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PII: S0264-410X(97)00159-X

Vaccine, Vol. 16, No. 1, pp. 62–67, 1998

Printed in Great Britain
0264–410X/98 $19+0.00

An efficacy trial of the malaria vaccine SPf66 in Gambian infants—second year of follow-up

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In 1994, 630 Gambian infants were immunized with three doses of the synthetic polypeptide malaria vaccine SPf66 or with a control vaccine. No significant protection against first or total attacks of malaria was observed among the children who received SPf66. However, the period of follow-up was short. Thus, 532 children were followed for a second malaria transmission season during which 291 episodes of malaria were detected. Protective efficacies of SPf66 against first attacks of malaria and against all attacks of malaria were 8% [95% CI −20%, 30%] and 2% [95% CI −26% 24%] respectively. SPf66 did not provide any significant degree of protection to Gambian infants during a second year of follow-up. © 1997 Elsevier Science Ltd.

Keywords: malaria; *Plasmodium falciparum*; malaria vaccine; SPf66

A malaria vaccine that could be given early in childhood and which would provide sustained protection would be a major advance in malaria control, especially in Africa where malaria continues to cause many deaths in children. The malaria vaccine SPf66 is a polymeric synthetic protein with amino acid sequences of three *Plasmodium falciparum* proteins linked by the Asn–Ala–Asn–Pro motifs derived from the circumsporozoite protein[1]. Reports that this vaccine provided protection against malaria in several parts of South America[2–4] generated a great deal of interest. This was heightened further when a trial undertaken in an area of high endemicity in Tanzania showed 30% protection against first attacks of malaria in children aged 1–5 yr[5].

In The Gambia, preliminary studies carried out in children aged 6–11 months showed that SPf66 was safe and immunogenic[6] in infants and an efficacy trial was, therefore, undertaken[7]. During the first half of 1994, 630 infants aged 6–11 months were immunized with three injections of either SPf66 or a control vaccine (inactivated polio vaccine; IPV) over a six-month period. The vaccine was safe and immunogenic but did not provide any significant degree of protection against first or total attacks of malaria. Thus, the results of this Gambian trial differ from those of the trial undertaken in Tanzania, although they are in agreement with the results of a trial of American manufactured SPf66 performed in Thailand[8]. Because malaria in The Gambia is seasonal, children in the trial were followed for only $3\frac{1}{2}$ months as malaria transmission then stopped, with the onset of the dry season. In Tanzania, the protective effects of SPf66 became apparent only about 3 months after the full course of vaccination had been completed, and a similar trend was noted in trials conducted in Colombia and in Venezuela[2,3], so the duration of follow-up in The Gambia might have been too short to detect an effect. Children in The Gambian trial have, therefore, been followed for a further malaria transmission season in case protection did not become apparent until several months after vaccination had been completed and to ensure that there were no harmful, long-term effects of vaccination. We report here the results from the second year follow-up of Gambian infants vaccinated with SPf66.

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MATERIAL AND METHODS

Vaccination and initial follow-up

The study area, study design, vaccination procedure and details of the first year follow-up results have been described previously[7]. In brief, 630 children aged 6–11 months resident in 210 villages in Upper River Division (URD), The Gambia, were recruited into the trial. After informed consent had been obtained from their parents, study children were given three injections of SPf66 at a dose of 1 mg on days 0, 30 and 180. Children were randomised individually to receive either malaria vaccine or IPV. Throughout the subsequent $4\frac{1}{2}$ months of the 1994 rainy season, each child was visited at home twice weekly by a field worker and families were encouraged to take any child who became ill to one of the six health centres in the area.

Two cross-sectional surveys were done, the first 3 weeks after the third dose had been given and the second at the end of November 1994.

Second year follow-up

In June 1995, parents were asked for consent to enrol their child in a second follow-up study. Five hundred and thirty-two (84%) children from the original cohort of 630 children were recruited.

From the start of the 1995 rainy season (July), all children were visited at home once a week by a project field assistant and parents were questioned about any sickness, attendances at a health centre or self-medication since the previous visit. The axillary temperature was recorded with an electronic thermometer; if it was 37.5°C or higher, two thick blood films were prepared. If a blood film showed malaria parasites, pyrimethamine+sulphadoxine was given within 24 h.

