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Summary

Background After rabies pre-exposure prophylaxis (PrEP) vaccination, scarcely available rabies immunoglobulins are not required for post-exposure prophylaxis (PEP). However, PrEP is not sufficiently accessible as it is cost-intensive and time-intensive. This study investigates whether rabies PrEP schedules can be shortened to one visit, removing some of these barriers.

Methods In a block-randomised (2:2:2:1) controlled, multicentre non-inferiority trial, healthy adult travellers (aged 18–50 years and >50 years) were randomly assigned to (A) single-visit intramuscular (1·0 mL); (B) single-visit intradermal (0·2 mL); (C) standard two-visit intramuscular (1·0 mL; day 0 and 7) PrEP; or (D) no rabies vaccination. 6 months later, participants received simulated intramuscular rabies PEP (1·0 mL; day 0 and 3). Rabies virus neutralising antibody (RVNA) concentrations were measured repeatedly. The primary outcome was the fold increase in geometric mean RVNA concentrations between day 0 and 7 after simulated PEP for all participants. The two main comparisons of this primary outcome are between the standard two-visit schedule and the one-visit intramuscular schedule, and between the standard two-visit schedule and the one-visit intradermal schedule. The non-inferiority margin was 0·67. This study is registered with EudraCT, 2017-000089-31.

Findings Between May 16, 2018, and March 26, 2020, 288 healthy adult travellers were randomly assigned and 214 participants were evaluated for the primary outcome. Single-visit intramuscular rabies PrEP induced an anamnestic antibody response non-inferior compared with the two-visit intramuscular schedule; single-visit intradermal PrEP did not. The fold increases in the single-visit intramuscular and the single-visit intradermal schedule were 2·32 (95% CI [1·43–3·77]) and 1·11 (0·66–1·87) times as high as the fold increase in the standard schedule, respectively. No vaccine-related serious adverse events were observed. Adverse events related to vaccination were mostly mild.

Interpretation Single intramuscular rabies vaccination can effectively prime travellers (aged 18–50 years), and potentially other populations, and could replace current standard two-visit rabies vaccination as PrEP.

Funding ZonMW.

Introduction

Rabies is a viral disease, accounting for an estimated 59 000 human deaths annually worldwide.[1] It is mainly transmitted through bites by infected animals and causes an untreatable and fatal encephalitis once the virus has spread to the central nervous system.[2] Canine bites are responsible for 99% of human rabies cases, making mass vaccination of dogs a highly effective control measure.[3] Moreover, rabies can be prevented through prompt administration of post-exposure prophylaxis (PEP) to exposed individuals.[3] However, the incidence of rabies remains high in Asia (60% of annual human cases worldwide) and Africa (36% of annual human cases worldwide), where access to these control measures is limited.[1]

Individuals residing in or visiting these areas can encounter rabies-infected animals. Although rabies in travellers is rare, bite wounds are reported to occur at a rate of 1 in 300 travellers per month of stay.[4] After such incidents, the bite victim must immediately seek PEP. PEP consists of thorough wound cleansing, administration of human or equine rabies immunoglobulin, and several intramuscular or intradermal rabies vaccinations within the space of 1–4 weeks depending on dose schedule.[3] However, rabies immunoglobulin is often not available due to costs, leaving many bite victims incompletely treated or requiring premature repatriation to receive rabies immunoglobulin in time.[5]

Rabies immunoglobulin is not required in the context of PEP if exposed individuals have previously received pre-exposure rabies prophylaxis (PrEP), consisting of two doses of rabies vaccine as PrEP (D0 and D7). This vaccine schedule induces long-lasting immunological memory that can be addressed by revaccination with just two doses (D0, D3) in the event of a bite, without the need for rabies immunoglobulin.[3,6] However, many travellers do not take PrEP because of high vaccine costs (currently between €36 and €87 per injection in the Netherlands)

Research in context

Evidence before this study

Rabies virus infections account for approximately 59 000 human deaths annually worldwide. Although rabies pre-exposure prophylaxis (PrEP) vaccination eliminates the need for scarcely available rabies immunoglobulins in case of possible rabies exposure, PrEP is not sufficiently accessible as it is expensive and requires multiple visits. If single-visit PrEP suffices, this will lead to increased accessibility and availability of rabies PrEP as a preventive measure.

We searched PubMed on April 17, 2023 for papers published from database inception, using the terms ("pre-exposure" OR "preexposure") AND "rabies" AND ("single" OR "abbreviated" OR shorten*), which rendered 52 articles, of which six reported data from single-visit PrEP schedules in humans. No language restrictions were applied, although all relevant articles were in English. Together, these studies suggest that single-visit priming results in adequate antibody responses upon revaccination (post-exposure prophylaxis), yet conclusive data from a randomised controlled clinical trial required for implementation are lacking.

Added value of this study

This study is the first randomised controlled trial to show that single-visit pre-exposure priming with rabies vaccine results in an adequate and effective antibody response after simulated rabies post-exposure prophylaxis.

Implications of all the available evidence

The increase in rabies virus neutralising antibody concentrations observed in participants with single-visit intramuscular priming was non-inferior compared with the standard, two-visit intramuscular schedule in adults aged 18–50 years, in addition to 100% seroconversion after booster vaccinations. Our data also suggest a similar adequate antibody response after single-visit intramuscular priming in an older group (>50 years). However, due to the limited number of participants in this age group, this observation requires further studies for confirmation. Overall, these results confirm earlier findings from observational studies indicating that PrEP can be reduced to a single visit. We therefore propose that the use of single-visit intramuscular rabies PrEP should be incorporated in rabies guidelines for travellers and groups at risk.

or insufficient time between travel clinic visits and departure.[7–9] In addition, current Dutch guidelines recommend PrEP only for specific risk groups (such as travellers with planned occupational exposure to animals or travellers to areas where PEP is not available within 24 h of a possible exposure) and long-term travellers (staying for more than 3 months).[10] Yet, a retrospective study[9] found that most travellers who required PEP did not fit these groups. Consequently, promoting rabies PrEP to a broader population of travellers could be necessary.

