A Phase 2 randomized, controlled trial of the efficacy of the GMZ2 malaria vaccine in African children

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ABSTRACT

BACKGROUND

GMZ2 is a recombinant protein malaria vaccine, comprising two blood-stage antigens of *Plasmodium falciparum*, glutamate-rich protein and merozoite surface protein 3. We assessed efficacy of GMZ2 in children in Burkina Faso, Gabon, Ghana and Uganda.

METHODS

Children 12–60 months old were randomized to receive three injections of either 100 μg GMZ2 adjuvanted with aluminum hydroxide or a control vaccine (rabies) four weeks apart and were followed up for six months to measure the incidence of malaria defined as fever or history of fever and a parasite density ≥5000/μL.

RESULTS

A cohort of 1849 children were randomized, 1735 received three doses of vaccine (868 GMZ2, 867 control-vaccine). There were 641 malaria episodes in the GMZ2/Alum group and 720 in the control group. In the ATP analysis, vaccine efficacy (VE), adjusted for age and site was 14% (95% confidence interval [CI]: 3.6%, 23%, p-value=0.009). In the ITT analysis, age-adjusted VE was 11.3% (95% CI 2.5%,19%, p-value=0.013). VE was higher in older children. In GMZ2-vaccinated children, the incidence of malaria decreased with increasing vaccine-induced anti-GMZ2 IgG concentration. There were 32 cases of severe malaria (18 in the rabies vaccine group and 14 in the GMZ2 group), VE 27% (95%CI -44%, 63%).

CONCLUSIONS

GMZ2 is the first blood-stage malaria vaccine to be evaluated in a large multicenter trial. GMZ2 was well tolerated and immunogenic, and reduced the incidence of malaria, but efficacy would need to be substantially improved, using a more immunogenic formulation, for the vaccine to have a public health role.

Keywords: Phase 2 clinical trial, GMZ2, GLURP, MSP3, vaccine, antibody, efficacy, *Plasmodium falciparum*.

Highlights:

- The GMZ2 fusion protein consist of the non-repeat region of *falciparum* GLURP genetically fused to a MSP3 fragment
- The GMZ2 fusion protein elicited functional antibodies in phase 1 studies
- In the ATP analysis, vaccine efficacy adjusted for age and site was 14% (95% CI: 3.6%, 23%)
- Vaccine efficacy was higher in older children
- In GMZ2-vaccinated children, the incidence of malaria decreased with increasing vaccine-induced anti-GMZ2 IgG concentration

1. Introduction

Malaria caused by *Plasmodium falciparum* infection continues to be a leading cause of morbidity and mortality in children in sub-Saharan Africa [1]. Following repeated infections, non-sterile immunity develops that protects against more severe forms of the disease [2]. This type of semi-immunity is directed against the asexual blood-stage of the parasite. Passive transfer experiments have shown that antibodies play an important role in this immunity, by inhibiting excessive parasite replication [3, 4]. In principle, all stages of parasite development within the human body are potential vaccine targets: the pre-erythrocytic-stage, (parasites inoculated by infected mosquitoes, circulate briefly and then develop in liver cells); the asexual blood-stage, which causes disease symptoms and complications; and the sexual-stages, when parasites differentiate into male and female gametes. The RTS,S vaccine, the first malaria vaccine to be evaluated in a phase 3 trial, elicits immunity against pre-erythrocytic stages and has shown consistent protection in clinical trials [5, 6]. The final results of the RTS,S phase 3 trial are an important landmark, but protective efficacy was moderate, and waned [7]. A number of approaches are being pursued to develop improved, second generation vaccines. These include asexual blood-stage vaccines, which seek to limit but not prevent parasitaemia. However, blood stage candidates that have been evaluated in clinical trials have shown no protection [8, 9] or protection limited to the vaccine parasite strain [10, 11]. Identifying the protective epitopes, and the variability in the antigens that are exposed to the immune system have posed major problems in the development of malaria vaccine candidates [12]. These challenges have led to the proposal that for a next generation malaria vaccine RTS,S should be complemented with conserved antigens from the blood-stage [13]. Some evidence of protection against blood-stage infection was seen in post-hoc

analyses of a phase 1 trial with a conserved part of the Merozoite Surface Protein 3 (MSP3) in Burkina Faso, suggesting a MSP3 vaccine might be effective [14].

The recombinant fusion protein, GMZ2, contains conserved fragments of two *P. falciparum* asexual blood-stage antigens, Glutamate-Rich Protein (GLURP) and MSP3 [15], both of which have been identified as targets of naturally acquired malaria immunity [16, 17] and specific IgG antibodies that are broadly inhibitory [18, 19]. Phase I clinical trials have shown good tolerability, safety and immunogenicity of GMZ2 adjuvanted with aluminum hydroxide (Alum) in malaria-naïve adults [20] as well as in African adults and children [21, 22]. Functional anti-GMZ2 antibodies at levels comparable to those observed in naturally exposed individuals were generated [19], but vaccine efficacy (VE) under natural exposure has not been evaluated.

Here, we report the first results of a multicenter phase 2 clinical trial to assess GMZ2/Alum vaccine efficacy in African children from Western, Central and East Africa.

2. Materials and methods

2.1. Study Populations

The trial was conducted in five sites with different transmission patterns. In Banfora and Sapone, Burkina Faso [23] and in Navrongo, Ghana, malaria transmission is intense and highly seasonal. In Iganga, Uganda [24], and Lambaréné, Gabon, transmission occurs throughout the year. Cohort studies were performed before the trial to assess the incidence of malaria. Additional details about the sites are provided in the Protocol (supplementary materials).

2.2. Study design and ethics

This was a randomized, controlled, multicenter, double blind phase 2 trial to measure VE of GMZ2/alum in African children. Participants were allocated in a 1:1 ratio to receive three doses of either GMZ2/Alum or the control vaccine (rabies, Verorab) four weeks apart, and were followed for six months. The study was performed in accordance with the protocol (Supplement), the International Conference on Harmonization, the Declaration of Helsinki in its 5th revision, and national regulatory requirements. The ethics committee and the regulatory authority of each country reviewed and approved the study (Supplement). An independent Data and Safety Monitoring Board reviewed the safety data (serious adverse events) during the trial. This trial is registered with PACTR, registration number: PACTR2010060002033537.

2.3. Study participants

Study participants were healthy children, aged 12–60 months, residing in the study areas and available for follow-up. They were not anemic (Hb<7g/dL) or malnourished (weight for age z-score <-2 - -3), and did not have signs of a chronic illness or renal or hepatic abnormality.

They had not taken immunosuppressive medication, immunoglobulin or blood products in the last three months, or another investigational drug or vaccine in the last month, and had no history of hypersensitivity to vaccines and no history of splenectomy. Routine vaccinations were given outside a 14 days interval before and after a dose of study vaccine. Written informed consent was obtained from the parents or legal guardian of each participant, signed by an impartial witness if they could not read. Families of participants were asked to report to the clinical team whenever a study-child was unwell. Blood samples were taken for microscopy at the health facilities from children with fever or history of fever. All disease episodes were treated according to national or international guidelines. Registered artemisinin combination therapies were used for treatment of uncomplicated malaria. Parenteral artesunate or quinine was used to treat severe malaria according to national guidelines.

2.4. Randomization and masking

Randomization at each site was done in randomly permuted blocks of 10 generated using Stata version 10. Children were screened and those who met eligibility criteria were assigned a randomization envelope in numerical sequence. Children were randomized to receive either 100 μg GMZ2 (Novasep, Belgium), reconstituted in 0.5 ml adjuvant (Alum, Statens Serum Institut, Denmark) or rabies vaccine (Verorab, Sanofi Pasteur). All vaccine doses were given as intramuscular injections into the deltoid muscle, alternately in the left and right arms. Syringes were masked and the vaccinating nurse was not involved in any other activity in the trial. Safety was assessed after the first 40 children were vaccinated at each site before continuing recruitment.

2.5. Outcomes

The primary endpoint was clinical malaria for 6-month after the first vaccination. Malaria was defined as asexual *P. falciparum* parasitemia of ≥5,000 parasites/μl with fever (tympanic temperature ≥38°C) or a history of fever in the previous 48 hours. Secondary endpoints included malaria with fever or history of fever and a parasite density >0, ≥500, ≥2,500, or ≥20,000/uL; incidence of solicited local and general symptoms within seven days of vaccination and unsolicited adverse events at any time; anemia (Hb<7g/dL) at six months; and severe malaria, defined as hospitalization for at least 24 hours with malaria as primary diagnosis. Passive follow-up was used to capture malaria episodes.

To assess safety, participants were directly observed for 30 minutes after each vaccine injection, followed by daily home visits for six days. On Day 7, a physician examined participants at the clinic. Following the third vaccine injection, monthly home visits were done to check that the child was still present in the study area and to capture adverse events (AE) and serious AEs (SAE) that were not actively reported to the clinical team.

2.6. Laboratory methods

Five ml of blood was collected by venipuncture into EDTA tubes for hematology and into dry tube for biochemistry. Hemoglobin was determined using a Hemocue analyzer at all sites. Full blood counts and biochemistry were done using calibrated automatic analyzers. *P. falciparum* parasitemia was assessed using two independent reads of Giemsa-stained thick blood smears at a 1000x magnification, followed by a third read in case of discordance (disagreement on positivity or a >2-fold difference in parasitemia). The number of parasites per μl of blood was calculated according to the measured leucocyte count. A slide was declared negative if no parasite was seen after microscopic examination of 200 high power fields. GMZ2-specific IgG antibody levels were determined by ELISA as previously described in detail using the vaccine antigen preparation for capture of antibodies [21].

