Asymptomatic Malaria Infection and the Immune Response to the 2-Dose Ad26.ZEBOV, MVA-BN-Filo Ebola Vaccine Regimen in Adults and Children

D. Ishola; The EBOVAC-Salone Malaria Infection (MALI) Sub-Study Team*

**Background.** Malaria infection affects the immune response to some vaccines. As Ebola virus (EBOV) outbreaks have occurred mainly in malaria-endemic countries, we have assessed whether asymptomatic malaria affects immune responses to the 2-dose Ad26.ZEBOV, MVA-BN-Filo Ebola vaccine regimen.

**Methods.** In this sub-study of the EBOVAC-Salone Ebola vaccine trial in Sierra Leone, malaria microscopy was performed at the time of Ebola vaccination. Participants with symptomatic malaria were treated before vaccination. Ebola vaccine responses were assessed post-dose 1 (day 57) and post-dose 2 (day 78) by the EBOV glycoprotein FANG enzyme-linked immunosorbent assay (ELISA), and responses expressed as geometric mean concentrations (GMCs). Geometric mean ratios (GMRs) of the GMCs in malaria-positive versus malaria-negative participants were derived with 95% confidence intervals (CIs).

**Results.** A total of 587 participants were studied, comprising 188 adults (≥18 years) and 399 children (in age groups of 12–17, 4–11, and 1–3 years). Asymptomatic malaria was observed in 47.5% of adults and 51.5% of children on day 1. Post-dose 1, GMCs were lower in 1–3-year-old malaria-positive compared with malaria-negative children (age group–specific GMR, 0.56; 95% CI, 0.39–0.81) but not in older age groups. Post-dose 2, there was no consistent effect of malaria infection across the different age groups but there was a trend toward a lower response (GMR, 0.82; 95% CI, 0.67–1.02).

**Conclusions.** The Ad26.ZEBOV, MVA-BN-Filo Ebola vaccine regimen is immunogenic in participants with asymptomatic malaria. Therefore, it is not necessary to screen for asymptomatic malaria infection prior to vaccination with this regimen.

**Keywords.** malaria; Ebola vaccination; immune suppression.

Ebola virus (EBOV) disease (EVD) is an infection of major global health importance. The world's largest EVD outbreak occurred in West Africa in 2014–2016, causing more than 28 600 cases and 11 300 deaths [1]. The second-largest outbreak occurred in 2018–2020 in the Democratic Republic of Congo (DRC), with over 3400 cases and nearly 2300 deaths [2]. Further outbreaks in 2021 in the DRC [3] and Guinea [4] underline that recurrent outbreaks of this devastating infection are likely in the coming years. Accordingly, research to identify safe and effective vaccines against EVD has been a major international scientific priority for the past few years. Two Ebola vaccine regimens have received regulatory marketing authorization (MA) [5-7] and World Health Organization (WHO) prequalification [8, 9]. A recombinant vesicular stomatitis virus Ebola vaccine (rVSVΔG-ZEBOV-GP) has received conditional MA in the European Union (EU) [7] and approval for use in adults in the United States [6] and several African countries [10]. Similarly, a heterologous 2-dose regimen consisting of an adenovirus vector expressing the Zaire Ebola virus glycoprotein (GP) (Ad26.ZEBOV) as dose 1, followed after 56 days by a modified vaccinia Ankara (MVA) viral construct (MVA-BN-Filo) as dose 2 (the 2-dose Ad26.ZEBOV, MVA-BN-Filo regimen), has received MA in the EU for prophylactic use for adults and children aged 1 year or older [5].

