Prevalence and Factors Associated with Malaria-Typhoid Co-infection among Febrile Children Aged Six Months to Twelve Years at Kampala International University Teaching Hospital in Western Uganda

Joanitor Nakisuyi[1], Melvis Bernis[1], Andrew Ndamira[1], Vicent Kayini[1], Richard Mulumba[2], Pius Theophilus[3], Ezera Agwu[3], Herman Lule*[4,]

1. Department of Paediatrics and Child Health, Faculty of Clinical Medicine and Dentistry, Kampala International University-Western Campus, P.o. Box 71 Bushenyi, Uganda
2. Department of Obstetrics and Gynecology, Faculty of Clinical Medicine and Dentistry, Kampala International University-Western Campus, P.o. Box 71 Bushenyi, Uganda
3. Departments of Medical Microbiology and Clinical Immunology, Faculty of Medicine, Kabale University, Uganda
4. Faculty of Medicine, Department of Clinical Neurosciences, Turku University Hospital and University of Turku, FI-20014, Turku, Finland

*Corresponding author: Dr. Herman Lule; Email Herman.h.lule@utu.fi

ABSTRACT

Background: Pediatric febrile illnesses pose diagnostic challenges in low income countries. Western Uganda is endemic for both Malaria and Typhoid but the true prevalence of each individual disease, their co-infections and associated factors are poorly quantified.

Objective: To determine the prevalence of malaria, Typhoid, their co-infection and associated factors amongst febrile children attending the paediatrics and child health department of Kampala International University Teaching Hospital (KIU-TH) in Western Uganda.

Methods: Cross-sectional study used a survey questionnaire covering demographics, clinical and behavioral variables. We obtained blood for peripheral films for malaria and cultures for Typhoid respectively; from 108 consecutively consented participants. Ethical approval was obtained from KIU-TH research and ethics committee (No. UG-REC-023/201834). Multivariate regression analysis was performed using Stata 14.0 (StataCorp. 2015) at 95% confidence interval, regarding $p<0.05$ as statistically significant.

Results: Majority of participants were males 62 % (n=67), cared for by their mothers 85.2% (n=92). The prevalence of malaria was 25% (n=27). The prevalence of Typhoid was 3.7% (n=4) of which 2.8% (n=3) had Malaria-Typhoid co-infection. Using treated water from protected public taps was associated with low Malaria-Typhoid co-infection [p=0.04; aOR=0.05, 95%CI [0.003-0.87], whereas drinking unboiled water from open wells increased the risk for the co-infection [p=0.037, cOR=17, 95%CI (1.19-243.25)].

Conclusions: The prevalence of blood culture confirmed Malaria-Typhoid co-infection was lower than previously reported in serological studies. Educational campaigns regarding use of safe water, hygienic hand washing and proper waste disposal should target mothers who mainly take care of these children.

Key words: Malaria, Typhoid, Co-infection, Febrile, Children, Uganda, Africa

INTRODUCTION

Febrile illnesses are still a global health challenge in the developing countries (Crump & Heyderman, 2015). Malaria and Typhoid fever are a major cause of febrile illness, responsible for 584,000 and 216,000 deaths annually, respectively (WHO, 2015a; Neil *et al.,* 2012). These deaths tend to double when there is dual infection (Shanks & White, 2013). Children below 15 years in Sub-Saharan Africa are at risk of these two infections due to possibility of common source spread from school settings (Baker, Hombach, & Marks, 2016).

Although current reports show declining global trends of Malaria, the disease burden is still high amongst low-income countries in the tropics (WHO, 2016). In such countries which have seasonal variations and contaminated ground water sources (Breiman *et al.*, 2012), the burden of malaria can be compounded with typhoid-salmonella co-infection at the interface of dry and wet seasons, linking the two disease entities (MacLennan, 2014). Besides, the social circumstances of both diseases can be driven by malnutrition, HIV, poverty and poor sanitation which are of public health concern in Uganda (Keddy *et al.,* 2016).

Uganda is endemic for both Malaria and Typhoid (Bulage *et al.*, 2017), thus a clinician practicing in small, resource-constrained health facilities should be able to anticipate this co-infection in children. Quite often, there is overlapping clinical features of the two diseases (Crump & Heyderman, 2015), which makes it difficult to diagnose them accurately, without routine laboratory facilities for blood culture (Slayton, Date, & Mintz, 2013). Such diagnostic challenges prompt the clinician to treat the suspected Malaria-Typhoid co-infection empirically amidst threats of higher levels of antibiotic resistance (Kariuki *et al.*, 2015). On the one hand, failure to prescribe relevant antibiotics on time poses a risk of diagnosing Typhoid fever only after complications such as bowel perforation have occurred (Bulage *et al.,* 2017). This has legal implication and impacts treatment outcome.

Whereas no lifelong protective vaccines have been developed for both Malaria and Typhoid due to their high rates of antigen variability (Crumpa & Heydermanb, 2014) and lack of immunogenicity in paediatric age group (Wain *et al*., 2015); the targets of the Global Technical Strategy (GTS) for Malaria 2016–2030 are to reduce its incidence and mortality rates globally by at least 90% compared with 2015 levels. This is in accordance with the sustainable development goals (WHO, 2015b). However, these targets may not be realised in presence of malaria-like-co-infections such as Typhoid, that can confound the diagnosis and hence increase the risk of morbidity and mortality resulting from late detection (Mogasale *et al.*, 2014).

