Risk factors for Burkitt lymphoma in East African children and minors: a case-control study in malaria-endemic regions in Uganda, Tanzania, and Kenya.

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Abstract

Endemic Burkitt lymphoma (eBL) is the most common childhood cancer in sub-Saharan African countries, however, few epidemiologic studies have been undertaken and none attempted enrolling cases from multiple countries. We therefore conducted a population-based case-control study of eBL in children aged 0–15 years old in six regions in Northern Uganda, Northern Tanzania, and Western Kenya, enrolling 862 suspected cases and 2,934 population-controls (response rates 98.5–100%), and processing ~40,000 vials of samples using standardized protocols. Risk-factor questionnaires were administered, and malaria period prevalence was measured using rapid diagnostic tests (RDTs). A total of 80.9% of the recruited cases were diagnosed as eBL; 61.4% confirmed by histology. Associations with eBL risk were computed using logistic regression models adjusted for relevant confounders. Associations common in at least two countries were

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emphasized. eBL risk was decreased with higher maternal income and paternal education and elevated with history of inpatient malaria treatment >12 months before enrollment, and with HIV seropositivity. Reporting malaria-attributed fever up to 6 months before enrollment and malaria-RDT positivity at enrollment were associated with decreased eBL risk. Conversely, reporting exposure to mass malaria suppression programs (e.g., indoor residual insecticide) was associated with elevated risk. HIV was associated with elevated eBL risk. The study shows that it is feasible to conduct networked, multisite population-based studies of eBL in Africa. eBL was inversely associated with socioeconomic status, positively associated with inpatient malaria treatment 12 months ago and with living in areas tageted for malaria suppression, which support a role of malaria in eBL.

Keywords

Burkitt lymphoma; non-Hodgkin lymphoma; epidemiology; Epstein-Barr virus; *Plasmodium falciparum* malaria; HIV/AIDS

Introduction

Endemic Burkitt lymphoma (eBL) is a life-threatening B-cell lymphoma that occurs relatively commonly ($\sim 50/10^6$)[1] in children in some countries in equatorial Africa and sporadically elsewhere ($\sim 1/10^6$)[2]. Deleterious chromosomal translocations that place coding regions of *c-MYC* under regulatory control of immunoglobulin enhancer elements[3] are considered primary genetic events both in endemic and sporadic BL[4]. Epstein-Barr virus (EBV) and *Plasmodium falciparum* (*Pf*) malaria are considered co-factors that either increase genetic instability in B cells or increase the systemic load of abnormal B cells, thereby increasing the incidence and skewing of the geographical distribution of BL cases[5]. Few epidemiologic studies of eBL have been conducted, highlighting rural residence[6], frequent malaria attacks, lack of access to mosquito bed nets[7, 8], having 3 or more siblings, sharing a bed with siblings, living in a non-monogamous family, and having a deceased parent[9] as risk factors for eBL. These risk factors share low socioeconomic status as a common characteristic in eBL children[9]. The mechanism of socioeconomic status for modulating eBL risk is by modulating the intensity of malaria exposure to the children[10] and/or the age of EBV infection[11]. However, these studies have suffered some limitations, notably, their small sample sizes, not enrolling representative cases or controls, not measuring malaria infection at the time of enrollment, and largely failing to collect biospecimens suitable for use with current proteomic assays or obtaining permission for genetic studies. None recruited multiple, networked sites, thus, the feasibility of this approach to study eBL in Africa is unknown.

Therefore, we enrolled suspected eBL cases and population-based controls in six rural malaria-endemic areas in Northern Uganda, Northeastern Tanzania, and Western Kenya (Figure 1) in the **E**pide**mi**ology of **B**urkitt **L**ymphoma in **E**ast African Children and **M**inors (EMBLEM) study during 2010–2016[12, 13].

Methods

Study area, design and population

Suspected eBL cases aged 0–15 years old were recruited at six local district or regional hospitals serving a population living in a defined geographic area illustrated in Figure 1. The ecosystem in this geographic area was characterized by lakes, rivers and swamps, and supports perennial but variable holoendemic malaria transmission for >6 months in the year[14], except in parts of Kenya where malaria is seasonal and lasts for shorter durations[14]. The selected geographic areas have historically had high BL endemicity[12, 15]. We considered several choices of controls, including health-facility-, school-, or neighborhood-controls. Health-facility controls were rejected because they were markedly younger than eBL cases (2.2 years versus 7.0 years) and were significantly more likely to have malaria symptoms when they were encountered at health facilities[16]. School-based controls were rejected because non-school going children, who represent a significant proportion of children at risk of eBL, would be excluded from this sampling space.[16] Neighborhood controls were considered but rejected because of logistical challenges envisaged to maintain consistent quality of work (a control can only be enrolled after a case has been encountered) across multiple sites in different countries and concerns about low response rates[16]. Therefore, we enrolled population-based controls of a similar age distribution from 300 random villages (100 villages per country) sampled from national census roster according to urban/rural status and proximity to water, as previously described[12, 13]. The key assumption of this approach was that children residing in the same area as the cases would be exposed to similar period experiences when broadly matched for age, geography, and sex[16]. Before sampling, villages in the census roster were categorized as "rural" based on having a census enumeration area (EA) population count of children below 15 years being less than the EA mean population count, otherwise as "urban"[12, 13]. Villages were further classified as near water (swamp, river, or lake) based on the EA boundary being within 500 m of surface water, otherwise as far from water based on geographical information maps.

To ensure that cases and controls had been exposed to malaria, eligibility was restricted to children aged 0–15 years old who were usual residents (≥ four months prior to enrollment) of the study area. Because our study was conducted in rural areas, where in- and out-migration is relatively low, we did not encounter children who were recent immigrants into the study area. Children who were usual residents of another village in the study area were considered eligible as defined in Figure 1. Cases were defined histologically or cytologically, and when this was not possible, according to clinical features, imaging and laboratory results compatible with a diagnosis of eBL. To increase ascertainment and encourage referral of suspected cases to the six participating hospitals, carefully designed, culturally appropriate health education messages about eBL were developed and disseminated in the study area, stressing the availability of facilitated pathology diagnosis and treatment[17].

The primary data collected for the study encompassed individual- and household-level risk factor questions, including age, sex, parental and household exposures, history of inpatient and outpatient malaria treatment, malaria-related and unrelated fevers, and use of indoor

residual spraying (IRS) in the house, insecticides, and mosquito bed nets elicited by interviewers following standardized protocols[12, 13]. The questionnaires were designed in English at the National Cancer Institute and approved by the Technical Evaluation of Questionnaires Committee of the Division of Cancer Epidemiology and Genetics. They were then translated into Swahili and three Luo languages (Acholi, Madi, and Lugbara) that are widely spoken in the region. The season of case or control enrollment was classified as wet or dry, based on country-specific calendars. All subjects provided venous blood specimens (collected pre-treatment in the cases) for research (10 ml) and for clinical tests (4 ml) in EDTA tubes. Clinical specimens were immediately examined by light microscopy for asexual malaria parasite forms and for malaria antigens (HRP-2 and pan-LDH) using commercial malaria-rapid diagnostic tests (malaria-RDTs)[12, 13]. Malaria antigens remain detectable by RDTs for 35–42 days after treatment of symptomatic malaria.[18] Human immunodeficiency virus (HIV) infection was assessed using three approved commercial RDTs (Determine HIV1/2, Stat Pack, Unigold commercial kits) following National Guidelines. Two concordant positive test results were required to give a participant a positive result and refer them to local counselling and treatment centers.

Research samples were transported in cold boxes to field laboratories within two hours of collection and centrifuged for 15 minutes at 1300g to separate into plasma, buffy coat, and red cell fractions for storage at −80°C. About 40,000 vials of samples were processed, and half of the vials (one half from each subject) were shipped under liquid nitrogen vapor to the Frederick National Cancer Laboratory, Frederick, MD.

Data quality

The success of the study was dependent of harmonization of procedures, taking into account prior lessons about eBL epidemiology[16, 19] and pathology[17]. Research infrastructure to support high-quality data and biological sample collection was established and nested within the local health systems.[20] Fulltime field staff were hired to implement the study in the three countries and trained by the same instructors at Makerere University College of Health Sciences in Uganda. Compliance with standard protocols was monitored through weekly teleconference calls, periodic field visits, and biennial joint investigator-staff meetings[20].

Questionnaires and laboratory forms were processed at the field offices using DataFax, a technology that uses character recognition software to extract data from customized, barcoded forms to generate electronic spreadsheets, to reduce data entry errors. This eliminated the need for double manual entry and reduced the number of computers and data clerks needed to process data. Computerized data were reviewed centrally and corrected before generating analysis files.

