vaccine was sufficient to induce an adequate anamnestic antibody response to rabies PEP in all subjects one year later, even in those in whom the RVNA threshold of 0.5 IU/mL was not reached after priming.

2.2 Introduction

Rabies is a fatal viral encephalitis that can infect all mammals. Ninety-nine percent of human cases result from dog bites. According to the most recent global estimates canine rabies causes 59,000 deaths in humans annually[1]. The vast majority occur in Asia (59.6%%) and Africa (36.4%), and are related to insufficient coverage of dog vaccination and absence of rabies post-exposure prophylaxis (PEP)[2]. For example in Indonesia, areas previously free of rabies are seeing a catastrophic re-emergence after initially successful elimination programs failed [3, 4] due to incomplete canine vaccination coverage[5].

The total cost of rabies has been estimated to be between 9 and 124 billion dollars per year[1, 6]. This cost can be mitigated in several ways, amongst which are reducing rabies disease prevalence through combating animal rabies, and immunization of vulnerable human populations. Although canine vaccination is the most cost-effective measure, this is still inadequately pursued [2] and the best option for the short to medium term is to improve availability and affordability of human rabies vaccination[1].

After a bite, scratch or lick of a rabid animal, rabies virus probably multiplies in the muscles surrounding the exposed site[7]. After a variable period of several days to even years, the virus enters the nervous system. This period is the only window of opportunity to stop disease and save the bite victim's life. Rabies virus can be stopped by thorough wound cleansing with soapy water, followed by wound disinfection with an iodine antiseptic solution and PEP[8]: the administration of a series of 4 or 5 doses of modern cell-derived rabies vaccine, combined with perilesional anti-rabies immunoglobulin (RIG) if necessary. Over 20 million people receive rabies PEP each year [9], mostly in resource-poor countries.

If the bite victim was vaccinated against rabies before the exposure occurred, revaccination with just 2 doses will suffice to boost a rapid and robust memory response. This memory response eliminates the need for RIG. It is the most important purpose of rabies pre-exposure prophylactic vaccination (PrEP): to build an immunological memory that provides a rapid and adequate anamnestic antibody response upon revaccination. PrEP vaccination also induces a transient antibody titer that may protect against (unnoticed) exposure.

The current standard 3-dose intramuscular PrEP schedule suffers from a number of disadvantages that severely limit accessibility and availability. The cost of a full PrEP course is often prohibitive, it requires 3 visits to the clinic, and there are regular vaccine shortages. Travelers in particular often don't have enough time to complete the PrEP series between their travel clinic visit and date of departure. This is becoming increasingly relevant as the number of PEP consultations is increasing year-on-year and the majority of those requiring PEP did not receive PrEP [10].

The administration of a fractional dose through the intradermal (ID) route in the standard schedule of 3 visits in 3 weeks may reduce costs significantly [11]. In addition, several studies indicate that PrEP can be shortened to 2 visits by using multi-site ID injection of the vaccine [12]. Such dose-sparing regimens may prove to be an excellent way to increase accessibility and availability of rabies PrEP [13]. There are indications that even one clinic visit using multi-site ID injection results in sufficient seroconversion: two ID injections of PCECV on day 0 resulted in 71-77% seroconversion rate at day 35 [14] and four ID injections of HDCV on day 0 resulted in 100% seroconversion rate at day 14[15]. A single intramuscular dose resulted in 100% seroconversion in 18 subjects 35 days after HDCV[16], and in 97% seroconversion in 33 subjects 35 days after PCECV[14]. The subjects in these studies demonstrated a memory response after a booster dose.

From these studies we hypothesize that a single rabies vaccine dose is sufficient to prime the immune system in such a way that it will result in a fast memory response after revaccination, even in the absence of seroconversion after primary vaccination. The primary objective of this dose finding study therefore is to determine the optimal pre-exposure vaccination regimen that would require only a single visit to the clinic in order to produce an adequate memory response in all subjects after one year.