According to World Health Organisation, the criterion standard for diagnosis of Malaria is a blood slide whereas Typhoid fever requires culture isolation of the organism, which is widely considered 100% specific (Keddy *et al*., 2011). Culture of the bone marrow aspirate is the most sensitive at 90% for Typhoid salmonella, but extremely painful; which may outweigh the benefits in paediatric population. It has been shown that multiple blood cultures (>3), yield sensitivities of 73-97%, particularly larger volume (10-30ml) (Keddy *et al.*, 2011). Despite though, it is not routine in Uganda to obtain the mandatory 3 blood samples in the pediatric population and such results are often not timely available to guide prescriptions.

Thus due to lack of standard diagnostic tools (Kariuki *et al.,* 2015), any fever in children in Uganda is primarily treated as Malaria (Mbonye *et al*., 2013; Chipwaza *et al*., 2015); only to think of other causes when there is no improvement on anti-malarial drugs (Bulage *et al.*, 2017). What is often available to diagnose Malaria and Typhoid infections in Ugandan context are rapid kits that have concerns of reduced specificity (Keddy *et al.,* 2011). Besides, late presentation of children with fever and possible exposure to an anti-malarial or antibiotic prior to hospital visit (Afema *et al.,* 2016), could result in missing such late infections even on blood smears and cultures. This has posed threat for irrational drug prescriptions and antibiotic resistance in our tertiary hospital settings (Kibuuka, 2015).

Although there are existing nation-wide interventions and published data to aid curbing Malaria in Uganda (Yeka *et al*., 2012), Malaria-Typhoid co-infection as a single disease entity is being overlooked in the paediatric population. Knowledge of the extent of this burden and factors associated with this co-infection are key to high index of suspicion, primary prevention, early detection and proper integrated case management. The main objectives of the present study therefore were to determine the prevalence of: (i) Malaria; (ii) Typhoid; (iii) Malaria-Typhoid Co-infection and (iv) Associated Factors; amongst febrile children attending the paediatrics department of Kampala International University Teaching Hospital (KIU-TH) in Western Uganda.

METHODS

Study design

This was a cross-sectional descriptive and analytical study conducted between March-November 2019.

Study participants and settings

The study involved children aged between 6 months to twelve years who presented with fever at the department of paediatrics and child care of Kampala International University Teaching Hospital (KIU-TH). This is the main teaching hospital for Kampala International University Schools of Medicine and Allied Health, located in Ishaka Municipality, Bushenyi District of Western Uganda. The hospital is a 700 bed capacity, providing emergency, out and in-patient specialised paediatrics and child health care. It provides diagnostic and therapeutic services to over 16,646 catchment population (UBOS, 2011). This Malaria endemic region has two rainy seasons beginning March to May and September to November, during which Malaria and diarrhoeal infections peak. According to the Uganda Bureau of Statistics, (2014), the population of children between six months to 12 years in Bushenyi district is about 45.9%; of which 7.2% do not attend school; 88.1% attend primary school, while the illiteracy rate is reported to be 12.1%. Reportedly over 95.8% of the district's population own at least one mosquito net; only 16.1% have access to piped water whereas 6.8% use bore holes (UBOS, 2014). Up to 0.6% of the districts' population do not have access to any toilet facility and practice open defecation while only 23.1% practice proper solid waste disposal and 95.7% are not living in descent dwellings (Uganda Bureau of Statistics, 2014).

Sample size calculation

Being across sectional study where the proportion (P) was the parameter of interest, and using non random sampling, the sample size was calculated using modified Daniel's formula (1999).

Objective 1: The prevalence of Malaria in children in Bushenyi District in Western Uganda had been reported to be 3.5% (Roh *et al.*, 2016) and therefore P=0.35.

$n = \frac{(z_\alpha + z_\beta)^2 p(1-p)}{e^2}$; α=0.05, e=5%; β=0.2 at 80% power;$z_\alpha = 1.96$ $z_\beta = 0.84$

By substitution; $n = \frac{(1.96 + 0.84)^2 x\ 0.035(1 - 0.035)}{0.05^2} = 108$

Objective 2: Based on the study done at KIU-TH in Western Uganda, the prevalence of Typhoid fever in children was reported to be 2.76% (Kiwungulo *et al.*, 2017). Substituting 0.0276 for P;

$n = \frac{(z_\alpha + z_\beta)^2 p(1-p)}{e^2}$; α=0.05, e=5%; β=0.2 at 80% power;$z_\alpha = 1.96$; $z_\beta = 0.84$

By substituting; $n = \frac{(1.96 + 0.84)^2 x\ 0.0276(1 - 0.0276)}{0.05^2} = 84$

Objective 3: Based on the Tanzania study the prevalence of Malaria-Typhoid Co-infection was reported to be 3.5% (Chipwaza e*t al*., 2015). Substituting 0.035 for P;

$n = \frac{(z_\alpha + z_\beta)^2 p(1-p)}{e^2}$; α=0.05, e=5%; β=0.2 at 80% power;$z_\alpha = 1.96$; $z_\beta = 0.84$

By substituting; $n = \frac{(1.96 + 0.84)^2 x\ 0.035(1 - 0.035)}{0.05^2} = 108$

Therefore the larger sample size of 108 was considered adequate to address all the study objectives.