By reducing costs and eliminating time constraints, important barriers can be removed for improving the uptake of rabies PrEP vaccinations. If PrEP with a single (fractional) dose of rabies vaccine would suffice to induce an adequate immune response upon administration of PEP, more travellers might opt for PrEP. WHO also recommends PrEP for populations in highly endemic settings with limited access to PEP.[3] Unfortunately, in practice, these recommendations cannot always be followed.[11] Single-visit PrEP would improve the accessibility of PrEP, as available resources could then be used more cost-effectively to protect larger groups in need, and logistics are easier to manage when a second visit is redundant.

Single-dose PrEP is a promising alternative to standard PrEP, with increasing evidence suggesting comparable effectiveness with fewer doses and visits.[12–16] In 2018, WHO endorsed two-dose PrEP (D0, D7) as the new standard to replace the previous three-dose schedule.[6] In a pilot study, a single intramuscular dose was found to induce a rapid and effective anamnestic antibody response 1 year later, even in individuals who lacked detectable rabies virus neutralising antibody (RVNA) concentrations at the time of revaccination. Similar responses were found in individuals who had received one-fifth fractional dose rabies vaccine through the intradermal route.[17,18] However, implementation of single-dose PrEP still requires more data.[11]

We conducted a randomised controlled non-inferiority trial to obtain more robust evidence for the implementation of single-visit PrEP. We aimed to show a rapid and adequate anamnestic antibody response upon revaccination against rabies 6 months after primary vaccination with a single intramuscular dose or one-fifth fractional intradermal dose of rabies vaccine.

Methods

Study design and participants

This multicentre randomised, non-inferiority study was conducted at three Dutch travel clinics (Leiden University Medical Centre, Leiden; Travel Clinic Erasmus Medical Centre, Rotterdam; and Amsterdam University Medical Centers, Amsterdam) between May, 2018, and December, 2021.

We recruited healthy travel clinic visitors aged 18 years or older who had not received previous rabies vaccination and did not require standard rabies PrEP according to national guidelines. Participants were excluded if their travel duration exceeded 8 weeks, or if they departed within 1 week. They were also excluded if they had a (suspected) allergy against egg protein or other vaccine components; were immunocompromised; had received blood products 3 months before inclusion; used

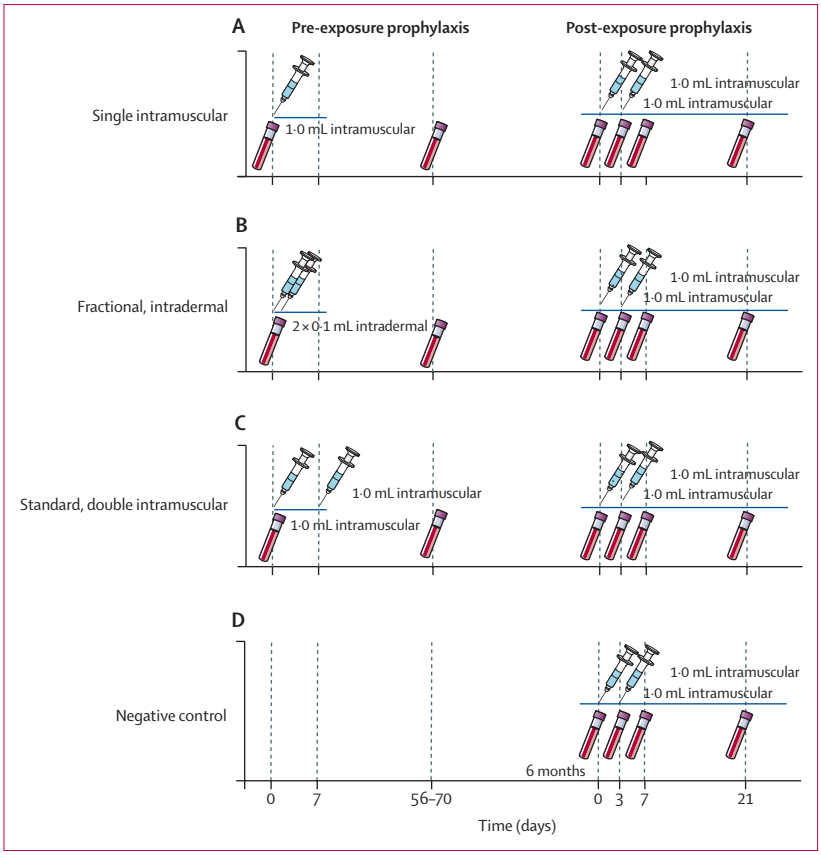

Figure 1: **Study overview**
Schematic overview of the study groups and their corresponding study procedures. Simulated post-exposure prophylaxis started 6 months after initial priming.

chloroquine or mefloquine; had a history of any neurological disorder; were pregnant or breastfeeding; had a concurrent infectious disease other than seasonal cold; or had a bleeding disorder or used anticoagulants.

The study was conducted in line with the International Council on Harmonization guidelines for Good Clinical Practice and the Declaration of Helsinki. Prospective participants were given information on the risks and burden of participation before the start of the study. Informed consent was obtained from participants before any study procedure was performed. Ethics approval was obtained from an independent ethics committee (Stichting Bebo, NL60550.056.17).

Randomisation and masking

Participants were randomly assigned by computer to one of the four study groups using permuted blocks (2:2:2:1) with varying block sizes (7 and 14) stratified by age group (18–50 years old or >50 years old) and study centre to receive either: (A) a single intramuscular dose of 1·0 mL rabies vaccine (on day 0); (B) two fractional, intradermal doses of 0·1 mL rabies vaccine (on day 0); (C) standard double intramuscular rabies vaccination of 1·0 mL (on day 0 and day 7); or (D) no rabies vaccination before travel (figure 1). Investigators and participants were not masked due to the nature of the intervention.