Passive case detection was centred on three health centres (Yorobawol, Fatoto, and Basse) where project nurses were deployed and at the Medical Research Council (MRC) clinic in Basse. Children in the trial who came to a health centre had their axillary temperature taken and a blood film was prepared if fever in the past 24 h was reported or if the temperature was 37.5°C or more. A clinical history was taken and a physical examination was performed. Diagnosis and treatment were recorded each time a child was seen at the health centre or at the MRC clinic. Active and passive surveillance ended on November 19,1995. All staff involved directly in the collection of clinical data or in making laboratory measurements were blind to the vaccine code.

Two cross-sectional surveys were carried out, one at the beginning and the other at the end of the rainy season. A short questionnaire was administered and parents were asked about bednet usage and, if bednets had been used, whether they had been treated with insecticide. Children were examined by a physician, anthropometric data collected, a blood film obtained, the packed cell volume (PCV) determined and a serum sample collected for antibody measurements. Children with a positive blood film and/or a PCV of $\leq 25\%$ were treated with pyrimethamine+sulphadoxine and/or iron.

Laboratory methods

Thick blood films were air-dried and stained with Giemsa. For each blood slide, 100 high-power fields (HPF) were examined and parasite densities were recorded as the number of parasites per HPF; one parasite per HPF was assumed to indicate a density of 500 parasites μl^{-1}, Ref. [9]. All slides were read by two laboratory assistants, and a third reading was made if there was disagreement between observers on positivity or if the difference of the log-densities recorded was more than 1.5. Agreement between observations was reached after the slides had been re-checked. The PCV was measured in a heparinised microcapillary tube using a microhaematocrit centrifuge.

Case definitions, data processing, statistical methods

Data were entered using Epi Info 6 software. All records were checked routinely for range and consistency at the MRC field station Basse. Analysis was done with SAS (SAS Institute, Cary, NC, USA) software. A clinical episode of malaria was defined as an illness associated with an axillary temperature of ≥ 37.5°C and with a *P. falciparum* parasite density of $\geq 6000\ \mu l^{-1}$, Ref. [10]. The estimated sensitivity and specificity for this case-definition were both 86%. Primary analysis considered cases detected by either active or passive surveillance during the 1995 rainy season, and was restricted to children who had received all three doses of SPf66 or IPV. A group of children who were vaccinated incorrectly at the beginning of the trial due to a mistake in randomisation and who received just one dose of SPf66 were excluded from calculations of vaccine efficacy[7]. Vaccine efficacy (VE) was determined on the basis of a comparison of incidence rates of clinical episodes between SPf66 and IPV groups ($VE = 100 \times [1-IRR]\%$, where *IRR* is malaria incidence in the SPf66 group/incidence in the IPV group). IRR was estimated by Poisson regression, relating the occurrence of each child's first or only malarial episode during the surveillance period to the number of child-days at risk. The regression allowed the incidence in each group to vary by calendar month, whilst preserving a constant rate ratio. Children did not contribute to either the denominator or the numerator following an episode of malaria. Children who were lost to follow-up, who withdrew or who died were included up to the date of loss, withdrawal or death. Children who received antimalarial treatment (chloroquine, pyrimethamine+sulphadoxine or quinine) without having a documented episode of malaria were excluded from the denominator for 28 days. In addition, children who were not found by the field assistant and who had not attended one of the health centres at the time of a home visit, but who were seen at a later visit were excluded for the period during which they were not under surveillance. Confidence intervals (CI) for vaccine efficacy with and without adjustment for confounders were calculated by Poisson regression. Kaplan–Meier survival curves were used to show timing of malaria episodes.

Vaccine efficacy was calculated separately on clinical episodes detected by active and/or active/passive surveillance (child referred by field assistant to the health centre), and for those detected by passive surveillance alone (mothers who spontaneously brought their child to a health centre). Total numbers of episodes of clinical malaria during the surveillance period were used to estimate the overall incidence rate ratio. After each recorded episode, a child was considered not to be at risk for the next 28 days, and was removed from the denominator for that period. Protective efficacy against episodes of documented fever associated with *P. falciparum* parasitaemia of any density was also calculated as above, excluding mixed infections with *P. ovale*, or *P. malariae*.