Sampling method

All eligible children with fever at the paediatric department of KIU-TH including; outpatients, in-patients and emergency wards; were consecutively recruited for the study until the desired sample size was realised. This was intended to generate a sample size large enough to relate the findings to the population.

Inclusion criteria

All children aged between 6 months and 12 years with fever were recruited into the study. Malaria cases were stratified as uncomplicated or severe based on clinical symptoms and number of Malaria parasites as observed under a microscope (Maltha *et al.,* 2014). This stratification was for the purposes of proper case management by the attending clinicians. Blood samples for Typhoid salmonella culture were collected from eligible participants with a positive blood slide for Malaria.

Exclusion criteria

Children whose parents or legally authorised representatives who declined consent during study period were excluded. Patients with a history of antibiotic /anti-malarial treatment within 2 weeks prior to admission and those on Malaria prophylaxis or long-term antibiotics were excluded from the study to minimise false negative results.

Study procedure

Recruitment of study participants was conducted at the paediatrics and child health (emergency, outpatient and inpatient) units of KIU-TH, after emergency resuscitation (if deemed necessary by the attending clinician). Every respondent or legally authorised representative was explained to the purpose of the study in order to endorse an informed consent document with a signature or thumb print. A pretested coded check list of parameters of interest specially designed for this purpose was then administered by the investigators. A complete history of associated symptoms such as nausea, loss of appetite, headache, abdominal and join pain, physical examination and relevant laboratory investigations was conducted and findings of interest were recorded on the data tool. In general, patients at paediatric department are received and triaged by the medical team on duty. The first contact clinician is a general doctor who then consults a paediatric resident, paediatrician or infectious disease specialist when there is need. The team routinely carries out several ward rounds in a day to review laboratory results and determine if there is need to amend the initial treatment decisions**.** The recruitment process and flow of participants is summarised in (Fig. 1).

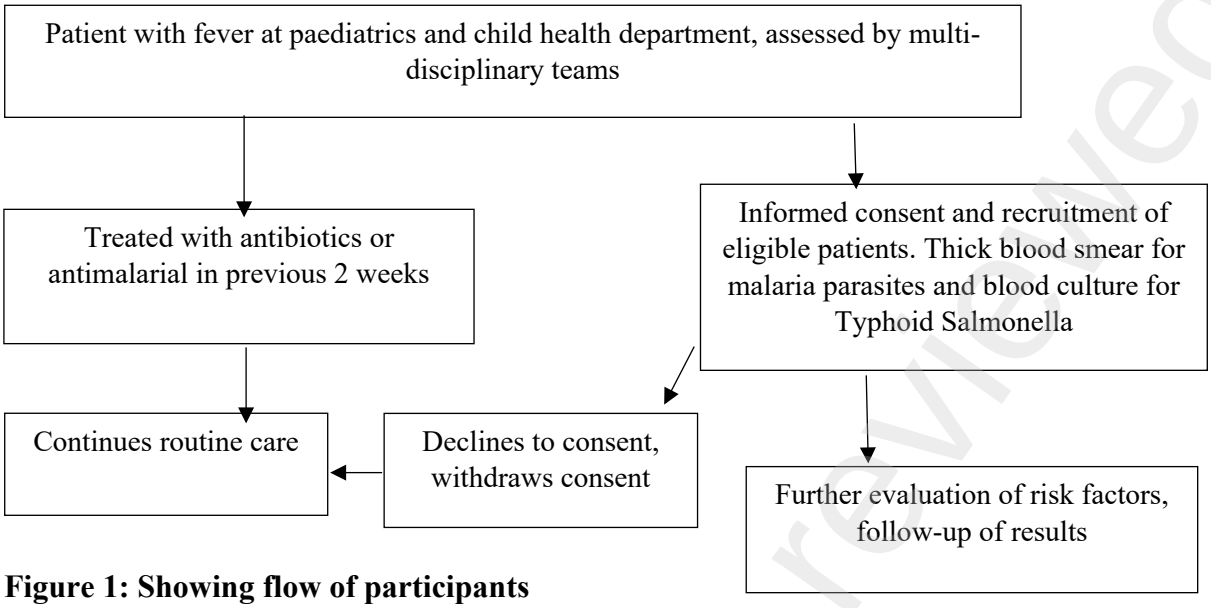

Figure 1: Showing flow of participants

Laboratory Procedures

All laboratory analyses were conducted at the microbiology laboratory of KIU-TH. Patients were sent at the laboratory reception where they were assigned a unique laboratory number after registration, followed by blood sample collection.

Specimen collection

Specimen was collected from patients with fever at the study site. Caretakers to participants or legally authorised representatives were asked to give written informed consent for both specimen collection and subsequently to answer a brief questionnaire in their local language for the illiterate.

Collection of sample for malaria blood slide

The ring finger was cleaned using an anti-septic solution (chlorhexidine) and allowed to dry, and then pricked with a sterile lancet. The first drop of blood was cleaned with a dried cotton wool and finger was squeezed to allow a drop of blood to flow on the centre of a clean, dry, grease free glass slide. A clean glass rod was used to spread the blood in a circular motion to make a thick blood film such that the back of the watch can be read through. The prepared thick blood film was allowed to air dry in accordance with (Liebman, 2015).

