Domestic river water use and risk of typhoid fever: results from a case-control study in Blantyre, Malawi

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Key points: This study identifies major water, hygiene, and social risk factors in Blantyre, Malawi, highlighting the importance of understanding our interactions with water sources beyond drinking water for better control of typhoid fever.

Abstract

Background: Typhoid fever remains a major cause of morbidity and mortality in low and middle-income settings. In the last 10 years, several reports have described the re-emergence of typhoid fever in southern and eastern Africa, associated with multidrug-resistant H58 *Salmonella* Typhi. Here, we identify risk factors for pediatric typhoid fever in a large epidemic in Blantyre, Malawi.

Methods: A case-control study was conducted between April 2015 and November 2016. Cases were recruited at a large teaching hospital, while controls were recruited from the community, matched by residential ward. Stepwise variable selection and likelihood ratio testing were used to select candidate risk factors for a final logistic regression model.

Results: Use of river water for cooking and cleaning was highly associated with risk of typhoid fever (OR 4.6 [CI: 1.6-12.5]). Additional risk factors included protective effects of soap in the household (OR 0.6 [CI: 0.4-0.98]) and more than one water sources used in the previous 3 weeks (OR 3.2 [CI: 1.6-6.2]). Attendance at school or other daycare was also identified as a risk factor (OR 2.7 [CI: 1.4-5.3]) and was associated with the highest attributable risk (51.3%).

Conclusions: These results highlight diverse risk factors for typhoid fever in Malawi, with implications for control in addition to the provision of safe drinking water. There is an urgent need to improve our understanding of transmission pathways of typhoid fever, both to develop tools for detecting *S.* Typhi in the environment, and inform water, sanitation, and hygiene interventions.

Keywords: Salmonella typhi, WASH, water, sanitation, environment

Introduction

Typhoid fever continues to be a major cause of morbidity and mortality in low and middle-income settings, with an estimated 10-20 million cases occurring annually, and approximately 200,000 deaths [1–3]. In south and southeast Asia, *Salmonella* Typhi was identified as the most common bacterial pathogen associated with bloodstream infection (BSI) among hospitalized patients between 1990 and 2010 [4]. In contrast, *S.* Typhi was not described as a major cause of BSI in southern and eastern African countries during the same period, even in centers with long term bacteraemia surveillance [5]. Instead, nontyphoidal serovars of Salmonella were much more prominent causes of BSI. Since 2012, the picture has changed dramatically, with multiple reports describing the emergence of typhoid as a major cause of BSI in southern and eastern Africa [6–9]. Though the drivers of this recent emergence remain unclear, typhoid is now acknowledged as a significant public health problem in both Africa and Asia[10].

S. Typhi is a human-restricted pathogen, and transmission occurs via the fecal-oral route. Its ecological niche after excretion remains poorly described, but there is evidence for heterogeneity in pathways of environmental exposure. For example, typhoid transmission has been linked to contamination of the water supply in Kathmandu, Nepal [11], whereas in Santiago, Chile, endemicity was maintained until the early 1990s through irrigation of salad crops with wastewater [12]. These contrasting data suggest that the critical intervention points at which typhoid transmission may be interrupted in the environment may be context-specific. In addition to transmission through an ecological niche, *S.* Typhi may also be transmitted within the household, most often through direct contamination of food by an infected individual. This type of transmission is not only present in endemic settings, but has led to outbreaks of typhoid after

endemicity has been interrupted through widespread sanitation improvements [13]. This poses an additional challenge for control.

Both transmission pathways are important in the spread of *S.* Typhi, but their relative importance in endemic settings is poorly understood. Risk factor studies have been conducted in a variety of locations, including both endemic and outbreak settings, to better understand the dominant drivers of transmission. Previously identified risk factors for typhoid include recent contact with individuals diagnosed with typhoid or enteric fever [14–16], food, including consuming flavoured ices [17] and ice cream [18] or ice cubes [19], buying lunch at school [17] or eating roadside or outdoor vended food [18–20], and drinking unsafe or untreated water at home [19,21,22] or drinking water at work[18]. Exposure to water used for purposes other than drinking has also been identified as a risk for typhoid, such as bathing and brushing teeth [19]. Findings on sanitation show lack of soap in the household and limited handwashing are associated with typhoid [15,19,20,23,24], while having a latrine in the household has been found to be protective in Indonesia[15], but a risk factor in Nepal [16]. In endemic locations, the majority of work has been done in Asian, Oceanian, and South American countries, and has so far been limited on the African continent [13-23].

These findings implicate a variety of water, sanitation, and hygiene factors, but the heterogeneity among locations indicates a need for site-specific investigation, particularly in regions that have been under-studied or where typhoid is re-emerging. Furthermore, although many food and water exposures have been previously identified, detailed studies describing where in the food preparation or production cycle, or through which aspect of water usage *S.* Typhi is entering and amplifying are lacking. This hampers the planning of effective intervention strategies at the source of contamination. Understanding the complexity of WASH factors in

transmission has assumed greater importance following the emergence of cephalosporin resistant typhoid in Pakistan [25], which threatens the role of antimicrobials in typhoid control. Whilst the typhoid conjugate vaccine offers a promising tool for control, targeted water and sanitation interventions are likely to be necessary too.