2.3 Methods

Study population

Volunteers were recruited through advertisements in Leiden University buildings. An incremental incentive was provided to all subjects who completed the entire study protocol. Volunteers between 18 and 65 years old were included if they were in good health, willing and able to adhere to the study regimen, and able to provide informed consent. Exclusion criteria were any previous rabies vaccination, known or suspected allergy against vaccine components, history of serious adverse

reactions after vaccination, history of syncope due to needle sticks, immunocompromized state either through medication or medical condition, receiving blood products in the last 3 months, hydroxychloroquine or mefloquine use, history of any neurological disorder, use of anticoagulants, breastfeeding, a positive urine pregnancy test, refusal to use contraceptives during the study period, high grade fever, acute infectious disease other than seasonal cold, and participation in another trial in the last 3 months.

Study design

This is a dose-finding study performed according to a non-blinded comparative randomized clinical trial design. The study was performed at the Travel Clinic of the Department of Infectious Diseases at Leiden University Medical Center (LUMC, Leiden, The Netherlands) from November 2014 until March 2016. Subjects were randomly assigned to 4 regimens for primary rabies vaccination using computer-generated permuted block randomization:

- A: 1-site 0.5 mL intramuscularly (IM) (standard dose)
- B: 1-site 0.1 mL ID (equivalent to 20% of standard dose)
- C: 2-site 0.1 mL ID (40% of standard dose)
- D: 3-site 0.1 mL ID (60% of standard dose).

All injections were given at the same visit on a single day. Multi-site injections were given at distinct body sites in order to address the maximum number of lymph node stations: the deltoid region for the 1- and 2-site regimens, and for the 3-site regimen also the quadriceps region. After 1 year, all subjects received 2 standard doses in the ipsilateral deltoid muscle on day 0 and day 3 to simulate rabies post-exposure prophylaxis (figure 1).

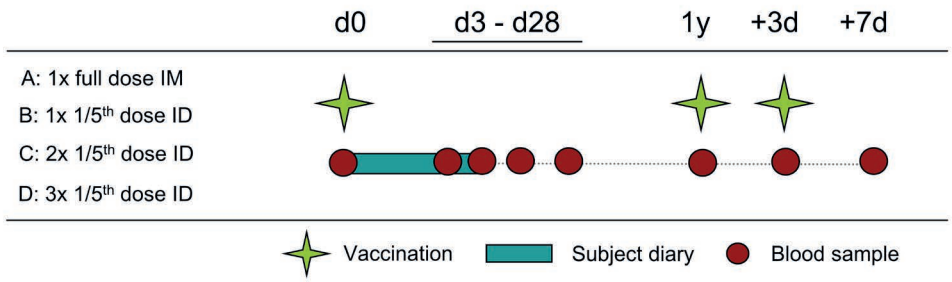

Figure 1: Subject timeline and study logistics. The 4 study arms presented on the left followed an identical course starting with the experimental single-visit vaccination at day 0, followed 1 year later by the standard intramuscular post-exposure vaccination schedule.

Vaccination, blood sampling and adverse events.

The vaccine used was a purified Vero cell rabies vaccine (PVRV, Verorab®, Sanofi Pasteur MSD). Verorab® in the original formulation contains per 0.5 mL dose ≥2.5 IU lyophilized inactivated rabies virus (strain PM/WI 38-1503-3M). All primary vaccinations were performed with the same vaccine batch (lot no. K1382-1, exp. date 6/2016; potency 3.2 IU/dose as determined by the manufacturer). The lowest priming dose used was 0.6 IU (group B; 1x 0.1 mL ID).Simulated PEP vaccinations were performed with different batches (lot no. 1126-3, exp. 3/2017 and L1446-1 exp. 8/2017). Between 2013 and 2016, Verorab® was the only rabies vaccine available in the Netherlands.