Collection of blood sample for culture of salmonella

The skin at a chosen site for venipuncture was cleaned using an antiseptic solution. The area was be allowed to dry prior to venipuncture. A non-touch technique was used to draw 3 mls of venous blood that was transferred into brain heart infusion broth after disinfection of the rubber

septum using an antiseptic solution. The culture bottle was labelled with the participant code number and then taken to the laboratory immediately. Following arrival at the laboratory, each specimen was registered in the appropriate record book and incubated at 37°C for 7days in accordance with (Baron *et al*., 2013). Samples collected in the night also underwent a similar process since the laboratory is easily accessible and within the hospital. The specimen were prepared as follows:

Thick blood smears staining

The dried thick blood film was dipped in water to lyse the red blood cells hence exposing the parasites and then stained with 4% Giemsa in a buffered solution at a pH of 7.2 for 20 minutes. After staining period the slide was rinsed in fresh water and allowed to drain dry. The slide was examined with X100 objective lens under microscope for the presence of malaria parasites which could be seen as a pinkish staining chromatin dot with a ring and gametocyte were seen as a crescent shaped structure (Sathpathi *et al.,*2014). The "plus subsystem" was used to quantify the malaria parasites as follows: + = 1-10 per 100 thick fields; ++ =11-100 per 100 thick fields; +++ =1-10 per thick field; and ++++ = > 10 per thick field in accordance with centre for disease control criteria (CDC, 2014). This was intended to guide clinical management.

Blood culture

After 7 days of incubation, blood samples with growth were sub-cultured on Salmonella-Shigella agar (SSA) under class II biosafety cabinet and incubated at 37°C for 18-24 hours. Cultures were re-incubated after first 24 hours without growth for up to 72 hours before reporting no growth. Cultures with growth were observed for colony characteristics. In Salmonella-Shigella Agar, *Salmonella* appear as black colonies with silver metallic sheen (De, Shetty, & Kelsey, 2014).

Gram staining for morphology

Colonies were picked with the help of sterile wire loop and smears were made by emulsifying the colony with a drop of normal saline on a clean dried slide. Gram-staining was done to observe the morphology features under a microscope. *Salmonella* colonies have a rod shape and are pink in colour and are arranged in pairs or singly. They are motile with flagellates and no spores (De *et al*., 2014).

Quality control

All slides and gram stains were interpreted by two independent laboratory technologist who were blinded of the patient's history. In case of controversy, a professor of medical microbiology and parasitology was consulted and his decision was considered final. Each of the slides were compared with a standard positive Malaria blood slide already available in the hospital laboratory. Each suspected salmonella isolate from research participant was compared with a standard salmonella organism. All the positive samples of isolated salmonella were taken for external quality control as blind duplicate samples at the nearby Mbarara Regional Referral Hospital for validation.

Data collection methods and study variables

We collected data using investigator administered pre-tested questionnaire designed in English and local language (Runyankole). We obtained data on independent variable including fever, abdominal pain, vomiting, and loss of consciousness. The data tool also captured information on social circumstances that were presumed to have an influence on disease transmission including: socio-demographic factors (age, maternal level of education, school going status of the child); behavioural factors (source of drinking water, hand washing practices, definitive human waste disposal); and awareness of preventive measures for the two infections. This method was earlier validated to be effective in similar study settings (Maltha *et al.,* 2014).

Validity and reliability of data collection instrument.

The pre-test study was conducted at Lugazi Health Centre IV. By using content validity index in which five participants who were not part of the sample population, were given the questionnaire and a measure the inter-participant agreement was determined. A Cronbach's co-efficient alpha of more than 0.8 was considered to imply that the items on the questionnaire were reproducible and consistent.

Data analysis

Data was entered into Microsoft Excel (version 2010) and exported to Stata software version 14.1 (StataCorp. 2015. Stata Statistical Software: Release 14. College Station, TX: StataCorp LP) for cleaning and analysis. The participants' socio-demographic, behavioral and clinical characteristics are summarised using frequencies and percentages in tables. The mean and standard deviation were used for continuous participant characteristics that were normally distributed otherwise the median and inter-quartile range were used. We used the modified Poisson regression (with robust standard errors) model to determine factors associated with Malaria-Typhoid co-infection. Factors with medical plausibility and those with $p<0.2$ at bivariate analysis were considered for multivariate analysis. At multi-variate analysis, confounding and effect modification (interaction) were assessed at cut off of 15%. The factors with $p<0.05$, in the final model were considered to be statistically significant. The measure of association are reported as odds ratios (OR), with corresponding 95% CI and p-values.

RESULTS

Socio-demographic characteristics of febrile children attending paediatric department at KIU-TH

Of the 108 participants, majority were below the age of 1 year 38.9% (n=42). Over 85.2% (n=92) of the legal guardians were married and it is largely mothers who took care of these children 86.1% (n=93). Male children dominated their female counter-parts (62 %; n=67) vs. 38%; n=41). Majority 73.2% (n=79) were school going, although their mothers were illiterate (40.7%; n=44); working as peasant farmers 43.5% (n=47) or house-wives 20.4% (n=22). Majority 55.6% (n=60) lived in semi-permanent houses as shown in (Table 1).