Ethical issues

The study was approved by Uganda Virus Research Institute Research and Ethics Committee, Uganda National Council for Science and Technology (H816), Tanzania National Institute for Medical Research (NIMR/HQ/R.8c/Vol. IX/1023), Moi University/Moi Teaching and Referral Hospital Institutional Research and Ethics Committee (000536), and in the US by National Cancer Institute Special Studies Institutional Review

Board (10-C-N133). Written informed consent was given by guardians; children aged 7 years or older assented.

Statistical methods

Analyses were performed separately for each country, and then with the data from the three countries combined. Associations of eBL risk with questionnaire variables and period prevalence for malaria, based on malaria- RDT positivity, were are based on odds ratios and 95% confidence intervals (ORs, 95% CIs) adjusted for age (0–2, 3–5, 6–8, 9–11, 12–15 years), sex, and village characteristics (rural/urban or proximity to surface water; "baseline models") and further for variables with a p<0.05 when added to the baseline models (SAS, Cary, North Carolina). Six other variables about animal exposures, not included in this paper but previously found to be associated with pfPR[12, 13], were additionally included in the multivariable models.

We minimized collinearity between variables by dropping one variable of any pair with a Spearman correlation coefficient (ρ)>0.6 (2 pairs in Uganda; 1 pair in Tanzania; and 2 pairs in Kenya), based on having a lower p-value or based on other *a priori* considerations. Stratified analyses were performed to evaluate the impact of including clinical cases and of enrolling cases from rural versus urban villages. This study was conducted to generate a new resource for eBL research, and the current analysis was conducted to generate baseline data to explore hypotheses; thus, we did not adjust for multiple comparisons, and a two-sided p <0.05 was considered significant. To reduce dangers of overinterpretation of our results, we place greater emphasis on findings that were consistent in at least two countries.

Results

Of 862 suspicious cases recruited, 697 (80.9%) were enrolled as eBL; 428 (61.4%) with histological confirmation. We excluded 165 suspicious cases (135 from Uganda, 12 from Tanzania, and 18 from Kenya) after histological or clinical review. Of the 2970 population-based controls approached, 2,934 (98.8%) were enrolled; 36 controls (Uganda=17, Tanzania=5, and Kenya=14) refused to participate[12, 13]. Response patterns are shown in Supplementary Figure 1.

The male:female ratio was 1.69:1 among cases and 1.13:1 among controls (Table 1). Cases and controls had similar mean ages in Uganda (8.0 versus 7.7 years) and Tanzania (6.8 years versus 7.4 years) but they were younger in Kenya (6.6 years versus 7.4 years; Table 1). The mean age of the cases in boys and girls was similar in the three countries; 9.5% (n=66) of all the cases were younger than 3 years old [Uganda: 3.1% (n=10); Tanzania: 10.2% (n=13); and Kenya: 17.4% (n=43)]. The proportion of cases younger than 3 years old in histologically confirmed cases was: 5.8% (n=25) [Uganda: 2.0% (n=5); Tanzania: 8.3% (n=3); and Kenya: 12.5% (n=17)]. In all the countries, most cases lived in villages near water (64.8–86.3%). Most cases in Uganda lived in rural villages (62.7%, n=151), but the proportion was slightly over one-third in Tanzania (37.7%, n=46) and slightly over one-half in Kenya (59.0%, n=125).

Associations of eBL with malaria-RDT and measures of mass malaria suppression

More cases were diagnosed during the wet season in Uganda (versus dry: OR=2.18, p<0.0001) and Tanzania (OR=1.49, p=0.05), but fewer in Kenya (OR= 0.45, p<0.0001; Table 2). Compared to controls, malaria-RDT positivity in blood was lower in eBL cases in Uganda (OR=0.44, p<0.0001), Tanzania (OR=0.36, p=0.0001), and Kenya (OR=0.26, p<0.0001). The markedly lower malaria-RDT positivity in eBL cases was not explained by exposure to IRS in the past 12 months (Figure 2A) or bed net ownership/use (Figure 2B), although eBL cases were more likely to report owning a bed net, particularly in Uganda (Figure 2C), suggesting a small bias.

Contrary to our expectations, exposure to malaria suppression interventions was associated with elevated eBL risk. Although reporting application of IRS in the house one year before enrollment was not different between eBL cases and healthy controls in Uganda (versus no IRS: OR=1.02, p=0.91), it was significantly associated with eBL risk in Tanzania (OR= 5.27, p<0.0001) and Kenya (OR=3.13, p<0.0001). Consistent with this pattern, reporting use of a mosquito bed net the night before interview was associated with eBL risk in children Uganda (versus not owning a mosquito bed net: OR=2.90, 95% CI 2.12–3.97), Tanzania (OR=2.19, 95% CI 1.31–3.67), and Kenya (OR=1.90, 95% CI 1.26–2.88). Moreover, the association with eBL risk was stronger among children who reported owning but not using their mosquito bed net the previous night (versus not owning: 22-fold in Uganda, 8-fold in Tanzania, and 7-fold in Kenya; Table 2). Despite regular use of insecticide being relatively rare, reported by only 1.6–4.8% of the controls, eBL risk was associated with reporting regular use of insecticide sprays in Uganda (versus non-use: OR=5.62, p<0.0001) and Tanzania (OR=2.31, p=0.02), but not in Kenya (OR=1.26, p=0.63).

Associations of eBL with fevers, inpatient or outpatient malaria treatment, and HIV

A history of fevers may provide clues about exposure and immunity to malaria as well as to other pathogens and the state of health in the children. When considering any fever experienced up to 12 months before enrollment, no difference was observed between eBL cases and controls in Uganda (versus no fever: OR=0.82, p=0.40), but eBL cases were less likely than controls to report these fevers in this period in Tanzania (OR=0.49, p=0.06) and Kenya (OR=0.22, p<0.0001). Considering fevers attributed to malaria in the period up to 6 months before enrollment eBL cases were less likely than controls to report these fevers in Uganda (versus none: OR=0.56, p 0.001) and Kenya (OR=0.47, p<0.0001) but not in Tanzania (OR=0.90, p=0.64, Table 2). Inpatient malaria treatment is necessary in lacking immunity to malaria and becomes less frequent in older children who have acquired immunity[21]. Inpatient malaria treatment >12 months before enrollment or lack of such treatment was reported more frequently by cases than controls in Uganda (versus 12 months before enrollment: OR=2.55 and OR=2.04, respectively, p=0.01) while lack of treatment was more frequently reported by cases in Tanzania (OR=1.94, 95% CI 0.94–3.99; p=0.07). However, the findings were heterogenous in Kenya with cases being less likely to report inpatient malaria treatment >12 months before enrollment or lack of receiving such treatment ever (OR=0.24 and OR=0.55, respectively, p=0.001).

In contrast to inpatient malaria treatment, outpatient malaria treatment is given to people with immunity to malaria when they experience break through infections and it is a surrogate of intensity of exposure. Compared to controls, cases frequently reported malaria outpatient treatment >12 months before enrollment in Uganda (versus 12 months: OR=3.69) and Kenya (OR=1.86). A history of no outpatient malaria treatment was associated with decreased eBL risk in Tanzania (OR=0.44 p=0.004; Table 2).

In all the three countries, fevers not attributed to malaria experienced up to 6 months before enrollment were associated with eBL risk in Uganda (OR=8.40, p<0.0001), Tanzania (OR=5.17, p<0.0001) and Kenya (OR=5.17, p<0.0001). This association was stronger for fever reported at enrollment (OR=49.7, p<0.0001, OR=32.1, p<0.0001, and OR=16.5, p<0.0001, for Uganda, Tanzania and Kenya, respectively, Table 2).

These non-malaria fevers were not explained by HIV infection, which was rare (25 cases, 3.6%; 23 controls, 0.78%; Table 2); it was less common in Uganda (2.0%) than in Tanzania (3.4%) and Kenya (6.9%). HIV infection was associated with eBL risk in Uganda (OR=5.49, p=0.01), Tanzania (OR=25.6, p=0.004), and in Kenya (OR=4.32, p=0.001).

Associations of eBL with parental and household characteristics

In all three countries, higher maternal income (Uganda: p-trend<0.0001; Tanzania: p-trend<0.0001; and Kenya: p-trend=0.021) and higher maternal- and paternal-education in Uganda and Tanzania were associated with decreased eBL risk (Table 2). Consistently, reporting lower status maternal occupations, like farming or manual labor, was associated with elevated eBL risk in Uganda (versus sales: OR=3.28) and Tanzania (OR=1.65) but not in Kenya (OR=1.48, p=0.21). Although living in house connected to electricity is an indicator of higher socioeconomic status, this was not associated with eBL risk.

