Journal of Travel Medicine
International Society of Travel Medicine
Established 1991
Promoting healthy travel worldwide
Journal of Travel Medicine, 2023, 1–10
https://doi.org/10.1093/jtm/taad053
Original Article

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Five accelerated schedules for the tick-borne encephalitis vaccine FSME-Immun® in last-minute travellers: an open-label, single-centre, randomized controlled pilot trial

Nicole Berens-Riha, PhD[1,2,*], Petra Andries, BSc[3], Annelies Aerssens, MD[4], Quentin Ledure, PharmD[5], Yolien Van Der Beken, BSc[6], Leo Heyndrickx, MSc[7], Els Genbrugge, MSc[8], Achilleas Tsoumanis, MSc[8], Yven Van Herrewege, PhD[8], Kevin K. Ariën, PhD[2,7], Martine Van Innis, PharmD[5], Peter Vanbrabant, BSc[3], Patrick Soentjens, PhD[1,3] and FASTBEPROTECT Research Team[‡]

[1]Department of Clinical Sciences, Institute of Tropical Medicine, Antwerp, Belgium, [2]Department of Biomedical Sciences, University of Antwerp, Antwerp, Belgium, [3]Centre for Infectious Diseases, Queen Astrid Military Hospital, Brussels, Belgium, [4]Department of Internal Medicine and Infectious Diseases, Ghent University Hospital, Belgium, [5]Hospital Pharmacy, Queen Astrid Military Hospital, Brussels, Belgium, [6]Clinical Laboratory, Queen Astrid Military Hospital, Brussels, Belgium, [7]Virology Unit, Institute of Tropical Medicine, Antwerp, Belgium and [8]Clinical Trials Unit, Institute of Tropical Medicine, Antwerp, Belgium

*To whom correspondence should be addressed. Email: nberens@itg.be

Preliminary results of the PRNT90 analysis only were presented as poster at NECTM 2022 (prize for best poster presentation).

‡An Spiessens[2], Hanne Landuyt[5], Emeline de Viron[5], Kristy Zaman[2], Eric Dooms[2], Sarah Djebara[2], Katrijn Ghoos[2], Johan Michiels[3], Betty Willems[3], Jozefien Buyze[5], Benjamin Damanet[2], Katrien De Koninck[2], Mony Hing[2], Annelies De Hondt[5]

[1]Department of Clinical Sciences, Institute of Tropical Medicine, Antwerp, Belgium, [2]Centre for Infectious Diseases, Queen Astrid Military Hospital, Brussels, Belgium, [3]Virology Unit, Institute of Tropical Medicine, Antwerp, Belgium, [4]Department of Internal Medicine and Infectious Diseases, Ghent University Hospital, Belgium, [5]Clinical Trials Unit, Institute of Tropical Medicine, Antwerp, Belgium, [6]Hospital Pharmacy, Queen Astrid Military Hospital, Brussels, Belgium, [7]Clinical Laboratory, Queen Astrid Military Hospital, Brussels, Belgium, [8]Department of Biomedical Sciences, University of Antwerp, Antwerp, Belgium.

Submitted 21 February 2023; Revised 4 April 2023; Editorial Decision 12 April 2023; Accepted 12 April 2023

Abstract

Background: The purpose of this exploratory study was to evaluate different accelerated tick-borne encephalitis (TBE) vaccine schedules for last-minute travellers.

Methods: In a single-centre, open-label pilot study, 77 TBE-naïve Belgian soldiers were randomized to one of the following five schedules with FSME-Immun®: group 1 ('classical accelerated' schedule) received one intramuscular (IM) dose at Day 0 and Day 14, group 2 two IM doses at Day 0, group 3 two intradermal (ID) doses at Day 0, group 4 two ID doses at Day 0 and Day 7 and group 5 two ID doses at Day 0 and Day 14. The last dose(s) of the primary vaccination scheme were given after 1 year: IM (1 dose) or ID (2 doses). TBE virus neutralizing antibodies were measured in a plaque reduction neutralization test (PRNT90 and 50) at Days 0, 14, 21, 28, Months 3, 6, 12 and 12+21 days. Seropositivity was defined as neutralizing antibody titres $\geq$10.

Results: The median age was 19–19.5 years in each group. Median time to seropositivity up to Day 28 was shortest for PRNT90 in ID-group 4 and for PRNT50 in all ID groups. Seroconversion until Day 28 peaked highest for PRNT90 in ID-group 4 (79%) and for PRNT50 in ID-groups 4 and 5 (both 100%). Seropositivity after the last vaccination after 12 months was high in all groups. Previous yellow fever vaccination was reported in 16% and associated with lower geometric mean titres of TBE-specific antibodies at all-time points. The vaccine was generally well tolerated.

However, mild to moderate local reactions occurred in 73–100% of ID compared with 0–38% of IM vaccinations, and persistent discolouration was observed in nine ID vaccinated individuals.
Conclusion: The accelerated two-visit ID schedules might offer a better immunological alternative to the recommended classical accelerated IM schedule, but an aluminium-free vaccine would be preferable.