Antibody concentrations are given as titers normalized to a highly positive pool. A titer of one would mean the same anti-GMZ2 antibody concentration as in the pool, 0.1 a 10-fold lower concentration.

2.7. Statistical methods

Sample size was calculated to have at least 90% power for the According to Protocol (ATP) analysis if the VE was at least 30%, using a 5% significance level, allowing for 15% loss to follow-up or incomplete vaccination. All children who were randomized were included in the Intention to Treat (ITT) analysis, those who received three doses of either GMZ2 or rabies vaccine were included in the ATP analysis. Time at risk was calculated from randomization (ITT) or from the date of the third dose of vaccine (ATP), until six months after the date for the third dose. VE, defined as the percentage reduction in the number of malaria episodes, was calculated as 100x(1-R), where R is the hazard ratio estimated by Cox regression, with site as a stratification factor, and confidence interval calculated using a robust standard error to account for repeated episodes in the same child. Cases that occurred within 14 days of a previous episode were not counted. A Statistical Analysis Plan (Supplement) was prepared before database lock and unblinding. Subgroup analyses by site, age group and use of insecticide-treated nets (ITN) was planned. Completed paper case record forms were quality checked and transcribed into a database using eClinical eDM and eDC system version 5.0. Single data entry was used, with verification of the data entry by proof reading of selected areas combined with full proof reading of selected CRFs to verify accuracy.

3. Results

3.1. Participants

Between November 2010 and September 2011, 1849 children were enrolled (Figure 1, and Supplementary Figure S1). Demographics and other baseline characteristics were similar in the two groups although there was a slightly higher proportion of older children in the rabies vaccine group (Table 1 and supplementary Table S1).

3.2. Efficacy

During the six-month follow up, there were 1361 episodes of malaria with parasite density of ≥5000 parasites per μL amongst those who received three doses of vaccine, 641 in the GMZ2/Alum group and 720 in the control group (Fig. 2). In the per-protocol analysis, VE, adjusted for age and site, was 13.6% (95% confidence interval [CI] 3.6%,23%, p-value=0.009). In the ITT analysis, there were 1925 episodes of malaria with parasite density of ≥5000 parasites per μL, 920 in the GMZ2/Alum group and 1005 in the control group with age-adjusted VE of 11.3% (95% CI 2.5%,19%, p-value=0.013). Similar estimates were obtained when different parasite density cut-offs were used (Table 2). Similar estimates were also obtained when age was not included in the model (Table 2). There was no evidence that efficacy varied by site (Supplementary Table S2). The number of children with one or more episodes of malaria is listed in supplementary Table S3. VE (ATP), adjusted for age and site, from the Kaplan Meier estimates of the proportion with malaria was 7.63% (95% CI, 0.95%,13.86%) and 6.36% (95% CI, 1.53 %,10.95%) in the ITT analysis. VE (ATP) was 20% (4%,33%) in children 3-4yrs of age and 6% (-8%,18%) in children 1-2yrs of age, interaction P-value 0.112. In the ITT analysis the VE was 18% (5%, 30%) in children 3-4yrs of age and 3% (-10%, 14%) in children 1-2yrs of age, interaction p-value=0.057. The proportion of children with fever, at a given parasite density, was lower in the older age-group than in younger children in both vaccine groups (odds ratio for fever adjusted for site 0.72 (95%CI 0.59,0.89) (supplementary Figure S2). The distribution of parasite density

among cases treated for malaria was similar in the GMZ2/Alum and rabies vaccine groups (supplementary Figure S2).

Thirty-two cases of severe malaria were reported in children who had received three doses of vaccine, 18 in the rabies group and 14 in the GMZ2/Alum group. In the ATP analysis, VE against severe malaria adjusted for age and site was 27% (95%CI -44%,63%) while in the ITT analysis, VE was 20% (95% CI -41%,55%). The prevalence of anemia after 6-months follow-up was similar (OR=2.96, 95% CI 0.60-14.7, p=0.185) in the GMZ2/Alum group (6/816) and in the rabies vaccine group (2/793).

3.3. Safety and reactogenicity

Vaccine doses were well tolerated (supplementary Tables S4 – S6). There were 17 individuals in the rabies group and 29 in the GMZ2/Alum group with solicited, grade 3 AE within seven days of a dose. Of these, four in the rabies group (two induration and two fever $\geq 39^{o}$C) and five in the GMZ2/Alum group (one swelling and four fever $\geq 39^{o}$C) were reported as related to the study vaccine. Three grade 3 unsolicited vaccine related AEs were reported within 28 days of a dose, two in the rabies group and one in the GMZ2/Alum group (supplementary Table S6). During the six months follow-up period post dose 3, five children died, two in the GMZ2/Alum group and three in the control group (Supplementary Table S7). There were 68 other SAEs (35 in the rabies group and 33 in the GMZ2/Alum group) most of these were malaria (Supplementary Table S8). Two of the SAEs were considered related to vaccination, both events in one individual who had received rabies vaccine.

3.4. Immunogenicity

The mean titer of anti-GMZ2 antibodies increased 8-fold (95%CI 6.1,11) from baseline in children who received three doses of GMZ2/Alum. There was a greater increase in children 1-2yrs old (14-fold increase, 95% CI 8.7, 23) compared to children 3-4yrs old (5.7-fold, 95% CI 4.0,8.2), however the smaller increase in older children was due to a higher baseline level of naturally acquired antibodies (Table 3) . At baseline, arithmetic mean GMZ2 IgG levels were 0.09 and 0.21 in the 1-2yrs and 3-4 yrs age groups, respectively (Table 3). A similar effect of age was also observed in the rabies vaccine group (Table 3). To investigate the association between the concentration of anti-GMZ2 IgG antibodies measured after the third vaccine dose (Day 84) and malaria incidence, children in the GMZ2/Alum group were divided into four groups based on the quartiles of the anti-GMZ2 antibody concentration (Table 4). Children with anti-GMZ2 antibody concentrations above the upper quartile had 23% (95%CI 1.8%,39%; p-value=0.035) lower incidence of clinical malaria, compared with children in the lowest quartile group. This association remains after adjusting for age-related exposure to *P. falciparum* malaria.

4. Discussion

GMZ2/Alum was well tolerated and immunogenic in children from West, Central, and East Africa. Although efficacy is too low for the vaccine to have a role in public health in its present form, the finding that the risk of acquiring clinical malaria decreased with increasing levels of GMZ2-specific antibodies, suggests that efficacy might improve if immunogenicity can be enhanced with improved formulations or a more potent adjuvant. Preclinical studies in *Saimiri sciureus* monkeys suggested that stronger adjuvants enhance both immunogenicity and protective efficacy of GMZ2 [25]. Whether novel formulations using stronger adjuvants may elicit significantly better protection against clinical disease remains to be investigated.

The worldwide dynamics of *P. falciparum* populations is complex and the distribution of different parasite strains differs from region to region and evolves over time possibly because of immune selection [26]. Multicenter trials in a range of different settings are therefore required to show that efficacy of vaccine candidates is not site dependent. We did not find evidence of variation in efficacy, although site effects cannot be ruled out, as the power for the interaction test was low. The limited antigenic variation observed in the GLURP and MSP3 regions included in the GMZ2 vaccine may facilitate the generation of broadly inhibitory antibodies as suggested in functional bioassays assay using geographically and genetically diverse *P. falciparum* isolates [18, 19]. These studies, together with a preclinical study using the GLURP component of GMZ2 [27], suggests that GMZ2 may generated a broad anti-parasitic immune response that covers more than a small number of isolates. This is particularly important in areas of high endemicity, where infections are usually complex. On the other hand, IgG antibodies against the components of GMZ2 may also control parasite multiplication through opsonic phagocytosis of *P. falciparum* merozoites [28]. Functional analyses of GMZ2 IgG from the present trial would be important to identify surrogate markers of protection [29].

GMZ2/Alum elicits higher protection in children 3-4 years of age compared to children 1-2 years of age. The interaction with age may suggest that vaccine-induced antibodies act in concert with protective antibodies acquired through natural exposure [30] and/or are more functionally competent in terms of avidity and IgG subclass profile [31]. We did not find any evidence that efficacy waned during the six months of follow-up, however analysis of longer term follow up, which is planned, will be required to give a better estimate of duration of protection. Numerous immune-epidemiological studies have demonstrated an association between the level of antibodies against GLURP and MSP3 and protection from clinical

malaria [16, 17, 32]. In this trial, we found a relationship between concentration of vaccine-induced antibodies and incidence of clinical malaria. Children in the GMZ2/Alum group with anti-GMZ2 IgG concentrations above the upper quartile had 23% fewer episodes of clinical malaria than children with in the lowest group This effect of high GMZ2 IgG antibodies was independent of age. In fact, there was an indication that children 1-2 yrs of age with low pre-vaccination GMZ2 IgG levels responded strongly to vaccination, whereas older children with more exposure to *P. falciparum* showed a smaller boost of GMZ2 IgG responses. This is in accordance with our previous findings [20-22] and suggests that prior exposure to *P. falciparum* might diminish subsequent boosting by vaccination. Together, these observations suggest that an increased immunogenicity of GMZ2 may improve VE. The present vaccine formulation is based on Alum which is not as potent as more recently developed adjuvants. Notably, a formulation of RTS,S with Alum was not efficacious [33]. New formulations such as oil-in-water emulsions [34-36] may improve immunogenicity and efficacy of GMZ2. Efficacy of the RTS,S vaccine against severe disease appeared to be limited by rebound effects that occurred due to delayed acquisition of natural immunity [7]. Blood-stage vaccines might be less likely than pre-erythrocytic vaccines to interfere with acquisition of natural immunity as they do not prevent infection but this would need to be evaluated in larger trials. The safety and reactogenicity profile of GMZ2/Alum was good, consistent with previous clinical trials [20-22]. GMZ2 could be combined with other vaccines to improve protection [13]. Combining GMZ2 with transmission blocking antigens [27] could help reducing the spread of parasites, including potential escape mutants, in the population. Our results show that a safe and broadly reacting blood-stage malaria vaccine is possible, but more immunogenic formulations need to be evaluated.