Study procedures

The rabies vaccine Rabipur (Bavarian Nordic A/S, Hellerup, Denmark; previously GSK Vaccines GmBH), which is a purified chick embryo cell vaccine, was administered either intramuscularly or intradermally. Each 1·0 mL dose contained 2·5 IU or more lyophilised inactivated rabies virus. Intramuscular vaccination was administered into the deltoid muscle, while the intradermal vaccination (0·1 mL) was delivered into the skin of the deltoid region of each arm. After each intradermal vaccination, wheal size was measured. Only if wheal size was 5 mm or more, was intradermal vaccination considered successful.

After 6 months, all participants received two intramuscular doses of 1·0 mL of Rabipur vaccine (on day 0 and day 3) to simulate PEP, as would have been used in the case of a possible rabies exposure, such as an animal-induced bite wound (figure 1). Adverse events were collected in a diary during the first 5 days after each vaccination. The timepoint of 6 months was chosen because this should be sufficient to cover the time at risk for rabies exposure for most travellers. If boostability is still shown at 6 months, there should also be boostability earlier in time.

To assess RVNA concentrations, blood was collected at baseline; 2 months after primary vaccination (only groups A–C); 6 months after baseline (before simulated PEP); and 3, 7, and 21 days after the start of simulated PEP. For group D, the baseline blood samples were collected at the 6 months timepoint. Blood samples were processed on the same day and serum was stored at –80°C until analysis.

Outcomes

The primary outcome of this study was boostability: the induction of a rapid, anamnestic antibody response after simulated PEP, measured as the fold increase in geometric mean RVNA concentrations from day 0 to day 7 after revaccination. In addition, our secondary outcomes included the proportion of RVNA seroconversion at various timepoints using cutoff values (ie, ≥0·5 IU/mL at baseline, 2 months after vaccination, and on day 0 and day 3 of PEP and ≥0·5 IU/mL, ≥3 IU/mL, and ≥5 IU/mL on day 7 of PEP). Seroconversion was a secondary and not a primary outcome in this study, because seroconversion rates 6 months after primary vaccination might still be too high to allow informative non-inferiority testing.

RVNA concentrations were measured by the Wageningen Bioveterinary Research Institute (Lelystad, Netherlands) using the fluorescent antibody virus neutralisation (FAVN) assay.[19] The upper limit of detection was 168·2 IU/mL.

Self-reported adverse events were assessed for relatedness and severity. Solicited adverse events

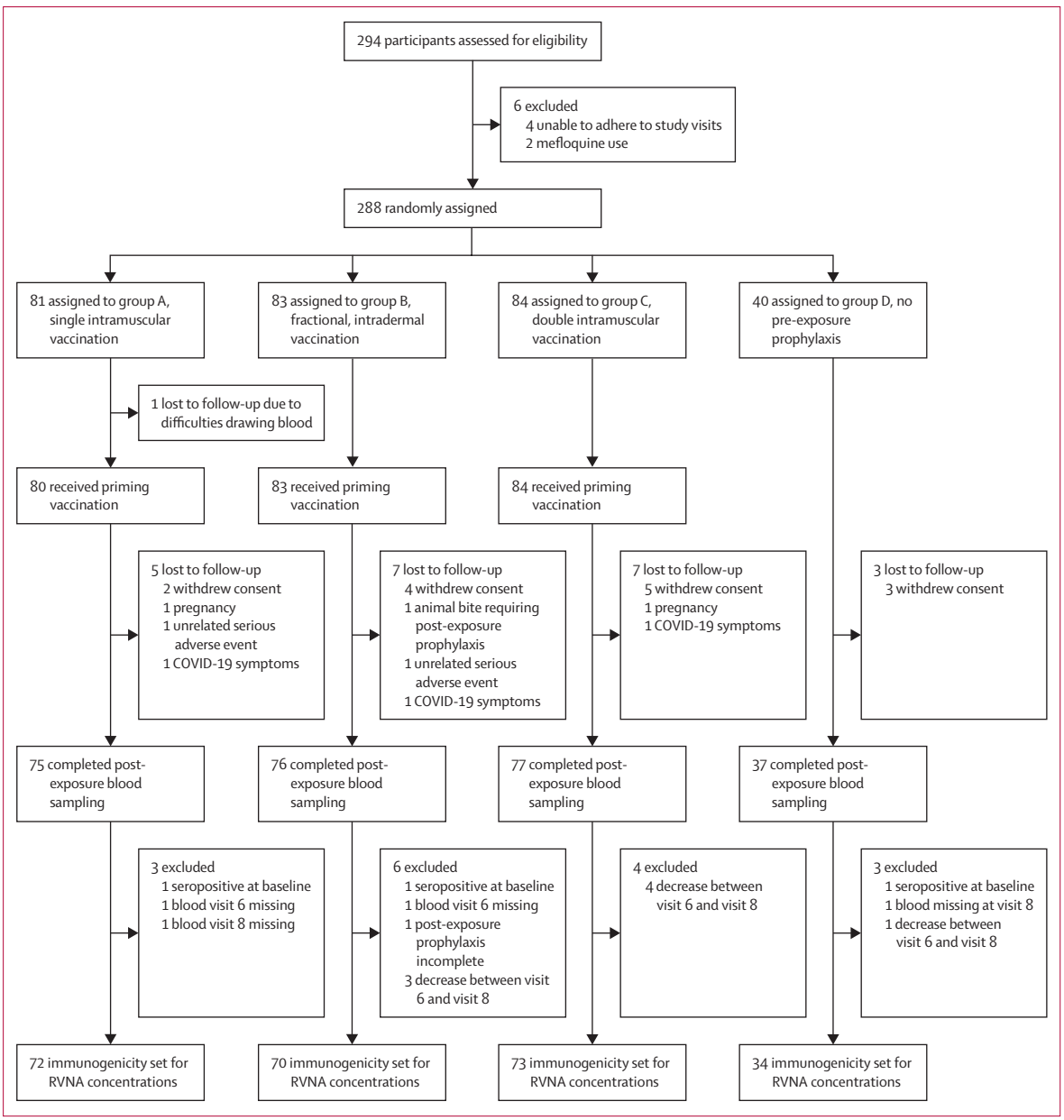

Figure 2: **Trial profile**
RVNA=rabies virus neutralising antibodies. Visit 6 corresponds to day 0 of the simulated post-exposure prophylaxis series. Visit 8 corresponds to day 7 of the simulated post-exposure prophylaxis series.

included pain at injection site, redness, muscle stiffness, headache, fatigue, and myalgia.