Location of ones' house, house size, and crowding within the house may be an indicator of socioeconomic status. Living in a house near a road (versus far: OR=3.37 and 2.15 in Tanzania and Kenya, respectively) or living in house in a town (OR=1.83, 2.30, and 3.30 in Uganda, Tanzania, and Kenya, respectively) were associated with elevated eBL risk. Living in a house with 3+ rooms (versus 1–2 rooms: OR=1.43, 2.43, and 1.46 for Uganda, Tanzania, and Kenya, respectively) or with 5–7 people (range OR=1.45 – 1.96) or 8 people (range OR=1.57 – 2.81, Table 2). Compared to children who obtained drinking water from an unprotected spring or well, eBL was associated with those whose drinking water was obtained from a protected spring or in Uganda (OR=1.58, p=0.07) or from piped-in/public tap in Kenya (OR=1.63, p=0.03). No difference in the sources of drinking water was observed between cases and controls in Tanzania (p=0.25). Distance of the home to the source of drinking water may be associated with greater exposure to environmental risk factors. Consistently, higher eBL risk in Uganda but lower eBL risk in Tanzania and Kenya were associated with increasing distance from home to the source of drinking water (all p<0.0001, Table 2).

Table 3 shows the results from the multivariable models and Figure 3 shows the associations that were significant in at least one country; we emphasize associations observed in at least two countries. Higher maternal income was associated with decreased eBL risk in Uganda

(p=0.0004) and Tanzania (p=0.001), whereas living in a house near the road or in a town or city were associated with elevated eBL risk in Tanzania (p<0.0001) and Kenya (p=0.03). Despite consistent evidence in our study that exposure to IRS was associated with reduced low-grade malaria prevalence whereas not such association was observed with use of mosquito bed nets or regular use of insecticide sprays[12, 13], all were associated with elevated eBL risk. Specifically, reporting IRS in the house in the past year was associated with eBL risk in all the three countries (Uganda: OR=1.71, p=0.026; Tanzania: OR=3.93, p<0.0001; and Kenya: OR= 6.78, p<0.0001), as was regular use of insecticide sprays was associated with eBL risk in Uganda (OR=5.62, p=0.002) and Tanzania (OR=3.47, p=0.026). Interestingly, the association with eBL was stronger in children who owned but did not use their mosquito bed net the night before interview in Uganda (OR=39.7, p=0.0001) and Kenya (OR=5.74, p=0.002).

The associations of elevated eBL risk with reporting a history of inpatient malaria treatment >12 months before enrollment remained significant in Uganda (p=0.007) and Tanzania (p=0.001), consistent with being exposed to malaria at an early age. The inverse associations between eBL and malaria-RDT positivity remained significant in Uganda (p=0.0003) and Kenya (p<0.0001) as did the associations with fever attributed to malaria up to 6 months before enrollment with eBL remained significant in Uganda (p=0.003) and Kenya (p=0.042). However, a history of fever not attributed to malaria in the period up to 6 months before enrollment was associated with eBL in all the three countries (Uganda: OR=8.82, p<0.0001; Tanzania OR=3.88, p<0.0001; Kenya OR= 6.98, p=<0.0001).

These results remained unchanged when we changed the analytic approach and used a combined data set (Supplementary Tables 1 and 2) or when we stratified analyses by diagnosis or rural/urban status of the case/control villages.

Discussion

Our EMBLEM study demonstrated the feasibility of conducting a networked, multisite population-based case-control study of eBL in in Uganda, Tanzania, and Kenya. The novel aspects of EMBLEM include implementing harmonized protocols to collect data and biospecimens, collecting local- and regional-area factors to adjust for macro- and micro-geographic factors, and conducting the study in rural areas where children are exposed from birth to intense malaria[12, 13]. Our study revealed significant associations both with elevated and decreased eBL risk, but it also exposed tremendous heterogeneity in many of the associations based on observation of significance in one, two, or all the three countries (Figure 3). The heterogeneity of associations may indicate false positive associations; thus, we emphasize associations found in at least two countries. Or it might indicate weakness in questionnaire-based methodology to accurately measure malaria and/or EBV exposures for eBL, hence there is a need to use other techniques to accurately measure these biologic exposures.

Focusing on the findings that were consistent in at least two countries, our obsevation that eBL risk is elevated with low maternal income and paternal education is not surprising. There is evidence that lower socioeconomic status is associated with higher malaria

intensity[12, 13], providing a mechanistic link with the elevated eBL risk[15]. Maternal income could be the reason for the long-term declines in trends in eBL incidence observed in Northern Tanzania during 2000–2009[22]. These declines in eBL incidence coincided, paradoxically, with increasing drug and insecticide resistance which led to worse malaria morbidity trends and it predated the introduction of mass malaria suppression programs in 2005[23]. However, because they the BL trends are concomitant with long-term increases in maternal income globally and in Tanzania[2425], we speculate that they may be causally related.

We found a heterogenous pattern in the relationship between eBL and some variables used as surrogates for malaria exposure and immunity. For example, inpatient malaria treatment > 12 months before enrollment or absence of that history were associated with a 2-fold higher risk of eBL among children in Uganda and Tanzania. This is consistent with the high and persistent malaria transmission experienced for more than 7 months in the year in those countries[14] and the notion that inpatient malaria treatment is required in younger children before they acquire immunity[26, 27], and such treatment is rarely required in older children who are immune despite being subject to heavy exposure to malaria[12, 13]. However, the association with this variable was opposite in Kenya, where 0.18–0.48-lower risk of eBL was observed. This may reflect seasonal and less persistent malaria in Kenya[13], associated with slower acquisition of immunity, such that exposures are likely to result in symptomatic infections that require treatment. In this setting, the same variable may be compatible with lighter malaria exposure. Definitive research using serological assays may help clarify these heterogenous patterns. Our findings with respect to HIV confirm the association between eBL and HIV and that it is not a major population factor for eBL in this region[8, 28].

Mass malaria suppression programs have been widely implemented in the study region. Unexpectedly, we found that exposure to mass malaria suppression variables, notably, use of mosquito bed nets, insecticide sprays, and IRS was associated with elevated eBL risk. This was surprising because IRS was associated with decreased pfPR[12, 13], whereas use of mosquito bed nets or regular use of insecticide sprays were not[12, 13]. The consistent association of these variables with eBL, on one hand, and lack of consistent association with pfPR, on the other, suggests that the associations with eBL risk are not mediated by alteration of malaria intensity[12, 13].

We suggest that these apparently paradoxical patterns reflect the preferential targeting for malaria suppression areas with both high malaria intensity and eBL incidence[15]. If so, then, we speculate that the continuing elevated eBL risk in these areas reflects heightened risk established prior to implementation of IRS. Because IRS successfully suppressed malaria in areas where it was applied[12, 13], our results indicate that the heightened risk may continue for up to 20 months, equivalent to IRS effects lasting 4–6 months per cycle and assuming 3–4 consecutive IRS cycles were implemented[29]. Plausibly, heavy chronic malaria before IRS led to a high burden of genetically unstable B cells[30] triggered by the historical malaria[3], followed by development of irreversible secondary mutations[31], oligoclonal expansion by recurrent malaria[5], and through Darwinian selection.[32] progression to eBL, despite absence of intense malaria pressure. The period of heightened risk of 1–2 years approximates the estimated latency period for eBL[33, 34].

Because we previously observed heterogenous malaria patterns in IRS areas in Uganda[12], it is possible that heightened eBL risk in IRS-areas is driven by incidence in areas where malaria intensity persists at levels compatible with eBL development. This hypothesis may be refuted or confirmed by careful geographic analysis of case activity in IRS areas.

The markedly reduced frequencies of malaria-RDT positivity and of reported malaria fevers up to 6 months before enrollment in eBL cases are paradoxical, considering the malaria model for eBL etiology[35]. Because cases are more likely to have contact with health facilities and be treated or receive mosquito bed nets, which would lower their malaria risk, stratified analysis by IRS and mosquito bed nets and observed similar patterns. An alternative explanation is that cases received malaria treatment before hospital admission. This was favored by authors of two reports that found lower malaria parasitemia in their eBL cases than controls in a case-control study in Kenya[36] and a prospective study in Uganda[37]. However, this does not apply to our study because the primary results are based on malaria antigens, which remain detectable in blood for 4–6 weeks after malaria treatment[18]. Furthermore, we collected data about malaria morbidity up to 6 months before enrollment and found it to be lower in eBL cases than controls. These results indicate that immunity acquired by eBL cases following an early exposure to malaria, before eBL onset[26, 27] critical to protecting these young children from severe malaria[38], continues to protect eBL children from the risk of malaria after disease onset.