Key words: TBE, TBE vaccination, accelerated schedule, ID vaccination, last-minute traveller, traveller, flavivirus vaccination

Background

Tick-borne encephalitis (TBE) is a viral disease mainly transmitted by the bite of an infected tick (Ixodes sp.) and is endemic in Asia and Central and Eastern Europe. Every year 10 000–15 000 new cases are reported with increasing numbers.[1] One reason for its prevalence might be the growing extent of tick habitats due to climate change and an increase in exposure prone activities.[2]

The tick-borne encephalitis virus (TBEV) consists of positive single-stranded RNA and belongs to the *Flaviviridae* family, genus Flavivirus. It enters through the skin via the tick saliva that contains components enhancing TBEV dissemination. The TBE aetiopathology comprises two phases. During the first viraemic phase, the patient suffers from non-specific symptoms such as fever, headache, fatigue, myalgia, nausea and vomiting.[3] In one to two-thirds of symptomatic patients, the virus crosses the blood–brain barrier, resulting in a second neurological phase with symptoms such as meningitis or neurological focal forms.[4] The case fatality rate reaches 2–3% in Siberia, where sporadically haemorrhagic forms were described.[5] Serological surveys on the other hand indicate asymptomatic courses accounting for 70–98% of all infections. Approximately, only one in every 100–300 tick bites results in symptomatic infection.[6]

Analysis of TBEV-specific IgM and IgG antibodies by enzyme-linked immunosorbent assay is performed in routine diagnostics, but cross-reactivity due to infection with or vaccination against other flaviviruses like West Nile (WN), Japanese encephalitis (JE), dengue or yellow fever (YF) can result in misinterpretation of results.[7] A former YF vaccination might even impair the efficacy of TBE vaccination.[8] The most sensitive method is the plaque reduction neutralization test (PRNT), which is only available in specialized laboratories.[1]

The lack of a standard effective treatment emphasizes the importance of disease prevention via vaccines.[9] Available vaccines are safe and effective. FSME-IMMUN® (Pfizer, Neudörfl strain) was first approved in 1976 for endemic regions. Encepur® (GlaxoSmithKline, K23 strain) was introduced in 1991 in Germany, and others followed.[4,7] For FSME-IMMUN®, the virus is produced in primary chicken embryo fibroblast cells and adsorbed on hydrated aluminium hydroxide.[10]

Since 2011, the World Health Organization recommends vaccination for travellers who plan outdoor activities during their travel in endemic regions.[11] The standard administration schedule consists of two intramuscular (IM) doses given 1–7 months apart and a third dose 1 year later, but it does not meet the needs of last-minute travellers, including soldiers who are sent on missions on short-term notice. The accelerated IM schedule with two vaccinations at Days 0 and 14 is approved for FSME-IMMUN® and Encepur® and recommended for rapid immunization.[12] The purpose of this pilot study was to evaluate different accelerated TBE vaccination schedules by reducing the number of visits and intervals. We parallelly investigated the intradermal (ID) administration route, as lower volumes could consequently reduce the vaccine costs in the case of group or mass vaccinations.

Methods

Study design and objectives

This study was an exploratory single-centre open-label randomized controlled trial with FSME-IMMUN® in TBE-naïve soldiers in a non-endemic area. Group 1 (classical accelerated schedule) received one IM dose at Day 0 and at Day 14 (thereafter '3^{1}IM'), group 2 two IM doses at Day 0 ('$2^{2,1}$IM'), group 3 two ID doses at Day 0 ('2^{2}ID'), group 4 two ID doses at Day 0 and at Day 7 ('3^{2}ID7') and group 5 two ID doses at Day 0 and at Day 14 ('3^{2}ID14'). A last vaccination to finalize the primary schedule was given after 1 year IM or ID (Figure 1).

The primary objective of this study was to estimate the median time to seroconversion of the different groups based on immunogenicity data up to 28 days after the first dose. Seroconversion was defined as the neutralizing antibodies $\geq$10 and was determined by the plaque reduction neutralization test 90 (PRNT90) and PRNT50 (sensitivity analysis). Furthermore, the proportion of subjects with seroconversion at each visit for each vaccination regimen, as well as the geometric mean titres (GMTs) at all visits in all groups, were estimated.

Solicited and unsolicited adverse events (AEs) were recorded after each vaccination session for 7 days, and serious adverse events (SAEs) were reported for 14 days after vaccination. An amendment for a further follow-up of vaccine-related local reactions was added.

Study site, subjects and inclusion criteria

The study was conducted at the Centre of Infectious Diseases, ID_4C, in the Military Hospital Queen Astrid, Brussels, Belgium, between May 2019 and December 2021. Participants were recruited in the Belgian defence personnel. Randomization was performed at the enrolment visit using a scratch list specifying the study group. The inclusion criteria were defined as age between 18 and 60 years, willingness to provide an informed consent and use of safe contraception methods during the study. Seropositive subjects (tested during the screening visit) or with a known allergy to one of the components of the vaccine were excluded from the study. Further exclusion criteria were: immunosuppression, intake of immune-depressant or -stimulant medication, pregnancy or active child wish, planned deployment to TBE endemic regions or an YF vaccination during the study period. Vaccinations with an inactivated vaccine within 2 weeks before or after each vaccination or with a live-attenuated vaccine within 1 month before or after each vaccination were not allowed.