Other secondary outcomes, not reported in this Article, included: knowledge, belief, and risk perception of animal bites and rabies vaccination, before and after travel; the percentage of participants with animal contact during their stay abroad; the type of animal and type of contact (eg, licking, scratching, or biting); the measures taken after animal contact during travel; the percentage of participants who applied wound care after animal contact; and the percentage of participants who started appropriate PEP after animal contact.

Statistical analysis

Due to the effect of ageing on immune responses, we stratified participants into two age groups: 18–50 years old, which was the group for our primary analysis, and older than 50 years, for which an immune response of smaller magnitude can be expected. The sample size was calculated based solely on the primary analysis group of 18–50 year olds.

	Group A, single intramuscular vaccination (n=81)	Group B, fractional intradermal vaccination (n=83)	Group C, double intramuscular vaccination (n=84)	Group D, no pre-exposure prophylaxis (n=40)
Sex				
Male	31 (38%)	37 (45%)	36 (43%)	14 (35%)
Female	50 (62%)	46 (55%)	48 (57%)	26 (65%)
Age, years	30 (12·7)	32 (13·9)	30 (13·7)	30 (13·5)
Age group, years				
18–50	69 (85%)	70 (84%)	72 (86%)	35 (88%)
>50	12 (15%)	13 (16%)	12 (14%)	5 (12%)
BMI	22·9 (3·06)	23·8 (3·47)	22·8 (3·25)	23·1 (3·57)
Smoking				
Yes	3 (4%)	12 (14%)	10 (12%)	7 (18%)
No	78 (96%)	71 (86%)	74 (88%)	33 (82%)
Received post-exposure prophylaxis between months 4 and 6				
Yes	59/75 (79%)	63/76 (83%)	61/77 (79%)	NA
No	16/75 (21%)	13/76 (17%)	16/77 (21%)	NA

Data are n (%), n/N (%), or mean (SD).

Table 1: **Baseline characteristics of study participants**

The primary objective of this study was to assess non-inferiority of a single intramuscular or fractional intradermal dose versus the standard two-visit intramuscular rabies vaccination by comparing the geometric mean fold increase in RVNA concentrations from day 0 to day 7 after revaccination. To allow geometric descriptive statistics, antibody concentrations of 0 IU/mL were set to 0·0256 IU/mL, which is the lower limit of detection of the assay. We log-transformed the concentrations and calculated the individual difference in log concentration between day 0 and day 7 in the simulated PEP series, which corresponds to log transformation of the fold increase in RVNA concentration ($\log[a]-\log[b]=\log[a/b]$). The antilog of the mean of these values corresponds to the geometric mean fold increase. As the FAVN assay consists of three-fold dilution steps, a logarithm base of 3 was chosen for log transformation.

Instead of defining non-inferiority margins on the basis of seroconversion after revaccination, we compared the geometric mean fold increase in RVNA concentrations between the different groups as described previously.[20] Non-inferiority was established if the lower bound of the mean log-transformed difference between groups was not less than –0·369 ($^3\log 1·5$), corresponding to half of one 3-fold dilution step in the FAVN assay. For the geometric mean fold increase, this means that the non-inferiority margin would be set at 0·67 ($3^{-0·369}$ or 1/1·5).

On the basis of an estimated SD of the $\log_3$ geometric mean concentration of 0·63, a one-sided alpha of 0·05, and a beta of 0·8, we calculated that the intervention groups and the positive control group should each consist of 59 participants. To show that the antibody response after PEP in participants receiving PrEP is truly a booster response, we included a negative control group that did not receive any PrEP.

Next, the individual fold increases in RVNA concentrations were plotted in histograms to identify high-responders and low-responders or non-responders before calculating a group-level geometric difference. To assess non-inferiority, between-group differences (with two-sided 95% CI) regarding the increase in log-transformed concentrations were calculated using independent *t*-tests for the stratified age groups, as well as for all participants. Baseline characteristics and adverse event data were described using proportions for categorical variables, means and 95% CI for normally distributed continuous data, or median and IQR for non-normally distributed continuous data. The amount of missing data was limited. Therefore, no further statistical methods were used to account for these.

Adverse events were analysed in the intention-to-treat group, which included all participants who had received at least one vaccination. The primary outcome, non-inferiority of single-visit rabies vaccination compared with standard double visit, was analysed in the per-protocol (immunogenicity) group that consisted of participants who received all vaccinations in the priming phase and the two vaccinations of the PEP schedule, who provided a blood sample on day 0 and day 7 of the PEP schedule, were RVNA seronegative (RVNA concentration <0·5 IU/mL) at baseline, and did not show a decrease in RVNA concentration between day 0 and day 7 of the PEP schedule.

All analyses were performed using R (4.1.0) and R studio (2023.03.0) with the following additional packages: tidyverse and naniar. Figures were either created using R and Rstudio or Graphpad (9.3.1). The study was registered in the Dutch Trial registry (NTR6817) and in EudraCT (2017-000089-31).

Role of the funding source

The funder of the study had no role in the study design, data collection, data analysis, data interpretation, writing of the manuscript, or the decision to submit for publication.

Results

Between May 16, 2018, and March 26, 2020, 294 participants were screened for eligibility, of whom 288 were enrolled and randomly assigned. 81 participants were randomly assigned to receive priming with a single intramuscular vaccination (group A); 83 with fractional intradermal vaccination (group B); 84 with two-visit intramuscular vaccination (group C); and 40 participants did not receive priming (group D). All but one participant received their allocated priming dose. All intradermal vaccinations were performed successfully (wheal size ≥5 mm). During the study, 23 (8%) of 288 participants were lost to follow-up and did not complete the post-exposure blood sampling (figure 2). 170 (59%) of 288 were female and the median age was 26 years (IQR 21–33); 42 (15%) participants were older than 50 years (table 1).