The sub-Saharan African regions affected by EVD are also areas affected by malaria, which remains a leading cause of morbidity and mortality. The WHO estimated that 229 million cases of malaria occurred in 2019, with 409 000 deaths, of which the vast majority occurred in Africa [11]. Malaria, including asymptomatic malaria, can impair the immune response to vaccination [12-14]. Although the mechanisms for this impairment have not been fully elucidated, they are likely to involve immune dysregulation of both T-cell and B-cell functions [15, 16]. The impact of malaria on vaccine responses appears to depend on the vaccine type. In children with symptomatic malaria, impaired humoral responses to tetanus toxoid and typhoid [15], *Haemophilus influenzae* type b conjugate [17], and meningococcal polysaccharide [18] vaccination have been observed, and children protected...
with malaria chemoprophylaxis showed a higher antibody response to a meningococcal polysaccharide vaccine than a control group [19]. Conversely, there was no evidence that malaria infection impaired immune responses to the HPV-16/18 recombinant AS04-adjuvanted vaccine in adolescents [20, 21] or adults [20] in East Africa. Considering that EVD outbreaks have occurred in countries where malaria is common, it is important to determine whether malaria infection could affect immune responses to Ebola vaccination, which might thus necessitate routine malaria screening before vaccination. Therefore, we have evaluated whether the presence of malaria parasitemia at the time of vaccination has an impact on the antibody response to the 2-dose Ad26.ZEBOV and MVA-BN-Filo vaccine regimen in healthy adults and children during an Ebola vaccine trial conducted in Sierra Leone, a country with a very high prevalence of malaria infection in both adults and children [22].

**METHODS**

**Study Design**

This observational cohort sub-study was nested within the EBOVAC-Salone trial, which evaluated the safety and immunogenicity of the 2-dose Ad26.ZEBOV, MVA-BN-Filo Ebola vaccine regimen in Sierra Leonean adults [23] and children between September 2015 and July 2018. The study was conducted in Kambia District in Sierra Leone, where malaria is a major public health issue [24] within a resource-restricted setting with socioeconomic limitations [25]. Information on the EBOVAC-Salone trial design is available on [www.clinicaltrials.gov (identifier: NCT02509494)], and has been published in detail elsewhere [23, 26]. Stage 1 of the trial was an open-label study in which 43 adults received the Ebola vaccine regimen. Stage 2 was an individually randomized trial in which adults (aged >18 years), adolescents (12–17 years), older children (4–11 years), and toddlers (1–3 years) were vaccinated in a sequential, age-de-escalated fashion, with individuals receiving either the Ebola vaccine regimen (Ad26.ZEBOV on day 1 as dose 1, and MVA-BN-Filo on day 57 as dose 2), or an active control. Only stage 2 participants were enrolled in this malaria sub-study, which was approved by the Sierra Leone Ethics and Scientific Review Committee and the London School of Hygiene and Tropical Medicine Ethics Committee.

**Participants**

All stage 2 trial participants were invited to join this sub-study. They comprised 400 adults (aged >18 years) and 576 children aged 1 year or older (192 adolescents aged 12–17 years, 192 children aged 4–11 years, and 192 toddlers aged 1–3 years). Sociodemographic information (eg, age and sex) was obtained as part of the main trial documentation, and additional information on participants’ exposure to risk factors for malaria was obtained specifically for this sub-study. After trial completion and data unblinding, only participants who received Ebola vaccination per protocol and consented to the malaria sub-study were included in this analysis.

**Consenting**

EBOVAC-Salone trial stage 2 participants who met the trial inclusion criteria were invited to join this sub-study on the day of dose 1 vaccination, and informed consent (plus informed assent in children aged 7–17 years) was obtained using a separate process from that used for the main trial. During this sub-study consenting, potential participants received full study details, including that they were free to withdraw consent at any time, without prejudice to their participation in the main trial.

**Malaria Prevention, Testing, and Treatment**

At screening, and on dose 1 and 2 vaccination days, clinical evaluation of participants included assessment for malaria, noting variations between adults and children, in accordance with national guidelines [27]. Those with findings suggestive of malaria were tested with the First Response Malaria Ag. (pLDH/HRP2) Combo Rapid Diagnostic Test (Premier Medical Corporation Private Limited, Mumbai), and if they tested positive, they were treated with a full course of an age-appropriate antimalarial regimen in line with the guidelines. Vaccination and sample collection were then deferred until they had completed treatment and became asymptomatic. All participants were provided with an insecticide-treated bednet at study enrollment and encouraged to sleep under this bednet.

**Clinical Samples**

Blood samples were collected from all participants to determine EBOV-specific immunoglobulin G (IgG) antibodies before dose 1 vaccination (on day 1, for baseline antibody measurements), before dose 2 vaccination (on day 57, for post-dose 1 antibody measurements), and on day 78 (for post-dose 2 antibody measurements).

On each occasion, an ethylenediaminetetraacetic acid (EDTA) blood sample was also obtained for a complete blood count (CBC) as part of the trial protocol. For participants who consented to the malaria sub-study, aliquots from the EDTA samples on days 1 and 57 (the dose 1 and 2 vaccination days) were used to prepare thin and thick blood films for malaria microscopy, including measurement of parasite density and speciation.