Vaccine efficacy against infection was estimated by comparing between groups *P. falciparum* asexual parasitaemia, high density parasitaemia (≥ 5000 parasites μl^{-1}), spleen rates found at the November clinical survey, the mean PCV and the mean change in PCV detected between the July and November surveys. Children who received antimalarial drugs within 28 days of the survey were excluded from these

analyses. Adjustments were made for potential confounding factors using multiple logistic or normal regression as appropriate.

RESULTS

Study cohorts

During the 1995 malaria transmission season, 532 children from the original vaccine cohort were recruited to the second follow-up study. Fifty-nine children who were incorrectly immunised at the beginning of the trial were excluded from the analysis of vaccine efficacy. Thus, the main analysis was based on 473 children (269 SPf66/204 IPV). The two groups had similar baseline characteristics at the beginning of the second follow-up period (*Table 1*).

Mortality and hospital admissions

During the 18 weeks of intense surveillance two children in the study cohort died at home, one in each group. Interviews with relatives suggest that neither death was due to malaria. One child in the incorrectly vaccinated group died of cerebral malaria at the MRC Basse clinic. Overall mortality was lower than would have been expected on the basis of data collected previously in URD; 5–10 deaths would have been expected during the rainy season in a group of children of the size and age of the vaccine cohort.

A total of 60 study children was admitted to one of the health facilities in the study area during the $4\frac{1}{2}$ months of follow-up: (40/269 SPf66 [14.9%] and 20/204 IPV [9.8%]) ($\chi^2 = 2.5$; ns). Clinical malaria was diagnosed in 44 children and was the most frequent cause of admission (28/269 children [10.4%] SPf66 and 16/204 children IPV [8.0%]) ($\chi^2 = 0.9$; ns). Three children in the SPf66 group received transfusion for severe malaria anaemia but none in the control group required transfusion.

Protective efficacy

Table 2 shows vaccine efficacy against first or only clinical episodes of malaria. One hundred and thirty-nine first or only clinical episodes of malaria were recorded in children who had received SPf66 (incidence 5.4 per 1000 child-days at risk) and 108 in children who received IPV (incidence 5.7 per 1000 child-days at risk) to give an adjusted vaccine efficacy of 8% [95% CI −20%,30%] ($P = 0.52$). Kaplan–Meier survival curves of the estimated proportion of children free of malaria by days of observation for the two-year follow-up period show very little difference between the two groups (*Figure 1*). Vaccine efficacy was −19% [95% CI −161%,45%] ($P = 0.65$) against episodes detected by passive case detection, and 12% [95% CI −23%,37%] ($P = 0.45$) against episodes detected by active or active/passive case detection. Analysis of episodes with or without fever and parasitaemia greater than 6000 μl^{-1} gave a vaccine efficacy of 7% [95% CI −21%,28%] ($P = 0.59$). There was no evidence of protection against episodes of fever associated with any level of parasitaemia (VE = −1%, [95% CI −30%,21%] ($P = 0.59$). *Table 3* shows all clinical episodes detected by active and passive surveillance. Incidence rates for all episodes of malaria among children in each group were identical at 5.8 per 1000 child-days at risk (adjusted vaccine efficacy 2%, [95% CI −26%,24%] ($P = 0.87$). *Table 4* shows total episodes of malaria for both years of follow-up. The combined adjusted vaccine efficacy for both years against all episodes of malaria was 5% [95% CI −15%,21%] ($P = 0.60$).

Cross-sectional surveys

Four hundred and sixty-nine children of the study cohort (266 SPf66, 203 IPV) were seen during the July 1995 survey at the beginning of the malaria transmission season and blood samples for determination of parasitaemia and PCV were collected. The proportion of children who had positive blood slides was similar in both groups (22.9% SPf66, 26.1% IPV); very few children had malaria parasitaemia of $\geq 5000\ \mu l^{-1}$ (2 SPf66/5 IPV). In contrast, 32 had enlarged spleens (14 SPf66/18 IPV). Mean PCV was similar in both groups (33.5% SPf66/33.7% IPV). There was no significant difference in the prevalence of splenomegaly, mean PCV and percentage of children with a PCV of 25% or less between the two groups at the time of this survey.