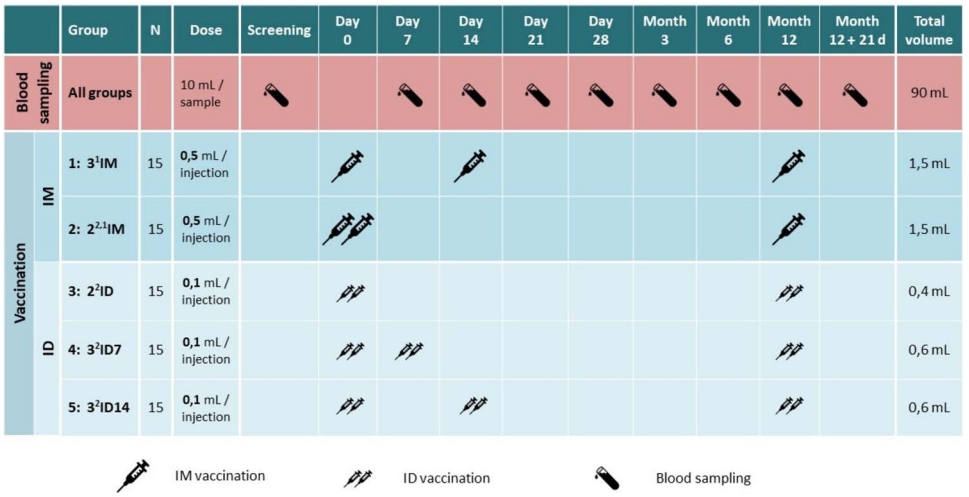

Figure 1. Study design. Legend Figure 1. Study design of all five groups. Group 1 (3^1IM) with three IM injections at Days 0 and 14 and Month 12. Group 2 ($2^{2,1}$IM) with three IM injections, two at Day 0 and one at Month 12. Group 3 (2^2ID) with two ID double injections at Day 0 and Month 12. Group 4 (3^2ID7) with three ID double injections at Days 0 and 7 and Month 12. Group 5 (3^2ID14) with three ID double injections at Days 0 and 14 and Month 12.

Laboratory procedures

The laboratory tests were performed at the Virology Unit of the Institute of Tropical Medicine. TBE virus neutralizing antibodies were measured in a PRNT. Six serial dilutions of heat-inactivated serum (1/10–1/320 in DMEM) were incubated during 1 h (37°C, 7% CO_2) with a pre-titrated amount of TBEV (Hypr strain). Sample virus mixtures were added to previously (Day-1) seeded A549 cells (adenocarcinomic human alveolar basal epithelial ce lls) in a 96-well plate and incubated for 2 h (37°C, 7% CO_2), after which a CMC overlay was added. After a 4-day incubation period (37°C, 7% CO_2) the supernatant was removed, cells were treated with formaldehyde (30 min) and stained with Naphthalene Blue Black (NBB) solution (30 min). After removal of the NBB, cells were rinsed with tap water and plaques were counted. The Reed-Muench method was used to calculate the neutralizing antibody titre that reduced the number of infected wells by 50 (PRNT50) and 90% (PRNT90), which was used as a proxy for the neutralizing antibody concentration in the sample.

All analyses of clinical trial samples were carried out in compliance with Good Clinical Laboratory Practice.

Vaccination procedure

The study vaccine FSME Immun® was stored in the fridge at a temperature between +2 and +8°C, as recommended by the manufacturer. It was brought to room temperature before administration. An ID dose consisted of 0.1 ml (or 1/5) of the vaccine vial, and one full vial (0.5 ml) was administered for an IM vaccination. Double doses were given at different vaccination sites (for IM vaccination in the left and right deltoid muscle; for ID vaccination in the left and right forearm).

Statistical analysis

The primary and secondary objectives were analysed using both an intention-to-treat (ITT) and per-protocol (PP) population, with ITT as primary approach. Due to the COVID19 pandemic, all participants had at least one out-of-window visit. It was therefore decided to exclude individual out-of-window visits in the PP population, so that the subjects would not be excluded entirely from the PP analyses. The visits at Months 6 and 12 were most affected by this, resulting in a low sample size at these visits.

Safety analyses were performed using an all-patients-treated approach, including all participants who received at least a single vaccination.

Participants were censored at the first occurrence of the following events: Day 28 after the start of the primary vaccination, lost-to-follow-up or withdrawal. The median time to seropositivity with a 95% confidence interval (CI) was estimated per schedule, accompanied by a Kaplan–Meier plot. Three participants had a missing intermediate serology result, and all other serology results (before and after missing result) were <10. Therefore, it was decided to impute the missing values as <10.

The number and proportion of participants who were seropositive at each visit were estimated with a 95% Wilson CI for the five vaccination regimens. The incidence of safety endpoints was estimated with 95% Wilson CI for each group separately. GMTs were calculated with 95% CI for each group for each visit. Values <10 were replaced by 5 for this purpose.

Due to recent findings[8] that former YF vaccination might potentially influence the outcome of TBE vaccination, an exploratory analysis was performed. Antibody titres and the proportion of subjects with neutralizing antibodies (≥10) were compared between those with and without previous YF vaccination, pooled over the arms. For the antibody titres, the geometric means and their ratio were calculated with 95% CIs (Cis). The *P*-value was obtained by means of a *t*-test for lognormal data (PROC TTEST Procedure with dist. = lognormal). All analyses were repeated with the PRNT50 results for sensitivity purposes.

All analyses were carried out in SAS/STAT® 12.3 (SAS Institute Inc, USA).