Table 1. Socio-demographic characteristics of febrile children attending paediatric department at KIU-TH

Variable	Frequency (n)	Percentage (%)
Religion		
Muslim	10	9.3
Christians	98	90.7
Marital status of guardian		
Single	9	8.3
Married	92	85.2
Divorced	4	3.7
Separated	2	1.9
Widowed	1	0.9

Sex		
Male	67	62.0
Female	41	38.0
Age category		
<1year	42	38.89
1-3	24	22.2
4-6	18	16.67
7-9	14	12.96
10-12	10	9.3
School going status		
Yes	79	73.2
No	29	26.9
Education level of mother		
None	44	40.7
Primary	12	11.1
Secondary	32	29.6
Tertiary	20	18.5
Education level of father		
None	19	17.6
Primary	5	4.6
Secondary	54	50.0
Tertiary	30	27.8
Occupation of mother		
Housewife	22	20.4
Peasant	47	43.5
Farmer	6	5.6
Civil servant	7	6.5
Businesswoman	16	14.8
Health worker	3	2.8
Others	7	6.5
Caretaker of child		
Father	1	0.93
Mother	93	86.1
Sibling	3	2.78
Maid	6	5.56
Others	5	4..63
Occupation of father		
No formal employment	4	3.7
Peasant	31	28.7
Farmer	28	26.0
Civil servant	8	7.4
Businessman	21	19.4
Health worker	7	6.5
Others	9	8.3
Type of house		
Permanent	48	44.4
Semi-permanent	60	55.6

Behavioural characteristics of febrile children attending paediatric department at KIU-TH

Over 61% (n=66) of the children and or their guardians seldom washed their hands before eating food whereas over 48% (n=52) of them frequently wash their hands without soap. Additionally, only 48.2% (n=52) of the children could wash their hand after use of toilet/latrine. Hygienically, about 2% practiced open defecation, while 82.4% (n=89) of the participants used latrine/toilets (Table 2).

Table 2: Behavioural characteristics of febrile children attending paediatric department at KIUTH

Variable	Frequency	Percentage
Washing hands before feeding		
No	1	0.93
Yes	41	37.96
Sometimes	66	61.11
What is used when washing hands		
None	4	3.7
Plain water only	15	13.89
Water with soap	37	34.26
Sometimes with plain water/without soap	52	48.15
Human waste disposal		
Open defecation	2	1.85
Latrine/toilet	89	82.4
Both open defecation and latrine/toilet	17	15.74
Washing hands after defecation		
No	18	16.67
Yes	52	48.15
Sometimes	38	35.19

Most participants, 30.6% (n=33) had their source of water from public taps followed by boreholes 26.9% (n=23). In our study population, 3.7% (n=4) used unboiled water. Approximately 83.3% (n=90) of the participants had heard of Malaria prevention whereas 71.3% (n=77) had heard about Typhoid prevention programs either on radio, television or community (Table 3).

Table 3: Source of water and media awareness about Malaria and Typhoid amongst febrile children attending paediatric department at KIU-TH

Variable	Frequency (n)	Percentage %)
Source of water		
Open well	11	10.19
Public borehole	29	26.86
Public tap	33	30.56

Family Tap	1	0.93
Borehole and public tap	23	21.3
Open well and borehole	4	3.70
Open well and shared public tap	4	3.70
Others	3	2.78
Status of drinking water		
Boiled	94	87.0
Unboiled	4	3.7
Both boiled and unboiled	10	9.3
Stagnant water around the house		
No	77	71.3
Yes	31	28.7
Ever heard about a programme on malaria prevention		
No	18	16.67
Yes	90	83.3
No		
Ever heard about a programme on Typhoid prevention		
No	31	28.7
Yes	77	71.3

Prevalence of Malaria, Typhoid and Malaria-Typhoid co-infections among febrile children attending paediatric department at KIU-TH

The prevalence of Malaria and Typhoid were 25% (27/108) and 3.7% (n=4) respectively whereas the co-infection was prevalent in 2.8% (n=3) (Table 4).

Table 4. Prevalence of Malaria, Typhoid and their co-infection among febrile children attending paediatric department at KIU-TH

Variable	Result	Frequency (%)	95%CI
Malaria	Negative	81(75.0)	65.8-82.4
	Positive	27(25.0)	17.6-34.2
Typhoid	Negative	104(96.3)	90.4-98.6
	Positive	4(3.7)	1.4-9.6
Malaria-Typhoid co-infection	Negative	105(97.2)	91.6-99.1
	Positive	3(2.8)	0.88-8.4

The most affected age groups for Malaria and Malaria-Typhoid co-infection were (7-9) and (10-12) years respectively (Table 5)

Table 5. Age specific prevalence of Malaria, Typhoid and Malaria-Typhoid co-infection among febrile children attending paediatric department at KIU-TH

Variable	Age group in yrs. (n)	Frequency (n)	Percentage (%)	95% CI	P-value
Malaria					
	<1(n=42)	9	21.4	11.2-37.0	1.00
	1-3(n=24)	6	25.0	11.0-47.4	0.74
	4-6(n=18)	4	22.2	7.7-49.4	0.95
	7-9(n=14)	5	35.7	13.7-66.0	0.29
	10-12(n=10)	3	30.0	7.6-69.0	0.57
Typhoid					
	<1(n=42)	0	-	-	-
	1-3(n=24)	0	-	-	-
	4-6(n=18)	0	-	-	-
	7-9(n=14)	1	7.1	0.007-0.44	0.17
	10-12(n=10)	3	30.0	0.08-0.69	-
Malaria-Typhoid co-infection					
	<1(n=42)	0	-	-	-
	1-3(n=24)	0	-	-	-
	4-6(n=18)	0	-	-	-
	7-9(n=14)	1	7.14	0.007-0.44	0.37
	10-12(n=10)	3	20.0	0.04-0.62	-

Factors associated with Malaria-Typhoid co-infection among febrile children attending Paediatric department at KIU-TH

Before adjustment, children who were being taken care of by their mothers were 96% less likely to have Malaria-Typhoid co-infection compared to those being taken care of by their fathers [p=0.028; cOR=0.04, 95% CI (0.003-0.71)], however this association did not remain valid upon adjustment for confounding [p=0.33; aOR=0.14, 95%CI (0.003-7.33)].

