Low-dose yellow fever vaccination in infants: a randomised, double-blind, non-inferiority trial

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Statistical analysis of secondary outcomes and missing data handling

Secondary outcome analyses included assessing non-inferiority using $PRNT_{90}$, and estimating the GMT, GMFI and GMT ratios and GMFI ratios of fractional dose to the standard dose using $PRNT_{50}$ and $PRNT_{90}$ at days 10, 28 and 365 after vaccination in both the PP and the ITT populations. The rate of evolutions of the antibody titres over the study period was compared using a linear mixed effects model. At the analysis stage, we rigorously explored missing data and observed acceptably low attrition rates. There were both intermittent and monotone missing data patterns. Logistic regression analysis was used to investigate the association of the missing primary outcome values with the dose group and baseline characteristics. The outcome variable in the logistic regression model was dropout defined such that it was a 0 if the blood sample result was available and 1 if the blood sample result was missing. The covariates were dose group, age, sex, temperature, baseline yellow fever status and previous medical illness. About 6% of participants missed day 10 (26 participants) and day 28 (27 participants) visits and 8% (n=36) participants did not have the last follow-up visit sample; 33 missed the last follow-up visit for unknown reasons, 1 (<1%) child had no sample due to blood sampling difficulty, 2 (<1%) day 365 samples had the same labels (see Figure 1). Thus, a total of 89 missing data values occurred and were distributed equally across the dose groups (Tables S1 and S2; Chi squared P=0.828), and the odds of having a missing value was not associated with any of the participant baseline characteristics (Table S2). There were no missing values for baseline characteristics and/or covariates. A sensitivity analysis was performed by analysing only those with complete data and our findings/conclusions did not change. To summarise, given the reasonable non-response rate, that the non-response was indifferentially distributed across the participant baseline characteristics and treatment group, and that our analyses used all available data, the bias due to non-response would not severely bias our findings. Thus, our findings are assumed valid under the missing at random mechanism [1].

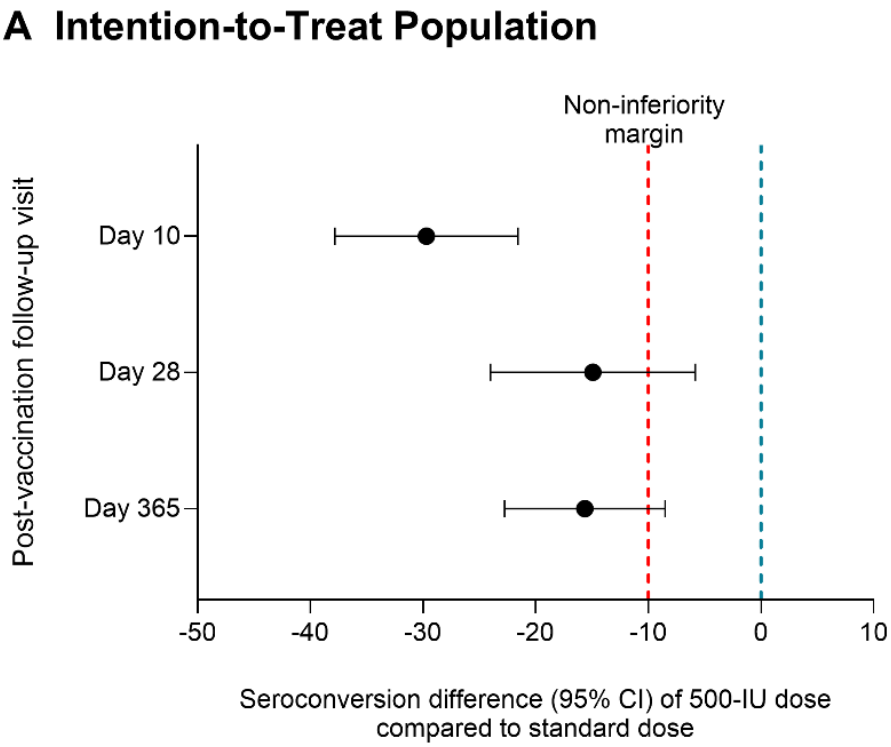
Table S1. Missing outcome data over study period by dose group

	Day of visit after first vaccination				
	0	10	28	365	Combined
Fractional dose	0	12	14	18	44
Standard dose	0	14	14	18	46
Total	0	26	28	36	90

Table S2. Association of missing outcome data with dose group and baseline characteristics.