Table 1 Characteristics of children in SPf66 and IPV groups at the time of entry into the second period of surveillance

	SPf66 ($n = 269$)		IPV ($n = 204$)	
	Number	(%)	Number	(%)
Male	139	51.7	122	59.8
Mean (SD) age at June survey (months)	27.4 (1.7)	—	27.3 (1.8)	—
Mean distance (km) from the nearest health centre (SD)	9.5 (5.3)	—	10.1 (5.6)	—
Bednet usage				
No bednet	145	57.3	91	47.2
Non-impregnated bednet	100	39.5	94	48.7
Impregnated bednet	8	3.2	8	4.1
Ethnic group				
Mandinka	95	35.3	64	31.4
Fula	140	52.0	117	57.4
Sarahule	28	10.4	16	7.8
Others	6	2.2	7	3.4
Hb genotype				
AA	199	76.2	155	77.5
AS	45	17.2	41	20.5
SS	1	0.4	0	0
AC	9	3.4	1	0.5
SC	2	0.8	1	0.5

Table 2 Vaccine efficacy against first or only episode of *P. falciparum* as established by passive and active case-detection during the second year of follow-up

	Group								
	SPf66			IPV			Vaccine efficacy (95%)		
Case definition	First (or only) episodes	Child-days	Incidence rate/1000	First (or only) episodes	Child-days	Incidence rate/1000	Unadjusted	Adjusted	*P*
Fever and $\geq$6000 parasites μl^{-1} (any detection)	139	25144	5.36	108	18877	5.72	6%	8% (−20,30)	0.52
Fever and $\geq$6000 parasites μl^{-1} detected by ACD/APCD[a]	90	27925	3.22	71	20783	3.42	6%	12% (−23,37)	0.45
Fever and $\geq$6000 parasites μl^{-1} detected by PCD[b]	18	31517	0.57	11	23901	0.46	−24%	−19% (−161,45)	0.65
Fever and parasitaemia any density	165	23372	7.06	121	17894	6.76	−4%	−1% (−30, 21)	0.92
Parasitaemia $\geq$6000 μl^{-1} with or without fever	150	24324	6.16	117	18513	6.30	2%	7% (−21, 28)	0.59

ACD: active case detection. PCD: passive case detection. APCD: active/passive case detection.
[a]Episodes detected by PCD are ignored.
[b]Episodes detected by ACD/APCD are ignored.

Five hundred and twelve study children were seen during the survey conducted at end of the rainy season. Blood samples were collected for determination of parasitaemia (512) and PCV (503). One hundred and

