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Rabies antibody response after two intradermal pre-exposure prophylaxis immunizations: An observational cohort study

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ABSTRACT

Background: Rabies is a lethal, but vaccine preventable disease. Vaccination uptake is however hampered by the time-consuming three-dose, 21/28-day schedule. The aim of this study was to examine whether adequate rabies antibody titers are reached after two intradermal (ID) doses of rabies vaccine, with a seven-day window.

Method: We conducted an observational cohort study with military personnel. A titer was assessed by RFFIT, on the day of the third vaccination, to ensure an adequate rabies antibody response after ID immunization.

Results: After this abbreviated two-dose, seven-day ID schedule, seroconversion was reached in 99.3% (427/430) with a geometric mean titer of 7.59 IU/mL (95% CI 7.04–8.17).

Conclusions: Implementation of this two-dose schedule will protect more people against Rabies. Travelers and military personnel under time constraints, who otherwise would remain unvaccinated, can be considered adequately protected after this two-dose schedule. For populations in endemic areas, local application of a two-dose schedule could provide an opportunity to vaccinate more people with less vaccine.

Given the paucity of published data, this study adds relevant evidence in support of the new policy (2017) of WHO, concerning a two-dose, seven-day schedule is approved for all healthy individuals.

1. Introduction

Rabies, a neglected disease with a case-fatality rate of almost 100% in humans who develop symptoms, is the cause of more than 59,000 annual deaths worldwide [1–3]. Unvaccinated individuals who suffer a transdermal wound from a potentially rabid animal need immediate post-exposure prophylaxis (PEP) by administration of rabies immunoglobulin (RIG), at the trauma site, followed by a WHO approved PEP schedule [4]. PEP by vaccination alone does not confer adequate protection in unvaccinated individuals. Because of costs and limited availability of RIG in large parts of the world, adequate PEP is largely confined to resource rich countries [5]. By contrast, no RIG is needed in individuals who once received rabies vaccinations as pre-exposure prophylaxis (PrEP), provided that two booster vaccinations are administered as soon as possible after exposure.

However, the multiple (three-dose) vaccination rounds and the duration (21/28 Days) of the PrEP schedule hampers vaccination-uptake. Local populations living in high burden, low income countries suffer the highest risk of death from rabies because of lack of availability of RIG and vaccines. People travelling to rabies endemic countries (e.g. tourist travelers, expats, military personnel) are also at risk when they abstain from rabies PrEP because of time constraints.

If an abbreviated, immunization schedule instead of the standard three-dose 21/28-day schedule would prove effective, more people could be protected by immunization in a shorter time at lower cost.

Most of the rabies immunogenicity data are based on the standard three-dose PrEP schedule. In the literature to date, there are few immunogenicity data supporting a two-dose schedule.

In 2015, the Ministry of Defence (MOD) suffered a nationwide shortage of rabies vaccine. It was therefore decided to switch from a three-dose intramuscular (IM) to a three-dose intradermal (ID) vaccination regime, as approved by WHO [2]. Because ID immunization is only registered for off-label use in the Netherlands and because this route had never been used before at the MOD, permission for this strategy was only granted by the MOD on the condition that post-vaccination titers proved to be adequate ($\geq$0.5 IU/mL according to WHO [2,4,6]). It was decided to measure the immune response after two vaccinations, on the day of the third vaccination (day 21 or 28). This

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study falls beyond the scope of the Act concerning Medical Research Involving Human Subjects, as confirmed by the Medical Ethics Committee of the AMC (Number: W17_459#17.531).

The aim of this study was to examine whether adequate rabies antibody titers are reached after two ID doses of rabies vaccine, with a seven-day window. In addition, this study explored possible relationships between the height of rabies antibody titers, and factors including: age, time interval between second vaccination and titer measurement, sex and intradermal wheal sizes measured immediately after ID vaccination.

2. Materials and methods

2.1. Study population

Military personnel of the Dutch marines were vaccinated against rabies before deployment. Only those marines without prior rabies immunization were included. We excluded marines suffering from autoimmune disease, using immunosuppressive drugs, using mefloquine, and those with an allergy to one of the components of the rabies vaccine.

2.2. Study design

We conducted an observational cohort study in the sickbay of the Marine base in Doorn, The Netherlands, between August 2015 and January 2017. All eligible marines were vaccinated by military registered nurses. To ensure an adequate rabies antibody response after ID immunization, according to MOD regulations, an antibody titer was assessed in all subjects, on the day of the third ID rabies vaccination (day 21 or 28 after the first vaccination). Subjects with an inadequate titer were scheduled for an additional titer measurement after the third vaccination.

2.3. Vaccine and wheal size

In this study, all marines received the commercially available rabies vaccine Verorab® (Sanofi Pasteur, United Kingdom; 0.5 mL inactivated WISTAR strain rabies PM/WI38-1503-3M). For each ID-injection, we used one-fifth (0.1 mL) of the full dose. A registered nurse measured the intradermal wheal size directly after each ID-injection. If the wheal size was 5 mm or less, we considered the vaccination as subcutaneous, and the participant would be excluded from the study and vaccinated with a conventional IM schedule.