	Odds ratio (95% CI)	P-value
Dose level group		
Standard dose	Reference	
Fractional dose	0.96 (0.62–1.46)	0.834
Mean age at enrolment (SD), months	1.1 (0.73–1.68)	0.644
Sex		
Female	Reference	
Male	1.17 (0.77–1.8)	0.463
Mean temperature (SD), ºC	0.74 (0.43–1.26)	0.261
Seropositive to yellow fever at baseline		
No	Reference	
Yes	1.38 (0.72–2.66)	0.336
Reported previous medical illness		
No	Reference	
Yes	0.55 (0.13–2.28)	0.410

* Data are adjusted odds ratios (95% confidence interval, CI). None of the covariates had missing values.



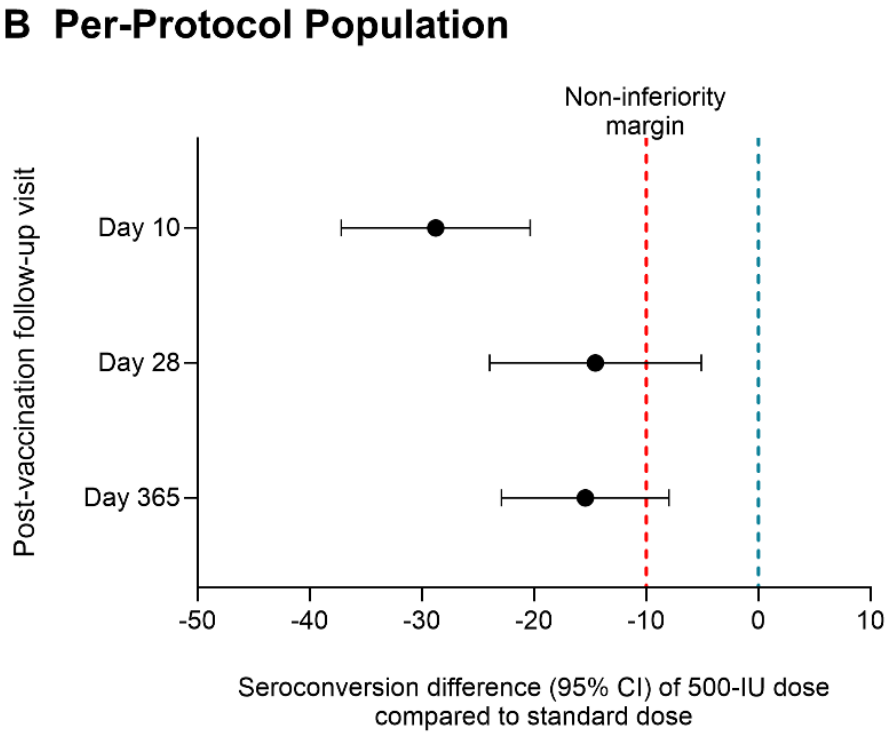


Figure S1. Noninferiority comparison of the seroconversion rate of the 500 IU dose with the full standard YF vaccine dose for the intention-to-treat (Panel A) and per-protocol (Panel B) population using $PRNT_{90}$.

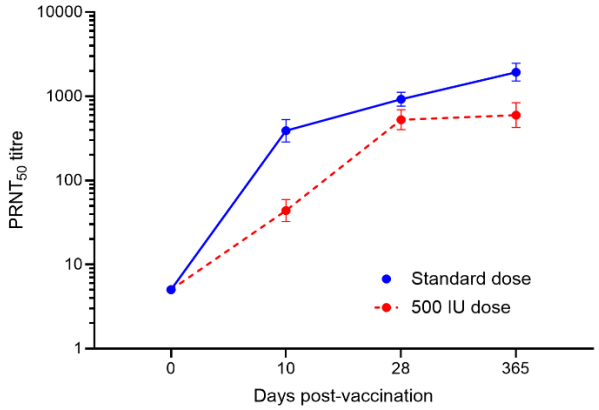
Table S3. Seroconversion and geometric mean titre in the per-protocol and the intention-to-treat populations by $PRNT_{90}$