Acknowledgments

We thank the children and their parents and guardians who volunteered to participate in the study, and without whose cooperation this study would have been impossible. We also thank the Data and Safety Monitoring Board (Trudie Lang (CHAIR), Brian Faragher, Blaise Genton, Angelina Kakooza, Grégoire Adzoda, John E. Williams, and Dao Fousseni) for their recommendations throughout the study.

This study was supported by grants from the European and Developing Countries Clinical Trials Partnership (grant IP.2007.31100.001), the German Federal Ministry of Education and Research (BMBF, grants 01KA0804 and 01KA1402) and the European Malaria Vaccine Initiative (now EVI).

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Figure legends

Figure 1. Trial profile. 1735 children received 3 doses of vaccine (867 Verorab and 868 GMZ2) and were included in the per-protocol analysis. 809/867 (%) and 827/868 (%) respectively were seen at the scheduled 6 month visit. There were three allocation errors: one child randomized to receive rabies vaccine was given three doses of GMZ2, (this child was included in per-protocol analysis); two children, one randomized to rabies vaccine and the other to GMZ2, received mixed doses, these children were excluded from the per-protocol analysis but were included in ITT analysis.

Figure 2. Timing of malaria episodes in each 2 vaccine group. The Nelson-Aalen estimate of the cumulative hazard (the mean number of episodes per child) in each group is shown against time since randomization. **A**) all age groups combined; **B**) children 1-2yrs of age; **C**) children 3-4 years of age. Time at risk for ITT analyses is from randomization until 3 months post dose 3, i.e. approximately 8 months.

Supplementary Figure S1. Trial profile for each of the five sites presented in figures A-E.

Supplementary Figure 2S. The probability of fever in the rabies vaccine (in blue) and GMZ2 group (in red) in relation to parasite density, in each age group in each site. Smoothed plots of the probability of fever were obtained using fractional polynomial logistic regression. Dashed lines show 90% confidence limits. The arrow shows the density associated with a 50% probability of fever to facilitate comparison between the plots. There is no density corresponding to 50% probability of fever in Saphone. The distribution of parasite densities in the rabies vaccine group in each site and age group is shown as a histogram.

Table 1: Characteristics of the trial participants at baseline.

Variable	Control	GMZ2
Number randomized	923	926
Age in months (mean, range)	36.7 (12-60)	35.6 (11-60)
Age group		
11-23 months	198 (22%)	201 (22%)
24-35 months	217 (24%)	226 (24%)
36-47 months	262 (29%)	307 (33%)
48-60 months	242 (26%)	190 (21%)
Gender: Male	502 (54%)	498 (54%)
Bednet use*	395 (50%)	367 (45%)
Temperature (mean, range)	36.7 (13-40.4)	36.7 (14-39.7)
Weight in kg (mean, range)	13.1 (0.9 - 95)	12.8 (7 -41)
Weight for age z-score** (mean, range)	-0.7 (-10.5 – 36.2)	-0.7 (-3.5 – 16.1)
Biochemical and haematological variables (mean, range)		
White blood cell count (10^3/uL)	9.5 (1.8 - 67.7)	9.8 (2.1 - 46.7)
Red blood cell count (10^6/uL)	4.5 (1.8 -9.5)	4.5 (1.8 - 10.1)
Haemoglobin (g/dL)	10.5 (6.4 - 14.2)	10.5 (6.2 - 14.1)
Haematocrit (%)	32.7 (8.4 - 41.9)	32.7 (11.3 - 44.5)
Platelets (10^3/uL)	328 (14 - 999)	327 (11 - 999)
Creatinine (umol/L)	30.5 (5-92)	30.3 (0-96.1)
Total Bilirubin (umol/L)	7.3 (1.1-64)	7.5 (0-47)
ALT** (IU/L)	18.6 (3.5-137)	19.0 (0-206)
ASAT (IU/L)	37.9 (7-278)	37.6 (0.5 - 181)
Alkaline Phosphatase** (IU/L)	268 (113 - 700)	261 (0-700)
Anti-GMZ2 IgG titre (mean EU per µl)[##]	0.153	0.153

*Slept under a treated net the night before the survey. Note bednet use was not measured at baseline but after the six-month visit.

*ALT was not measure in Uganda

[**]Alkaline Phosphatase was not measured in the two Burkina Faso sites or Gabon

[##] At baseline, 51% of participants had detecTable anti-GMZ2 antibodies (mean 0.153 enzyme-linked immunosorbent assay units (EU) per µl), this varied by site with highest levels in Sapone (mean 0.3) and lowest in Gabon (mean 0.04).

Table 2: Vaccine efficacy at different parasitemia threshold densities ITT and ATP populations

	Rabies Vaccine		GMZ2		Vaccine efficacy (95%CI)		
	No. of malaria episodes	Mean no. episodes per child	No. of malaria episodes	Mean no. episodes per child	unadjusted, site as strata	adjusted for age (site as strata)	age interaction p-value
Malaria with documented fever or history of fever and parasitaemia at any density >0/μL							
ITT	1264	1.37	1204	1.30	6.1% (-1.9% - 13.5%)	7.2% (-0.6% - 14.4%)	0.062
ATP	894	1.03	842	0.97	6.3% (-3.1% - 14.7%)	7.9% (-1.1% - 16.1%)	0.136
Malaria with documented fever or history of fever and parasite density >5000/μL							
ITT	1005	1.09	920	0.99	9.7% (0.4% - 18.1%)	11.3% (2.5% - 19.3%)	0.057
ATP	720	0.83	641	0.74	11.3% (0.8% - 20.7%)	13.6% (3.6% - 22.5%)	0.112
Malaria with documented fever or history of fever and parasite density >20,000/μL							
ITT	777	0.84	718	0.78	8.7% (-2.1% - 18.6%)	10.6% (0.1% - 21%)	0.043
ATP	563	0.65	501	0.58	11.3% (-1.1% - 22.1%)	13.7% (2.0% - 24.1%)	0.075

Table 3: Anti-GMZ2 IgG responses in children 1-2 yrs and 3-4 yrs of age in the two vaccine groups. Arithmetic mean titres (SD) at baseline and on day 84 (ATP population).

	Rabies vaccine mean(SD)		**Ratio (95%CI)**	**GMZ2 mean (SD)**		**Ratio (95%CI)**
Age group	Day 0	Day 84	Day84/Day0	Day 0	Day 84	Day84/Day0
1-2yrs	0.07 (0.24)	0.15 (0.47)	2.2 (1.4,3.7)	0.09 (0.38)	1.21 (2.53)	14 (8.7,23)
3-4yrs	0.22 (0.85)	0.35 (1.32)	1.6 (0.95,2.6)	0.21 (0.72)	1.21 (2.01)	5.7 (4.0,8.2)
Ratio (95%CI)	3.3 (2.0,5.5)	2.3 (1.4,3.8)		2.5 (1.4,4.2)	1.0 (0.77,1.3)	

Table 4: Association between GMZ2 IgG titre measured on day 84, and the incidence of malaria in each vaccine group.

	GMZ2			Rabies vaccine		
GMZ2 IgG titre*	No. of children	Hazard ratio[#] (95%CI)	P-value	No. of children	Hazard ratio[#] (95%CI)	P-value
1	201	1		654	1	
0.21-0.54	201	0.85 (0.69,1.1)	0.147	70	1.1 (0.82,1.4)	0.696
0.54-1.3	201	0.94 (0.75,1.2)	0.606	39	0.84 (0.54,1.3)	0.423
≥1.3	200	0.77 (0.61,0.98)	0.035	34	0.68 (0.44,1.1)	0.083

*4 groups defined by quartiles of GMZ2 IgG titre in the GMZ2 group.

[#] adjusted for age and site.