After each intradermal injection, wheal size and amount of leakage was measured. Wheal size was quantified as wheal diameter across 2 perpendicular axes, the average of which was taken as single value wheal size.

Subjects were asked to complete a diary for 5 days after primary vaccination. The following adverse events were solicited in the diary: local tenderness, swelling, itching, myalgia, erythema (grouped together in the analysis as 'local reactogenicity'), headache, fatigue, medication taken and extent to which symptoms influenced day-to-day functioning. After PEP vaccination, these events were solicited orally.

Venapunctures were performed on days 0, (2-)3, (6-)7, 14(-16), and 28(-35). After 1 year, venapunctures were performed at baseline (day 0), before the second booster dose (day 3) and at day 7. Blood samples were processed on the same day and serum was stored at -80 °C until analysis.

Fluorescent Antibody Virus Neutralization (FAVN)

The FAVN is a virus neutralization microtest adapted from the rapid fluorescent focus inhibition test (RFFIT) by Cliquet et al[17]. Both the FAVN and the RFFIT are recognized by WHO and OIE (World Organization for Animal Health) as the gold standard for rabies serology. As reference serum, the OIE dog serum calibrated against WHO's 1994 human reference serum was used (2nd international reference serum) [18]. The actual antibody level necessary for protection is unknown, but a level above the validated cut-off of 0.5IE/mL is definitive proof of seroconversion [18]. For this trial, the FAVN was performed in the high containment unit (HCU) of the Central Veterinary Institute (CVI), Wageningen University and Research Center (WUR), Lelystad, The Netherlands.

In brief, sera to be tested were complement inactivated for 30 minutes at 56 ˚C. Serial 3-fold dilutions from 1:3 to 1:81 of controls and test sera were mixed with 100 TCID50 rabies virus (CVS-11 strain/ ATCC VR959, ANSES, Nancy, France) and assayed in quadruplicate. The mixture was incubated for 1 hour at 37 ˚C and transferred to 96-well plates (Greiner Bio One BV, Alphen aan den Rijn, The Netherlands). BHK-21 cells in monolayer culture were trypsinized, suspended in DMEM + Glutamax (Invitrogen, subsidiary of Thermo Fisher Scientific, Waltham, Massachusetts, USA) and added to the virus-serum mixtures. The 96-well plates were then incubated for 2 days at 37 ˚C in 5% CO2. After incubation, the plates were fixed with 80% acetone, air-dried and stained with fluorescein isothiocyanate conjugated (FITC) anti-rabies serum (Fujirebo Diagnostics, Philadelphia, USA).

Fluorescent wells were counted quantitatively under an inverted fluorescence microscope. The decimal log dilution at which 50% of wells were neutralized (logD50) was calculated according to the Spearman-Kärber method and the titer reported in international units according to the following formula: ((10^logD50 of tested sample)/10^(logD50 of OIE reference))*0.5.

Sample size and analysis

This was a dose-finding trial. As such no formal sample size calculation was performed. In consultation with the medical statistician, it was decided to include 5 subjects per arm and an additional 5 per arm in the two arms with the lowest seroconversion rates one month after primary vaccination. The lowest seroconverting arms were chosen for expansion in order to facilitate demonstration of the primary hypothesis: 100% booster response after 1 year even in the absence of seroconversion after primary vaccination. A booster response was defined as seroconversion within 7 days post booster,

or a 4-fold increase of RVNA titer in those groups with a pre-booster RVNA >0.5 IU/mL.

Participant demographics and adverse events were analyzed using descriptive statistics. Reverse cumulative distributions were compared using the 2-sample Kolmogorov-Smirnov Z-test. Geometric titers and fold increase were analyzed using students t-test. Correlations were analyzed visually using scatter plots. Analyses were performed using SPSS Statistics version 23 (IBM Corp., Armonk, New York, USA), Excel version 2010 (Microsoft Corp., Redmond, Washington, USA) and Prism version 6 (GraphPad Software, La Jolla, California, USA).