**Malaria Microscopy**

Blood films were Giemsa-stained, stored, and subsequently examined in batches by 2 experienced microscopists. Where there was discordance between the results of the 2 readers in terms of positivity or parasite density, a third microscopist read the slide, and a final result was obtained according to preset criteria [28], following the WHO malaria microscopy guide [29]. Interrater reliability was analyzed using the kappa coefficient to
estimate consistency and degree of agreement between the microscopists who independently read each slide. External quality control was established for the reading of blood films through independent cross-checking of a 10% randomly selected sample of slides by an experienced external microscopist.

**Ebola Antibody Assay**

EBOV GP–specific antibody concentrations were measured at Q2 Solutions Vaccine Testing Laboratory (San Juan Capistrano, CA, USA), using the EBOV GP (Kikwit) Filovirus Animal Non-Clinical Group (FANG) enzyme-linked immunosorbent assay (ELISA) [30]. The antibody geometric mean concentration (GMC) in arbitrary ELISA units (EU)/mL was calculated for each group, with 95% confidence intervals (CIs).

**Data Management**

Demographic information and Ebola antibody data were imported from the database of the main clinical trial. Additional
demographic information on malaria study participants, data on risk factors for malaria infection, and the malaria microscopy readings were recorded on paper forms and double-entered in an OpenClinica database (OpenClinica LLC, Waltham, MA, USA). The separate databases were merged into a composite dataset.

### Statistical Analysis

Formal sample-size calculations for the malaria subanalysis were not done because analysis was restricted to samples from participants enrolled in the EBOV-AC-Salone trial. We performed log-normal power calculations on enrollment data for the trial intervention arm and determined that, with at least 550 participants receiving the 2-dose Ebola vaccine regimen, we would have 90% power to detect a ratio of GMCs of 0.87 for EBOV GP–specific antibody concentrations between participants with and without malaria parasitemia, assuming a prevalence of 50% for malaria parasitemia among trial participants (based on the most recent Malaria Indicator Survey data for Kambia District) [22], a coefficient of variation of 0.5 within each of the 2 groups (participants with and without parasitemia), and an alpha (type I) error of 0.05. As the antibody concentrations showed a skewed distribution, the EBOV GP–specific antibody concentrations were log-transformed using log base 10 for regression modeling of the GMCs, and the corresponding 95% CIs were estimated. The age-specific effect and the overall effect of malaria on antibody response were determined by calculating age-specific and overall GMCs (95% CI) for malaria-negative and -positive participants. Linear regression was used to compare mean log-transformed EBOV GP antibody concentrations between participants with and without malaria in each age group and overall. Since the prevaccination antibody concentrations differed between participants, adjusted coefficients for the effect of malaria on vaccine-induced immune responses were obtained by including prevaccination EBOV GP–specific antibody concentrations as an independent variable in the linear regression models. These adjusted regression coefficients and 95% CIs were back-transformed to obtain geometric mean ratios (GMRs) (ie, ratios of GMCs in participants with malaria parasitemia compared with those without parasitemia). Post-dose 2 antibody concentrations were compared between individuals with malaria infection at both vaccination time points and those with malaria infection on only 1 of the vaccination days. Finally, correlation analysis was used to examine the quantitative association between malaria parasite density at the time of vaccination and the postvaccination antibody concentration. Pearson correlation coefficients were obtained after each dose and in each age group.

### RESULTS

#### Study Participants

The study flow chart is presented in Figure 1. A total of 140 of the 730 trial participants in the Ebola vaccine group did not meet the requirements for inclusion in this study, including 55 who did not receive dose 2 and 72 individuals who received dose 2 outside the protocol window due to a temporary regulatory pause in the trial. Overall, 587 participants were included in the malaria study analysis (Figure 1 and Table 1), of whom 188 were adults aged 18 years or older and 399 were children (125 aged 1–3 years, 133 aged 4–11 years, and 141 aged 12–17 years). Overall, 368 participants (63%) were male. Details on other sociodemographic characteristics of the participants are shown in Table 1.