	Seroconversion*, n/N (%, 95% CI)	Seroconversion difference†, percentage points (95% CI)	Geometric mean titre (95% CI)	Geometric mean titre ratio‡ (95% CI)	Geometric mean fold increase titre (95% CI)	Geometric mean fold increase ratio‡: (95% CI)
Per-protocol analysis						
Day 10		-28.76 (-37.19 to -20.34)		0.43 (0.35 to 0.53)		0.43 (0.35 to 0.53)
500 IU dose	18/180 (10%, 6–15)	-	6.3 (5.8 to 6.9)	-	1.3 (1.2 to 1.4)	-
Standard dose	69/178 (39%, 32–46)	-	14.7 (12.3 to 17.6)	-	2.9 (2.5 to 3.5)	-
Day 28		-14.53 (-23.97 to -5.08)		0.66 (0.52 to 0.83)		0.66 (0.52 to 0.83)
500 IU dose	112/179 (63%, 55–69)	-	24 (20 to 28)	-	5 (4 to 6)	-
Standard dose	138/179 (77%, 70–83)	-	36 (31 to 43)	-	7 (6 to 9)	-
Day 365		-15.43 (-22.89 to -7.97)		0.46 (0.35 to 0.59)		0.46 (0.35 to 0.59)
500 IU dose	134/174 (77%, 70–83)	-	38 (31 to 45)	-	8 (6 to 9)	-
Standard dose	159/172 (92%, 87–96)	-	82 (69 to 98)	-	16 (14 to 20)	-
Intention-to-treat analysis						
Day 10		-29.7 (-37.82 to -21.58)		0.42 (0.35 to 0.52)		0.42 (0.35 to 0.51)
500 IU dose	21/198 (11%, 7–16)	-	6.5 (6 to 7.1)	-	1.3 (1.2 to 1.4)	-
Standard dose	79/196 (40%, 34–47)	-	15.4 (12.9 to 18.4)	-	3.1 (2.6 to 3.7)	-
Day 28		-14.92 (-24.02 to -5.82)		0.65 (0.52 to 0.81)		0.65 (0.52 to 0.81)
500 IU dose	120/196 (61%, 54–68)	-	24 (20 to 28)	-	5 (4 to 5)	-
Standard dose	150/197 (76%, 70–82)	-	36 (31 to 42)	-	7 (6 to 8)	-
Day 365		-15.63 (-22.76 to -8.49)		0.47 (0.37 to 0.59)		0.46 (0.36 to 0.59)
500 IU dose	147/192 (77%, 70–82)	-	38 (31 to 45)	-	7 (6 to 9)	-
Standard dose	177/192 (92%, 87–95)	-	81 (69 to 95)	-	16 (14 to 19)	-

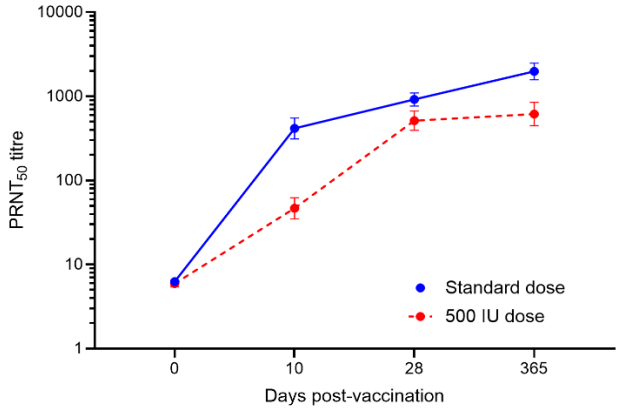
*Seroconversion is defined as a ≥4-fold rise in $PRNT_{90}$ at each timepoint from baseline; N is the number of children in the per-protocol or intention-to-treat population; n is the number seroconverted; seroconversion rate, %=n/N times 100. †Seroconversion

difference=500 IU−Standard. ‡Geometric mean titre ratio, Geometric mean fold increase ratio=500 IU÷Standard. A GMT ratio or GMFI ratio less than 1 favours the standard dose a ratio great than 1 favours the 500 IU dose.