Children who reported taking unboiled drinking water from open wells were 17 times more likely to get Malaria-Typhoid co-infection [p=0.037, cOR=17, 95%CI (1.19-243.25)]. Children whose source of water was public taps were 97% less likely to have Malaria-Typhoid co-infection compared to those who used open wells [p=0.015, cOR=0.03, 95%CI [0.02-0.51]. This association remained statistically significant even after adjustment for confounding [p=0.04; aOR=0.05, 95%CI (0.003-0.87)]. There was no statistically significant association between Malaria-Typhoid co-infection and gender, level of education, type of accommodation and school going status (Table 6).

Table 6. Bivariate and Multivariate analysis of factors associated with Malaria-Typhoid co-infection among febrile children attending paediatric department at KIU-TH

Variable	Cor	95%CI	p-value	aOR	95%CI	p-value
Sex						
Male	1.00	-	-			
Female	3.38	0.30-38.56	0.33			
School going status						
No	1.00	-	-			
Yes	1.38	0.12-15.76	0.80			
Parent education level						
None	1.00	-	-			
Primary	1.73	0.10-30.45	0.71			
Secondary	0.61	0.04-10.39	0.74			
Tertiary	-	-	-			
Caretaker						
Father	1.00	-	-	1.00	-	-
Mother	0.04	0.003-0.71	0.028	0.14	0.003-7.33	0.33
Type of house						
Semi-permanent	1.00	-	-			
Permanent	0.39	0.003-4.43	0.45			
Source of drinking water						
Open well	1.00	-	-	1.00	-	-
Public taps	0.03	0.02-0.51	0.015	0.05	0.003-0.87	0.040
Status of drinking water						
Boiled	1		-			
Unboiled	17	1.19-243.25	0.037			
Own mosquito net						
NO	1.00	-	-			
YES	0.21	0.12-2.53	0.22			
Uses a mosquito net						
NO	1.00	-				
YES	0.03	0.003-0.43	0.009			
Holes in mosquito net						
NO	1.0	-				
YES	1.21	0.11-13.94	0.88			
Treated mosquito net						
YES	1.0	-	-			
NO	2.11	0.18-25.14	0.55			
Stagnant water around home?						
NO	1.00	-				
YES	5.24	0.46-60.03	0.18			
Prevention for malaria						
Insecticides	1.00	-	-			

Mosquito nets only	0.09	0.005-1.67	0.107
Both insecticides and mosquito nets	0.27	0.015-5.03	0.33
What time are windows closed?			
4-6pm	1.00	-	
6-7pm	-	-	
7-8pm	3.11	0.19-52.08	0.43
>8pm	9.83	0.54-178.0	0.12
Ever heard of typhoid program?			
NO	1.00	-	-
YES	0.08	0.07-9.16	0.86

DISCUSSION

Prevalence of malaria amongst febrile children attending paediatric department of KIU-TH

The first objective of the study was to determine the prevalence of malaria amongst febrile children attending the paediatric department of KIU-TH which was found to be 25%. This prevalence is lower than 36.5% reported in an Ethiopian study (Meseret *et al*., 2014a), though higher than the 3.5% (Roberts, 2016) and 12% (Yeka *et al*., 2012) previously reported in Western and South Western Uganda respectively. The current figure is also higher than the Ugandans National average of 19% (Uganda Bureau of Statistics and ICF, 2018). This discrepancy could be arising from difference in inclusion criteria, our study having recruited only those who were febrile; including those above 5 years. There has been also concerns that regular reports from Uganda Health Management and Information System (HMIS) suffer inaccuracies; including underreporting of fevers, since only episodes covered by the national public health system are captured amidst lack of laboratory confirmation (Yeka *et al*., 2012).

However our findings show a significant reduction in malaria prevalence from a previously reported Ugandan National average of 42% in 2009 (UBOS & ICF, 2011). This reduction could depict successful National Malaria Control Programmes such as indoor residual spray and distribution of free insecticide treated mosquito nets for vector control. The most affected age group in our study population was 7-9 years. In a similar study in Northern Uganda, this age group was the most affected at 61.8% (Maziarz et al., 2017). This could be due to increased

outdoor activity in this age group but whether much of the exposure is at school or home deserves further investigation.

Prevalence of Typhoid amongst febrile children attending paediatric department of KIU-TH

The second objective of the study was to determine the prevalence of Typhoid amongst febrile children attending the paediatric department of KIU-TH, based on blood cultures. The study established the prevalence at 3.7% and all affected participants were aged 7-12 years. This blood culture based prevalence is comparable to 2.76% (Kiwungulo *et al.*, 2017) previously reported at KIU-TH and to 2.3% reported at Mulago National Referral Hospital (Mutanda, 1998). Also our low prevalence compares well to 0.5% to 5% reported by (Meseret *et al*., 2014a) and (Habte *et al*., 2018) respectively in Ethiopia.