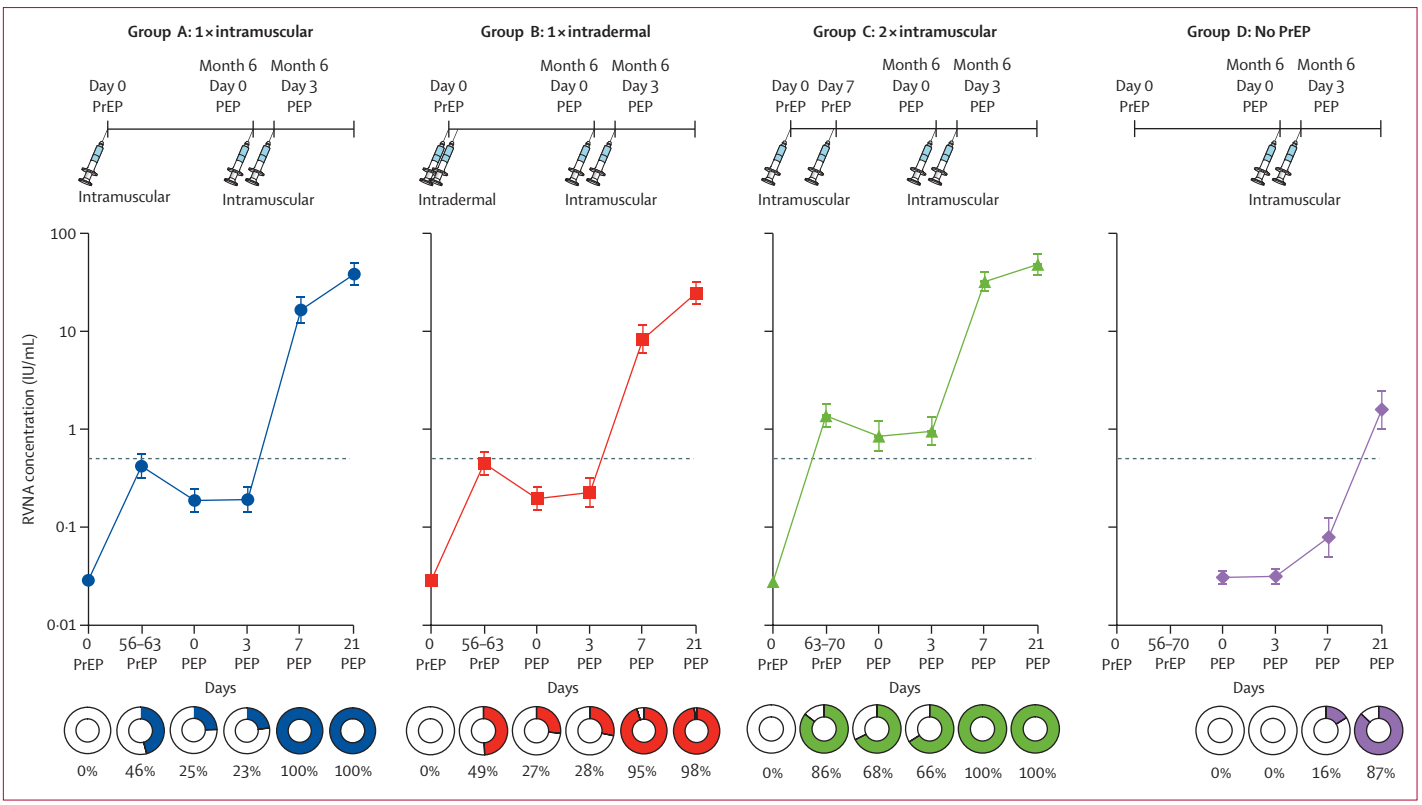

Figure 3: **Kinetics of geometric mean RVNA concentrations in participants aged 18–50 years**
Kinetics of the RVNA response to rabies vaccination for all study groups. All PrEP-receiving groups (ie, single intramuscular, depicted in blue; fractional intradermal, depicted in red; and standard double intramuscular, depicted in green) show an adequate booster response. The group that did not receive any PrEP (depicted in purple) shows a slower and less profound response, typical of a primary response to a neoantigen. Markers indicate geometric mean concentrations and their asymmetric 95% CI. Below the graphs, seroconversion rates (RVNA concentration ≥0·5 IU/mL) are displayed. The dotted grey line indicates an RVNA concentration of 0·5 IU/mL. PEP=post-exposure prophylaxis. PrEP=pre-exposure prophylaxis. RVNA=rabies virus neutralising antibodies.

Due to COVID-19 restrictions, simulated PEP was delayed in 45 (20%) of 228 participants in group A, B, and C. The maximum time between priming and PEP was 10·7 months.

For immunogenicity analyses, 72 participants were included in group A (single intramuscular), 70 in group B (fractional intradermal), 73 in group C (standard, two-visit intramuscular), and 34 in group D (no priming). In the primary analysis group (participants between 18–50 years old, n=214), there was a clear distinction in RVNA concentration kinetics between a primary response (as shown by the group without PrEP), and booster responses, elicited in the groups that received any form of PrEP (figure 3). Eight participants were excluded from the immunogenicity analyses because they showed a biologically inexplicable sharp drop in antibody concentrations from day 0 to day 7 (three in the fractional intradermal group, four in the two-visit intramuscular PrEP group, and one in the group without PrEP).

2 months after PrEP, participants primed with standard two-visit intramuscular vaccination had higher mean RVNA concentrations (geometric mean concentration 1·38; 95% CI 1·04–1·85) than those primed with single intramuscular or fractional intradermal vaccination (0·42; 0·31–0·57 and 0·45; 0·33–0·60, respectively; appendix 2 p 8). At 6 months, a similar difference was observed before the start of simulated PEP.

See Online for appendix 2

After PEP, a rapid and robust increase was observed in all participants who had received any form of PrEP. On day 7 after PEP initiation, high RVNA concentrations were observed in all PrEP-receiving groups (single intramuscular geometric mean concentration 16·54; 95% CI 12·00–22·80; fractional intradermal 8·23; 5·85–11·57; and two-visit intramuscular 32·61; 25·47–41·77; appendix 2 pp 2, 8). Kinetics in the older group (ie, those >50 years old) followed a similar course, albeit with lower RVNA concentrations (appendix 2 p 3).