#### Malaria Parasitemia

The prevalence of malaria parasitemia was high in all age groups at both vaccination time points. On day 1, the overall prevalence of parasitemia was 50.3%: 22.5% in the 1–3-year age group, 51.1% in children aged 4–11 years, 77.5% in those aged 12–17 years, and 47.5% in adults. On day 57, the overall prevalence of parasitemia was 38.6%: 17.9% in the 1–3-year age group, 42.7% in children aged 4–11 years, 64.1% in those aged 12–17 years, and 31.4% in adults (Table 2). An analysis of risk factors for malaria infection is provided in Supplementary Tables 1 and 2.
As expected, young age (P < .001) and not using an insecticide-treated bednet (P = .029) were predictive of malaria parasitemia in the adjusted analysis (Supplementary Table 2). *Plasmodium falciparum* accounted for a large majority of the infections; 1 infection with *P. ovale* and 5 with *P. malariae* were detected. The percent agreement and interrater reliability between the 2 independent slide readers was high, with 95.8% agreement (kappa = 0.9164) for day 1 microscopy and 93.1% agreement (kappa = 0.8569) for day 57 microscopy (Supplementary Table 3).

**Effect of Malaria Infection on the EBOV GP–Specific Antibody Response**

EBOV GP–specific antibody concentrations were measured and compared in malaria-positive and malaria-negative individuals after each vaccine dose. After adjusting for prevaccination baseline antibody concentrations (Figure 2), a GMR for antibody concentration post-dose 1 of .72 (95% CI, 0.61–0.84) was found for all age groups combined. Age group–specific GMRs at this time point were .56 (95% CI, 0.39–0.81) in the 1–3-year age group, .99 (95% CI, .74–1.31) in children aged 4–11 years, 1.15 (95% CI, .83–1.58) in those aged 12–17 years, and .89 (95% CI, .70–1.12) in adults. For antibody concentrations after dose 2 vaccination, the GMR for all ages combined was .82 (95% CI, .67–1.02), and the age group–specific GMRs were .80 (95% CI, .35–1.79) in the 1–3-year age group, .63 (95% CI, .43–.92) in the 4–11-year age group, 1.24 (95% CI, .80–1.93) in the 12–17-year age group, and .87 (95% CI, .65–1.17) in adults. The lower antibody concentrations found in malaria-positive 1–3-year-olds following dose 1 were no longer observed after dose 2.

### Table 2. Effect of Malaria Infection on the Immune Response to the 2-Dose Ad26.ZEBOV, MVA-BN-Filo Ebola Vaccine Regimen

| Age Group   | Malaria Infection Pre-Dose 1 (Day 1) and Ebola Antibody Post-Dose 1 (Measured on Day 57) | Malaria Infection Pre-Dose 2 (Day 57) and Ebola Antibody Post-Dose 2 (Measured on Day 78) |
|-------------|-----------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------|
|             | n = 120                                                                                 | n = 123                                                                                 |
| 1–3 years   | Positive (%) n = 27 (22.5%) Negative (%) n = 93 (77.5%)                                 | Positive (%) n = 22 (17.9%) Negative (%) n = 101 (82.1%)                                 |
|             | Day 57 EBOV GP–specific antibody GMC (95% CI)                                           | Day 78 EBOV GP–specific antibody GMC (95% CI)                                           |
|             | 475 (340 663)                                                                          | 18.874 (8344, 42 692)                                                                 |
|             | 779 (658 922)                                                                          | 23.428 (19 428, 28 133)                                                                |
| 4–11 years  | n = 133                                                                                 | n = 117                                                                                 |
|             | Positive (%) n = 68 (51.1%) Negative (%) n = 65 (48.9%)                                 | Positive (%) n = 50 (42.7%) Negative (%) n = 67 (57.3%)                                  |
|             | Day 57 antibody GMC (95% CI)                                                          | Day 78 antibody GMC (95% CI)                                                            |
|             | 399 (321 498)                                                                          | 7755 (6664, 10 619)                                                                    |
|             | 381 (304 477)                                                                          | 13 860 (11 060, 17 369)                                                                |
| 12–17 years | n = 139                                                                                 | n = 131                                                                                 |
|             | Positive (%) n = 107 (77.0%) Negative (%) n = 32 (23.0%)                                 | Positive (%) n = 84 (64.1%) Negative (%) n = 47 (35.9%)                                  |
|             | Day 57 antibody GMC (95% CI)                                                          | Day 78 antibody GMC (95% CI)                                                            |
|             | 329 (278 390)                                                                          | 10 933 (8559, 13 109)                                                                  |
|             | 274 (195 406)                                                                          | 8994 (5991, 13 504)                                                                    |
| 18+ years   | n = 181                                                                                 | n = 175                                                                                 |
|             | Positive (%) n = 86 (47.5%) Negative (%) n = 95 (52.5%)                                 | Positive (%) n = 55 (31.4%) Negative (%) n = 120 (68.6%)                                |
|             | Day 57 antibody GMC (95% CI)                                                          | Day 78 antibody GMC (95% CI)                                                            |
|             | 206 (169 250)                                                                          | 3513 (2724, 4532)                                                                      |
|             | 260 (213 319)                                                                          | 4037 (3396, 4800)                                                                      |
| Overall     | n = 573                                                                                 | n = 546                                                                                 |
|             | Positive (%) n = 288 (50.3%) Negative (%) n = 285 (49.7%)                                 | Positive (%) n = 211 (38.6%) Negative (%) n = 335 (61.4%)                                |
|             | Day 57 antibody GMC (95% CI)                                                          | Day 78 antibody GMC (95% CI)                                                            |
|             | 310 (278 348)                                                                          | 7837 (6630, 9264)                                                                      |
|             | 408 (362, 461)                                                                         | 9817 (8586, 11 227)                                                                    |