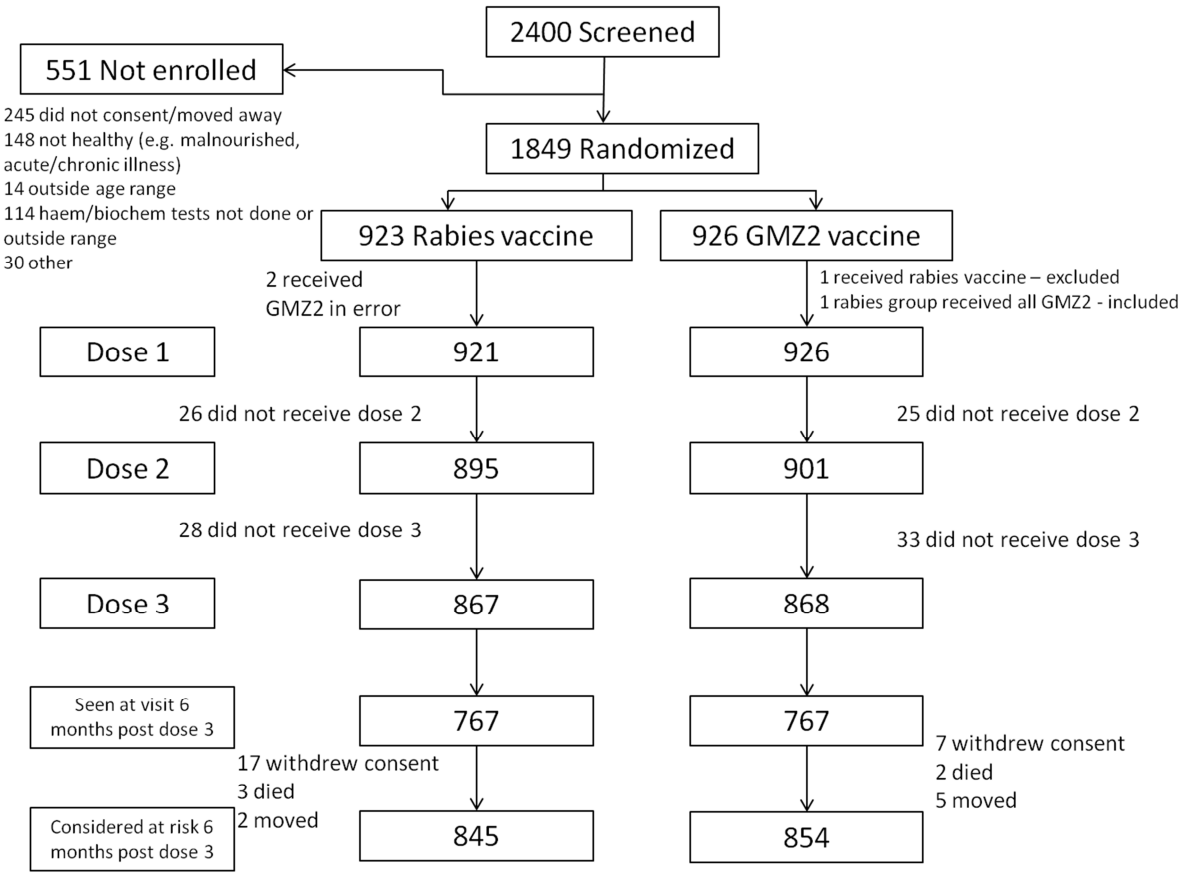


Figure 1: Trial profile. 1735 children received 3 doses of vaccine (867 Verorab and 868 GMZ2) and were included in the per-protocol analysis. 809/867 (%) and 827/868 (%) respectively were seen at the scheduled 6 month visit. There were three allocation errors: one child randomized to receive rabies vaccine was given three doses of GMZ2, (this child was included in per-protocol analysis); two children, one randomized to rabies vaccine and the other to GMZ2, received mixed doses, these children were excluded from the per-protocol analysis but were included in ITT analysis.

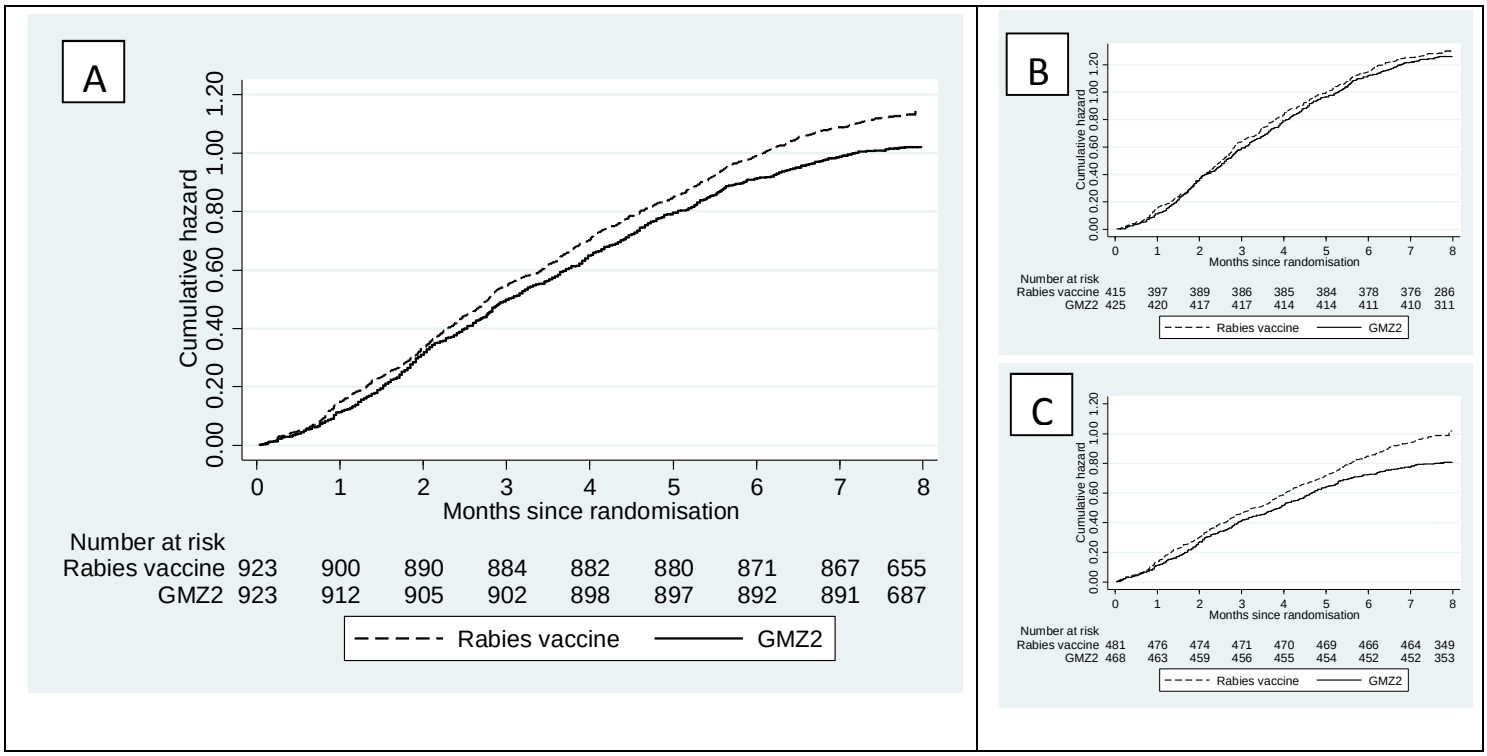


Figure 2. Timing of malaria episodes in each vaccine group.

The Nelson-Aalen estimate of the cumulative hazard (the mean number of episodes per child) in each group is shown against time since randomization. **A**) all age groups combined; **B**) children 1-2yrs of age; **C**) children 3-4 years of age. Time at risk for ITT analyses is from randomization until 3 months post dose 3, i.e. approximately 8 months.

Supplementary Table S1: Baseline characteristics by randomisation arm per site

	Banfora		Sapone		Gabon		Ghana		Uganda	
Variable	Control	GMZ2	Control	GMZ2	Control	GMZ2	Control	GMZ2	Control	GMZ2
Number randomized	290	290	150	150	256	256	100	100	127	130
Age in months (mean,range)	36.2 (12-59)	34.6 (12-59)	35.6 (12-59)	34.7 (12-58)	35.9 (12-59)	34.4 (11-59)	37.6 (12-60)	37.5 (12-60)	39.7 (12 - 59)	39.7 (13 - 59)
Age group (number (%)): 11-23 months	53 (18%)	53 (18%)	37 (25%)	44 (29%)	69 (27%)	68 (27%)	27 (27%)	24 (24%)	12 (9%)	12 (9%)
24-35 months	84 (29%)	102 (35%)	34 (23%)	27 (18%)	55 (22%)	55 (22%)	13 (13%)	15 (15%)	31 (24%)	27 (21%)
36-47 months	93 (32%)	90 (31%)	41 (27%)	43 (29%)	53 (21%)	82 (32%)	28 (28%)	34 (34%)	47 (37%)	58 (45%)
48-60 months	60 (21%)	45 (16%)	38 (25%)	36 (24%)	76 (30%)	50 (20%)	31 (31%)	26 (26%)	37 (29%)	33 (25%)
Gender: Male	152 (52%)	151 (52%)	87 (58%)	80 (53%)	143 (56%)	138 (54%)	49 (49%)	55 (55%)	71 (56%)	74 (57%)
Bednet use, sleep under a treated net*: Yes	163 (64%)	165 (63%)	60 (47%)	51 (38%)	74 (39%)	71 (37%)	78 (79%)	70 (71%)	20 (18%)	10 (8%)
Temperature (mean, range)	36.7 (36-39.4)	36.6 (36-38.4)	36.9 (36.1 – 38.1)	36.8 (36 – 37.6)	37.2 (35.2 – 40.4)	37.2 (36-39.7)	36.3 (35.6 – 37.3	36.3 (36 – 37.7)	36.2 (13 – 38.9)	36.2 (14 – 38.2)
Weight in kg (mean,	12.9 (6.9-95)	12.3 (7.6 –	12.4 (7.6 –	12.5 (7.4 –	13.2 (7.4 –	12.7 (7.5 –	13.1 (6.2 –	13.3 (7-41)	14.3 (0.9 -	14.4 (8 -