Our results highlight non-malaria-related fevers as a common problem associated with eBL. These fevers are often referred to as "B" symptoms, fever of undetermined origin in patients with neoplasia[39]. Determining the causal factors could inform clinical management of eBL cases or lead to discovery of infections that may play role in the late stages of progression to eBL.

Our results have some limitations. We relied on questionnaire data, which are subject to multiple errors, including recall bias, inaccurate responses, and variability in their distributions in different geographic, social, and political contexts. Despite its population-based design, the study is susceptible to differential case ascertainment within and between region, and the incompleteness in obtaining pathology diagnosis was a concern[17]. The impact of geographic and diagnostic distortions is minimal, based qualitatively similar results from analyses stratified by rural/urban status and histological diagnosis. We performed multiple comparisons, thus some of the associations should be considered for hypothesis-generation. The strengths of our study are collecting data under a uniform protocol in three countries, and performing analyses following a common approach are strengths of our study.

To conclude, we show the feasibility of conducting population-based studies of eBL in multiple countries using uniform protocols. We observed elevated risk of eBL with non-malaria-related fevers up to before 6 months of enrollment, with exposure to mass malaria suppression variables, and HIV status; and decreased risk with indicators of higher socioeconomic status, current malaria antigenemia, and malaria history within 6 months of admission. The other associations identified likely reflect biological and ecological relationships, including effects mediated by malaria, HIV, or unknown infections.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

Abbreviations:

CI	confidence interval
eBL	Endemic Burkitt lymphoma
EA	enumeration area
EMBLEM	Epidemiology of Burkitt Lymphoma in East African Children and Minors
EBV	Epstein-Barr virus
HIV	human immunodeficiency virus
IRS	indoor residual spraying
OR	odds ratio
Pf	Plasmodium falciparum
pfPR	Plasmodium falciparum prevalence
RDTs	rapid diagnostic tests

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Novelty and Impact:

Endemic Burkitt lymphoma (eBL) is the commonest childhood cancer in sub-Saharan Africa, but few epidemiologic studies have been conducted, and none have attempted recruiting from multiple countries using harmonized methods. We recruited population-based cases and controls from six regions in Uganda, Tanzania, and Kenya using harmonized protocols to investigate infectious, environment and genetic risk factors for eBL. Our results confirm the feasibility of multi-site population-based enrolment of eBL and provide new baseline data about eBL epidemiology.
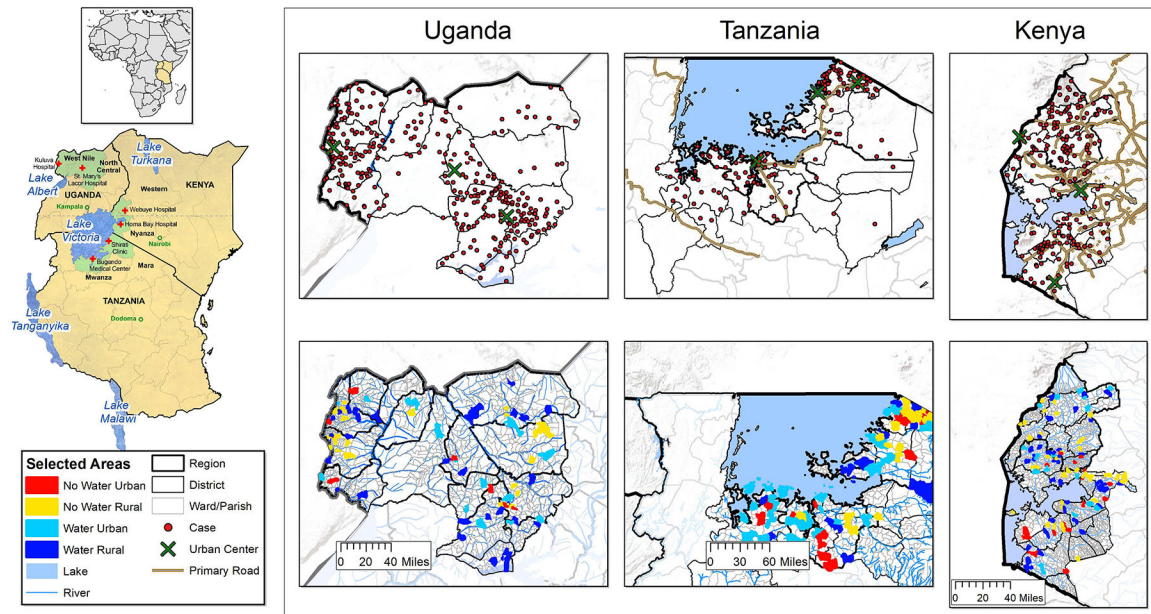
Figure 1.

Map showing the EMBLEM study area marked with green shading and the participating hospitals marked with a red cross (St. Mary's Hospital, Lacor and Kuluva Hospital in Northern Uganda; Bugando Medical Center and Shirati District Hospital in Northern Tanzania; Homa Bay District and Webuye District Hospitals and Moi University Teaching and Referral Hospital in Western Kenya). Two regions were selected per country. A locator map shows East Africa within Africa. Multi-panel maps show the zoom out of the study areas for each country. The bottom row shows the relief features, including rivers and the location of 100 villages randomly sampled per country as a source of matched population controls. The sampled villages are indicated according to their stratification category, that is, proximity to water and population density (see "Methods" section). The upper row shows the geographical distribution of the cases that were enrolled in the EMBLEM study plotted within their district of origin (large urban centers are marked on the map). The primary all season roads serving the study areas are included to give a rough idea of the geographical dispersion of cases in relation to the villages where the matched population controls were sampled and the transport infrastructure in the study areas.
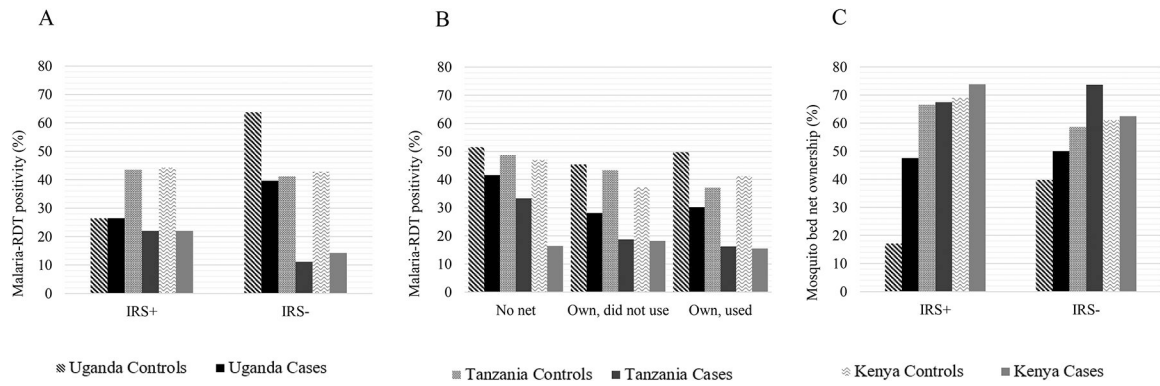
Figure 2.
Figure shows malaria-RDT positivity among cases and controls by indoor residual spraying (IRS) use (IRS+) or not (IRS−) in the past year (Panel A) and by mosquito bed net ownership (Panel B) and mosquito bed net ownership by IRS use in the past year (Panel C). The results are ordered as controls first followed by cases for Uganda, Tanzania and Kenya.
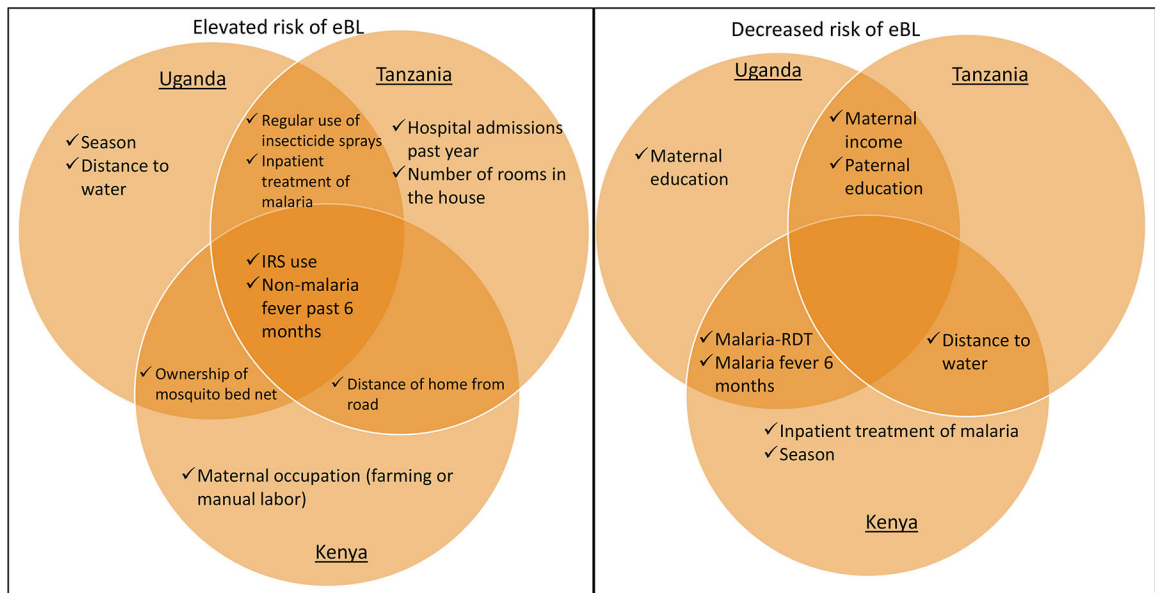
Figure 3.
Venn diagram showing the characteristics associated with elevated or decreased risk of eBL in Uganda, Tanzania, and Kenya, highlighting findings common in the three or two countries.