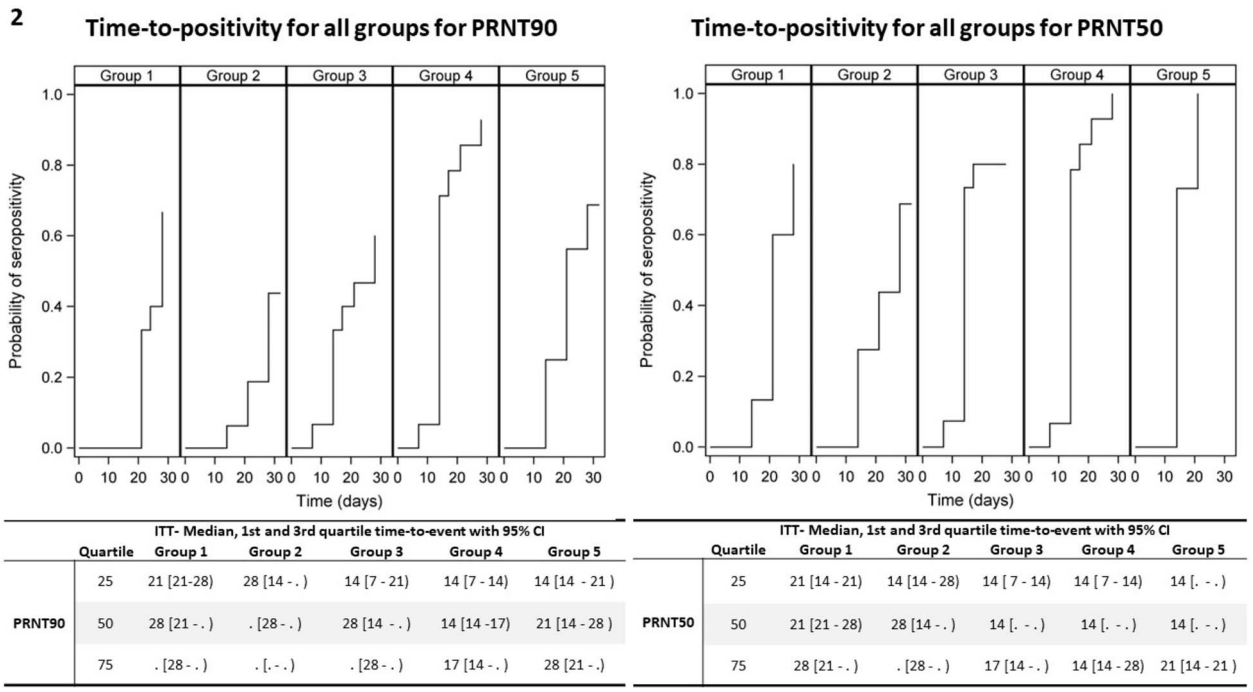

Figure 2. Time to seropositivity for all groups for PRNT90 and PRNT50. **Legend Figure 2.** The Kaplan–Meier graphs show the time to seropositivity in days for the different vaccine schedules between Day 0 and Day 28. The curves describe the cumulative incidence of seroconversion (seropositivity) until Day 28: every patient turned seropositive, stayed seropositive in this analysis, and reconversion to seronegativity is not captured. Group 1 (3^1IM), group 2 ($2^{2,1}$IM), group 3 (2^2ID), group 4 (3^2ID7), group 5 (3^2ID14), ITT analysis.

Ethics and registration

Written informed consents were obtained at screening. The Institutional Review Board of the ITM, the Ethics Committee of University Hospital of Antwerp (UZA) and the Competent Authorities of Belgium (FAMPH) approved the trial. The study was carried out in compliance with the Declaration of Helsinki and according to the most recent Good Clinical Practice guidelines, it was registered in the EudraCT public registry as Eudra-CT 2019-000801-61.

Results

Demographics

Ninety-six Belgian soldiers were screened. Of these, 77 TBE-naïve participants were enrolled and completed the first dose (Day 0). To each of the five groups, 15–16 participants were assigned. Sixty-seven (87%) participants completed the full vaccination schedule, four were lost to follow-up and six withdrew consent due to the local side effects after ID vaccination (Supplemental Table 1).

Participants were aged between 18 and 49 years. Median age was 19–19.5 years in each group. Male subjects were over-represented (83%) compared with female subjects (17%) (Supplemental Table 2).

Serology PRNT90

All serology data presented refer to the ITT-analysis since there was no marked difference with the results from the PP analysis. Median time to seropositivity was shortest for 3^2ID7 with 14 days and for 3^2ID14 with 21 days (Figure 2). Seropositivity was first observed at Day 7 for 2^2ID and 3^2ID7. The 3^2ID7 group peaked the highest and earliest in terms of percentage seropositivity at Day 14 (78.6%), whereas the other ID groups peaked at Days 21 (56.3%, 3^2ID14) and 28 (46.7%, 2^2ID), and the IM groups 3^1IM and $2^{1,2}$IM peaked at day 28 with 53.3 and 25.0%, respectively. No group showed seroconversion of all participants before the last foreseen dose(s) of the primary vaccination schedule at Month 12. The decline of detectable antibodies above the seropositivity threshold after day 28 was substantial in all groups. All but two participants were seroconverted after the last dose(s) (Table 1, Supplemental Figures 1 and 2).

Serology PRNT50 (sensitivity analysis)

The PRNT50 analysis showed results similar to those with PRNT90, but the lower cut-off resulted in higher seropositivity levels in all groups (Figure 2). Median time to seropositivity was short with 14 days in all ID groups. 3^2ID7 and 3^2ID14 showed 100.0% seroconversion at Days 28 and 21, respectively. 2^2ID peaked with 80.0% at Day 21, whereas 3^1IM and $2^{1,2}$IM peaked at Day 28 with 66.7 and 56.3% only. Seropositivity was 100.0% in all groups after the last dose(s) (Table 1, Supplemental Figures 1 and 2).