range)		18.7)	36.7)	37.4)	21.6)	19.6)	19.3)		25)	22)
Biochemical and haematological variables (mean, range)										
White blood cell count (10^3/uL)	9.5 (4.4 - 67.7)	9.4 (4.3 – 21.4)	9.7 (5 - 22.6)	10.1 (4.5 - 46.7)	10.0 (1.8 - 21.6)	10.5 (2.1 - 29.9)	8.8 (4.3 - 15.7)	9.4 (5.6 – 20.9)	8.9 (3.2 - 31.9)	9.4 (4.8 - 18.6)
Red blood cell count (10^6/uL)	4.5 (2.9 - 5.9)	4.5 (3.2 – 6.7)	4.5 (3.5 - 5.6)	4.5 (3.0 - 6)	4.5 (1.8 - 5.9)	4.5 (1.8 - 7.4)	4.4 (3.3 – 6.1)	4.4 (3.3 - 5.9)	4.4 (2.8 - 9.5)	4.4 (2.9 - 10.1)
Haemoglobin (g/dL)	10.5 (7.3 - 13.7)	10.5 (7.4 - 13.5)	11.0 (7.9 - 14.2)	10.9 (7.6 - 14.1)	10.2 (7.3 - 12.9)	10.1 (7.4 - 13.6)	10.4 (6.4 - 12.6)	10.4 (6.2 - 12.5)	10.5 (7 - 13.6)	10.8 (8 - 13.6)
Haematocrit (%)	33.4 (21.8 - 40.3)	33.3 (23.9 - 42.4)	33.2 (24.8 - 40.3)	33.0 (25.1 - 40.4)	31.8 (14.2 - 38.9)	31.6 (14.2 - 44.5)	32.9 (23 - 40)	33.1 (23 - 40)	32.1 (8.4 - 41.9)	32.9 (11.3 - 42)
Platelets (10^3/uL)	359 (40.2 - 999)	344 (77.8 - 814)	293 (64.9 - 566)	302 (10.8 - 835)	336 (14-658)	343 (27-999)	335 (82 - 807)	331 (127 - 688)	277 (40 - 637)	287 (72 - 628)
Creatinine (umol/L)	29.5 (6.3 - 55.3)	29.1 (4.6 - 55)	35.4 (16.9 - 63)	35.1 (14 - 69)	23.4 (5-44.2)	22.9 (5-45)	54.1 (9 - 92)	55.7 (31.7 - 96.1)	22.3 (11.3 - 41.9)	22.2 (0 - 37.3)
Total bilirubin (umo l/L)	7.8 (2.5 - 26.7)	8.0 (1.0 - 28.9)	7.2 (2.5 - 19.8)	7.8 (2.5 - 25.7)	7.6 (3-18)	8.2 (2-47)	7.1 (1.1 - 64)	6.1 (1.5 - 39.8)	5.9 (1.3 - 54)	6.0 (0 - 24.1)
ALT** (IU/L)	17.6 (3.5 - 63.2)	16.5 (0-68.7)	24.1 (8 - 137)	24.8 (6 - 154)	14.3 (6-67)	14.3 (6-73)	24.0 (5.7 - 104.4)	28.6 (7.5 - 206.1)	-	-
ASAT (IU/L)	42.1 (23.3 -	41.6 (1.1-	41.5 (20 -	42.4 (8 -	31.0 (7-74)	31.6 (15-	38.1 (22.9 -	40.2 (19.9 -	36.4 (18.4 -	32.6 (0.5 -

	93.7)	86.9)	151)	138)		122)	96.2)	181.4)	278)	52.8)
Alkaline Phosphatase*** (IU/L)	-	-	-	-	-	-	311 (167 - 613)	285 (141- 483)	236 (113 - 700)	243 (0 - 700)

*Slept under a treated net the night before the survey. Note bednet use was not measured at baseline but after the 6 month visit.

**ALT was not measured in Uganda

***Alkaline Phosphatase was not measured in the 2 Burkina Faso sites or Gabon

Supplementary Table S2. Vaccine efficacy by site at primary outcome, 6 months: Fever/history of fever and parasite density >5000/uL

	ITT				ATP			
Site	Rabies vaccine	GMZ2	Hazard Ratio (95%CI)	VE (95%CI)	Rabies vaccine	GMZ2	Hazard Ratio (95%CI)	VE (95%CI)
	Rate (No episodes/PYAR):				Rate (No episodes/PYAR):			
Banfora	2.72 (497/182.7)	2.42 (444/183.4)	0.89 (0.77 - 1.02)	11% (-2%-23%)	3.01 (403/133.8)	2.59 (348/134.3)	0.86 (0.74 - 1.01)	14% (-1% - 26%)
Lambarene	0.45 (70/155.2)	0.44 (71/161.0)	0.98 (0.68 - 1.41)	2% (-41% - 32%)	0.41 (45/109.4)	0.34 (38/110.8)	0.83 (0.54 - 1.29)	17% (-29% - 46%)
Navrongo	1.04 (67/64.4)	0.95 (61/64.5)	0.91 (0.63 - 1.31)	9% (-31% - 37%)	0.98 (49/50.0)	0.92 (45/49.0)	0.94 (0.61 - 1.45)	6% (-45% - 39%)
Sapone	2.29 (214/93.5)	2.18 (210/96.1)	0.96 (0.78 - 1.17)	4% (-17% - 22%)	1.93 (137/70.9)	1.88 (133/70.9)	0.97 (0.76 - 1.24)	3% (-24% - 24%)
Iganga	1.79 (147/82.2)	1.57 (132/84.3)	0.88 (0.68 - 1.14)	12% (-14% - 32%)	1.30 (81/62.1)	1.17 (75/63.9)	0.90 (0.65 - 1.25)	10% (-25% - 35%)
Pooled	1.72 (995/578.1)	1.56 (918/589.3)	0.91 (0.83 - 1.00)	9% (0% - 17%)	1.68 (715/426.3)	1.49 (639/428.8)	0.89 (0.80 - 0.99)	11% (1% - 20%)

Supplementary Table S3: Number of malaria episodes experienced by children during 6-month follow-up.

Number of episodes Parasitaemia >5000	ITT Number of children Rabies Vaccine	ITT GMZ2	ATP Number of children Rabies Vaccine	ATP GMZ2
0	408	435	451	466
1	237	246	219	255
2	139	126	118	82
3	87	72	54	44
4	34	33	23	16
5	15	10	1	4
6	3	4	1	1
Total	923	926	867	868

Adverse Events

Supplementary Table S4: Solicited local symptoms: (within 7 days of the dose) by severity

	Rabies vaccine				GMZ2			
	Dose 1 N=921	Dose 2 N=895	Dose 3 N=867	After any dose N=921	Dose 1 N=925	Dose 2 N=900	Dose 3 N=867	After any dose N=925
Any solicited local adverse event	Number of individuals affected				Number of individuals affected			
	140 (15%)	89 (10%)	85 (10%)	217 (24%)	188 (20%)	109 (12%)	95 (11%)	268 (29%)
Grade 3	1	1	0	2	0	0	1	1
Pain: Any	114 (12%)	67 (7%)	77 (9%)	180 (20%)	156 (17%)	92 (10%)	74 (9%)	224 (24%)
Grade 3	0	0	0	0	0	0	0	0
Swelling: Any	26 (3%)	15 (2%)	13 (1%)	49 (5%)	43 (5%)	14 (2%)	13 (2%)	64 (7%)
Grade 3	0	0	0	0	0	0	1	1
Induration: Any	23 (2%)	11 (1%)	8 (1%)	41 (4%)	33 (4%)	18 (2%)	20 (2%)	63 (7%)
Grade 3	1	1	0	2	0	0	0	0
Erythema: Any	1 (<1%)	0 (0%)	0 (0%)	1 (<1%)	4 (<1%)	0 (0%)	0 (0%)	4 (<1%)
Grade 3	0	0	0	0	0	0	0	0
Pruritus: Any	1 (<1%)	6 (1%)	0 (0%)	6 (1%)	3 (<1%)	2 (<1%)	1 (<1%)	6 (1%)
Grade 3	0	0	0	0	0	0	0	0

Supplementary Table S5: Solicited general symptoms: (within 7 days of the dose)

	Rabies Vaccine				GMZ2			
	Dose 1 N=921	Dose 2 N=895	Dose 3 N=867	After any dose N=921	Dose 1 N=925	Dose 2 N=900	Dose 3 N=867	After any dose N=925
Any solicited general adverse event	Number of individuals affected				Number of individuals affected			
	81 (9%)	65 (7%)	68 (8%)	183 (20%)	98 (11%)	85 (9%)	65 (7%)	216 (23%)
Any with information on relation	65 (7%)	53 (6%)	51 (6%)	147 (16%)	81 (9%)	67 (7%)	53 (6%)	179 (19%)
Related to study vaccine	11	14	12	35	15	15	14	39
Grade 3 related	1	0	1	2	1	3	0	4
Fever: Any	26 (3%)	32 (4%)	36 (10%)	88 (10%)	37 (4%)	35 (4%)	47 (5%)	110 (12%)
Related to study vaccine	5	12	10	27	12	14	13	36
Grade 3 related	1	0	1	2	1	3	0	4
Irritability: Any	4 (<1%)	6 (1%)	1 (<1%)	11 (1%)	8 (1%)	8 (1%)	2 (<1%)	17 (2%)
Related to study vaccine	1	0	0	1	1	2	0	3
Grade 3 related	0	0	0	0	0	0	0	0
Drowsiness: Any	8 (1%)	6 (1%)	4	17 (2%)	14	8 (1%)	2 (<1%)	23 (2%)