Ethical considerations

The study was approved by the local Medical Ethics Committee (METC) of the Leiden University Medical Center (LUMC, Leiden, the Netherlands) and registered in clinicaltrials.gov under NCT02276625. All participants provided informed consent before enrollment.

2.4 Results

In total, 30 subjects were enrolled, all of whom completed the study including the simulated PEP one year after primary vaccination. Subjects were between 18 and 31 years of age (median 21.9) and were predominantly female (70%) (table 1).

One month after priming (experimental primary vaccination), there was an overall 93% (95% CI 84-100%) seroconversion rate for the one-visit priming schedules. Nine out of 10 subjects seroconverted in arm A (1-IM) and 9 out of 10 in arm B (1/5th-ID). Five out of 5 subjects seroconverted in arm C (2/5th-ID) and 5 out of 5 in arm D (3/5th-ID) (table 2). One month after priming, the geometric mean titers (GMT) were not different between groups, and there was no dose-response relationship with regards to antigen dose at priming (table 2; figure 2).

Table 1: Participant demographics, group size and comparative characteristics at baseline

	A 1 dose IM	B 1/5th dose ID	C 2x 1/5th ID	D 3x 1/5th ID
No of subjects	10	10	5	5
Age (yrs, [median, range])	21.5 (20-31)	20.5 (19-25)	23 (18-28)	22 (19-25)
BMI (mean, range)	23.9 (21-34)	23.4 (21-27)	25.2 (20-38)	22.3 (21-24)
Sex (# female)	8/10	8/10	2/5	3/5
Vaccine priming dose (IU)	3.2	0.6	1.2	1.8

Table 2: Serology, wheal size and side effects.

	A 1 dose IM	B 1/5th dose ID	C 2x 1/5th ID	D 3x 1/5th ID
Interval primary serology (mean days)	29.1	29.5	29.8	28.6
Interval primary-booster (mean days)	372	369	368	369
Average wheal diameter (mm)	NA	8.5	8.7	10.0
Serology				
GMT at baseline (IU/mL)	0.0 [0.0-0.0]	0.0 [0.0-0.0]	0.0 [0.0-0.0]	0.0 [0.0-0.0]
GMT at 7 days post primary (IU/mL, [95% CI])	0.0 [0.0-0.0]	0.0 [0.0-0.0]	0.0 [0.0-0.0]	0.0 [0.0-0.0]
RVNA range (IU/mL)	0.0-0.1	0.0-0.1	0.0-0.1	0.0-0.1
GMT 1 month post primary (IU/mL, [95% CI])	2.2 [1.3-3.9]	2.0 [1.1-3.8]	6.7 [2.9-15.4]	4.2 [1.4-13.0]
RVNA range (IU/mL)	0.5-11.6	0.4-7.4	2.5-29	0.8-16.8
Seroconversion, no. of subjects	9/10	9/10	5/5	5/5
GMT pre-booster baseline (IU/mL, [95% CI])	0.4 [0.2-0.6]	0.0 [0.0-2.0]	0.2 [0.1-0.4]	0.5 [0.3-0.8]
RVNA range (IU/mL)	0.1-1.3	0.0-1.3	0.1-0.6	0.2-1.0
Seroconversion, no. of subjects	3/10	2/10	1/5	2/5
GMT 3 days post booster (IU/mL, [95% CI])	0.3 [0.2-0.6]	0.0 [0.0-1.5]	0.3 [0.1-0.6]	0.5 [0.9-0.3]
RVNA range (IU/mL)	0.1-1.3	0.0-1.3	0.1-0.7	0.2-1.3
GMT 7 days post booster (IU/mL, [95% CI])	63.9 [45.1-90.6]	22.6* [10.8-47.0]	13.0* [7.7-22.0]	20.1* [12.9-31.5]
Fold increase in GMT vs pre-booster [95% CI]	252 [114-390]	86 [28-144]	67* [38-96]	48* [26-70]
RVNA range (IU/mL)	26.6-239.2	3.0-239.2	5.1-26.6	11.7-34.8
Seroconversion, no. of subjects	10/10	10/10	5/5	5/5
Side effects and AEs after primary vacccination				
Myalgia	3/10	2/10	2/5	1/5
Localized erythema	0/10	4/10	3/5	4/5
Headache	3/10	3/10	2/5	0/5
Fatigue	3/10	1/10	1/10	1/10