The EBOV-specific binding antibody GMC (95% CI), in each age group and at each vaccination time point in relation to the presence or absence of malaria parasitemia at the time of vaccination is shown.

Abbreviations: CI, confidence interval; EBOV, Ebola virus; GMC, geometric mean concentration; GP, glycoprotein.

As expected, young age (P < .001) and not using an insecticide-treated bednet (P = .029) were predictive of malaria parasitemia in the adjusted analysis (Supplementary Table 2). *Plasmodium falciparum* accounted for a large majority of the infections; 1 infection with *P. ovale* and 5 with *P. malariae* were detected. The percent agreement and interrater reliability between the 2 independent slide readers was high, with 95.8% agreement (kappa = 0.9164) for day 1 microscopy and 93.1% agreement (kappa = 0.8569) for day 57 microscopy (Supplementary Table 3).

**Effect of Malaria Infection on the EBOV GP–Specific Antibody Response**

EBOV GP–specific antibody concentrations were measured and compared in malaria-positive and malaria-negative individuals after each vaccine dose. After adjusting for prevaccination baseline antibody concentrations (Figure 2), a GMR for antibody concentration post-dose 1 of .72 (95% CI, .61–.84) was found for all age groups combined. Age group–specific GMRs at this time point were .56 (95% CI, .39–.81) in the 1–3-year age group, .99 (95% CI, .74–1.31) in children aged 4–11 years, 1.15 (95% CI, .83–1.58) in those aged 12–17 years, and .89 (95% CI, .70–1.12) in adults. For antibody concentrations after dose 2 vaccination, the GMR for all ages combined was .82 (95% CI, .67–1.02), and the age group–specific GMRs were .80 (95% CI, .35–1.79) in the 1–3-year age group, .63 (95% CI, .43–.92) in the 4–11-year age group, 1.24 (95% CI, .80–1.93) in the 12–17-year age group, and .87 (95% CI, .65–1.17) in adults. The lower antibody concentrations found in malaria-positive 1–3-year-olds following dose 1 were no longer observed after dose 2.
Antibody concentrations were compared in those who were malaria-positive (any species, and at 1 or both visits), using the GMC in malaria-negative participants as the reference at each time point. The age-adjusted GMR was \(0.75\) (95% CI, \(0.56–1.01\)) in those who were malaria-positive at both time points (Table 3).

The potential impact of malaria parasite density at the time of vaccination on the antibody concentrations after each dose and in each age group was determined. Although a negative correlation was observed between antibody concentration and parasite density in children aged 1–3 years after dose 2 (\(r = -0.401\)), no clear pattern emerged overall (Figure 3).

**DISCUSSION**

As Ebola outbreaks occur in tropical African settings where malaria remains highly prevalent, widespread Ebola vaccination is most likely to be undertaken in areas with a high malaria prevalence. Malaria is recognized to impact some vaccine immune responses [14, 15, 17, 18]. In this study, we observed lower antibody concentrations 57 days after dose 1 in young children with asymptomatic malaria at the time of vaccination compared with aparasitemic children, but this is likely of limited impact as this effect on antibody concentrations was not observed at 21 days post-dose 2, when the prevalence of malaria parasitemia was lower than at the time of the first dose. Moreover, at both time points, parasitemia prevalence in these 1–3-year-old children was notably lower than in all others, further moderating the significance of the finding in this age group. There were no significant differences between parasitemic and aparasitemic participants among older children and adults at either time point.