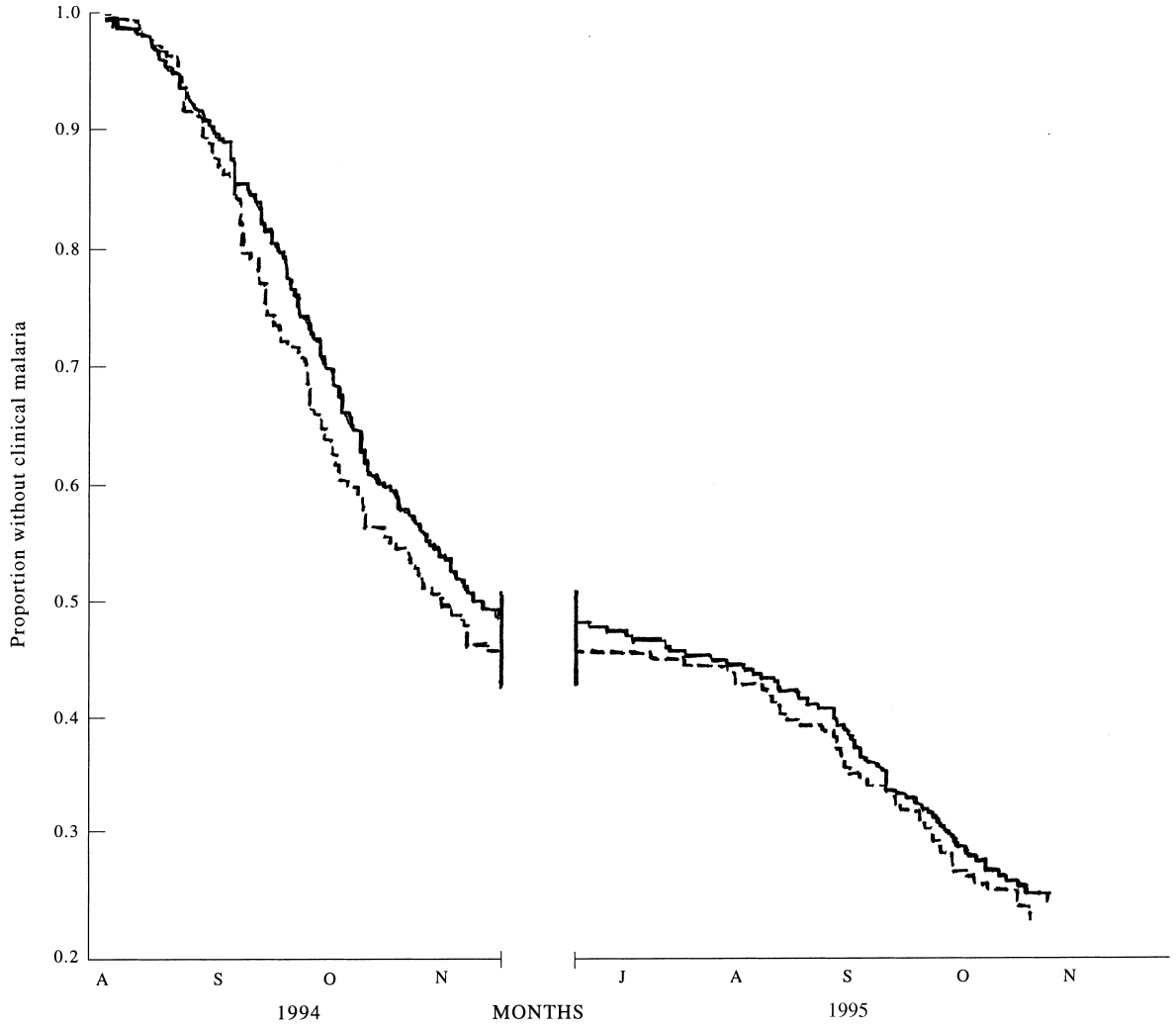

Figure 1 Kaplan–Meier curves established from active and passive case detection during two follow-up periods, (——— SPf66, - - - IPV)

Table 3 Clinical episodes of malaria detected by active and passive surveillance during the second year of follow-up

	Group	
Episodes per child[a]	SPf66 ($n = 266$)	IPV ($n = 203$)
0	126	94
1	115	93
2	25	15
3	0	1
Total episodes	165	126
Child-days at risk	28548	21419
Incidence rate 1000^{-1}	5.78	5.80

[a]Episodes 28 days apart or less have not been considered as new clinical episodes.
VE against all episodes: unadjusted 0%; adjusted 2% [−26%, 24%] (P = 0.87) (allowing for over-dispersion).

forty-three children who had received antimalarial treatment (85 SPf66/58 IPV) 28 days or less before the survey and those who had been wrongly vaccinated were excluded. The prevalences of parasitaemia, high density parasitaemia, splenomegaly and low PCV ($\leq 25\%$) were similar in both groups (*Table 5*). Similarly the mean PCV and change in PCV over the transmission season were the same in both groups. These results did not change after adjusting for confounding factors (*Table 6*).

DISCUSSION

The initial phase of The Gambian SPf66 trial was disappointing in that, in contrast to findings in Tanzania, it did not show any significant degree of protection against first or total attacks of malaria. One possible explanation for the different outcomes of the two trials was that children were not followed up for long enough in The Gambian study to detect an effect. However, results from the second year proved to be very similar to those of the first year, the protective efficacy against first attacks of malaria was 8% [95% CI −20%,30%] and against all attacks 2% [95% CI −26%,24%]. No beneficial effect was observed on the PCV. Thus, the second year of follow-up has established that short duration of follow-up was not the cause of different results found in the Tanzanian and Gambian studies. What are the other possible explanations?

In Tanzania, malaria morbidity was determined mainly by passive case detection at a health centre; this approach can detect most of the potentially severe cases but only a proportion of mild cases. Thus, it is possible that the apparent reduction in malaria morbidity recorded in vaccinees in Tanzania was due to a decrease in the number of moderately severe cases rather than in the number of attacks. In contrast, the Gambian study relied predominantly on active case detection. Passive case detection might be a better method of case detection for a malaria vaccine trial if the vaccine has its main effect on severe disease or on mortality. However, there was no suggestion in the Gambian study that vaccine efficacy was any better when assessed by passive rather than by active case detection and no significant difference in the number of cases with malaria sufficiently severe to warrant admission to a health centre was detected between groups.