Table 1:

Characteristics of endemic Burkitt lymphoma cases and controls the EMBLEM Study in Uganda, Tanzania, and Kenya, 2010 – 2016.

	Uganda		Tanzania		Kenya		All countries combined*	
	Cases, n (%)	Controls, n (%)	Cases, n (%)	Controls, n (%)	Cases, n (%)	Controls, n (%)	Cases, n (%)	Controls, n (%)
Demographics								
Age, years								
0–2	10 (3.1)	56 (4.9)	13 (10.2)	45 (5.5)	43 (17.4)	92 (9.5)	66 (9.5)	193 (6.6)
3–5yr	78 (24.2)	262 (22.8)	45 (35.2)	198 (24.2)	63 (25.5)	242 (25.1)	186 (26.7)	702 (23.9)
6–8yr	101 (31.4)	376 (32.7)	25 (19.5)	281 (34.3)	67 (27.1)	261 (27.1)	193 (27.7)	918 (31.3)
9–11yr	78 (24.2)	286 (24.9)	26 (20.3)	181 (22.1)	44 (17.8)	203 (21.0)	148 (21.2)	670 (22.8)
12–15	55 (17.1)	170 (14.8)	19 (14.8)	114 (13.9)	30 (12.2)	167 (17.3)	104 (14.9)	451 (15.4)
Mean age, (standard deviation)	8.0 (3.4)	7.7 (3.3)	6.8 (3.8)	7.4 (3.3)	6.6 (3.8)	7.4 (3.7)	7.3 (3.7)	7.5 (3.5)
Sex								
Female	120 (38.0)	541 (47.0)	56 (44.1)	387 (47.3)	80 (32.5)	448 (46.4)	256 (37.2)	1376 (46.9)
Male	196 (62.0)	609 (53.0)	71 (55.9)	432 (52.8)	166 (67.5)	517 (53.6)	433 (62.8)	1558 (53.1)
Missing/Unknown	6		1				8	
Male:female ratio	1.6	1.1	1.3	1.1	2.1	1.2	1.7	1.1
Design variables								
Proximity to water†								
Far	33 (13.7)	486 (42.3)	43 (35.3)	411 (50.2)	55 (26.1)	500 (51.8)	131 (22.8)	1397 (47.6)
Near	208 (86.3)	664 (57.7)	79 (64.8)	408 (49.8)	156 (73.9)	465 (48.2)	443 (77.2)	1537 (52.4)
Missing/Unknown	81		6		36		123	
Population density of children 0–15 years‡								
Low	151 (62.7)	753 (65.5)	46 (37.7)	418 (51.0)	125 (59.0)	652 (67.6)	322 (56.0)	1823 (62.1)
High	90 (37.3)	397 (34.5)	76 (62.3)	401 (49.0)	87 (41.0)	313 (32.4)	253 (44.0)	1111 (37.9)
Missing/Unknown	81		6		35		122	
Year of study enrollment								
2010–2012	109 (33.9)	68 (5.9)	20 (15.6)	178 (21.7)	21 (8.5)	369 (38.2)	150 (21.5)	68 (2.3)
2013–2015	177 (55.0)	1082 (94.1)	96 (75.0)	641 (78.3)	206 (83.4)	596 (61.8)	479 (68.7)	1629 (55.5)
2016	36 (11.2)		12 (9.4)		20 (8.1)		68 (9.8)	1237 (42.2)

*This column shows the observed characteristics based on data from all the three study countries combined; the percentages are for columns.

[†]Villages were classified as 'near water' if any part of their boundary was within 500 meters of surface water; otherwise they were classified as 'far from water'.

[‡]Villages were classified as 'high population-density', a surrogate for urban areas, if the population count of children aged 0–15 years was greater than or equal to the average population count for census enumeration areas in the regions studied for each country; otherwise as 'low population-density'. (See Methods).

Table 2:

Associations between endemic Burkitt lymphoma and patient characteristics in Uganda, Tanzania, and Kenya, 2010–2016.

Characteristics	Uganda			Tanzania			Kenya		
	Cases, n (%)	Controls, n (%)	OR* (95% CI)	Cases, n (%)	Controls, n (%)	OR* (95% CI)	Cases, n (%)	Controls, n (%)	OR* (95% CI)
Season[†]									
Dry	138 (42.9)	731 (63.6)	Ref	47 (36.7)	395 (48.2)	Ref	108 (43.7)	267 (27.7)	Ref
Wet	184 (57.1)	419 (36.4)	2.18 (1.63, 2.92)	81 (63.3)	424 (51.8)	1.49 (1.00, 2.23)	139 (56.3)	698 (72.3)	0.45 (0.33, 0.62)
p-value			<0.0001			0.050			<0.0001
Measures of malaria									
Indoor residual insecticide sprayed in house									
No	191 (61.0)	752 (65.7)	Ref	38 (30.7)	551 (68.2)	Ref	190 (81.9)	880 (92.5)	Ref
Yes	122 (39.0)	393 (34.3)	1.02 (0.75, 1.38)	86 (69.4)	257 (31.8)	5.27 (3.43, 8.09)	42 (18.1)	71 (7.5)	3.13 (1.97, 4.96)
Missing/Unknown	9	5		4	11		15	14	
p-value			0.906			<0.0001			<0.0001
Mosquito net ownership and use the night before									
No	125 (39.8)	767 (67.1)	Ref	21 (16.7)	289 (35.4)	Ref	44 (18.9)	322 (33.8)	Ref
Yes, but not used	35 (11.2)	11 (1.0)	22.5 (9.94, 51.0)	17 (13.5)	30 (3.7)	8.29 (3.81, 18.1)	38 (16.3)	43 (4.5)	7.09 (3.91, 12.8)
Yes, and used	154 (49.0)	366 (32.0)	2.90 (2.12, 3.97)	88 (69.8)	497 (60.9)	2.19 (1.31, 3.67)	151 (64.8)	589 (61.7)	1.90 (1.26, 2.88)
Missing/Unknown	8	6		2	3		14	11	
p-value			<0.0001			<0.0001			<0.0001
Regularly uses mosquito insecticide sprays									
No	298 (95.2)	1126 (98.4)	Ref	114 (89.8)	775 (95.2)	Ref	226 (97.0)	924 (97.5)	Ref
Yes	15 (4.8)	18 (1.6)	5.62 (2.62, 12.0)	13 (10.2)	39 (4.8)	2.31 (1.15, 4.62)	7 (3.0)	24 (2.5)	1.26 (0.49, 3.26)
Missing/Unknown	9	6		1	5		14	17	
p-value			<0.0001			0.019			0.628