GMTs

All ID groups showed higher GMTs than the IM groups at Days 14–28, with 3^2ID7 presenting the highest titres at Day 14 [PRNT90: 16.4 (95% CI 8.39–32.2)] and Day 21 (PRNT50:

Table 1. Seropositivity (ITT analysis) in the five different groups at all visits for PRNT90 and PRNT50 (sensitivity analysis)

Seropositivity (ITT analysis) in the five different groups										
	Schedule									
	Group 1 3 [1]IM *N* = 15 *n*% (95% CI)		Group 2 2 [2,1]IM *N* = 16 *n*% (95% CI)		Group 3 2 [2]ID *N* = 15 *n*% (95% CI)		Group 4 3 [2]ID7 *N* = 15 *n*% (95% CI)		Group 5 3 [2]ID14 *N* = 16 *n*% (95% CI)	
	PRNT90	PRNT50	PRNT90	PRNT50	PRNT90	PRNT50	PRNT90	PRNT50	PRNT90	PRNT50
Day 7	0/15 0.0 (0.0–20.4)		0/16 0.0 (0.0–19.4)		1/14 7.1 (1.3–31.5)		1/15 6.7 (1.2–29.8)		0/16 0.0 (0.0–19.4)	
Day 14	0/15 0.0 (0.0–20.4)	2/15 13.3 (3.7–37.9)	1/15 6.7 (1.2–29.8)	4/15 26.7 (10.9–52.0)	6/15 40.0 (19.8–64.3)	12/15 80.0 (54.8–93.0)	11/14 78.6 52.4–92.4)	12/14 85.7 (60.1–96.0)	4/15 26.7 (10.9–52.0)	11/15 73.3 (48.0–89.1)
Day 21	6/15 40.0 (19.8 – 64.3)	9/15 60.0 (35.7–80.2)	3/16 18.8 (6.6–43.0)	6/16 37.5 (18.5–61.4)	4/15 26.7 (10.9–52.0)	11/15 73.3 (48.0–89.1)	10/14 71.4 (45.4–88.3)	13/14 92.9 (68.5– 98.7)	9/16 56.3 (33.2–76.9)	16/16 100.0 (80.6–100.0)
Day 28	8/15 53.3 (30.1 – 75.2)	10/15 66.7 (41.7–84.8)	4/16 25.0 (10.2–49.5)	9/16 56.3 (33.2–76.9)	7/15 46.7 (24.8–69.9)	10/15 66.7 (41.7–84.8)	9/13 69.2 (42.4–87.3)	13/13 100.0 (77.2–100.0)	9/16 56.3 (33.3–76.9)	14/16 87.5 (64.0– 96.5)
Month 3	0/15 0.0 (0.0–20.4)	1/15 6.7 (1.2–29.8)	1/16 6.3 (1.1–28.3)	4/16 25.0 (10.2–49.5)	1/15 6.7 (1.2–29.8)	1/15 6.7 (1.2–29.8)	1/15 6.7 (1.2–29.8)	5/15 33.3 (15.2–58.3)	0/16 0.0 (0.0–19.4)	3/16 18.8 (6.6–43.0)
Month 6	0/15 0.0 (0.0–20.4)	1/15 6.7 (1.2–29.8)	1/16 6.3 (1.1–28.3)	2/16 12.5 (3.5–36.0)	2/15 13.3 (3.7–37.9)	3/15 20.0 (7.0–45.2)	2/15 13.3 (3.7–37.9)	5/15 33.3 (15.2–58.3)	1/15 6.7 (1.2–29.8)	3/15 20.0 (7.0–45.2)
Month 12	0/15 0.0 (0.0–20.4)	5/15 33.3 (15.2–58.3)	1/15 6.7 (1.2–29.8)	3/15 20.0 (7.0- 45.2)	1/13 7.7 (1.4–33.3)	2/13 15.4 (4.3–42.2)	5/11 45.5 (21.3–72.0)	6/11 54.5 (28.0–78.7)	4/13 30.8 (12.7–57.6)	7/13 53.8 (29.1–76.8)
Month 12 + 21 days	15/15 100.0 (79.6–100.0)		14/15 93.3 (70.2–98.8)	15/15 100.0 (79.6–100.0)	13/13 100.0 (77.2–100.0)		10/11 90.9 (62.3–98.4)	11/11 100.0 (74.1–100.0)	13/13 100.0 (77.2–100.0)	

50.8 (95% CI 27.1–95.1). GMTs for both cut-offs were markedly higher 21 days after the third vaccination compared with results after the first two vaccinations. Vaccination schedules with three vaccination visits in total (3^1IM, 3^2ID7, 3^2ID14) showed higher titres than those with only two vaccination visits. Schedules with an interval of 14 days (3^1IM, 3^2ID14) developed the highest titres (Figure 3, Supplemental Table 3).

Serology and previous yellow fever vaccination

Twelve (15.6%) of the 77 participants had received a previous yellow fever (YF) vaccination. They showed lower GMTs of neutralizing antibodies than YF naïve participants at each visit. The difference was statistically significant at Days 21 and 28, Month 12 and 21 days later for both cut-offs (pooled analysis over all groups) (Table 2). Two participants in the PRNT90 analysis were non-responders (female, 33 years, group 2 / male, 33 years, group 4) throughout the study, they had received an YF vaccination in the past. With the PRNT50 cut-off, both were seropositive after the last vaccination but had very low titres (10 and 12). The YF vaccinations were given between 1994 and 2018, and no specific pattern was observed between antibody response and the date of vaccination (Supplemental Figure 3). None of the vaccinees had a documented JE vaccination. Other flavivirus infections were not actively asked. None of the participants had been deployed to a dengue fever endemic country, but past private exposure to flaviviruses cannot be excluded.

Safety

The vaccine was generally well tolerated. Until Day 28, any vaccine-related AEs including general symptoms were observed in 4 (26.7%), 0 (0%), 1 (6.7%), 2 (13.3%) and 2 (12.5%) participants for group 3^1IM, $2^{2,1}$IM, 2^2ID, 3^2ID7 and 3^2ID14, respectively. However, mild to moderate local reactions occurred in 100.0% of the ID groups compared with 0.0–37.5% in the IM groups after the first dose(s), with lower prevalence after the last dose(s) (Supplemental Table 4A and 4B). Sequelae at the injection site after ID administration were further followed up, 9 of 46 (19.6%) ID participants developed persistent discolouration. Four participants are still followed-up for >3 years and recently presented with reddish-brown spots (diameter 0.5–1 cm) at the injection site (Supplemental Figure 4). No vaccine-related SAEs were reported in the study.