These findings are similar to those found in another study of the impact of malaria on the immune response to an Ebola

---

Table 3. Post-Dose 2 EBOV GP–Specific Binding Antibody Concentrations (ELISA Units/mL) on Day 78, Categorized According to Microscopy-Confirmed Malaria Parasitemia on Day 1 and Day 57

| Malaria Parasitemia (Microscopy) | EBOV GP–Specific Binding Antibody (Day 78) |
|---------------------------------|--------------------------------------------|
| Time Point                      | n            | %  | GMC (95% CI) | Crude GMC Ratio (95% CI) | Age-Adjusted GMC Ratio (95% CI) |
| Negative (both day 1 and day 57) | 209          | 378| 11 552 (9769–13 660) | Reference | Reference |
| Positive day 1 and negative day 57 | 137          | 24.8| 7251 (5905–8904) | .63 (.48–.82) | .74 (.59–.94) |
| Negative day 1 and positive day 57 | 75           | 13.6| 7909 (5936–10 537) | .68 (.49–.95) | .7 (.53–.93) |
| Positive (both day 1 and day 57)  | 132          | 23.9| 7814 (6331–9645) | .68 (.52–.88) | .75 (.56–1.01) |

N = 553. Those who tested malaria microscopy negative at both time points are taken as the reference group.

Abbreviations: CI, confidence interval; EBOV, Ebola virus; ELISA, enzyme-linked immunosorbent assay; GMC, geometric mean concentration; GP, glycoprotein.
Figure 3. Correlation between malaria parasite density at the time of vaccination, and postvaccination antibody concentrations by age group: dose 1 (upper panels), dose 2 (lower panels). Abbreviations: EBOV, Ebola virus; GP, glycoprotein; IgG, immunoglobulin G.
vaccine undertaken in adults in Sierra Leone [31]. In that previous study, which, apart from including only adults, has several other differences from the study reported in this paper, the impact of asymptomatic malaria parasitemia was detected by polymerase chain reaction (PCR) rather than microscopy, and was evaluated over a period of 12 months in 506 adult subjects immunized with the single-dose rVSVΔG-ZEBOV-GP Ebola vaccine; children were not included. Antibody responses were measured by both FANG ELISA and a plaque neutralization (PRNT) assay. Although a sensitive malaria PCR assay was used, the prevalence of parasitemia (14.6%) in the previous study was substantially less than the 47.5% found in adults in the study described in this paper, perhaps because the former was undertaken in an urban area while our study population is from a rural setting with higher malaria transmission. The previous study also observed lower measurements in parasitemic subjects at the 1-, 6-, and 9–12-month time points in the PRNT assay, and at the 6- and 9–12-month time points with the ELISA, but differences between groups were not marked and 95% confidence limits overlapped at all time points. The findings from these 2 studies are reassuring, indicating that either Ebola vaccine could be used successfully in malaria-endemic areas, with no need to routinely screen for malaria before vaccination (or for presumptive malaria treatment at vaccination).

The strengths of this study include the inclusion of young children as well as adolescents and adults, because malaria disproportionately affects children compared with older age groups. Its limitations include measuring post-dose 1 antibody at 2 months after vaccination, thus possibly missing an impact of malaria on the early immune response. However, as this is a 2-dose vaccine regimen, the post-dose 2 antibody is of primary importance. Although we did not include virus neutralizing antibody measurement in this study, we and others have shown that binding and neutralizing antibody responses to the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen correlate well [23, 32]. Therefore, a similar effect of malaria infection on both binding and neutralizing antibody responses would be expected, as seen with the rVSVΔG-ZEBOV-GP vaccine [31]. Our study only included asymptomatic individuals, so the impact of symptomatic malaria at the time of vaccination on this Ebola vaccine regimen remains unknown.