Previously in Uganda, Typhoid infection based on blood cultures has been largely studied only during outbreaks rather than routine in the paediatric population. Ranges between 2.6% to 22.6% were reported among adults during Kasese outbreak in Western Uganda (Kabwama *et al*., 2017). In a retrospective study amongst all febrile patients attending clinics in Bushenyi district, the overall prevalence was reported to be 36.6%, affecting mainly 10-29 year olds of low income class (Agwu, Wambua, & Titus, 2009), however this was a Widal Agglutination serological based study with sensitivity and specificity concerns amidst data quality constraints of retrospective studies. Serological tests as opposed to blood cultures have been found to give higher prevalence rates of Typhoid fever resulting from false positive results in Nigeria (Igharo *et al*., 2012b), India (Shukla *et al.,* 2014) and Pakistan (Khan *et al*., 2013). In an Ethiopian study, Typhoid fever was prevalent in 19% based on serological Widal test as opposed to 0.5% based on blood culture (Meseret *et al*., 2014a), emphasising the need for extending laboratories with capacity to do blood cultures for proper diagnosis. In conformity with our study findings, a blood culture study in Cameroon found Typhoid fever prevalence of 2.5% amongst febrile patients. Other studies in low and middle income countries have found Typhoid prevalence lowest amongst children below 4 years and above 15 years (Antillón *et al.,* 2017; Igharo *et al*., 2012b) and highest amongst school going age group of 5-10 years (Khan *et al*., 2013). The higher Typhoid burden in the later age group could be due to common source infections from public boreholes in our primary schools setting; alongside poor hand hygienic practices.

Prevalence of Malaria-Typhoid co-infection amongst febrile children attending paediatric department at KIU-TH

The third objective of the study was to determine the prevalence of Malaria-Typhoid co-infection amongst febrile children attending the paediatric department of KIU-TH. The prevalence of Malaria-Typhoid co-infection based on microscopy and blood culture respectively in our study population was found to be at 2.8%. Our co-infection rates are comparable to 3.5% reported in a Tanzanian study among children below 15 years (Chipwaza e*t al*., 2015) and to 2.5% in an Indian study (Shukla *et al.,* 2014). Contrary to the findings of (Meseret *et al*., 2014a), all cases in the present study were between 7-12 years as opposed to 2-5 years. However, the prevalence of this co-infection in our study is lower than 6.5% reported in an Ethiopian study of blood cultures (Meseret *et al*., 2014a), although the later involved a general population; including participants above 12 years.

The co-infection rates in our study is far lower that what has been reported previously in serological studies. In their serological study in Western Uganda, Agwu *et al*., (2009) reported a co-infection rate of 20.9% which was comparable to 18.3% in a Nigerian study that used serological tests in a general population (Igharo *et al*., 2012b). The inclusion of general population should control for confounding from comorbidities such as HIV/AIDs that have been shown to be associated with higher rates of both malaria and Typhoid (Agwu, *et al*., 2009), otherwise the resulting high prevalence of Malaria-Typhoid co-infection could be overestimated. In a similar study in Sierra Leon, there was no association between having Malaria and Typhoid fever, but presence of fever was more associated with Salmonella Typhi compared to Plasmodium parasites (Kargbo, Massaquoi, Samura, Meng, & Zou, 2014). A consensus on age specific-blood culture based reporting of this co-infection amongst researchers, could thus address variability of findings in the future studies.

Factors associated with Malaria-Typhoid co-infection amongst febrile children attending paediatric department at KIU-TH

The fourth objective of the study intended to determine the factors associated with Malaria-Typhoid co-infections amongst febrile children. Upon adjusting for confounders, we found that the most important factor influencing this co-infection was the source of water. Using treated water from protected public taps was associated with low Malaria-Typhoid co-infection ($p=0.04$) whereas drinking unboiled water from open public wells increased the risk for the co-

infection (p=0.037). Capturing such history could be made a routine element of screening children presenting with febrile illnesses in our settings.

Other Ugandan authors have pinned contaminated water and food as main driving factors for Typhoid infection (Kabwama *et al*., 2017), although their main focus had been on adult population. In a similar Ethiopian study, using non treated water from open sources such as springs and wells was associated with blood culture confirmed Typhoid fever, especially amongst rural dwellers (Habte *et al*., 2018). Contrary to findings of Khan *et al*., (2013) in Pakistan, we found no significant association between the child's school going and Malaria-Typhoid co-infection in the present study despite the fact that all cases were within 7-12 years; a typical school going age group. Other studies attribute Malaria-Typhoid co-infection in school going age to increased outdoor activity as well as poor hand washing habits in absence of parental supervision (Meseret *et al*., 2014a). In our study, over 61% of the children seldom washed their hands before handling food, whereas 48% of them did so but without soap. These statistics coupled with open defecation and failure to wash hands after visiting toilets demonstrated in the present study, warranty urgent behavioural change campaigns.

Study limitations

There were some limitations in this study. Although blood slide and blood culture are considered gold standard for diagnosing Malaria and Typhoid respectively, in exceptional cases, Malaria parasites may not be captured in peripheral blood smears even in presence of severe infection either due to sequestration of parasitized cells in deep capillary beds. A bone marrow aspirate analysis that is recommended for such cases (Wickramasinghe & Abdalla, 2000) was beyond the scope of this study, thus we could have missed such cases. As such, the reported isolated case of Typhoid fever in the present study was included based on presence malaria pigment in circulating neutrophils and monocytes despite having no Malaria parasites in setting of suspected malarial infection. The sensitivity of blood cultures for Typhoid salmonella is intrinsically moderate at only 85-90% (Darton *et al*., 2017), yet the more invasive bone marrow cultures were not ethically justifiable for our study population. Also our consecutive recruitment could limit generalisability of the findings.