			(<1%)		(2%)			
related	1	0	0	1	2	2	0	4
Grade 3 related	0	0	0	0	0	0	0	0
Loss of appetite:					29			
Any	17 (2%)	10 (1%)	6 (1%)	32 (3%)	(3%)	25 (3%)	6 (1%)	57 (6%)
Related to study								
vaccine	2	1	1	4	4	2	1	7
Grade 3 related	0	0	0	0	0	0	0	0
					21			
Diarrhoea: Any	29 (3%)	13 (1%)	14 (2%)	48 (5%)	(2%)	16 (2%)	5 (1%)	40 (4%)
Related to study								
vaccine	4	1	2	7	1	1	1	3
Grade 3 related	0	0	0	0	0	0	0	0

Supplementary Table S6: Unsolicited adverse events by vaccine group and dose (within 28 days of the dose):

Any unsolicited adverse event	Rabies vaccine				GMZ2			
	Dose 1 N=921	Dose 2 N=895	Dose 3 N=867	After any dose N=921	Dose 1 N=925	Dose 2 N=900	Dose 3 N=867	After any dose N=925
	Number affected with at least one event (%)				Number affected with at least one event (%)			
Any	552 (60%)	458 (51%)	484 (56%)	810 (88%)	540 (58%)	481 (53%)	483 (56%)	814 (88%)
Related	14 (2%)	11 (1%)	6 (1%)	29 (3%)	29 (3%)	14 (2%)	12 (1%)	51 (6%)
Grade 3 related	1	0	1	2	0	0	1	1

Supplementary Table S7: Line listing of volunteers who died.

Subject id	Randomisation group	Event start date	Date last vaccination	Time since last vaccination (days)	Age	Sex	Term
0000364	Rabies Vaccine	13/10/2011	05/07/2011	100	25	Male	SUDDEN DEATH
0000376	Rabies Vaccine	11/11/2011	07/07/2011	127	18	Female	DROWNING
L1250-8	Rabies Vaccine	02/06/2011	03/01/2011	150	56	Male	MALARIA, CONVULSIONS
L1701-3	GMZ2	19/12/2011	06/09/2011	104	12	Female	PNEUMONIA
M0032-9	GMZ2	25/08/2011	07/07/2011	49	17	Female	SEVERE MALARIA

Supplementary Table S8: Line listing of all volunteers with severe malaria.

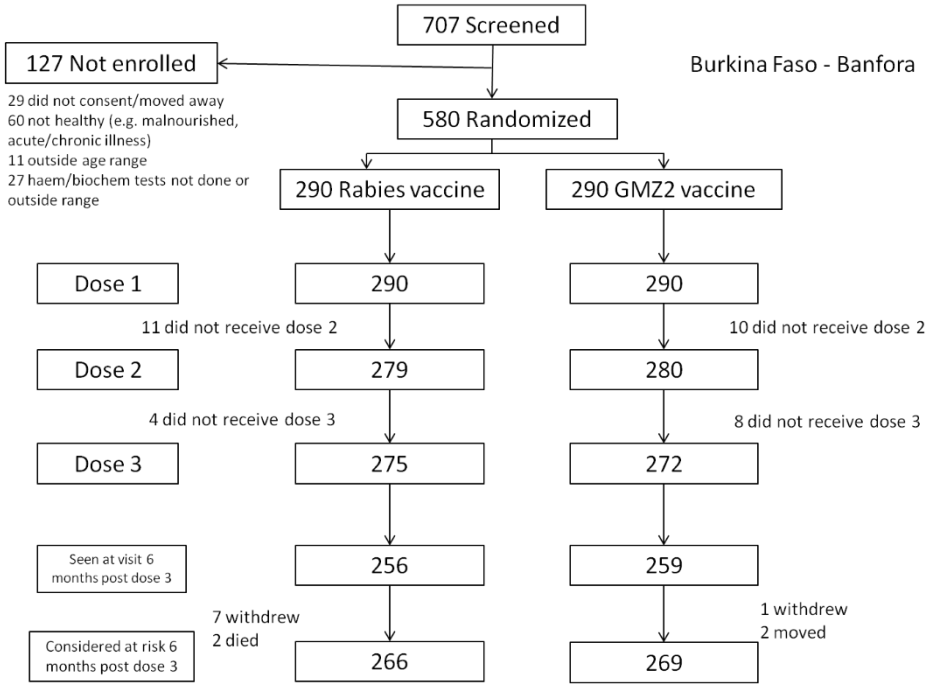
Subject id	Site	Randomisation group	Event start date	Date last vaccination	Time since last vaccination (days)	Age (yrs)	Sex	Main condition	Additional conditions reported	Outcome
0000012	Banfora	Rabies Vaccine	19/10/2011	28/06/2011	113	2	M	Malaria Malaria	Pneumopathy/	Recovered/ Resolved
0000013	Banfora	GMZ2	16/09/2011	28/06/2011	80	2	F	Malaria Severe Malaria	Convulsions/	Recovered/ Resolved
0000095	Banfora	GMZ2	17/06/2011	31/05/2011	17	2	M	Malaria Malaria	Vomiting/	Recovered/ Resolved
0000118	Banfora	Rabies Vaccine	03/09/2011	29/06/2011	66	2	F	Malaria Malaria (failure of oral medication)	/	Recovered/ Resolved
0000245	Banfora	GMZ2	22/11/2011	02/07/2011	143	2	F	Malaria Uncomplicated malaria associated to bronchitis with inability to take treatment per os	Bronchitis/	Recovered/ Resolved
0000262	Banfora	Rabies Vaccine	28/05/2011	04/05/2011	24	3	M	Malaria Severe malaria	Convulsions/	Recovered/ Resolved
0000298	Banfora	Rabies Vaccine	03/09/2011	02/07/2011	63	5	M	Malaria Uncomplicated malaria associated to generalizes edema syndrom	Edema/	Recovered/ Resolved
0000454	Banfora	GMZ2	03/10/2011	05/07/2011	90	2	M	Malaria Severe Malaria	/	Recovered/ Resolved
0000478	Banfora	GMZ2	02/06/2011	14/05/2011	19	3	M	Malaria Malaria	/	Recovered/ Resolved
0000541	Banfora	Rabies Vaccine	30/06/2011	09/06/2011	21	2	M	Malaria Severe malaria	Lethargic/	Recovered/ Resolved
0000616	Banfora	Rabies Vaccine	17/07/2011	11/07/2011	6	2	M	Malaria Severe malaria	Lethargic/	Recovered/ Resolved
0000649	Banfora	Rabies Vaccine	07/09/2011	08/08/2011	30	1	M	Malaria Severe malaria	Anemia/Pneumonia	Recovered/ Resolved
0000658	Banfora	GMZ2	13/01/2012	08/08/2011	158	2	M	Inguinal hernia strangulated Inguinal hernia strangulated	Malaria/Fever	Recovered/ Resolved

0000678	Banfora	GMZ2	04/07/2011	27/06/2011	7	3	M	Malaria Severe malaria	Hemoglobinuria/	Recovered/ Resolved
0000685	Banfora	GMZ2	24/10/2011	24/08/2011	61	0	M	Malaria Severe malaria	Anemia/	Recovered/ Resolved
0000736	Sapone	Rabies Vaccine	17/09/2011	16/08/2011	32	3	M	Malaria Severe malaria	Fever/Convulsions	Recovered/ Resolved
0000737	Sapone	Rabies Vaccine	10/11/2011	16/08/2011	86	0	M	Malaria Severe malaria	Fever/Convulsions	Recovered/ Resolved
0000780	Sapone	Rabies Vaccine	20/09/2011	20/08/2011	31	3	F	Malaria Severe malaria	Fever/Respiratory distress	Recovered/ Resolved
0000796	Sapone	GMZ2	10/09/2011	17/08/2011	24	4	M	Malaria Severe malaria	Vomiting/Fever	Recovered/ Resolved
0000857	Sapone	GMZ2	26/09/2011	19/08/2011	38	3	M	Malaria Malaria	/	Recovered/ Resolved
0000935	Sapone	GMZ2	12/01/2012	19/08/2011	146	3	F	Malaria Malaria	Fever/Vomiting/Convulsions/	Recovered/ Resolved
0000986	Sapone	Rabies Vaccine	06/12/2011	19/08/2011	109	1	M	Malaria Malaria	Fever/Vomiting	Recovered/ Resolved
0001047	Sapone	GMZ2	11/07/2011	24/06/2011	17	2	M	Malaria Malaria	Respiratory distress/Convulsions	Recovered/ Resolved
0001069	Sapone	GMZ2	14/08/2011	20/07/2011	25	4	M	Malaria Malaria	Bronchitis/	Recovered/ Resolved
0001079	Sapone	Rabies Vaccine	12/11/2011	20/08/2011	84	3	M	Malaria Malaria	Anemia/Respiratory distress/Convulsion/	Recovered/ Resolved
L1223-3	Lambarene	Rabies Vaccine	26/03/2011	04/01/2011	81	4	F	Malaria Malaria	Fever/	Recovered/ Resolved
L1309-0	Lambarene	Rabies Vaccine	15/02/2011	28/01/2011	18	3	F	Malaria Malaria	Tachycardia/Tachypnoea/Runny nose/Fever/Vomiting/Cough	Recovered/ Resolved
L1364-1	Lambarene	Rabies Vaccine	23/01/2011	12/01/2011	11	4	F	Malaria Malaria	Fever/	Recovered/ Resolved
L1386-2	Lambarene	Rabies Vaccine	21/01/2011	12/01/2011	9	3	M	Malaria Malaria	Fever/	Recovered/ Resolved
L1435-9	Lambarene	Rabies Vaccine	21/09/2011	28/03/2011	177	5	F	Malaria Malaria	Fever/	Recovered/ Resolved
L1462-7	Lambarene	Rabies Vaccine	13/09/2011	24/03/2011	173	4	F	Malaria Malaria	Fever/Vomiting/Diarrhoea/Appetite lost	Recovered/ Resolved
L1499-8	Lambarene	Rabies Vaccine	02/06/2011	08/04/2011	55	4	F	Malaria Malaria	Gastroenteritis/Ear infection	Recovered/ Resolved
L1639-0	Lambarene	GMZ2	20/05/2011	28/04/2011	22	2	F	Malaria Malaria	Bronchitis/Fever	Recovered/ Resolved
L1649-4	Lambarene	GMZ2	13/09/2011	03/06/2011	102	0	F	Malaria Malaria	Fever/Convulsion	Recovered/ Resolved