Discussion

This non-commercial pilot study investigated five accelerated IM and ID schedules of TBE vaccination for last-minute travellers. The ID schedules showed, in general, a short median time to seropositivity and acceptable to excellent seroconversion until Day 28. However, local side effects were frequent, and sometimes long-lasting discoloration was observed.

First reports about ID TBE vaccination in few individuals were published by an Austrian group in the 1980s. A single multisite ID vaccination of the same dose used for IM administration resulted in seroconversion of all vaccines compared with only one-third with single IM dose.[13,14] Similarly, the ID groups showed better early immune responses than the IM groups in our study. ID administration seems to evoke fast seroconversion. All vaccines with 3^2ID7 and 3^2ID14 were seropositive until Days 28 and 21, respectively. The single-visit ID 2^2ID schedule peaked early at Day 14 (80%) (PRNT50).

The classical accelerated IM schedule (3^2IM) resulted in a slow and insufficient antibody response, only half (PRNT90) to two-third (PRNT50) of the participants in our study had seroconverted at Day 28. Data are consistent compared with a published trial from the Czech Republic, in which seropositivity at Day 21 after two IM doses (Days 0 and 14) was achieved by only half of the participants (53%, PRNT50).[15]

The double dose IM (2 × 0.5 ml) at Day 0 (group 2) showed a weaker response, with only 25 (PRNT90)–56.3% (PRNT50) of the participants having antibodies at Day 28, and very low GMTs in general. As in the Czech study one IM dose at Day 0 resulted in 28–29% seropositivity, the question could be raised whether a double dose has any benefit compared with a single dose.

After Day 28, antibody levels waned in all groups, leading to the question of whether short-term seroconversion confers full mid-term protection. In any case, the last dose of the primary vaccination schedule showed an excellent booster effect.

Reduction of intervals and visit frequency seemed unfavourable when looking at the final GMTs. Higher frequency of visits and longer intervals in between showed the best results, as expected (schedules with vaccinations at day 0, 14 and 365).

FSME Immun® was exclusively vaccinated in our study. Encepur® was used in the Czech study and showed similar results IM. Other new vaccines might evoke different immune responses or side effects. Although local reactions were mostly mild and short-term, the frequency after ID administration was remarkable and, for nine participants, even persistent.

This is most likely attributable to the aluminium content of the vaccine.[16] Aluminium-free TBE vaccines might increase ID administration acceptance. The Russian aluminium-free vaccine Evervac® was found non-inferior to a commercial TBE vaccine in a phase I/II study. However, ID administration was not tested.[17]

Cross-reactions of the TBE virus with other flaviviruses are not broadly studied. TBEV is closely related to the Omsk haemorrhagic fever virus, which is endemic in Russia and can cause gastrointestinal or bleeding problems and sometimes encephalitis.[18] Flavivirus post-infection or post-immunization sera contain a subset of strongly cross-reactive antibodies that show low-affinity binding to virions, possibly resulting in low cross-reactive neutralizing activity and antibody binding enhancement.[19] A recent study by Bradt and colleagues showed that a pre-existing YF immunity could impair and modulate the antibody response to TBE vaccination. A TBE vaccination resulted in a strong boost of broadly flavivirus cross-reactive antibodies in YF pre-vaccinated participants but lower TBE neutralizing antibodies compared with YF-naïve participants. The effect was most pronounced after the second vaccination (Day 28) and decreased over time, with neutralizing antibodies equalizing after the third vaccination.[8] These observations could be confirmed in our study. Due to the exploratory nature of our study, conclusions can only be drawn very cautiously, but a previous YF vaccination corresponded with lower TBE GMTs at all visits. The time interval between the YF and