2.4. Measurement

According to WHO, the gold standard for determination of rabies antibodies is the Rapid Fluorescent Focus Inhibition Test (RFFIT) [2]. The Belgian National Rabies Reference Laboratory at the Scientific Institute of Public Health (WIV-ISP), in Brussels, performed all RFFITs. The results were expressed in International Units per milliliter (IU/mL), which reflects the level of virus-neutralizing antibodies in serum. In accordance with WHO recommendations, we present the total number and percentage of participants in whom titers were $\geq$0.5 IU/mL, and the geometric mean titer (GMT) with 95% confidence interval (CI) and range [6].

2.5. Statistical analysis

All drop-outs (wheal size < 5 mm, blood collection error) were excluded in the analysis (see Fig. 1). A base-10 log transformation was performed on all titers for calculation. We used an unpaired *t*-test to examine whether there was a difference in GMTs tested on days 21 or 28. We examined relationships between log transformed titers and age, interval, sex, and wheal sizes with univariable linear regression. We included variables with a *p*-value less than 0.1 in the multivariable analysis. We performed the analysis using SPSS version 24.0, present 95% CIs and regarded a *p*-value of less than 0.05 as statistically significant.

3. Results

During the study eight participants were excluded, seven because of inadequate wheal sizes and one because of a blood collection error. In total, 430 participants were included for analysis. The study population consisted of 419 men (97%) and 11 women (3%). The median age was 23 years [range 18–48].

After two vaccinations, seroconversion was reached in 427 of the 430 participants (99.3%) (shown in Table 1). Three participants, males aged 21, 25 and 27 years with adequate wheal size, did not seroconvert on day 21. All three seroconverted after the third vaccination. After two ID vaccinations, in the seroconverted group (n = 427), the GMT was 7.59 IU/mL (95% CI 7.04 to 8.17). In 381 (88.6%) participants, the rabies antibody titer was above 3.0 IU/mL; in 199 participants (46.3%) titer levels were above 10.0 IU/mL.

The immunogenicity data for days 21 and 28 are shown separately in Table 1. Both in univariable and multivariable analysis (shown in Table 2), there was a significant difference between GMTs measured on days 21 vs. 28 (*p*-values: < 0.0001). The median size of the wheals on days 0 and 7 was 7 mm (range: 5 to 13) and 8 mm (range: 6 to 13), respectively. In univariable linear regression, wheal size had a significant correlation with the height of antibody titer (*p*-value: < 0.0001 and 0.029). Sex and age did not correlate significantly with the height of antibody titers.

4. Discussion

The results of our study show that an intradermal two-dose, seven-day vaccine strategy provides a high seroconversion rate (SCR) of 99.3% in a large cohort, with a mean titer above 7 IU/mL, suggesting this strategy is equally effective to an intramuscular three-dose vaccination schedule. Based on the observed rise of GMTs from day 21–28, we would expect to achieve a SCR even closer to 100% if all titers would have been measured on day 28.

We performed a post-hoc power calculation on the SCR. Assuming that 99% of subjects would have a titer $\geq$0.5 IU/mL and using exact Clopper-Pearson limits, a sample size of 400 was considered adequate to estimate the percentage with limits of the 95% CI equal to 97.5% and 99.7% [7,8].

Antibody titers after an ID PrEP-schedule are generally lower compared to an IM schedule [9]. However, because booster vaccinations (PEP) in previously vaccinated individuals are always indicated after exposure to a potentially rabid animal, the speed and strength of this booster response are more relevant parameters than the height of the post-PrEP titer [10]. This so called boostability does not differ for both routes of administration in case of rabies PEP treatment with vaccinations on day 0 and 3 [9,11]. Thus, ID immunization is considered equally effective to IM.

Few studies evaluating two-dose schedules have been reported [12]. One study by Mills et al. (2011) reported a two-dose, seven-day ID schedule in 420 persons between 10 and 65 years with a SCR of 94.5% on day 21 [13]. In addition, Kamoltham et al. (2007) used a two-dose, 28-day ID schedule in 43 schoolchildren (5–8 years) with 97.7% SCR on day 49 [14].

Our sample consisted mainly of young adult males (97.3%) and was not representative of travelers seeking advice on rabies prophylaxis. Although, we found no association between sex and the level of antibody titers, the low number of women meant that we had little statistical power to detect such an association. However, because previous more heterogeneous studies [13] showed similar SCRs, we do not expect a clinically relevant effect of sex.