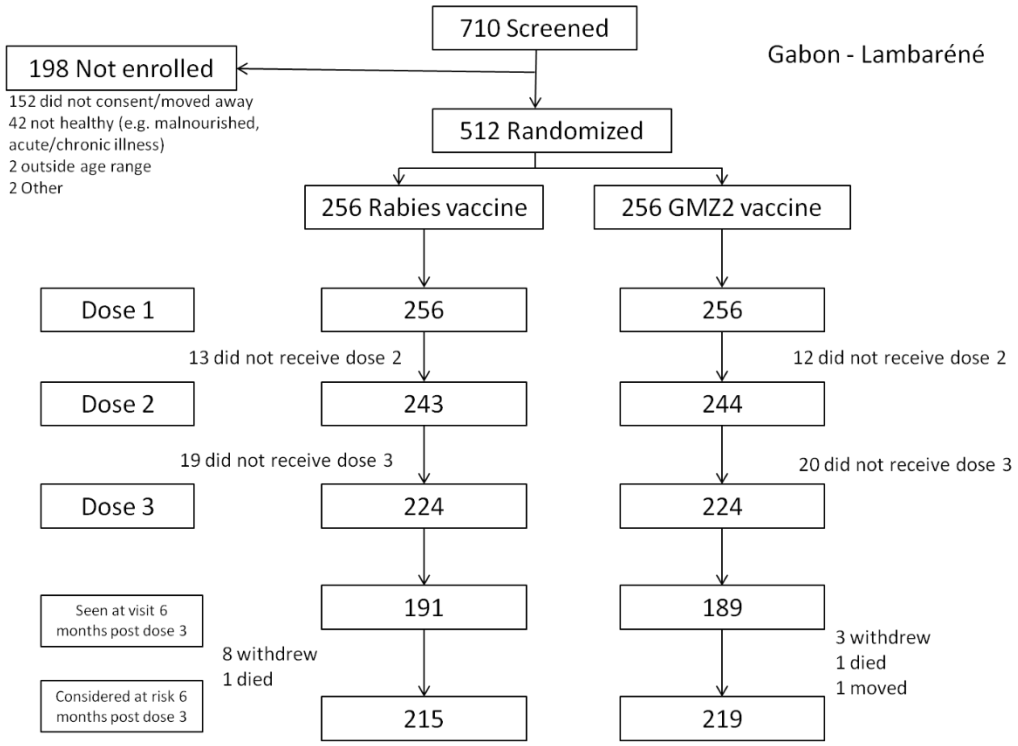
L1691-2	Lambarene	Rabies Vaccine	23/12/2011	25/08/2011	120	1	F	Malaria Malaria	Fever/Pallor/Dyspnea/	Recovered/ Resolved
L1707-1	Lambarene	Rabies Vaccine	11/12/2011	05/09/2011	97	2	M	Malaria Malaria	Bronchitis/Cough/Febrile convulsion/	Recovered/ Resolved
M0009-4	Iganga	GMZ2	24/11/2011	25/07/2011	122	0	M	Malaria severe malaria with URTI	URTI (upper respiratory tract infection)/Fever/Cough/Convulsion	Recovered/ Resolved
M0027-6	Iganga	GMZ2	28/11/2011	25/07/2011	126	3	M	Malaria Severe Malaria	Anemia/Fever/Anorexia/Vomiting	Recovered/ Resolved
M0065-7	Iganga	Rabies Vaccine	16/05/2011	13/05/2011	3	1	F	Malaria severe Malaria with dehydration	Dehydration/Fever/Vomiting/	Recovered/ Resolved
M0084-9	Iganga	GMZ2	26/10/2011	25/07/2011	93	1	M	Malaria clinical Malaria with URTI	URTI (upper respiratory tract infection)/Fever/Cough/	Recovered/ Resolved
M0091-8	Iganga	Rabies Vaccine	01/10/2011	08/07/2011	85	2	F	Malaria Malaria	Cough/Labored breathing/Nasal flaring/	Recovered/ Resolved
M0188-0	Iganga	Rabies Vaccine	31/10/2011	27/07/2011	96	3	M	Malaria severe Malaria with dehydration	Dehydration/Fever/Vomiting/Diarrhoea	Recovered/ Resolved
M0253-0	Iganga	GMZ2	26/09/2011	29/07/2011	59	2	M	Malaria Malaria	Pneumonia/Fever/Cough/	Recovered/ Resolved
N1877-4	Navrongo	GMZ2	23/10/2011	29/07/2011	86	3	M	Malaria Severe Malaria	/	Recovered/ Resolved
N1928-3	Navrongo	Rabies Vaccine	03/07/2011	07/06/2011	26	0	F	Malaria Malaria	Diarrhea/Fever/Vomiting/Appetite lost	Recovered/ Resolved
N1950-1	Navrongo	GMZ2	14/07/2011	06/07/2011	8	2	F	Malaria Malaria	Fever/	Recovered/ Resolved

Supplementary Figure S1: Trial profile for each of the five sites presented in figures A-E