Table 2. Influence of previous YF vaccination on the vaccination outcome

A.PRNT90	**A.PRNT90 YF vaccination: Geometric mean ab titres, ratio, and percentages**					
	Geometric mean (95% CI)		**Ratio geometric means (95% CI and *P*-value)**		***n* seropositive % seropositive (95% Wilson CI)**	
Visit	**No YF vaccination**	**YF vaccination**	**Ratio (95% CI)**	***P*-value**	**No YF vaccination**	**YF vaccination**
Day 0	5.00 (5.00–5.00)	5.00 (5.00–5.00)	1.00 (1.00–1.00)	.	0/65 0.0 (0.0–5.6)	0/12 0.0 (0.0–24.2)
Day 7	5.29 (4.87–5.74)	5.00 (5.00–5.00)	1.06 (0.97–1.15)	0.18	2/64 3.1 (0.9–10.7)	0/12 0.0 (0.0–24.2)
Day 14	8.43 (6.61–10.7)	5.85 (4.13–8.29)	1.44 (0.96–2.17)	0.08	21/63 33.3 (22.9–45.6)	1/11 9.1 (1.6–37.7)
Day 21	10.4 (8.22–13.2)	5.41 (4.53–6.46)	1.92 (1.44–2.55)	**<0.0001**	31/65 47.7 (36.0–59.6)	1/11 9.1 (1.6–37.7)
Day 28	10.9 (8.62–13.8)	5.41 (4.53–6.46)	2.02 (1.52–2.68)	**<0.0001**	36/64 56.3 (44.1–-67.7)	1/11 9.1 (1.6–37.7)
Month 3	5.57 (4.85–6.38)	5.00 (5.00–5.00)	1.11 (0.97–1.28)	0.12	3/65 4.6 (1.6–12.7)	0/12 0.0 (0.0–24.2)
Month 6	5.64 (5.03–6.33)	5.00 (5.00–5.00)	1.13 (1.01–1.27)	**0.04**	6/65 9.2 (4.3–18.7)	0/11 0.0 (0.0–25.9)
Month 12	6.50 (5.56–7.61)	5.00 (5.00–5.00)	1.30 (1.11–1.52)	**<0.01**	11/55 20.0 (11.6–32.4)	0/12 0.0 (0.0–24.2)
Month 12 + 21 days	82.2 (66.2–102)	43.7 (22.2–85.9)	1.88 (1.09–3.23)	**0.02**	55/55 100.0 (93.5–100.0)	10/12 83.3 (55.2–95.3)
B.PRNT50	**B.PRNT50 YF vaccination: Geometric mean ab titres, ratio, and percentages**					
	Geometric mean (95% CI)		**Ratio geometric means (95% CI and *P*-value)**		***n* seropositive % seropositive (95% Wilson CI)**	
Visit	**No YF vaccination**	**YF vaccination**	**Ratio (95% CI)**	***P*-value**	**No YF vaccination**	**YF vaccination**
Day 0	5.00 (5.00–5.00)	5.00 (5.00–5.00)	1.00 (1.00–1.00)	.	0/65 0.0 (0.0–5.6)	0/12 0.0 (0.0–24.2)
Day 7	5.40 (4.83–6.04)	5.00 (5.00–5.00)	1.08 (0.97–1.21)	0.17	2/64 3.1 (0.9–10.7)	0/12 0.0 (0.0–24.2)
Day 14	15.4 (11.5–20.7)	7.38 (3.90–14.0)	2.09 (0.99–4.41)	0.05	39/63 61.9 (49.6–72.9)	2/11 18.2 (5.1–47.7)
Day 21	28.1 (20.9–37.8)	7.26 (4.70–11.2)	3.87 (2.34–6.42)	**<0.0001**	52/65 80.0 (68.7–87.9)	3/11 27.3 (9.7–56.6)
Day 28	27.2 (20.3–36.5)	8.66 (5.09–14.7)	3.14 (1.51–6.54)	**<0.01**	52/64 81.3 (70.0–88.9)	4/11 36.4 (15.2–64.6)
Month 3	6.68 (5.58–7.98)	5.00 (5.00–5.00)	1.34 (1.12–1.60)	**<0.01**	14/65 21.5 (13.3–33.0)	0/12 0.0 (0.0–24.2)
Month 6	6.67 (5.62–7.90)	5.86 (4.62–7.44)	1.14 (0.86–1.51)	0.35	12/65 18.5 (10.9–29.6)	2/11 18.2 (5.1–47.7)
Month 12	9.43 (7.44–12.0)	5.00 (5.00–5.00)	1.89 (1.49–2.39)	**<0.0001**	23/55 41.8 (29.7–55.0)	0/12 0.0 (0.0–24.2)
Month 12 + 21 days	151 (124–183)	83.6 (43.0–163)	1.80 (1.09–2.98)	**0.02**	55/55 100.0 (93.5–100.0)	12/12 100.0 (75.8–100.0)

P-values >0.05 are marked in bold.

the TBE vaccination varied highly and seemed to show no specific pattern concerning TBE antibody response. For both non/low-responders, an YF vaccination was documented. Possible factors influencing vaccine success, such as gender imparity or old age, could be ruled out.[20–22] For European travellers, cross reactions between TBE, YF, JE and now dengue vaccinations are most relevant. Also imported dengue, WN or Zika virus infections are likely to increase in the future, more dengue vaccines for travellers will come on the market. Interactions between flavivirus post-infection or post-vaccination antibodies need to be considered in future vaccine schedules.

The randomized controlled design and the overall good follow-up rate of 87% were strengths of the trial, which was conducted by a team that has substantial experience