	Uganda			Tanzania			Kenya		
	Cases, n (%)	Controls, n (%)	OR* (95% CI)	Cases, n (%)	Controls, n (%)	OR* (95% CI)	Cases, n (%)	Controls, n (%)	OR* (95% CI)
Malaria rapid diagnostic test									
Negative	204 (65.4)	561 (49.0)	Ref	95 (80.5)	473 (58.7)	Ref	192 (84.2)	542 (57.0)	Ref
Positive	108 (34.6)	583 (51.0)	0.44 (0.32, 0.60)	23 (19.5)	333 (41.3)	0.36 (0.22, 0.59)	36 (15.8)	409 (43.0)	0.26 (0.17, 0.39)
Missing/Unknown	10	6		10	13		19	14	
p-value			<0.0001			0.0001			<0.0001
History of fevers, malaria treatment and hospital admission									
Has fever at enrollment									
No	136 (43.5)	1122 (97.9)	Ref	41 (32.3)	767 (94.0)	Ref	70 (31.1)	836 (87.5)	Ref
Yes	177 (56.6)	24 (2.1)	49.7 (30.4, 81.2)	86 (67.7)	49 (6.0)	32.1 (19.6, 52.7)	155 (68.9)	120 (12.6)	16.5 (11.1, 24.4)
Missing/Unknown	9	4		1	3		22	9	
p-value			<0.0001			<0.0001			<0.0001
1 fever up to 12 months before enrollment									
No	31 (22.8)	254 (22.6)	Ref	11 (26.8)	115 (15.0)	Ref	54 (76.1)	320 (38.2)	Ref
Yes	105 (77.2)	868 (77.4)	0.82 (0.51, 1.31)	30 (73.2)	652 (85.0)	0.49 (0.23, 1.03)	17 (23.9)	517 (61.8)	0.22 (0.12, 0.40)
Missing/Unknown	186	28		87	52		176	128	
p-value			0.401			0.059			<0.0001
1 fever due to malaria up to 6 months before enrollment									
No	113 (36.2)	313 (27.3)	Ref	42 (33.6)	250 (30.7)	Ref	108 (48.7)	288 (30.1)	Ref
Yes	199 (63.8)	832 (72.7)	0.56 (0.41, 0.76)	83 (66.4)	565 (69.3)	0.90 (0.60, 1.37)	114 (51.4)	668 (69.9)	0.47 (0.34, 0.65)
Missing/Unknown	10	5		3	4		25	9	
p-value			0.0002			0.636			<0.0001
1 fever not due to malaria up to 6 months before enrollment									
No	181 (59.5)	1062 (92.9)	Ref	44 (34.9)	599 (73.6)	Ref	100 (44.6)	772 (81.1)	Ref
Yes	123 (40.5)	81 (7.1)	8.40 (5.79, 12.2)	82 (65.1)	215 (26.4)	5.17 (3.42, 7.82)	124 (55.4)	180 (18.9)	5.17 (3.67, 7.28)
Missing/Unknown	18	7		2	5		23	13	

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	Uganda			Tanzania			Kenya		
	Cases, n (%)	Controls, n (%)	OR* (95% CI)	Cases, n (%)	Controls, n (%)	OR* (95% CI)	Cases, n (%)	Controls, n (%)	OR* (95% CI)
≥1 hospital admission									
p-value			<0.0001			<0.0001			<0.0001
No	112 (35.7)	626 (54.7)	Ref	31 (24.4)	516 (63.2)	Ref	71 (30.3)	697 (73.2)	Ref
Yes	202 (64.3)	519 (45.3)	2.01 (1.49, 2.70)	96 (75.6)	300 (36.8)	5.45 (3.50, 8.51)	163 (69.7)	255 (26.8)	6.67 (4.70, 9.47)
Missing/Unknown	8	5		1	3		13	13	
p-value			<0.0001			<0.0001			<0.0001
Inpatient malaria treatment									
Yes, past 12 months	24 (7.6)	150 (13.1)	Ref	10 (7.9)	106 (13.0)	Ref	35 (15.0)	95 (10.0)	Ref
Yes, > 12 months	82 (26.1)	269 (23.5)	2.55 (1.39, 4.67)	13 (10.2)	136 (16.7)	1.15 (0.47, 2.82)	12 (5.1)	117 (12.3)	0.24 (0.11, 0.50)
Never	208 (66.2)	725 (63.4)	2.04 (1.17, 3.57)	104 (81.9)	574 (70.3)	1.94 (0.94, 3.99)	187 (79.9)	743 (77.8)	0.55 (0.35, 0.86)
Missing/Unknown/Unknown	8	6		1	3		13	10	
p-value			0.010			0.066			0.001
Outpatient malaria treatment									
Yes, past 12 months	153 (48.9)	532 (46.5)	Ref	62 (48.8)	255 (31.3)	Ref	106 (45.3)	539 (56.4)	Ref
Yes, > 12 months	73 (23.3)	90 (7.9)	3.69 (2.44, 5.56)	14 (11.0)	69 (8.5)	0.87 (0.45, 1.68)	23 (9.8)	64 (6.7)	1.86 (1.05, 3.28)
Never	87 (27.8)	523 (45.7)	0.88 (0.63, 1.24)	51 (40.2)	492 (60.3)	0.44 (0.29, 0.67)	105 (44.9)	352 (36.9)	1.49 (1.06, 2.09)
Missing/Unknown	9	5		1	3		13	10	
p-value			<0.0001			0.0004			0.021
HIV status									
Negative	302 (98.1)	1135 (99.4)	Ref	114 (96.6)	775 (99.9)	Ref	203 (93.1)	936 (98.4)	Ref
Positive	6 (2.0)	7 (0.6)	5.49 (1.52, 19.9)	4 (3.4)	1 (0.1)	25.6 (2.76, 236)	15 (6.9)	15 (1.6)	4.32 (1.90, 9.86)
Missing/Unknown	14	8		10	43		29	14	
p-value			0.009			0.004			0.001
Parental characteristics									

	Uganda			Tanzania			Kenya		
	Cases, n (%)	Controls, n (%)	OR* (95% CI)	Cases, n (%)	Controls, n (%)	OR* (95% CI)	Cases, n (%)	Controls, n (%)	OR* (95% CI)
Mother's education									
Up to standard 4	200 (63.7)	581 (50.8)	Ref	41 (32.3)	201 (24.6)	Ref	36 (15.7)	182 (19.0)	Ref
Standard 5–7	100 (31.9)	446 (39.0)	0.70 (0.51, 0.95)	82 (64.6)	570 (69.9)	0.74 (0.48, 1.13)	105 (45.7)	455 (47.6)	1.36 (0.84, 2.21)
Senior secondary school	14 (4.5)	117 (10.2)	0.51 (0.28, 0.96)	4 (3.2)	45 (5.5)	0.43 (0.14, 1.29)	89 (38.7)	319 (33.4)	1.58 (0.96, 2.61)
Missing/Unknown	8	6		1	3		17	9	
p-value			0.019			0.193			0.201
p-trend			0.005			0.070			0.079
Father's education									
Up to standard 4	85 (27.5)	212 (18.7)	Ref	35 (27.8)	111 (13.7)	Ref	27 (11.8)	151 (16.1)	Ref
Standard 5–7	148 (47.9)	547 (48.3)	0.73 (0.51, 1.05)	76 (60.3)	619 (76.1)	0.37 (0.23, 0.60)	86 (37.6)	371 (39.6)	1.80 (1.02, 3.15)
Senior secondary school	76 (24.6)	373 (33.0)	0.59 (0.39, 0.89)	15 (11.9)	83 (10.2)	0.55 (0.28, 1.11)	116 (50.7)	416 (44.4)	1.93 (1.11, 3.36)
Missing/Unknown	13	18		2	6		18	27	
p-value			0.040			<0.001			0.063
p-trend			0.013			0.011			0.040
Mother's occupation									
Trader/Sales	11 (3.5)	94 (8.2)	Ref	13 (10.2)	62 (7.6)	Ref	45 (19.4)	254 (26.5)	Ref
Peasant farmer	286 (90.8)	1009 (88.1)	1.30 (0.65, 2.61)	103 (81.1)	724 (88.7)	0.64 (0.34, 1.23)	145 (62.5)	570 (59.6)	1.39 (0.94, 2.07)
Manual laborer	18 (5.7)	43 (3.8)	3.28 (1.28, 8.42)	11 (8.7)	30 (3.7)	1.65 (0.64, 4.21)	42 (18.1)	133 (13.9)	1.48 (0.87, 2.52)
Missing/Unknown	7	4		1	3		15	8	
p-value			0.021			0.026			0.207
Father's occupation									
Trader/Sales	40 (12.7)	173 (15.1)	Ref	22 (17.3)	100 (12.3)	Ref	54 (23.4)	243 (25.4)	Ref
Peasant farmer	243 (77.4)	831 (72.7)	0.83 (0.54, 1.27)	97 (76.4)	669 (82.1)	0.60 (0.36, 1.02)	111 (48.1)	422 (44.1)	1.11 (0.75, 1.65)