The primary outcome of the study was boostability, defined as the fold increase in RVNA concentrations between day 0 and 7 after simulated PEP. Histograms displaying the frequencies of individual increases in concentration did not show a clear distinction between responders and non-responders (appendix 2 p 4) regarding the increase in RVNA concentration after PEP, and thus a cutoff value for a responder status could not be determined. A small number of participants showed no or little increase in RVNA concentration, which can

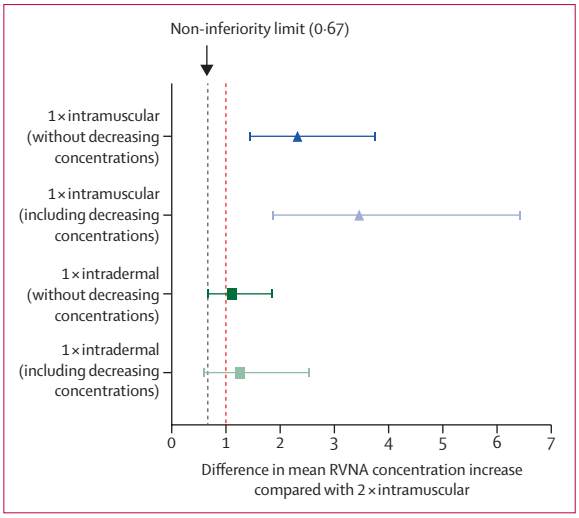

Figure 4: **Mean difference in RVNA increase between single-visit and two-visit schedules in participants aged 18–50 years**

The fold difference in the geometric mean increase in RVNA concentrations from day 0 to day 7 after booster vaccination between single-visit intramuscular (blue) or intradermal (green) PrEP and two-visit intramuscular PrEP (red dashed line), plotted with their corresponding 95% CIs. The non-opaque lines indicate the primary analysis study population without decreasing concentrations from day 0 to day 7 after booster vaccination. The opaque lines indicate a post-hoc analysis that additionally includes participants with decreasing concentrations. The figure shows the ratio of the fold geometric mean increases, in which group C (two intramuscular) is taken as the standard and therefore set to 1 (the x-coordinate of the red dashed line). If the fold change is the same for the compared groups (eg, fold increase in group A/fold increase in group C), then the ratio would be 1. The plotted data are limited to the age group of 18–50 years. The lower bound of the CI of the single intramuscular group does not cross the non-inferiority margin of 0·67, indicated by the vertical black line in the figure, indicating non-inferiority. PrEP=pre-exposure prophylaxis. RVNA=rabies virus neutralising antibodies.

be expected as a consequence of natural variation in immune responses. However, the majority of participants in PrEP-receiving groups showed strong increases from day 0 to day 7 after simulated PEP (appendix 2 p 5).

On day 7 after PEP, priming with single intramuscular, but not with fractional intradermal rabies PrEP had induced an anamnestic antibody response that was non-inferior compared with the standard two-visit intramuscular schedule (geometric mean fold increase in RVNA concentration 88·4 [95% CI 62·3–125·4]; 42·4 [28·6–62·8]; and 38·0 [27·4–52·8], respectively) in adults (18–50 years old). The ratio of the fold increase between single intramuscular PrEP and the standard two-visit intramuscular schedule was 2·32 (95% CI 1·43–3·77), whereas for fractional intradermal PrEP and the standard schedule, it was 1·11 (0·66–1·87). The lower bound of the CI of the fold increase ratio between the single intramuscular and the standard two-visit intramuscular schedule did not exceed the non-inferiority margin of 0·67, indicating non-inferiority (p<0·0001, one-sided; figure 4). Fractional intradermal PrEP was statistically inferior (p=0·026, one-sided), with the lower bound of the CI passing the non-inferiority margin, albeit barely. In a post-hoc sensitivity analysis that also included the participants with decreased concentrations at day 7, the non-inferiority criterion was still met for the single intramuscular group (3·46; 95% CI 1·87–6·41, p<0·0001, one-sided), but again not for the fractional intradermal group (1·25; 0·61–2·53, p=0·041, one-sided; figure 4).

When all participants, also those older than 50 years, were included in the non-inferiority analysis, single intramuscular, but not fractional intradermal, rabies PrEP still induced a non-inferior, anamnestic antibody response (RVNA concentration fold increase 86·0 [95% CI 63·3–116·9] and 39·8 [27·6–57·3], respectively) compared with the standard two-visit intramuscular schedule (37·4 [27·6–50·6]) after PEP. The ratio in fold RVNA concentration increase between single intramuscular PrEP and the standard two-visit intramuscular schedule was 2·30 (95% CI [1·49–3·55], p<0·0001, one-sided), whereas for fractional intradermal PrEP and the standard schedule, it was 1·06 (0·66–1·72, p=0·027, one-sided). Even though this study was not powered for the age group including those older than 50 years, non-inferiority was statistically shown for the single intramuscular schedule (ratio with standard two-visit intramuscular 2·18 [95% CI 0·77–6·19]; p=0·014, one-sided), but not for the single fractional dose intradermal schedule (ratio with standard double intramuscular 0·80 [0·20–3·21]; p=0·39, one-sided; appendix 2 p 6).

In 212 (99%) of 215 participants in PrEP-receiving groups, seroconversion (RVNA concentration ≥0·5 IU/mL) occurred on day 7 of PEP, irrespective of age (figure 3). In the group of those older than 50 years, 32 (100%) of 32 participants receiving PrEP had seroconverted by day 7 (appendix 2 p 3). Combining the two age groups, seroconversion rates were 72 (100%) of 72 in the single intramuscular group, 67 (96%) of 70 in the fractional intradermal group, and 73 (100%) of 73 in the standard two-visit intramuscular groups, whereas this was only 5 (15%) of 34 in the group without PrEP. Using a stricter cutoff of 3 IU/mL on day 7, seroconversion occurred in 68 (94%) of 72 participants primed with single intramuscular, 56 (80%) of 70 participants primed with fractional intradermal, and 72 (99%) of 73 participants primed with standard two-visit intramuscular PrEP (appendix 2 p 7). When using an even more stringent cutoff of 5 IU/mL on day 7, seroconversion occurred in 60 (83%) of 72, 47 (67%) of 70, and 67 (92%) of 73 participants, respectively (appendix 2 p 7).