Further questions remain regarding the potential influence of malaria on Ebola vaccine responses. We did not include children under the age of 1 year in this study, but the possible impact of malaria on the antibody response to the 2-dose Ad26.ZEBOV, MVA-BN-Filo Ebola vaccine regimen in infants is currently being addressed in a separate study in Sierra Leone (www.clinicaltrials.gov; identifier: NCT03929757). Furthermore, in this study, the presence of malaria parasitemia was measured at 2 time points, while it is possible that the degree of past exposure to malaria over months or years could influence the immune response to the 2-dose Ad26.ZEBOV, MVA-BN-Filo Ebola vaccine regimen. This possibility is currently being explored in a separate study by measuring antibody responses to a panel of malaria antigens at the time of vaccination.

Overall, this study has shown that there is no indication that asymptomatic malaria infection at the time of vaccination has a meaningful impact on the immunogenicity of the 2-dose Ad26.ZEBOV, MVA-BN-Filo Ebola vaccine regimen, and the observed modest effect should not impair the efficacy of this vaccine regimen in areas where malaria is highly endemic and where the vaccine may be needed most in the future.
References
1. Centers for Disease Control and Prevention (CDC). 2014–2016 Ebola outbreak in West Africa. Available at: https://www.cdc.gov/vhf/ebola/history/2014-2016-outbreak. Accessed 24 February 2022.
2. World Health Organization. Ebola: North Kivu/Ituri, Democratic Republic of the Congo, August 2018–June 2020. Available at: https://www.who.int/emergencies/situations/ebola/2019-drc.. Accessed 24 February 2022.
3. Khan FMA, Hasan MM, Kazimi Z, et al. Ebola and COVID-19 in Democratic Republic of Congo: grappling with two plagues at once. Trop Med Health 2021; 49:65–70.
4. Keita AK, Koundouno FR, Faye M, et al. Resurgence of Ebola virus in 2021 in Guinea suggests a new paradigm for outbreaks. Nature 2021; 597:539–43.
5. European Commission. Vaccine against Ebola: Commission grants new market authorisations. Available at: https://ec.europa.eu/commission/presscorner/detail/en/ip_20_1248. Accessed 24 February 2022.
6. US Food and Drug Administration. First FDA-approved vaccine for the prevention of Ebola virus disease, marking a critical milestone in public health preparedness and response. Available at: https://www.fda.gov/news-events/press-announcements/first-fda-approved-vaccine-prevention-ebola-virus-disease-marking-critical-milestone-public-health. Accessed 24 February 2022.
7. European Commission. Vaccine against Ebola: Commission grants first-ever market authorisation. Available at: https://ec.europa.eu/commission/presscorner/detail/en/ip_19_6246. Accessed 24 February 2022.
8. Johnson & Johnson. Johnson & Johnson joins World Health Organization in efforts to prevent spread of Ebola in West Africa. 13 May 2021. Available at: https://www.jnj.com/johnson-johnson-joins-world-health-organization-in-efforts-to-prevent-spread-of-ebola-in-west-africa. Accessed 24 February 2022.
9. Burke T. Ebola virus vaccine receives prequalification. Lancet 2019; 394:1893.
10. Merck. ERVEBO® (Ebola Zaire Vaccine, Live) now registered in four African countries, within 90 days of reference country approval and WHO prequalification. Available at: https://www.merck.com/news/erobo-ebola-zaire-vaccine-live-now-registered-in-four-african-countries-within-90-days-of-reference-country-approval-and-who-prequalification. Accessed 24 February 2022.
11. World Health Organization. World malaria report 2020: 20 years of global progress and challenges. (30 November 2020). ISBN 978-92-4-001579-1 (electronic version). Geneva, Switzerland: World Health Organization, 2020. License: CC BY-NC-SA 3.0 IGO. Available at: https://www.who.int/publications/i/item/9789240015791. Accessed 24 February 2022.
12. Cunningham AJ, Riley EM. Suppression of vaccine responses by malaria: insignificant or overlooked? Expert Rev Vaccines 2010; 9:409–29.
13. Williamson WA, Greenwood BM. Immune response to vaccination after acute malaria. Lancet 1978; 1:1328–9.