	Uganda			Tanzania			Kenya		
	Cases, n (%)	Controls, n (%)	OR* (95% CI)	Cases, n (%)	Controls, n (%)	OR* (95% CI)	Cases, n (%)	Controls, n (%)	OR* (95% CI)
Manual laborer	31 (9.9)	139 (12.2)	1.00 (0.56, 1.79)	8 (6.3)	46 (5.6)	0.75 (0.30, 1.84)	66 (28.6)	291 (30.4)	0.92 (0.60, 1.42)
Missing/Unknown	8	7		1	4		16	9	
p-value			0.535			0.161			0.602
Mother's income, US dollars‡									
7.5	213 (66.2)	565 (49.1)	Ref	81 (63.3)	279 (34.1)	Ref	88 (35.6)	244 (25.3)	Ref
7.6– 15.0	76 (23.6)	247 (21.5)	1.05 (0.74, 1.48)	30 (23.4)	260 (31.8)	0.43 (0.27, 0.69)	55 (22.3)	222 (23.0)	0.76 (0.50, 1.17)
>15.0	33 (10.3)	338 (29.4)	0.33 (0.20, 0.52)	17 (13.3)	280 (34.2)	0.23 (0.13, 0.41)	104 (42.1)	499 (51.7)	0.65 (0.46, 0.93)
p-value			<0.0001			<0.0001			0.004
p-trend			<0.0001			<0.0001			0.021
Home characteristics									
Distance of home from main road									
Far from the main road	239 (75.9)	812 (70.9)	Ref	73 (57.5)	642 (78.8)	Ref	84 (36.2)	518 (54.1)	Ref
Near the main road	53 (16.8)	281 (24.5)	0.86 (0.58, 1.28)	40 (31.5)	114 (14.0)	3.37 (2.13, 5.33)	96 (41.4)	332 (34.7)	2.15 (1.50, 3.08)
In town or city	23 (7.3)	53 (4.6)	1.83 (1.02, 3.28)	14 (11.0)	59 (7.2)	2.30 (1.17, 4.50)	52 (22.4)	108 (11.3)	3.30 (2.08, 5.25)
Missing/Unknown	7	4		1	4		15	7	
p-value			0.078			<0.0001			<0.0001
Number of rooms in house									
1–2 room	273 (86.9)	987 (86.1)	Ref	47 (37.0)	481 (59.0)	Ref	116 (50.2)	539 (56.3)	Ref
3 rooms	41 (13.1)	159 (13.9)	1.43 (0.94, 2.17)	80 (63.0)	335 (41.1)	2.43 (1.62, 3.63)	115 (49.8)	419 (43.7)	1.46 (1.06, 2.01)
Missing/Unknown	8	4		1	3		16	7	
p-value			0.094			<0.0001			0.021
Number of children and adult resident									
2–4 people	173 (55.1)	703 (61.3)	Ref	28 (22.1)	302 (37.0)	Ref	48 (20.8)	257 (26.8)	Ref

	Uganda			Tanzania			Kenya		
	Cases, n (%)	Controls, n (%)	OR* (95% CI)	Cases, n (%)	Controls, n (%)	OR* (95% CI)	Cases, n (%)	Controls, n (%)	OR* (95% CI)
5–7 people	115 (36.6)	385 (33.6)	1.45 (1.06, 1.96)	56 (44.1)	334 (40.9)	1.96 (1.18, 3.24)	125 (54.1)	500 (52.2)	1.49 (0.99, 2.24)
8 people	26 (8.3)	58 (5.1)	2.19 (1.21, 3.97)	43 (33.9)	180 (22.1)	2.81 (1.62, 4.86)	58 (25.1)	201 (21.0)	1.57 (0.97, 2.54)
Missing/Unknown	8	4		1	3		16	7	
p-value			0.006			0.001			0.120
p-trend			0.002			0.0002			0.060
Number of people sleeping in the same room as child									
0–2 people	49 (15.6)	202 (17.6)	Ref	28 (22.1)	269 (33.0)	Ref	92 (39.8)	389 (40.6)	Ref
3 people	99 (31.5)	275 (24.0)	1.55 (0.99, 2.42)	46 (36.2)	288 (35.3)	1.41 (0.84, 2.37)	83 (35.9)	259 (27.0)	1.28 (0.88, 1.85)
4 people	166 (52.9)	669 (58.4)	1.07 (0.71, 1.63)	53 (41.7)	259 (31.7)	1.88 (1.13, 3.13)	56 (24.2)	310 (32.4)	0.71 (0.47, 1.06)
Missing/Unknown	8	4		1	3		16	7	
p-value			0.058			0.049			0.022
p-trend			0.691			0.014			0.142
Connected to electricity grid									
No	306 (97.1)	1092 (95.4)	Ref	122 (96.1)	745 (91.3)	Ref	202 (86.7)	835 (87.6)	Ref
Yes	9 (2.9)	53 (4.6)	0.75 (0.26, 2.17)	5 (3.9)	71 (8.7)	0.44 (0.17, 1.13)	31 (13.3)	118 (12.4)	1.22 (0.77, 1.93)
Missing/Unknown	7	5		1	3		14	12	
p-value			0.598			0.087			0.397
Source of drinking water									
Unprotected spring/well	244 (77.5)	882 (77.0)	Ref	59 (46.5)	453 (55.5)	Ref	130 (55.6)	572 (59.9)	Ref
Protected spring/well	61 (19.4)	202 (17.6)	1.58 (1.07, 2.34)	42 (33.1)	218 (26.7)	1.45 (0.93, 2.27)	46 (19.7)	171 (17.9)	1.43 (0.95, 2.16)
Public tap/piped household	10 (3.2)	61 (5.3)	1.05 (0.47, 2.31)	26 (20.5)	145 (17.8)	1.25 (0.74, 2.11)	58 (24.8)	212 (22.2)	1.63 (1.10, 2.40)
Missing/Unknown	7	5		1	3		13	10	
p-value			0.073			0.254			0.029

	Uganda			Tanzania			Kenya		
	Cases, n (%)	Controls, n (%)	OR* (95% CI)	Cases, n (%)	Controls, n (%)	OR* (95% CI)	Cases, n (%)	Controls, n (%)	OR* (95% CI)
Distance to water source, meters									
<500	38 (12.1)	383 (33.5)	Ref	52 (40.9)	136 (16.7)	Ref	149 (64.5)	356 (37.2)	Ref
500–999	63 (20.0)	126 (11.0)	4.86 (2.93, 8.08)	22 (17.3)	159 (19.5)	0.37 (0.21, 0.65)	33 (14.3)	230 (24.0)	0.32 (0.20, 0.49)
1000–4999	122 (38.7)	511 (44.6)	2.85 (1.85, 4.37)	32 (25.2)	440 (53.9)	0.16 (0.09, 0.26)	38 (16.5)	268 (28.0)	0.38 (0.25, 0.59)
5000	92 (29.2)	125 (10.9)	11.4 (6.83, 19.1)	21 (16.5)	81 (9.9)	0.69 (0.38, 1.27)	11 (4.8)	104 (10.9)	0.18 (0.08, 0.41)
Missing/Unknown	7	5		1	3		16	7	
p-value			<0.0001			<0.0001			<0.0001
p-trend			<0.0001			<0.0001			<0.0001

Abbreviation: CI = confidence interval

Note: p-values are for heterogeneity

*
Associations are minimally adjusted for age, sex, proximity to water and population density of children 0–15 years.

[†]The months of April to June and September to December were classified as wet season months, while the months of January to March and July to August were classified as dry season months.

[‡]Income was categorized based on the international poverty line of $1.90 per a day to calculate the average 30-day monthly income. Total household income or father's income were not analyzed because the results for these were considered unreliable.

Table 3:

Multivariate associations with endemic Burkitt lymphoma the EMBLEM Study, 2010–2016.