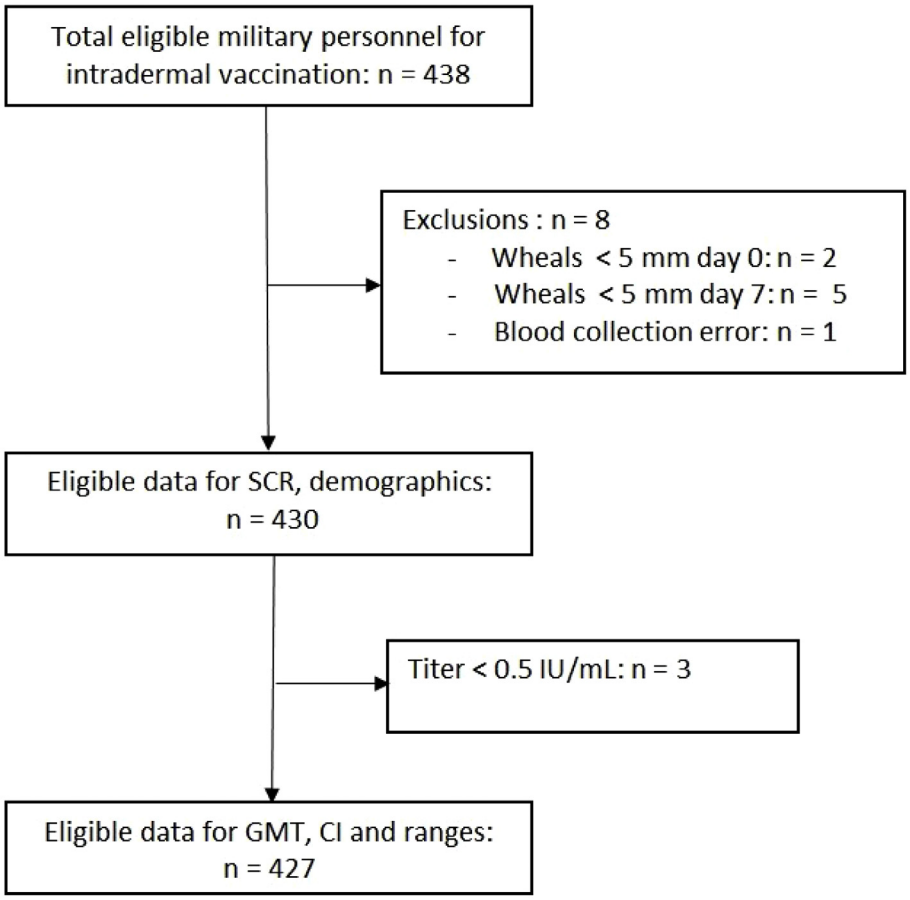

Fig. 1. Study participants selection.
Abbreviations: CI, confidence interval; GMT, geometric mean titer; SCR, seroconversion rate.

Table 1
Rabies immunogenicity data after a two-dose, seven-day intradermal schedule.

Days after first immunization	N	SCR[a]	N[b]	GMT[b]	95% CI[b]	Range[b]
21 and 28	430	99.3	427	7.59	7.04–8.17	0.79–112.51
21	340	99.1	337	6.25	5.80–6.74	0.79–49.99
28	90	100	90	15.65	13.84–17.70	3.19–112.51

Abbreviations: CI, confidence interval; GMT, geometric mean titer; SCR, seroconversion rate.
[a] In percent.
[b] In seroconverted group with international units per milliliter.

A two-dose schedule can be completed in one week. Travelers and military personnel under time constraints, who would otherwise be left unvaccinated, can be considered as adequately protected by this schedule. For populations in endemic areas, local application of a two-dose schedule could provide an opportunity to vaccinate more people with less vaccine, thus reducing the number of rabies deaths. If an exposure to rabies occurs after receiving two-dose PrEP, the post-exposure treatment will consist of prompt wound cleaning and vaccine administration without RIG. This is independent of the time interval between exposure and PreP [15].

At the time of writing, on 1st December 2017, WHO published a recommendation stating that a two-dose, seven-day schedule is to be approved for all age groups of healthy individuals [16]. Given the paucity of published data, this study adds relevant evidence in support of this new policy.

Conflicts of interest

The authors declare no conflict of interest.

Author contribution

The study was designed by JB, CS, SVG and MPG; CADP and JB conducted the study. The RFFIT was performed by ST and SVG; CADP drafted the first version of the manuscript; all authors critically revised

Table 2
Statistics: univariable and multivariable linear regression; relationships between log transformed titers and age, interval, sex, wheal size day 0 and 7.

Characteristic	Univariable (parameter + 95% CI)	*p*-value	Multivariable[a] (parameter + 95% CI)	*p*-value
Age (per 5 years)	−0.016 (−0.046 to 0.013)	0.280	–	–
Interval	0.399 (0.329 to 0.468)	< 0.0001	0.380 (0.304 to 0.455)	< 0.0001
Sex (male)	0.023 (−0.180 to 0.226)	0.825	–	–
Wheal day 0	0.049 (0.030 to 0.069)	< 0.0001	0.013 (−0.007 to 0.032)	0.202
Wheal day 7	0.029 (0.003 to 0.055)	0.029	−0.001 (−0.025 to 0.023)	0.953

[a] We included variables with a *p*-value less than 0.1 in the multivariable analysis.

the draft and approved the final version of the manuscript for submission.

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