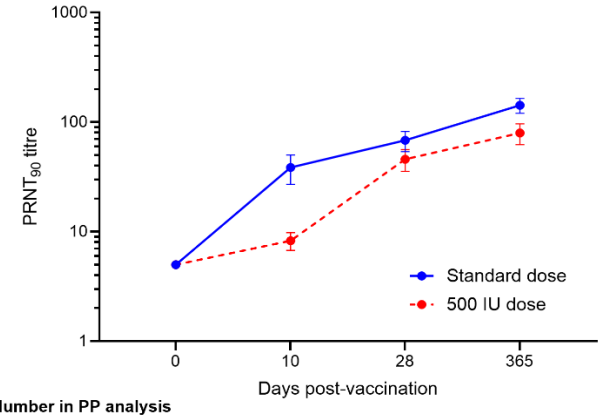
A PRNT$_{50}$ in per-protocol population



B PRNT$_{50}$ in intention-to-treat population



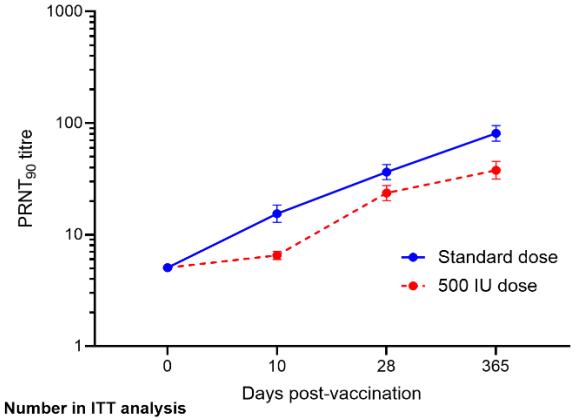
C PRNT$_{90}$ in per-protocol population



Number in PP analysis

500 IU dose	192	180	179	174
Standard dose	188	178	179	172

D PRNT$_{90}$ in intention-to-treat population



Number in ITT analysis

500 IU dose	210	198	196	192
Standard dose	210	196	197	192

Figure S2. Geometric mean titres at different timepoints in the per-protocol population using $PRNT_{50}$ (Panel A) and $PRNT_{90}$ (Panel C) and in the intention-to-treat population using $PRNT_{50}$ (Panel B) and $PRNT_{90}$ (Panel D)