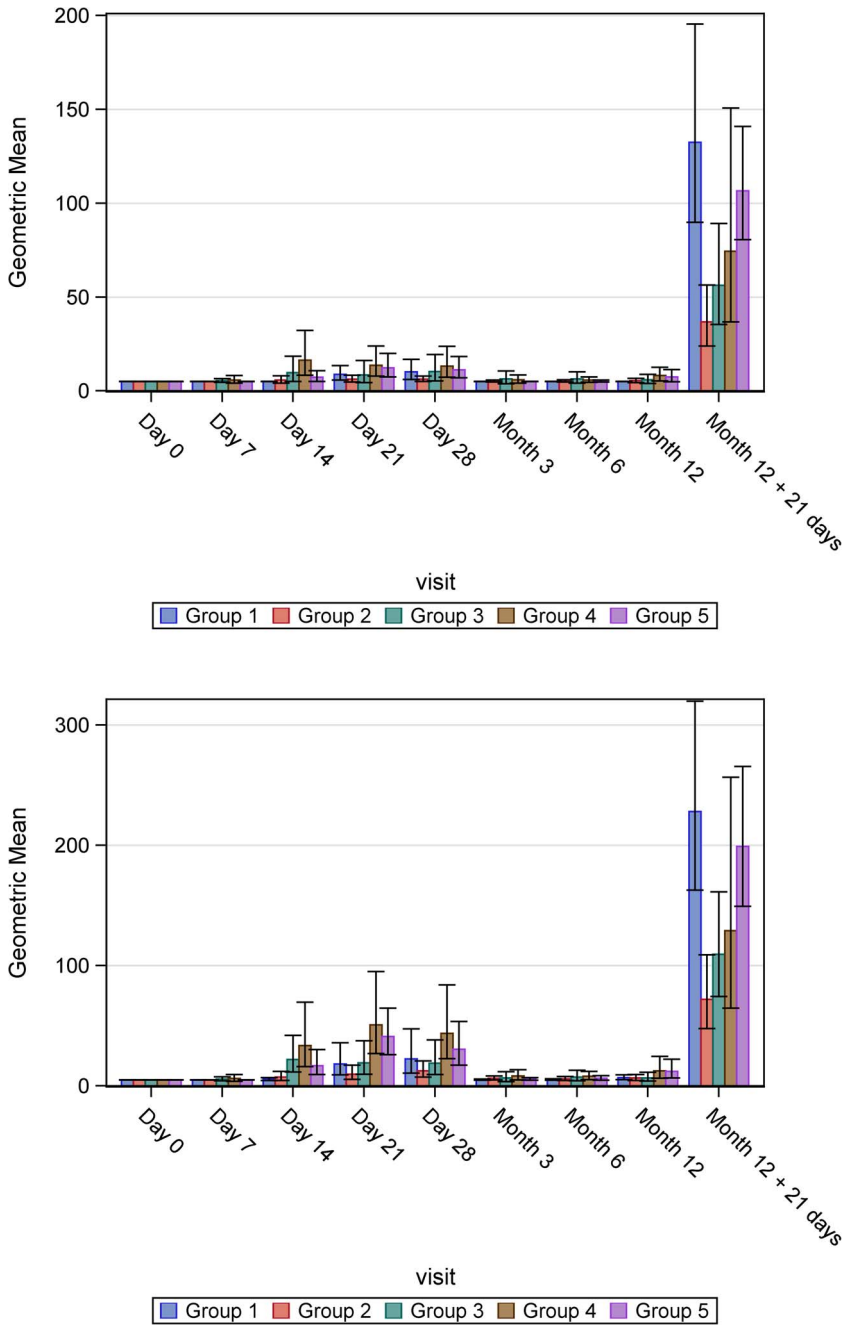

Figure 3. GMT levels over time for the groups 1–5 for PRNT90 (A) and PRNT50 (B). **Legend Figure 3.** GMT levels at each visit with CIs in the groups 1–5. Group 1 (3^{1}IM), group 2 ($2^{2,1}$IM), group 3 (2^{2}ID), group 4 (3^{2}ID7), group 5 (3^{2}ID14), ITT analysis

in performing vaccine trials and appropriate ID injections. However, evident limitations of this trial were the low sample size, the explorative nature and the fact that most participants were very young males, making them not representative of the whole population. The trial was not designed to make formal comparisons between the schedules; *P*-values from the exploratory analyses should be interpreted with care. The study was intended as a two-step approach: first an exploratory study with a low sample size to assess safety and plausible immunogenicity effect sizes without comparing the groups formally. A full and formally powered non-inferiority trial would be planned afterwards. Although the immunogenicity data look promising, the persistent dermal discolouration after ID injection made us hesitate to vaccinate larger groups ID. To provide consultants with well-founded information for last-minute travellers, larger TBE vaccine studies with ID administration in a more diverse, gender-equal and especially older population are needed. But trial participants need to be informed about the potential persistent local discolouration if aluminium-containing TBE vaccines are to be used.

Conclusions

The accelerated TBE ID schedules 3^2ID7 and 3^2ID14 might offer a better immunological alternative for last-minute travellers at risk to the recommended classical accelerated IM schedule according to this exploratory pilot study. ID schedules with two visits before Day 28 showed short median time to seropositivity of 14 days and 100% seroconversion until Day 28 for PRNT50. Powered RCTs with a more diverse study population are needed to demonstrate the non-inferiority of the accelerated ID schedules compared with the standard IM schedule. However, the evaluation of aluminium-free alternatives would be preferable and their development should be encouraged.

Supplementary data

Supplementary data are available at *JTM* online.

Funding

The study was financed by a grant by the Royal Higher Institute for Defense Brussels ('HFM 18-1: ALL-IN-ONE: FASTBEPROTECT'). The Institute of Tropical Medicine of Antwerp (ITM) acted as sponsor of the study.

Acknowledgements

We thank the participants for their involvement in the study and all helping hands of the medical team of the Queen Astrid Military Hospital in Brussels during the vaccinations and administrative processes.

Authors' contributions

Nicole Berens-Riha (data processing, data analysis, drafting of first manuscript, correspondence), Petra Andries (coordination of patient care, vaccinations and data entry), Annelies Aerssens (study design and manuscript writing), Quentin Ledure (coordination and performance of vaccine handling), Yolien Van Der Beken (supervision of sample collection and transport from the study site to the ITM laboratory), Leo Heyndrickx (Performance of PRNTs), Els Genbrugge (design of the statistical analysis plan, performance of the statistical analysis), Achilleas Tsoumanis (design of the statistical analysis plan, performance of the statistical analysis) Yven Van Herrevege (supervision of data management and sponsoring tasks), Kevin K. Ariën (coordination and supervision of the PRNTs), Martin Van Innis (supervision of the vaccine handling), Peter Vanbrabant (sub-investigator, clinical trial physician), The FASTPROTECT research team (design and writing of the protocol, statistical analysis, data and patient management), Patrick Soentjens (PI of the study, design of the study, grant application and overall coordination) All authors revised the manuscript.

Conflict of interest

None declared.

Data availability statement

Anonymised data can be shared in agreement with the ITM data sharing policy.

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