* significant difference compared to arm A, students t-test

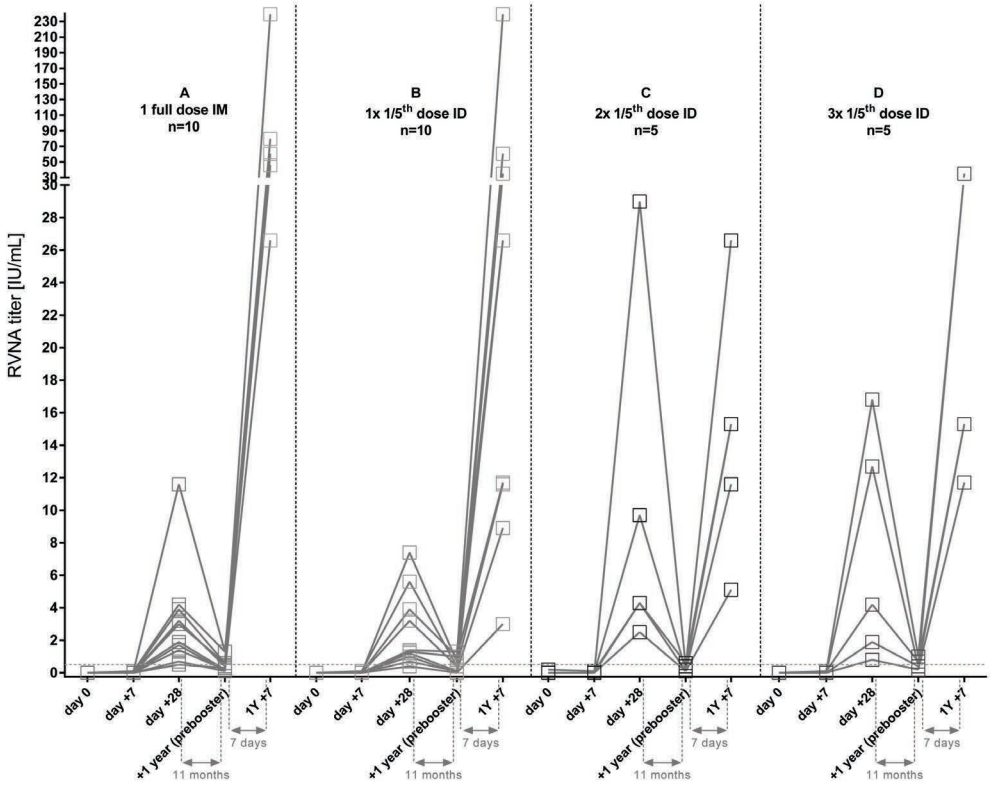

Figure 2: Individual titers plots. Individual titer plots showing per subject RVNA titers at baseline, day 7 and day 28 after primary vaccination, titers after 1 year (prebooster) and 7 days post booster. Note the lack of response 7 days post primary as opposed to 7 days post booster and the high post-booster titers in the group primed with 1 intramuscular injection.

One year after primary vaccination, 8 out of 30 subjects still had RVNA titer >0.5 IU/mL before simulated PEP. The distribution was as follows: 3 out of 10 in arm A; 2 out of 10 in arm B; 1 out of 5 in arm C, and 2 out of 5 in arm D (table 2).