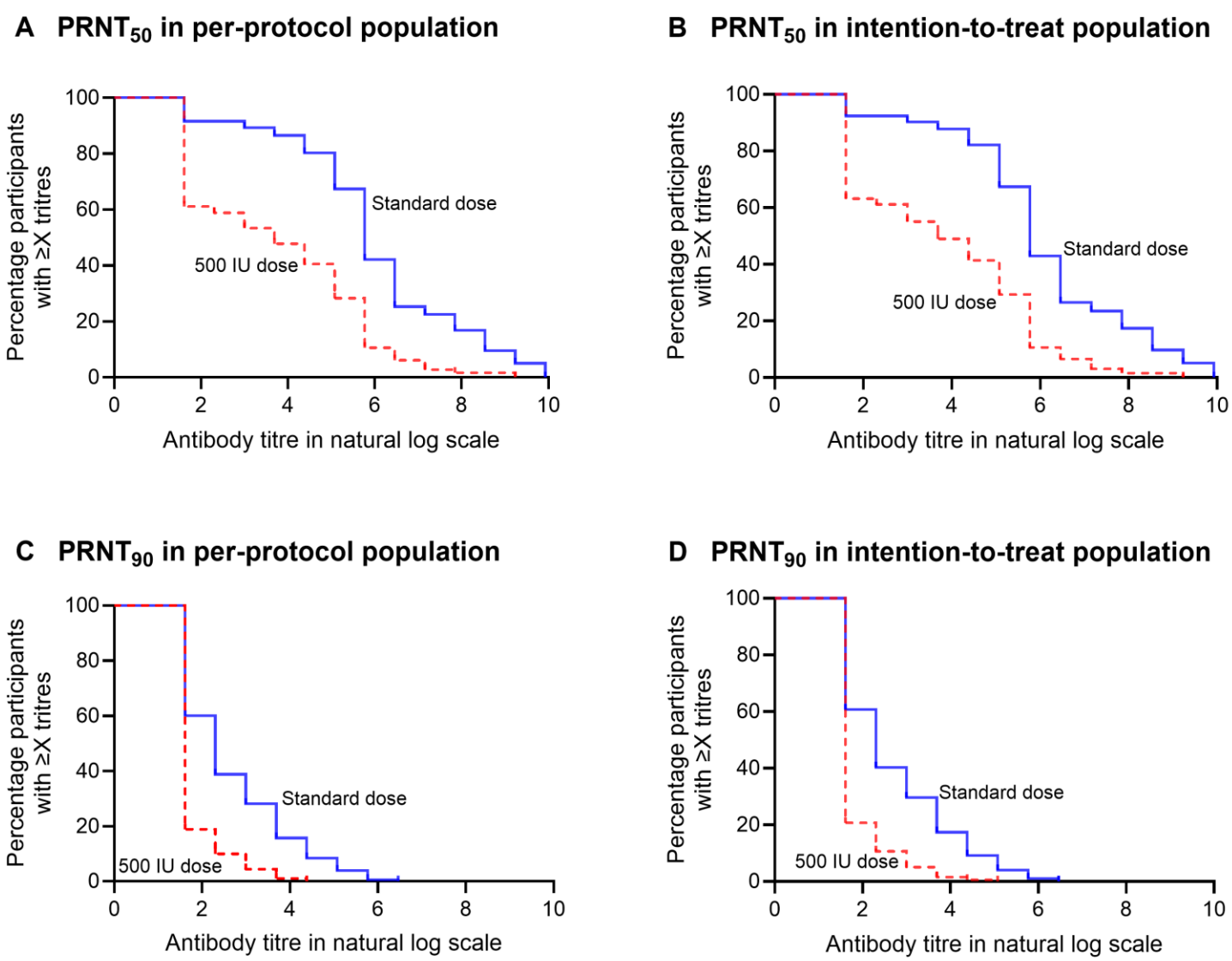


Figure S3. Reverse cumulative distribution curves for antibody tires by day 10 for the Per-protocol and intention-to-treat populations.