No vaccine-related serious adverse events were reported. During the first 5 days after initial vaccination, several vaccine-related adverse events were reported, which were mostly of mild severity in these healthy travellers (table 2). 120 (73%) of 164 participants in the intramuscular groups and 62 (75%) of 83 in the intradermal group had at least one vaccine-related adverse event. In the intramuscular groups, the most frequently reported adverse events were: pain at the injection site reported by 82 (50%) of 164 participants;

	Intradermal vaccination (n=83)	Intramuscular vaccination (n=164)
Pain at the injection site		
Mild	17 (20%)	80 (49%)
Moderate	2 (2%)	2 (1%)
Severe	0	0
Any	19 (23%)	82 (50%)
Swelling at the injection site		
Mild	8 (10%)	1 (1%)
Moderate	0	0
Severe	0	0
Any	8 (10%)	1 (1%)
Axillary lymphadenopathy		
Mild	1 (1%)	1 (1%)
Moderate	0	0
Severe	0	0
Any	1 (1%)	1 (1%)
Redness at the injection site		
Mild	35 (42%)	2 (1%)
Moderate	0	0
Severe	0	0
Any	35 (42%)	2 (1%)
Malaise		
Mild	1 (1%)	1 (1%)
Moderate	0	0
Severe	0	0
Any	1 (1%)	1 (1%)
Fatigue		
Mild	18 (22%)	46 (28%)
Moderate	11 (13%)	7 (4%)
Severe	0	1 (1%)
Any	29 (35%)	54 (33%)
(Table 2 continues in next column)		
(Continued from previous column)		
Headache		
Mild	16 (19%)	29 (18%)
Moderate	4 (5%)	5 (3%)
Severe	1 (1%)	1 (1%)
Any	21 (25%)	35 (21%)
Myalgia		
Mild	13 (15%)	57 (35%)
Moderate	2 (2%)	9 (5%)
Severe	0	0
Any	15 (18%)	66 (40%)
Stiffness		
Mild	8 (10%)	45 (27%)
Moderate	1 (1%)	3 (2%)
Severe	0	0
Any	9 (11%)	48 (29%)
Pain (general)		
Mild	0	2 (1%)
Moderate	0	0
Severe	0	0
Any	0	0
Other		
Mild	7 (8%)	2 (1%)
Moderate	0	0
Severe	0	0
Any	7 (8%)	2 (1%)

Data are n (%).

Table 2: **Vaccine-related adverse events by severity in the first 5 days after initial vaccination**

myalgia by 66 participants (40%); fatigue by 54 participants (33%); and stiffness by 48 participants (29%). After intradermal vaccination, the most frequently observed adverse events were: redness at the injection site reported by 35 (42%) of 83 participants; fatigue by 29 participants (35%); headache by 21 participants (25%); and pain at the injection site by 19 participants (23%). Other reported adverse events in the intradermal group consisted mainly of mild itching at the injection site, reported by 7 participants (8%).

Discussion

In this multicentre, randomised trial, we showed that priming with a single intramuscular dose of rabies vaccine resulted in an anamnestic rabies virus neutralising antibody response upon revaccination non-inferior to the standard two-visit intramuscular vaccination in adults up to 50 years old. These results are in line with several observational studies that have provided evidence in support of single-visit rabies PrEP,[12,13,21,22] although more conclusive data from a randomised controlled trial were missing until now.[17] Single-visit schedules are likely to increase uptake of rabies PrEP.

Unlike other non-inferiority studies on rabies vaccination schedules, we chose the fold increase in antibodies after booster vaccination as our primary outcome rather than seroconversion rates. Seroconversion rates become non-informative if the seroconversion threshold of 0·5 IU/mL has already been reached in a substantial proportion of the study participants before revaccination.[23] The increase in antibody concentrations is a more appropriate outcome measure, clinically indicating that a rapid and adequate immune response can be elicited after possible exposure to rabies, and statistically allowing more informative testing for non-inferiority. The non-inferiority margin was set to half of the 3-fold dilution step in the FAVN assay, which falls within the inter-test variability of the assay.

It might seem counter-intuitive that the increases in RVNA concentration observed in the single-visit rabies PrEP groups appeared higher than in the standard

two-visit group. This finding can partly be explained by the lower pre-booster geometric mean concentration of the single-visit groups. This pre-booster difference is, however, small and of little clinical significance. Most notably, participants with single-visit PrEP showed strong antibody increases after boosting that were non-inferior to the standard two-visit schedule. Moreover, seroconversion was achieved in 96–100% of PrEP-receiving participants on day 7 after booster vaccination. Three participants in PrEP-receiving groups did not seroconvert by day 7; however, these had adequate RVNA concentrations (≥0·5 IU/mL) on day 21 after PEP. The combination of non-inferior increases in RVNA concentrations and high seroconversion rates 7 days after the start of PEP provides strong evidence in support of implementation of a single-visit rabies vaccination schedule.

Although this study draws strength from its randomised, large-scale set-up, there were also limitations. Firstly, we were unable to recruit as many participants older than 50 years as anticipated. Therefore, we are unable to make well substantiated statements about the efficacy of single-visit rabies PrEP in this age group, even though non-inferiority was observed for the single-visit intramuscular, but not fractional intradermal, schedule. In addition, both single intramuscular and fractional intradermal PrEP resulted in 100% seroconversion (≥0·5 IU/mL) on day 7 after booster vaccination in participants older than 50 years. On the basis of these limited data, we would also carefully advise single-visit intramuscular PrEP for people older than 50 years; however, single-visit intradermal PrEP needs additional research in an older population. In addition, we would be hesitant in other vulnerable populations, such as pregnant women, immunocompromised travellers, and children, as evidence from this study cannot be directly extrapolated to these specific groups.