All 30 subjects seroconverted within 1 week of the first booster dose (table 2; figure 2), even those who did not seroconvert after primary vaccination. Therefore, all experimental study arms satisfied the primary endpoint. At day 7 after revaccination GMT increased 251-fold in arm A, and between 48- to 86-fold in the intradermal arms (table 2). The difference in fold increase was significant ($p<0.03$) for arm A compared to arms C and D. Although the 1-IM arm (A) showed the highest GMT post-booster, no dose-response relationship was found in our study either when all groups were compared nor in the ID arms separately. Serology performed at day 3 post-booster did not show a difference in GMT

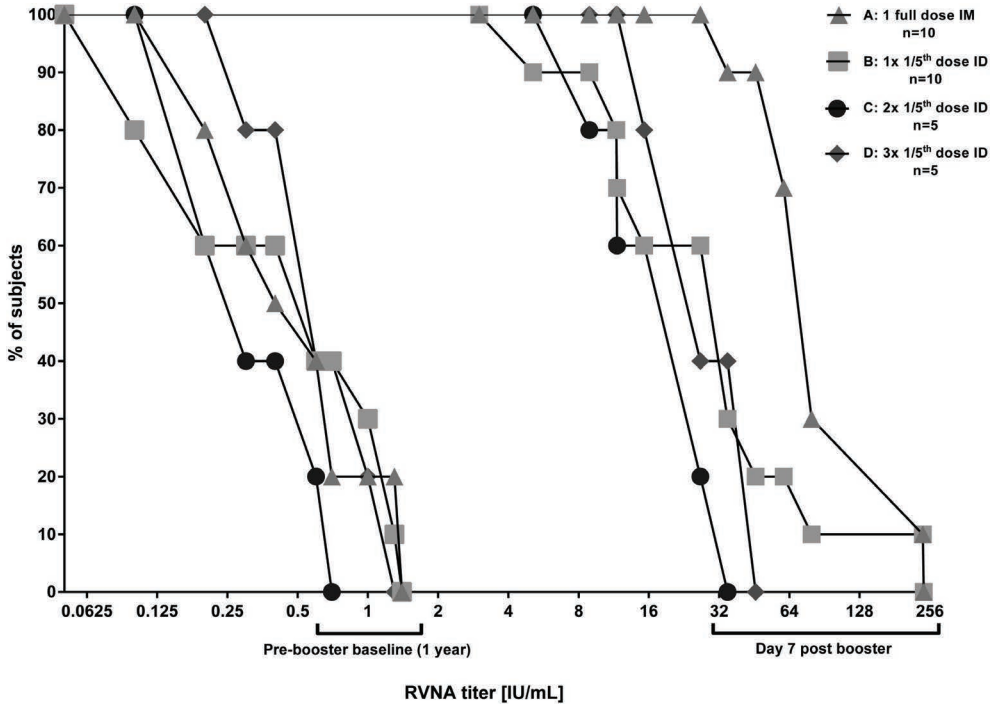

Figure 3: Reverse cumulative distribution of RVNA antibody titers before and after booster vaccination. Reverse cumulative distribution curves of RVNA titers per group, shown for the pre-booster baseline at 1 year (on the left) and 7 days after the post-exposure boosters (on the right). Note the highest post-booster titers in the group primed with 1 intramuscular injection ($p<0.015$, 2-sample Kolmogorov-Smirnov Z-test).

from the pre-booster baseline.

Reverse cumulative distribution curves of RVNA titers at day 7 post booster showed the highest titers in the 1-IM group (figure 3, $p<0.015$ for each intradermal group versus intramuscular, Kolmogorov-Smirnov Z-test), while no difference between the intradermal groups was found. There was no correlation between local reactogenicity and RVNA titer at day 28 post primary or day 7 post booster (data not shown). The average wheal diameter after intradermal injection was 9.2 mm (table 2).