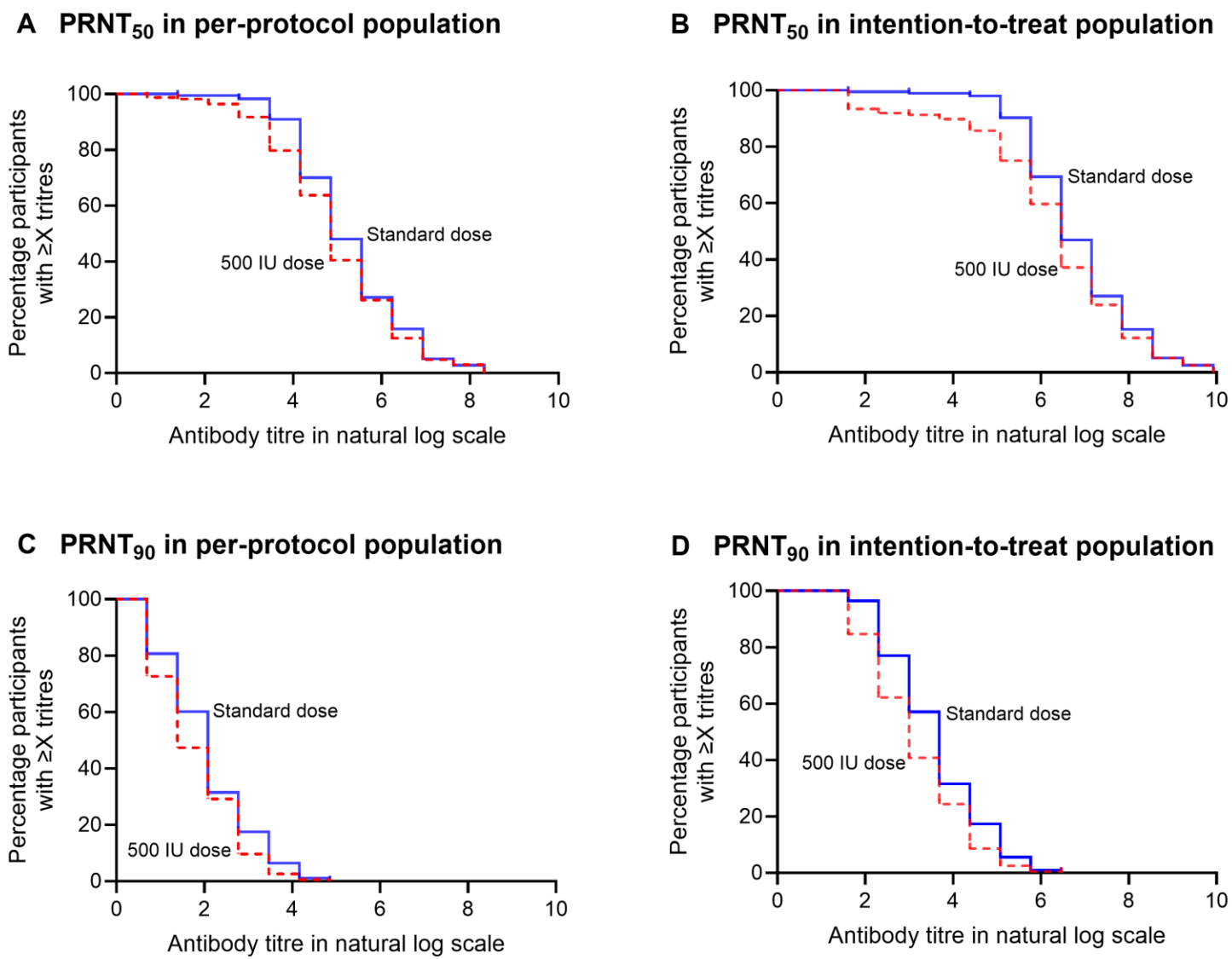


Figure S4. Reverse cumulative distribution curves for antibody tires by day 28 for the Per-protocol and intention-to-treat populations.