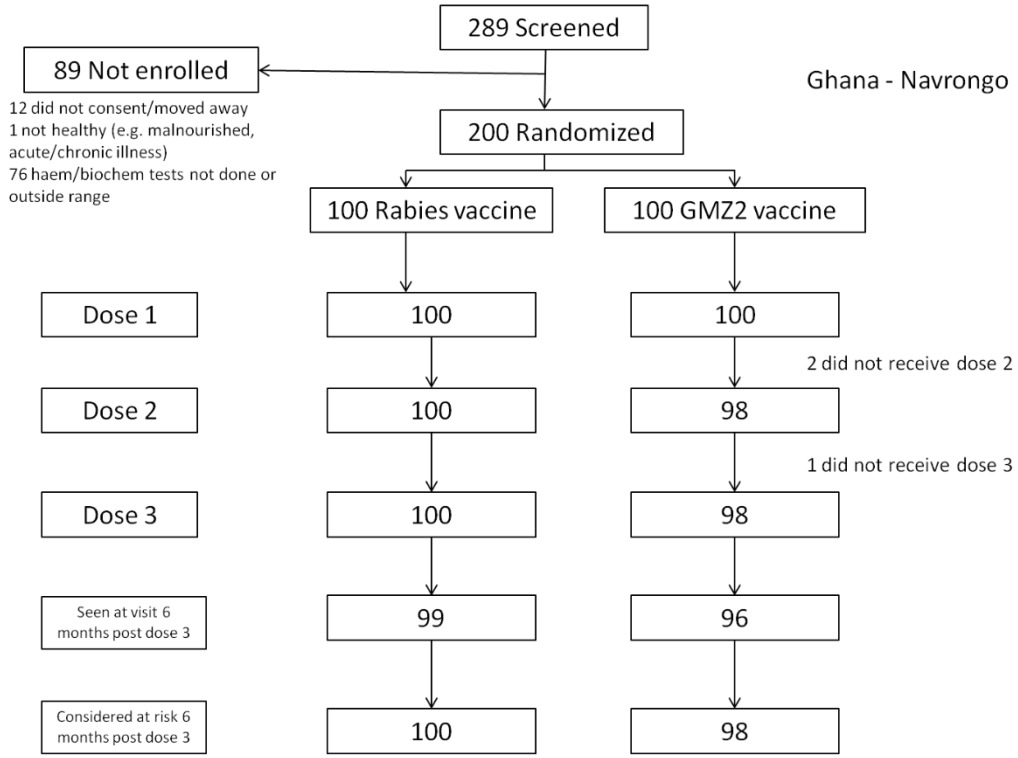
A: Burkina Faso – Banfora



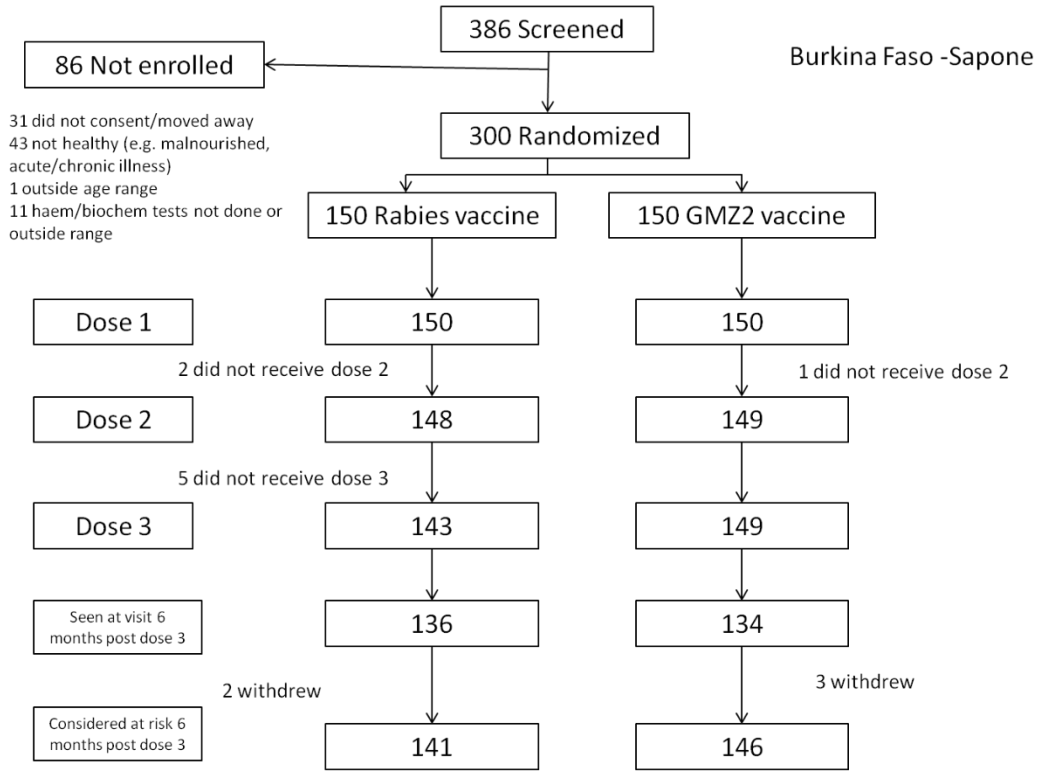
B: Gabon - Lamberéné



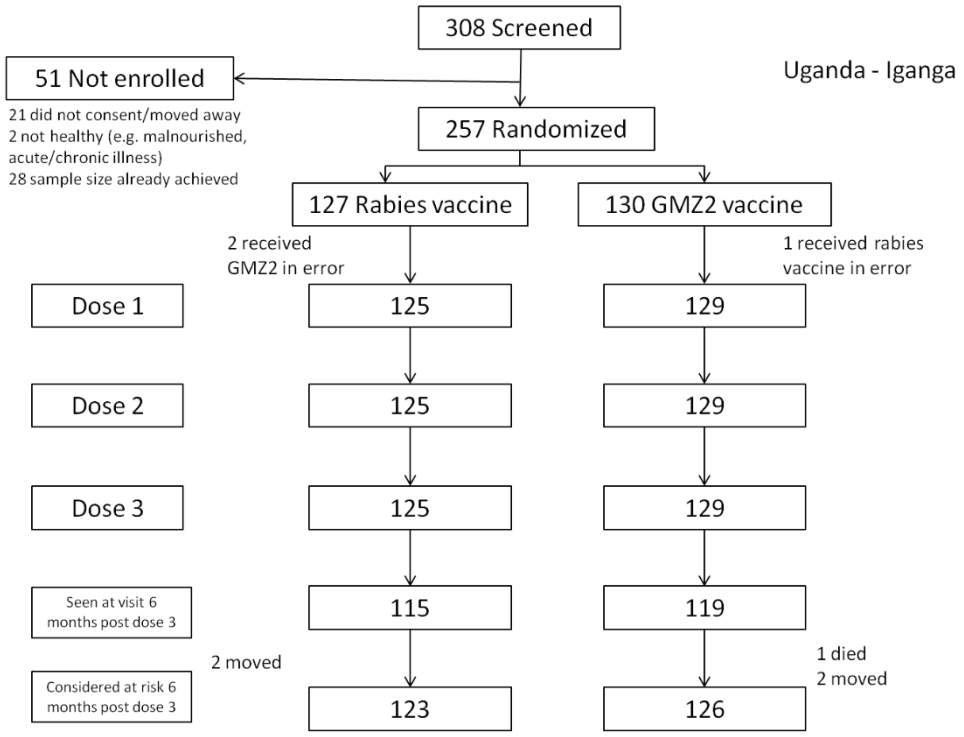
C: Ghana - Navrongo



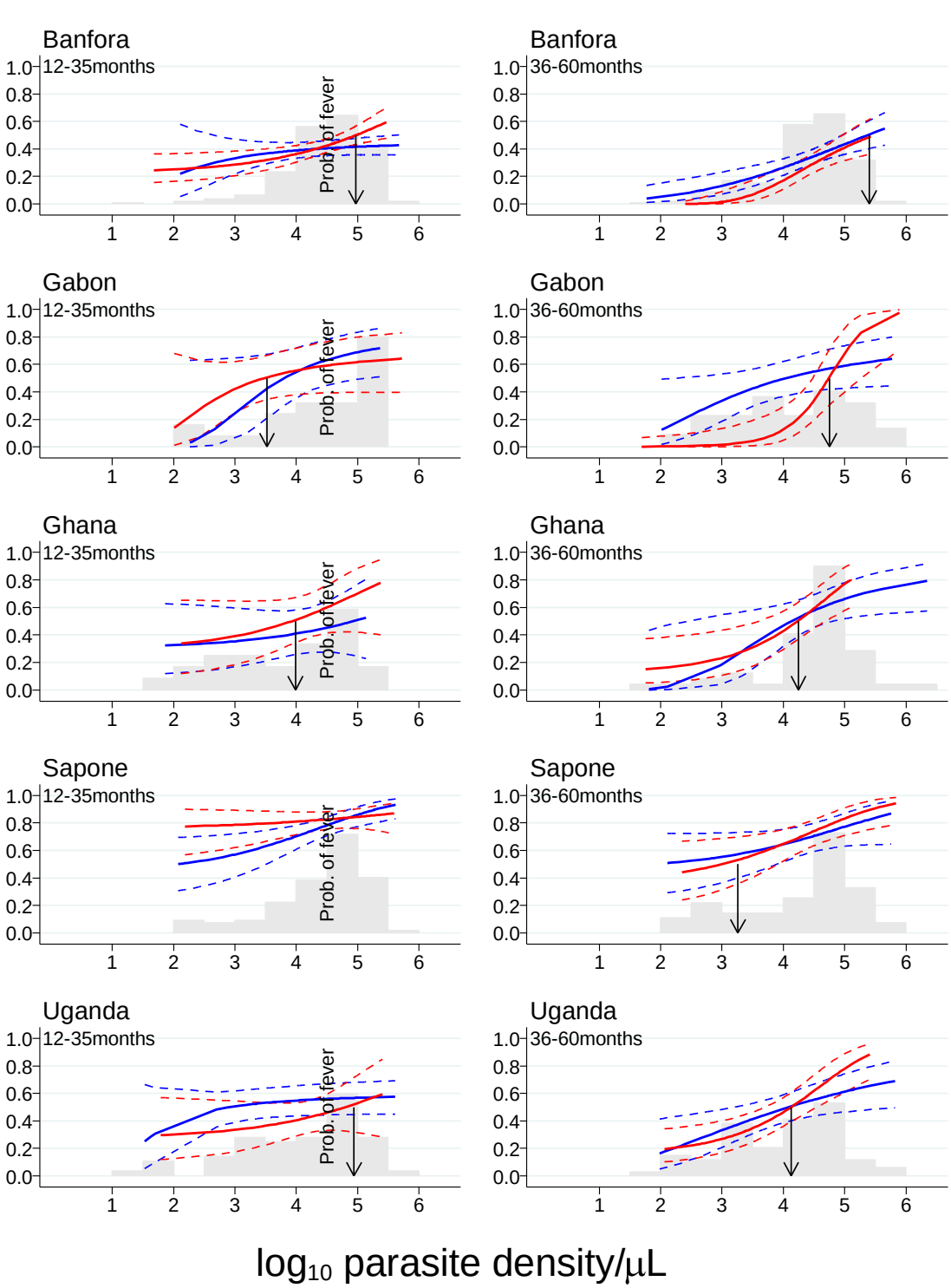
D: Burkina Faso – Sapone



E: Uganda – Iganga



Supplementary Figure 2S: The probability of fever in the rabies vaccine (in blue) and GMZ2 group (in red) in relation to parasite density, in each age group in each site.



Smoothed plots of the probability of fever were obtained using fractional polynomial logistic regression. Dashed lines show 90% confidence limits. The arrow shows the density

associated with a 50% probability of fever to facilitate comparison between the plots. There is no density corresponding to 50% probability of fever in Saphone. The distribution of parasite densities in the rabies vaccine group in each site and age group is shown as a histogram.