14. Zimmermann P, Curtis N. Factors that influence the immune response to vaccination. Clin Microbiol Rev 2019; 32:e00884–18.
15. Portugal S, Tipton CM, Sohn HJ, et al. Malaria-associated atypical memory B cells exhibit markedly reduced B cell receptor signaling and effector function. Elife 2015; 4:e07218.
16. Ryg-Cornejo V, Ioannidis LJ, Ly A, et al. Severe malaria infections impair germinal center responses by inhibiting T follicular helper cell differentiation. Cell Rep 2016; 14:68–81.
17. Uen S, Milligan P, Ethevenoux C, Greenwood B, Mulholland K. Effect of fever on the serum antibody response of Gambian children to Haemophilus influenzae type b conjugate vaccine. Pediatr Infect Dis J 2000; 19:444–9.
18. Greenwood BM, Bradley AK, Blakebrough JS, Whitte HC, Marshall TF, Gilles HM. The immune response to a meningococcal polysaccharide vaccine in an African village. Trans R Soc Trop Med Hyg 1980; 74:340–6.
19. Bradley-Moore AM, Greenwood BM, Bradley AK, et al. Malaria chemoprophylaxis with chloroquine in young Nigerian children. II. Effect on the immune response to vaccination. Ann Trop Med Parasitol 1985; 79:563–73.
20. Brown J, Baisley K, Kavishe B, et al. Impact of malaria and helminth infections on immunogenicity of the human papillomavirus-16/18 AS04-adjuvanted vaccine in Tanzania. Vaccine 2014; 32:611–7.
21. Nakalembe M, Banura G, Namujzi PB, Mirembe FM. Immunogenicity to the biivalent HPV-16/18 vaccine among adolescent African students exposed to helminths and malaria. J Infect Dev Ctries 2015; 9:197–205.
22. National Malaria Control Programme (NMCP); Statistics Sierra Leone; University of Sierra Leone; Catholic Relief Services; ICF. Sierra Leone Malaria Indicator Survey 2016. Freetown, Sierra Leone: National Malaria Control Programme, Statistics Sierra Leone, University of Sierra Leone, Catholic Relief Services, ICF, 2016.
23. Ishola D, Manno D, Afable MO, et al. Safety and long-term immunogenicity of the two-dose heterologous Ad26.ZEBOV and MVA-BN-Filo Ebola vaccine regimen in adults in Sierra Leone: a combined open-label, non-randomised stage 1, and a randomised, double-blind, controlled stage 2 trial. Lancet Infect Dis 2022; 22:979–109.
24. Howells S. Preventing malaria in Sierra Leone (Tropical Health and Education Trust [THET] blog). 25 April 2013. Available at: https://www.thet.org/preventing-malaria-sierra-leone/. Accessed 24 February 2022.
25. Reliefweb. Sierra Leone: Kambia District Profile (29 December 2015). Produced by OCHA Sierra Leone. Available at: https://reliefweb.int/sites/reliefweb.int/files/resources/district_profile_kambia_29_dec_2015.pdf. Accessed 24 February 2022.
26. Afable MO, Ishola D, Manno D, et al. EBL3001 Study Group. Safety and immunogenicity of the human papillomavirus-16/18 AS04-adjuvanted vaccine in 1354–60.
27. Government of Sierra Leone, Ministry of Health and Sanitation, National Malaria Control Programme. Guidelines for case management of malaria. 4th ed. Sierra Leone: Ministry of Health and Sanitation, 2015.
28. Swyzen C, Yekemans J, Bruls M, et al. Development of standardized laboratory methods and quality processes for a phase III study of the RTS, S/AS01 candidate malaria vaccine. Malar J 2011; 10:223.
29. World Health Organization. Basic malaria microscopy (2010). 2nd ed. Geneva, Switzerland: World Health Organization; 2010. Available at: https://apps.who.int/iris/handle/10665/44208. Accessed 24 February 2022.
30. Rudge TL, Jr. Sankovich KA, Niemuth NA, et al. Development, qualification, and validation of the filovirus animal nonclinical group anti-Ebola virus glycoprotein immunoglobulin G enzyme-linked immunosorbent assay for human serum samples. PLoS One 2019; 14:e0215457.
31. Mahon BE, Simon J, Widdowson MA, et al. Baseline asymptomatic malaria infection and immunogenicity of recombinant vesicular stomatitis virus—Zaire Ebola virus envelope glycoprotein. J Infect Dis 2021; 224:1907–15.
32. Goldstein N, Bockstal V, Bart S, et al. Safety and immunogenicity of heterologous Ad26- and MVA-vectored Ebola vaccines: a randomized, controlled phase 1 study. J Infect Dis 2022; 226:595–607.