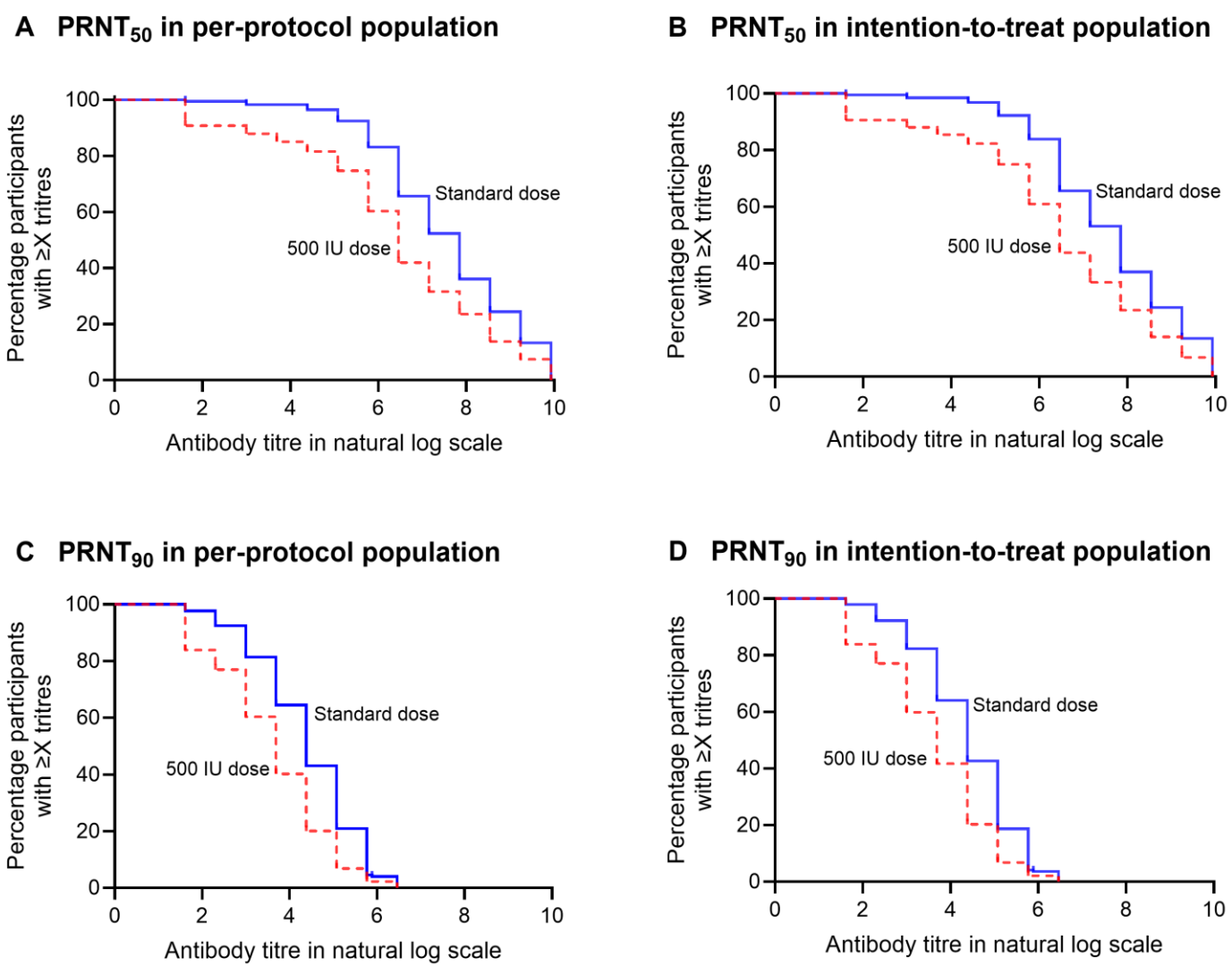


Figure S5. Reverse cumulative distribution curves for antibody tires by day 365 for the Per-protocol and intention-to-treat populations.

Table S4. Incidences of adverse events (preferred terms) for 500 IU and Standard dose groups listed from the most common.

Adverse event	500 IU dose	Standard dose	Relative risk† (95% CI)
Respiratory tract infection	77	64	1.20 (0.92–1.58)
Diarrhoea	25	21	1.19 (0.69–2.06)
Conjunctivitis	9	10	0.90 (0.37–2.17)
Rash	11	8	1.38 (0.56–3.35)
Fever	11	6	1.83 (0.69–4.87)
Cough	8	7	1.14 (0.42–3.09)
Gastroenteritis	6	8	0.75 (0.26–2.12)
Abscess	2	6	0.33 (0.07–1.63)
Eczema	4	2	2.00 (0.37–10.8)
Wound	2	3	0.67 (0.11–3.95)
Dermatitis allergic	3	1	3.00 (0.31–28.61)
Bronchiolitis	0	3	0.00
Oral candidiasis	0	3	0.00
Otitis externa	2	1	2.00 (0.18–21.89)
Body tinea	0	2	0.00
Chicken pox	0	2	0.00
Decreased appetite	0	2	0.00
Folliculitis	2	0	2.00 (0.18–21.89)
Malaria	1	1	1.00 (0.06–15.88)
Otitis media	0	2	0.00
Rhinorrhoea	2	0	2.00 (0.18–21.89)
Scabies	1	1	1.00 (0.06–15.88)
Tinea capitis	2	0	2.00 (0.18–21.89)
Atopy	0	1	0.00
Ear infection	1	0	1.00 (0.06–15.88)
Eye infection	1	0	1.00 (0.06–15.88)
Haematoma	0	1	0.00
Iron deficiency anaemia	0	1	0.00
Parotitis	1	0	1.00 (0.06–15.88)
Pneumonia	1	0	1.00 (0.06–15.88)
Rhinitis	1	0	1.00 (0.06–15.88)
Septic spots	1	0	1.00 (0.06–15.88)

Tinea corporis	1	0	1.00 (0.06–15.88)

† 500 UI dose versus standard dose.

Table S5. Demographics and baseline characteristics by site

	Kilifi	Mbarara
Number enrolled	210	210
Mean age at enrolment (SD), months	9.2 (0.4)	9.3 (0.5)
Sex		
Female	98 (47%)	116 (55%)
Male	112 (53%)	94 (45%)
Mean temperature (SD), ^{0}C	36.3 (0.4)	36.4 (0.4)
Seropositive to yellow fever at baseline	35 (17%)	5 (2%)
Reported previous flavivirus infection	0	0
Reported previous medical illness	2 (1%)	15 (7%)
HIV exposed at baseline	1 (<1%)	0