Characteristics	Uganda aOR* (95% CI)	Tanzania aOR* (95% CI)	Kenya aOR* (95% CI)	All countries combined† aOR* (95% CI)
Season‡				
Dry	Ref	Ref	Ref	Ref
Wet	2.54 (1.67, 3.86)	1.51 (0.79, 2.86)	0.28 (0.18, 0.45)	1.28 (0.99, 1.66)
p-value	<0.0001	0.211	<0.0001	0.058
Measure of malaria				
Indoor residual insecticide sprayed in house				
No	Ref	Ref	Ref	Ref
Yes	1.71 (1.07, 2.73)	3.93 (2.03, 7.62)	6.78 (3.51, 13.10)	2.32 (1.75, 3.09)
p-value	0.026	<0.0001	<0.0001	<0.0001
Mosquito net ownership and use the night before				
No	Ref	Ref	Ref	Ref
Yes, but not used	39.7 (13.3, 118)	4.09 (1.25, 13.35)	5.74 (2.48, 13.3)	9.23 (5.59, 15.24)
Yes, and used	4.12 (2.64, 6.42)	1.32 (0.60, 2.90)	1.47 (0.86, 2.53)	2.36 (1.76, 3.18)
p-value	<0.0001	0.058	0.0002	<0.0001
Regularly uses mosquito insecticide sprays				
No	Ref	Ref		Ref
Yes	5.62 (1.85, 17.01)	3.47 (1.16, 10.37)		2.97 (1.65, 5.32)
p-value	0.002	0.026		0.0003
Malaria rapid diagnostic test				
Negative	Ref	Ref	Ref	Ref
Positive	0.43 (0.28, 0.68)	0.56 (0.27, 1.16)	0.33 (0.20, 0.55)	0.46 (0.35, 0.60)
p-value	0.0003	0.120	<0.0001	<0.0001
History of fevers, and malaria treatment				
1 fever due to malaria up to 6 months before enrollment				
No	Ref		Ref	Ref
Yes	0.48 (0.29, 0.77)		0.59 (0.35, 0.98)	0.63 (0.47, 0.84)
p-value	0.003		0.042	0.001
1 fever not due to malaria up to 6 months before enrollment				
No	Ref	Ref	Ref	Ref
Yes	8.82 (5.37, 14.49)	3.88 (2.05, 7.36)	6.98 (4.39, 11.09)	5.66 (4.31, 7.42)
p-value	<0.0001	<0.0001	<0.0001	<0.0001
1 hospital admission				
No		Ref		Ref
Yes		21.29 (9.61, 47.17)		9.35 (6.85, 12.77)

Characteristics	Uganda	Tanzania	Kenya	All countries combined[†]
	aOR[*] (95% CI)	aOR[*] (95% CI)	aOR[*] (95% CI)	aOR[*] (95% CI)
p-value		<0.0001		<0.0001
Inpatient malaria treatment				
Yes, past 12 months	Ref	Ref	Ref	Ref
Yes, > 12 months	3.97 (1.66, 9.51)	2.89 (0.80, 10.41)	0.18 (0.07, 0.48)	0.99 (0.62, 1.57)
Never	3.41 (1.52, 7.67)	26.43 (7.73, 90.36)	0.48 (0.25, 0.91)	5.59 (3.61, 8.66)
p-value	0.007	<0.0001	0.003	<0.0001
Outpatient malaria treatment				
Yes, past 12 months	Ref	Ref	Ref	Ref
Yes, > 12 months	2.17 (1.17, 4.03)	0.45 (0.14, 1.48)	1.61 (0.70, 3.72)	2.23 (1.50, 3.31)
Never	0.59 (0.35, 0.99)	0.54 (0.27, 1.07)	1.48 (0.87, 2.53)	0.96 (0.71, 1.29)
p-value	0.0001	0.154	0.277	<0.0001
Parental characteristics				
Mother's education				
Up to standard 4	Ref			Ref
Standard 5–7	0.81 (0.52, 1.26)			0.81 (0.60, 1.08)
Senior secondary school	0.46 (0.19, 1.12)			0.77 (0.50, 1.20)
p-value	0.197			0.319
p-trend	0.082			0.227
Father's education				
Up to standard 4	Ref	Ref	Ref	Ref
Standard 5–7	0.75 (0.44, 1.26)	0.25 (0.11, 0.55)	1.13 (0.54, 2.37)	0.71 (0.51, 1.00)
Senior secondary school	0.53 (0.28, 0.99)	0.19 (0.05, 0.68)	1.03 (0.49, 2.17)	0.70 (0.47, 1.04)
p-value	0.136	0.002	0.908	0.116
p-trend	0.033	0.002	0.943	0.068
Mother's occupation				
Trader/Sales	Ref	Ref	Ref	Ref
Peasant farmer	0.64 (0.23, 1.75)	0.47 (0.17, 1.34)	2.80 (1.53, 5.11)	1.31 (0.86, 1.97)
Manual laborer	0.79 (0.19, 3.21)	0.75 (0.18, 3.02)	2.36 (1.07, 5.22)	1.54 (0.89, 2.69)
p-value	0.668	0.334	0.004	0.272
Mother's income, US dollars[§]				
7.5	Ref	Ref	Ref	Ref
7.6- 15.0	0.97 (0.60, 1.58)	0.68 (0.31, 1.46)	0.78 (0.42, 1.43)	0.88 (0.65, 1.20)
>15.0	0.27 (0.14, 0.52)	0.20 (0.08, 0.47)	0.70 (0.40, 1.24)	0.46 (0.33, 0.64)
p-value	0.0004	0.001	0.468	<0.0001
p-trend	0.0003	0.001	0.249	<0.0001
Home characteristics				
Distance of home from main road				
Far from the main road		Ref	Ref	Ref
Near the main road		4.33 (2.00, 9.37)	1.88 (1.15, 3.07)	1.66 (1.23, 2.24)
In town or city		9.17 (2.84, 29.63)	1.94 (0.97, 3.90)	2.92 (1.83, 4.67)

Characteristics	Uganda aOR* (95% CI)	Tanzania aOR* (95% CI)	Kenya aOR* (95% CI)	All countries combined† aOR* (95% CI)
p-value		<0.0001	0.026	<0.0001
Number of rooms in house				
1–2 room		Ref	Ref	Ref
3 rooms		1.33 (0.67, 2.65)	1.46 (0.91, 2.33)	1.19 (0.88, 1.62)
p-value		0.419	0.117	0.256
Number of children and adult resident				
2–4 people	Ref	Ref		Ref
5–7 people	1.51 (0.99, 2.31)	1.12 (0.49, 2.58)		1.23 (0.92, 1.66)
8 people	1.47 (0.62, 3.49)	1.35 (0.56, 3.29)		1.21 (0.81, 1.80)
p-value	0.144	0.788		0.365
p-trend	0.08	0.497		0.428
Number of people sleeping in the same room as child				
0–2 people			Ref	Ref
3 people			1.27 (0.75, 2.16)	1.42 (1.02, 1.97)
4 people			1.10 (0.63, 1.93)	1.31 (0.94, 1.84)
p-value			0.673	0.109
p-trend			0.649	0.177
Source of drinking water				
Unprotected spring/well			Ref	Ref
Protected spring/well			1.44 (0.82, 2.53)	1.53 (1.11, 2.10)
Public tap/piped household			0.97 (0.53, 1.76)	1.08 (0.72, 1.63)
p-value			0.395	0.032
Distance to water source, meters				
< 500	Ref	Ref	Ref	Ref
500–999	5.58 (2.81, 11.08)	0.32 (0.13, 0.78)	0.57 (0.32, 1.02)	1.30 (0.91, 1.86)
1000–4999	3.48 (1.99, 6.10)	0.10 (0.05, 0.24)	0.38 (0.21, 0.70)	0.84 (0.61, 1.14)
5000	14.75 (7.34, 29.66)	0.46 (0.17, 1.28)	0.19 (0.07, 0.50)	2.39 (1.60, 3.57)
p-value	<0.0001	<0.0001	0.0004	<0.0001
p-trend	<0.0001	<0.0001	<0.0001	0.034

Abbreviation: aOR= adjusted odds ratio; CI = confidence interval

Note: p-values are for heterogeneity

* In addition to the adjustment for age, sex, proximity to water and population density of children 0–15 years, the associations are mutually adjusted for all variables included in the multivariate model. In addition, the multivariate models were adjusted for keeping animals inside the house or nearby (chicken, pigs, goat, cows, birds, dogs).

† This column shows the observed associations based on data from all the three study countries combined, adjusted for each country

‡ The months of April to June and September to December were classified as wet season months, while the months of January to March and July to August were classified as dry season months.

§Income was categorized based on the international poverty line of $1.90 per a day to calculate the average 30-day monthly income. Total household income or father's income were not analyzed because the results for these were considered unreliable.