Conclusions and implication of results

The prevalence of Malaria was high in our study population compared to the national average. The prevalence of blood culture confirmed Typhoid fever and Malaria-Typhoid co-infection

were not as high as previously reported based on serological studies. Using treated water from protected public taps was protective whereas consuming unboiled water from open public wells was the main risk factor for Malaria-Typhoid co-infection. These findings justify the routine standardised testing for either infections amongst febrile children, to avoid irrational antibiotic prescriptions (Kibuuka *et al*., 2015) and complications related to late diagnosis. Health worker and community driven educational campaigns regarding use of safe water, hygienic hand washing practices and proper waste disposal should target mothers who mainly take care of these children.

List of abbreviations

CDC	Centre for Disease Control and Prevention
GTS	Global Technical Strategy for Malaria
Hb	Haemoglobin
HIV	Human Immunodeficiency Virus
ICCM	Integrated Community Case Management
KIU-TH	Kampala International University Teaching Hospital
MIS	Malaria Indicator Survey
P.I	Patient Information
SDG	Sustainable Development Goals
SSA	Salmonella Shigella Agar
UBOS	Uganda Bureau of Statistics
WHO	World Health Organisation
LMICs	Low to Middle Income Countries

Operational Definitions

Fever: Refers to rectal temperature equals to or above 38^{o}C (Kargbo *et al*., 2014). However, in this study, because most guardians reported self-prescription of antipyretics for their children prior hospital visits, all children with temperatures above 37.4 ^{o}C were considered febrile.

Malaria: An acute or chronic illness characterized by paroxysms of fever, chills, fatigue, anaemia and splenomegaly in presence of Malaria parasites (WHO, 2015a).

Uncomplicated Malaria: A patient presenting with symptoms of Malaria and a positive blood slide for Malaria but with no features of severe Malaria (WHO, 2015a).

Severe Malaria: A patient with one or more of the following features occurring in absence of an identified alternative cause and in the presence of *P. falciparum*: impaired consciousness (Blantyre coma score<3 in children); prostration (generalised weakness so that the person is unable to sit, stand or walk without assistance); multiple convulsions (more than two episodes within 24 hours); Acidosis (A base deficit of >8mEq/L or if not available, plasma bicarbonate level of <15mmol/L or venous plasma lactate≥5mmol/L, or severe acidosis manifesting clinically as respiratory distress (rapid deep laboured breathing); hypoglycaemia (blood or plasma glucose<2.2mmol/L(<40mg/dL), severe Malarial anaemia (Hb≤5g/dL or hematocrit of ≤15% in children <12 years of age (<7g/dL and <20% respectively in adults with a parasite count of >10,000/µL); renal impairment (plasma or serum creatinine >265µmol/L (3mg/dL) or blood urea> 20mmol/L; jaundice(plasma or serum bilirubin>50µmol/L (3mg/dL) with a parasitic density> 100,000/µL; pulmonary edema (radiological confirmation or oxygen saturation<92% on room air with respiratory rate> 30/min often with chest in drawing and crepitation on auscultation; significant bleeding (including recurrent or prolonged bleeding from the nose, gums or vein puncture sites, hematemesis or melena); shock (capillary refill ≥3s or temperature gradient on leg, systolic blood pressure of <70mmHg with evidence of impaired perfusion (cold peripheries); hyper-parasitemia (>10% *P.falciparum,* > 20,000/µL *P.vivax* or >100,000/µL *P.knowlesi)* (World Health Organization, 2015a).

Typhoid fever: A patient presenting with history of persistent gradual fever, abdominal pain, bloating, red spotted rash and positive blood culture suggestive of *Salmonella Typhi* (Keddy *et al*., 2011).

Confirmed Malaria-Typhoid co-infection: A patient meeting criteria for Malaria based on microscopy with a positive blood culture suggestive of *Salmonella typhi or paratyphi* (Keddy *et al.*, 2011).

DECLARATIONS

Ethics approval and consent to participate

The study strictly followed the Uganda National Courncil for Science and Technolgy Guidelines (2014) on research involving use of human participants and in accordance with the Declaration of Helsinki (DoH) by World Medical Association (DoH, 2013). Ethical approval was obtained from School of Medicine Research and Ethics Committee of Kampala International University, Western Campus (No. UG-REC-023/201834). Informed consent was sought from all participants and or their legally authorized representatives who endorsed their signatures or thumb prints on the consent form document, having been made to understand the risks and benefits of the study. All participants were free to withdraw their consent at any stage of the study. Withdrawal of consent by any patient did not affect the quality of treatment or impinge on their entitlements. All laboratory results were immediately availed to the guardians and attending clinicians to guide treatment.

Consent for publication

Not applicable

Availability of data and materials

The datasets generated and/or analysed during the current study are not publicly available due to ethical restrictions but are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

JN and RM conceived the study, reviewed the relevant literature and collected the data. PT and EA processed the samples. HL designed the study, analysed and interpreted the data and drafted the manuscript. MB, AN, AE and HL supervised the study. VK reviewed the manuscript. All authors read and approved the final manuscript for submission.

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