There were no serious adverse events (SAE). Adverse events occurred after both the primary and booster vaccinations (table 2). After the primary vaccination, 12 out of 30 subjects did not report any side effect. The remaining subjects reported localized erythema (only ID groups, 11/30), myalgia (8/30), headache (8/30), and fatigue (6/30). In the intradermal groups, a little over half of subjects experienced painful erythema of around 5 mm at the site of injection for the first few days.

In some instances, a small red spot remained until 4 weeks after injection, predominantly over the quadriceps area. After 1 year, no evidence of intradermal injection could be found on the skin of any of the subjects. After booster vaccination, myalgia occurred in 8/30 subjects with one subject also reporting swollen axillary lymph nodes and another reporting fatigue.

Two subjects finished the study outside the pre-defined time limits; one subject 4 months early and one 4 months late, both in arm A (1-IM). Removing their data from analysis did not change the results (data not shown).

2.5 Discussion

This dose finding study demonstrates that priming with a single dose of rabies vaccine was sufficient to induce an adequate anamnestic antibody response to rabies PEP one year later. A robust memory response was seen in all subjects across all experimental priming regimens, regardless of dose or route of administration, even in those in whom the RVNA threshold of 0.5 IU/mL was not reached after priming.

Although most subjects seroconverted after primary vaccination, it was short-lived, as RVNA titers dropped significantly during the first year. Only 27 percent of subjects had a titer >0.5 IU/mL after one year. The rapid drop in RVNA titers is most likely explained by the lower number of effector cells that are formed after a single injection, in comparison to the standard 3-dose PrEP where further expansion of the pool of effector cells is expected to occur after the second and third dose [19]. After standard 3-dose PrEP vaccination, RVNA persist beyond the first year[20-22], and only start to drop below 85% after the second year[23]. Therefore, repeated vaccinations over several weeks or months are needed if prolonged persistence of RVNA after vaccination is required[24, 25]. Single dose primary vaccination however did induce adequate numbers of memory cells given the robust memory response that was seen after booster vaccination.

A booster response is characterized by a rapid and strong anamnestic antibody response after revaccination. Although straightforward in principle, the quantitative definitions of 'rapid' and 'strong' used in literature are quite diverse. We chose seven days after revaccination to differentiate a secondary or booster response from a primary antibody response, which generally occurs between 14 and 28 days after vaccination [19]. A significant rise in antibody titres is classically defined as seroconversion or an increase in antibody titres of more than two dilution steps, to account for the

inter-assay variability of serological tests. In this study all subjects had at least a 10-fold increase in RVNA titres at day 7 after revaccination. In contrast, no serological response was detectable in any of the subjects 7 days after the initial priming dose.

Our study has several strengths including the randomized controlled design, use of gold standard serology, blinding of laboratory personnel who performed the serology, and no loss to follow-up. Limitations include the small sample size and the fact that only young healthy adults were included. This may impact external validity, as it is known that the elderly have lower seroconversion rates after rabies vaccination [26]. In addition, it is unknown how long immunologic memory persists beyond one year. Lastly, standard 3-dose PrEP vaccination was not included for comparison. In future studies, a control arm with the current standard schedule should be included.

Intradermal administration of vaccines is thought to enhance immunogenicity because of the high density of antigen presenting cells (APC) in the papillary dermis [27]. Furthermore, the papillary dermis facilitates rapid trafficking of antigen and activated APC to draining lymph nodes where subsequent T-cell and B-cell activation and initiation of an adaptive immune response can occur [28] [29]. As a consequence, intradermal vaccination allows for the use of less vaccine than intramuscular or subcutaneous administration to obtain similar antibody responses, saving costs and increasing availability of vaccine in resource-poor regions of the world [30].