Difference in the age-range of the children who were vaccinated could be important although no age effect on the level of protection was observed in the initial Tanzanian trial within the age range 1–5 yr[11]. It is possible that early vaccination, before natural exposure to malaria has occurred, diminishes the immune response to SPf66 as may have been the case in some Gambian children. Some vaccines, such as bacterial polysaccharide vaccines, lack efficacy when given to very young children, although they are effective in older children, but age-dependency is unlikely to influence markedly the response to a peptide vaccine. Studies in progress in Tanzania in which infants are being vaccinated with SPf66 should clarify whether this is the case for SPf66.

Difference in the genetic characteristics of children in the Gambian and Tanzanian trials might have affected their immune response to SPf66 and the outcome of the trials. It is known that humoral immune responses against SPf66 are influenced by

Table 4 Total clinical episodes of malaria detected by active and passive surveillance for the two years of follow-up

	Group	
Episodes per child[a]	SPf66 ($n = 266$)	IPV ($n = 203$)
0	69	52
1	95	75
2	69	52
3	31	18
4	2	5
5	0	1
Total episodes	334	258
Child-days at risk	52280	39612
Incidence rate 1000^{-1}	6.39	6·5.

[a]Episodes 28 days apart or less have not been considered as new clinical episodes.
VE against all episodes: unadjusted 2%; adjusted 5% [95% CI −15%,21%] (P = 0.60).

Table 5 Results of November, 1995 clinical survey

	Group		
Finding	SPf66	IPV	*P*-value
P. falciparum parasitaemia (any density)	69/184 (37.5%)	54/146 (37.0%)	0.99
P. falciparum parasitaemia ≥ 5000 parasites μl^{-1}	36/184 (19.6%)	30/146 (20.5%)	0.93
Geometric mean density (SD)	5820	6509	0.76
IQR	(1500–50000)	(1500–80000)	—
Splenomegaly	44/184 (23.9%)	36/146 (24.7%)	0.98
Mean PCV (%) (SD)	29.7 (10.4)	30.1 (9.5)	0.90
PCV < 25%	29/184 (15.8)	17/146 (11.6)	0.36
Mean PCV (%) difference between July and November surveys (SD)	−3.9 (10.3)	−3.1 (9.8)	0.49

Table 6 Adjusted odds ratios for November 1995 survey, SPf66-vaccinated compared with control children

	OR	95% CI	*P*-value
P falciparum parasitaemia	1.03	0.67–1.59	0.90
High density parasitaemia (*P. falciparum*)	1.08	0.64–1.83	0.76
Splenomegaly	1.09	0.67–1.81	0.72
Anaemia (PCV < 25%)	1.33	0.58–3.04	0.50

genetic factors[12–14]. It is also possible that the occurrence of variants among the parasite population in the study area affects the efficacy of SPf66, because one constituent of SPf66 corresponds to a variable region of malaria antigen MSP1[15]. The MSP1 profile of parasites obtained from Gambian children vaccinated with SPf66 or with IPV is now being studied.

Although the results of the trial are disappointing, overall mortality was lower than would have been expected during the periods of intensive surveillance. Thus, all the children in the trial obtained some benefit from the trial regardless of whether they received SPf66 or polio vaccine.

ACKNOWLEDGEMENTS

We thank Professor M.E. Patarroyo and his colleagues at the Instituto de Imunologia, Bogota, Colombia for provision of SPf66 and Mr G. Fegan for help in establishing the data-management system. We thank also the children and their parents; field staff Kebba Keita, Alhagie Darboe, Pa Amadou Susso, Waza Njie, Famalang Camara, Dembo Fatty, Pa Camara, Kebba Naban and Seedy Singateh; Nurse Karamo Bayo, Abu Khan Jallow and Famara Wally; Helene Obaro, Momodou Hydara and Fatou Jagne for help with data entry; Dr Margaret Pinder, Momodou Jallow, and Simon Correa for help with microscopy. This study was supported by the UK Medical Research Council.

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