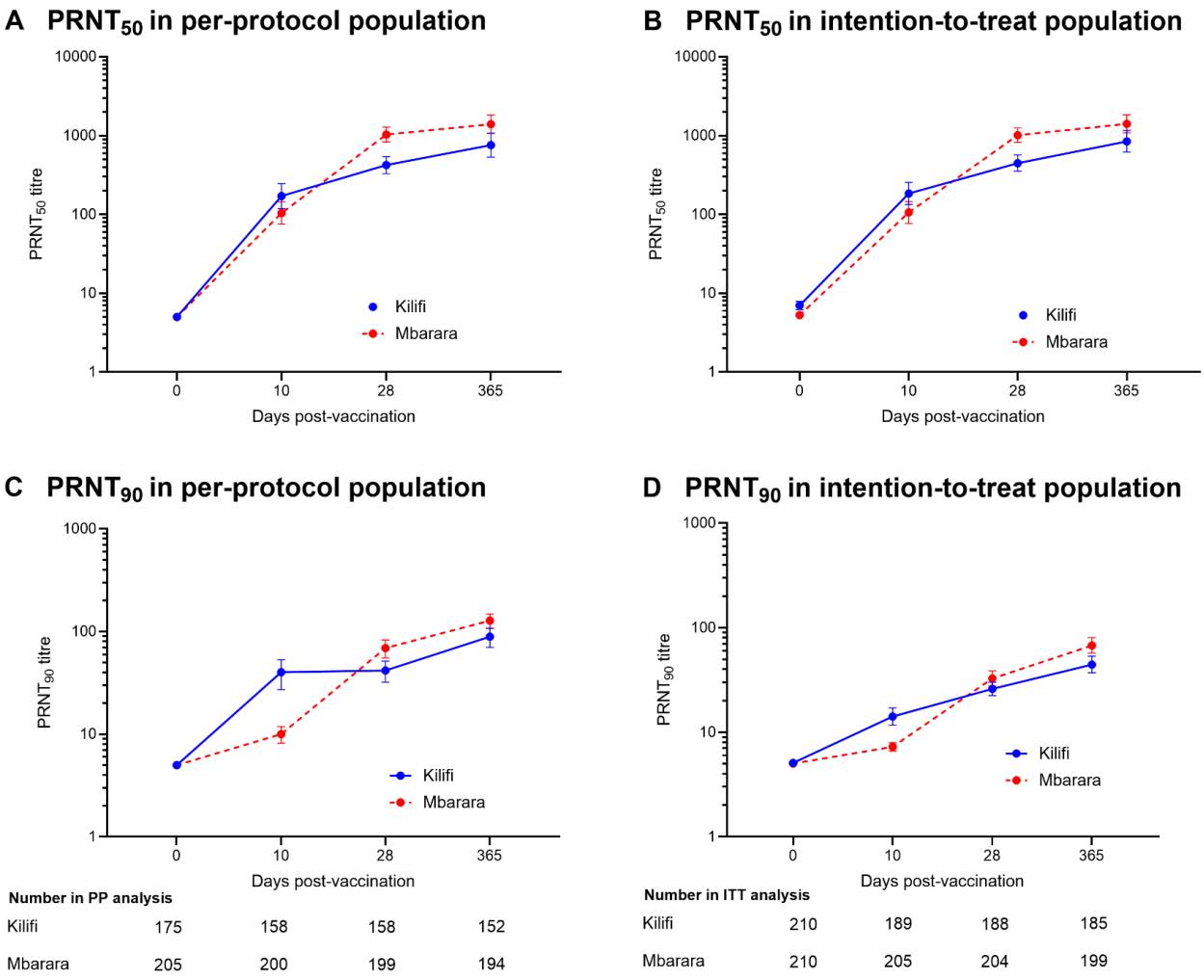


Figure S6: GMTs over time by site

Listing 1. Serious adverse events

Screen ID	Age at SAE onset (months)	Preferred Term	System Organ Class	Outcome	Relationship to study product	Study group
K0436	9	Abscess	Infections and infestations	Recovered without sequelae	Not related	Standard
K0513	14	Burns	Injury, poisoning and procedural complications Investigations	Recovered without sequelae	Not related	500 IU
K3016	16	Gastroenteritis	Infections and infestations	Recovered without sequelae	Not related	Standard
K3074	12	Lower respiratory tract infection	Infections and infestations	Recovered without sequelae	Not related	Standard
K3094	17	Lower respiratory tract infection	Infections and infestations	Recovered without sequelae	Not related	500 IU
K3104	16	Gastroenteritis	Infections and infestations	Recovered without sequelae	Not related	500 IU
M0323	19	Rectal prolapse	Gastrointestinal disorders	Recovered without sequelae	Not related	Standard
M0335	9	Malaria	Infections and infestations	Recovered without sequelae	Not related	500 IU
M0379	9	Pneumonia	Infections and infestations	Recovered without sequelae	Not related	500 IU
M0394	20	Pneumonia	Infections and infestations	Recovered without sequelae	Not related	500 IU
M0480	11	Bronchiolitis	Infections and infestations	Recovered without sequelae	Not related	500 IU
M0520	10	Malaria	Infections and infestations	Recovered without sequelae	Not related	500 IU

Reference

1. Little RJA, Rubin DB. Statistical Analysis with Missing Data. 3rd ed; 2019.