The feasibility of an abbreviated schedule of intradermal rabies vaccination was first demonstrated by Turner et al in 1976[31], later followed by Warrell and others[12, 32-34]. Turner also demonstrated that a single intradermal injection of 0.1 mL HDCV could prime the immune system leading to 100% seroconversion 28 days after a single intramuscular booster six months later [31, 35]. Although it was not the primary objective of their study, Brinkman et al found that a single intramuscular injection of HDCV resulted in 100% booster response within 7 days following a single intramuscular booster three months later[16]. Our study extends these findings, demonstrating that priming with a single intradermal fractional dose or intramuscular standard dose of a modern Vero cell-based rabies vaccine results in a robust memory response in all subjects, one year later.

We specifically chose to include the intramuscular route in this study as well, because it is technically less demanding than intradermal injection. In addition, in many countries the intramuscular route is the only licensed route of administration of rabies vaccine. Although individual RVNA titers varied substantially, we found that priming by the intramuscular route with a standard dose of rabies

vaccine resulted in a significantly higher fold-increase of post-booster GMT compared to priming by the intradermal route with a fractional dose. RVNA titers achieved post booster in this study were similar to those found after boosting subjects that received a standard pre-exposure vaccination [36].

If antibody responses in secondary immune responses are related to the number of memory cells, it would be reasonable to conclude that more memory cells were formed in the intramuscular group. Whether this is explained by the higher vaccine dose or by the intramuscular route remains to be determined. We did not observe a dose-response relationship between post-booster GMT and the fractional dose of rabies vaccine used for priming by the intradermal route. Possibly, this is because of the small sample size. Tauber et al. did find a dose-response relationship after single visit multi-fractional dose ID vaccination despite similar small numbers of subjects [34]. Beran et al also found a clear and significant linear correlation between vaccine dilution and resultant GMT, both early and late after intradermal vaccination with a single lot of purified chick embryo cell rabies vaccine (PCECV) according to the Thai Red Cross post-exposure regimen[37].

If confirmed, our findings may have a profound impact on preventive rabies vaccination strategy, especially in travelers. Although bite wounds occur at a rate of 1 in 300 travellers per month of stay [27, 29, 38], most travellers do not receive standard 3-dose pre-travel PrEP because of high costs and insufficient time between visit to the travel clinic and departure. On the other hand, travelers only run a risk of rabies exposure over a limited period of time. If preventive rabies pre-exposure priming with a single dose of rabies vaccine would suffice to cover this period, PrEP rabies vaccination would come within reach of many more travellers. This simplified schedule could be repeated for future travels. After three vaccine doses a lifelong rapid and adequate anamnestic antibody response after revaccination can be expected as with the standard 3-dose PrEP vaccination schedule. It is important to stress that vaccinated travelers must seek immediate medical attention after a bite accident. In a recent case series on rabies PEP it was found that 50 % of the travelers had received their first injection of rabies vaccine in the destination country within 24 hours [10] and 60% within 48 hours [personal communication with Wieten RW].

Before implementation, we would like to summarize the way forward as follows. First we need to demonstrate that, within a specified time window, the anamnestic RVNA response to simulated rabies PEP after single-dose priming is non-inferior to standard 3-dose PrEP. Secondly, we have to establish for which age groups single-dose priming would provide RVNA titres >0.5 IU/mL for a

sufficient time period (for example 3 or 6 months). Finally, we need to determine how the financial and logistical advantages of the single-dose priming relate to those of the standard 3-dose PrEP in specific risk groups.

In conclusion, effective rabies pre-exposure vaccination for travelers may be achieved in a single visit using a modern vaccine, with 100% booster response after 1 year even in those who do not seroconvert after the priming dose. Adequately powered non-inferiority studies should follow up on the results from this dose finding study and should include the standard intramuscular PrEP schedule as a control arm.

Author contributions

LV conceived of the research idea; EJ researched, designed and executed the trial under supervision of LV; EJ and LV analyzed the data and wrote the manuscript.

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Disclosure

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