Secondly, the FAVN assay had an upper limit of detection of 168·2 IU/mL. These concentrations were reached in nine (4%) of 214 participants (three in the single-visit intramuscular group and six in the standard double-visit intramuscular group) on day 7 after booster vaccination, which is slightly more than anticipated assuming a normal distribution. Nevertheless, the effect of these high responders on our primary outcome is limited, given they only made up 4% of total observations. Lastly, we were unable to show non-inferiority of the single-visit intradermal schedule, as the lower bound of the CI exceeded the non-inferiority margin. However, with a slightly larger sample size, we believe we could have shown non-inferiority for two reasons. First, we calculated a more stringent 95% CI around the estimate corresponding to a two-sided alpha of 0·5, rather than the one-sided alpha of 0·5 (and 90% CI) as used in the sample size calculation. Second, eight participants could not be included in the primary outcome evaluation because they showed a marked decrease in RVNA concentrations after booster vaccination. These biologically implausible values are highly unlikely to reflect true RVNA concentrations at the time of sampling. Human errors could have occurred during the large-scale sample processing, and these erroneous samples were therefore excluded, further reducing the effective sample size. However, our post-hoc sensitivity analyses that included participants with decreasing RVNA concentrations showed that single intramuscular vaccination was still non-inferior.

This study has several practical implications, but the framework in which they should be implemented should be carefully defined. The timing of vaccination remains crucial because sufficient numbers of rabies-specific memory B cells need to be present to successfully respond to PEP and induce an adequate anamnestic antibody response.[24] Although the single-visit schedule is ideal for travellers, as it reduces costs and increases efficiency, it could lead to travellers thinking they can get their vaccine at the last minute before travel. If they were to be bitten by a stray dog only 2 days later, PEP would not be boosting any immune memory cells at that moment, and they should be treated as if they were not vaccinated at all. This raises the question of how much time between the first vaccination and exposure is needed, to safely assume that someone is adequately vaccinated and can be boosted with simple PEP without rabies immunoglobulin. From an immunological viewpoint, we would advise 14 days between the first vaccination and possible exposure before someone can be treated as if they had completed their PrEP schedule.[24–27] At that point, a germinal centre reaction should have occurred, resulting in boostable memory B cells that can rapidly differentiate into plasma cells upon PEP vaccination.

Implementing shorter schedules will result in lower RVNA concentrations than multi-visit schedules, which requires knowledge on which RVNA concentrations protect against disease, and how long this protection lasts. Although WHO assumes that an antibody concentration of 0·5 IU/mL indicates an adequate vaccination response, protection at this level has never been proven in humans.[28–30] For this reason, we also included stricter cutoffs for seroconversion, such as 3 IU/mL or 5 IU/mL. Although seroconversion concentrations are fairly similar between the PrEP-receiving groups and all groups show an adequate increase in RVNA concentrations, there are considerable differences in post-booster antibody concentrations in the different groups. In some individuals, RVNA concentrations increased very little or not at all after booster vaccination, which can be expected due to natural variation. Yet, most of them had RVNA concentrations more than 0·5 IU/mL 7 days after booster vaccination. Therefore, knowing how many antibodies are sufficient for protection against disease is vital. We can only assume that the high geometric mean concentrations we observed, far higher than 0·5 IU/mL, are sufficient. Additionally, the window between PrEP and simulated

PEP in our study was only 6–10·7 months. The long-term efficacy of single-visit PrEP should be prioritised as a topic of future studies, as knowledge about the duration of boostability should guide the timing of further booster vaccinations. In the meantime, we would advise travellers to receive a second intramuscular dose before their next travel to complete the currently recommended two-visit PrEP vaccination series.

Our findings have clear implications for travellers and risk groups in non-endemic settings. Vaccinating these groups will become more cost-efficient and time-efficient, and travellers are likely to be more encouraged to take rabies PrEP before travel if just one visit is sufficient, which means that the demand for rabies immunoglobulin should decrease. However, the ultimate goal would be to implement single-visit rabies PrEP in countries where rabies is endemic. These countries face a much larger rabies morbidity and mortality burden. Unfortunately, the results of this study, in a primarily Dutch urban population, cannot be directly extrapolated to endemic settings. Still, we envisage that these positive results will stimulate future studies in rural, rabies-endemic settings that also explore the feasibility of more widespread vaccine distribution. Single-visit, fractional intradermal rabies vaccination might be the most efficient schedule, optimising the use of available rabies vaccine to protect at-risk populations. As an additional advantage, enhanced access to PrEP would create opportunities to build more awareness about rabies risk and treatment, such as when PEP could be required.

In conclusion, we propose that the use of single-visit intramuscular rabies PrEP should be incorporated in rabies guidelines for travellers and other at-risk groups. However, vaccination in travellers should take place as far in advance of departure as possible—preferably at least 2 weeks in advance. The effect of single-visit rabies PrEP can only be guaranteed if there is sufficient time separating PrEP and possible exposure.

Contributors

FH, PJJvG, MPG, and LGV prepared the research protocol and were involved in study design. JPRK, CP, PHV-M, CADP, and PLE generated the data. JPRK, CP, and FH were involved in the data management. LAO and JPRK performed the data analysis. All authors contributed to data interpretation and reviewed the manuscript. LAO and JPRK accessed and verified the data and prepared the first version of the manuscript. All authors have confirmed full access to all the data in the study and were responsible for the decision to submit the manuscript for publication.

Declaration of interests

LAO and LGV are conducting a study sponsored by Bavarian Nordic, the manufacturer of the Rabipur vaccine. Bavarian Nordic had no role in designing, conducting, analysing, or reporting the trial on which this manuscript reports. All other authors declare no competing interests.

Data sharing

De-identified individual participant data that underlie the results reported in this Article will be made available online immediately after publication and can be accessed without investigator support. The study protocol, informed consent form, and clinical study report will also be made available online.

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For the **de-identified individual participant data** see https://dans.knaw.nl

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