Blantyre is the second-largest city in the country of Malawi, located in the Southern region (Figure 1). WASH-related interventions over the last 10 years in Blantyre have focused on water access, with an increase in kiosks, trials of delivery systems, and protection of open sources, but interventions on household water treatment and improved sanitation have been limited. Blantyre has experienced a sharp increase in typhoid, increasing from an average of 14 cases per year between 1998 and 2010, to over 700 in 2013 [6]. Typhoid has remained endemic in Blantyre, and the mechanism of this sustained transmission is currently unknown. We therefore conducted a case-control study to investigate risk factors for typhoid in this setting.

Methods

Queen Elizabeth Central Hospital (QECH) in Blantyre, Malawi, provides free healthcare to urban Blantyre and the surrounding district, and tertiary care to the Southern region of Malawi. Laboratory surveillance for BSI has been routine since 1998, and is conducted through the Malawi-Liverpool-Wellcome Trust Clinical Research Programme (MLW), based at QECH[26]. Pediatric patients are eligible for routine blood culture if they present to the hospital with non-specific febrile illness and test negative for malaria, have persistent febrile illness after treatment for malaria, or are severely ill with suspected sepsis. Blood was drawn for each eligible patient (2-4mL), followed by automated culture (BacT/ALERT, Biomerieux) and serotyping for identification of *Salmonella* Typhi [26].

Cases were defined as children under 9 years of age with blood culture confirmed *S.* Typhi infection diagnosed between April 2015 and November 2016 at QECH, and who originated from the Blantyre urban area. Eligible controls were healthy children under 9 years of age and were recruited at a 4:1 ratio throughout the study period. Children under 9 years of age were prioritized for the study because of the known frequency of typhoid in this age group[6]. A high-resolution census sub-divided urban Blantyre into 393 enumeration areas (EAs), each with an estimated population size (Figure 1)[27]. To avoid spatial over-matching, which would have made it impossible to identify small-scale spatial heterogeneity of risk, controls were matched by larger residential wards rather than by EA. To approximate the random selection of controls within a ward, we selected EAs with probability proportional to population size. Within each sampled EA, households were approached along a random path until an eligible control was identified and consent was taken from legal guardians.

Exclusion criteria specific to cases after initial recruitment was living outside EA boundaries. For both cases and controls, individuals were excluded if they had a household member who previously was diagnosed with typhoid during the period of the study.

A standardized questionnaire was administered to the guardians of participants, where guardian was defined as a caregiver for the child, above 18 years of age The questionnaire recorded both demographic and socioeconomic indicators, as well as potential risk factors for typhoid. The incubation period for typhoid in outbreak settings can be highly variable, but is not known to frequently extend longer than three weeks [13]. Therefore, questions distinguished exposure in the last three weeks from exposure in the last year. Sources of water for drinking and water used for cooking and cleaning were separately surveyed. The location and altitude of households and identified water sources were collected using Garmin Etrex 30 GPS devices.

Controls were requested to provide a stool sample to describe asymptomatic shedding of *S.* Typhi, described in Supplement 1.

Statistical analysis

Logistic regression was used to assess potential risk factors in the study. Residential ward was included as a fixed effect for all analyses, to take account of the stratified sampling design for controls. The majority of predictor variables were assessed directly from the questionnaire, while distance to hospital, distance to primary water source, and elevation change between the household and water source were calculated for each individual, using the recorded household locations, water source locations, and ascertained GPS coordinates of QECH. Due to the large number of questions in the initial survey, stepwise forward variable selection was conducted to reduce the number to an interpretable size. This process began with the base model, defined as the fixed effect of residential ward, plus intercept. At each iteration, likelihood ratio tests were conducted to compare the base model with each potential variable addition. The variable addition resulting in the lowest p-value from the likelihood ratio test was then added to the model. The process was repeated with the base model now updated with the added variable. The process stopped when no variable addition improved the model at a significance level of $p<0.05$.

The final logistic regression model was fitted using the resulting selected variables. Odds ratios with 95% confidence intervals were calculated using coefficients and standard errors estimated from the fitted model. Unadjusted individual odds ratios were also calculated for each selected variable to assess dependence of multivariate model findings on the combination of included parameters. To enable comparison between continuous variables in the study, we rescaled each so an increase in scaled value is equal to one standard deviation increase in the

unscaled value. Due to only one individual reporting more than one febrile family member, and one individual reporting more than two water sources, for the final model fit these continuous variables were converted to categorical variables.

Finally, we extend the multivariate logistic regression model to estimate the potential percentage reduction in cases in our population attributed to removing reported exposures. Detailed methods are described (Supplement 2). Because we do not know the null exposure value of continuous variables, these calculations were only made for variables that were binary, and those were estimated to be significant in the model.

To investigate spatial correlation in risk within residential wards, we assessed the residuals of the fitted logistic regression model [28]. All statistical analyses were conducted using R statistical software, version 3.5.1[29].

Ethics

This study was approved by the University of Malawi, College of Medicine Research and Ethics Committee[P.08/14/1617], the Liverpool School of Tropical Medicine Research Ethics Committee[14.042] and the Lancaster University Faculty of Health and Medicine Ethics Committee[FHMREC17014].

Results

During the study period, 189 children were diagnosed with blood culture confirmed typhoid (Figure 2). There were no cases of *Salmonella* Paratyphi A. 125 cases were included in the study, with a median age of 5(IQR 3-7); 60 patients were not recruited, amongst whom 35 declined participation, 24 could not be reached after diagnosis, and 1 patient died from

complications of perforation prior to recruitment. After recruitment, two patients were excluded because they were secondary cases in households that had previously been surveyed, and two cases were excluded from the analysis because their household location fell outside the study boundary. One control was excluded, due to another household member having culture-confirmed typhoid during the week of recruitment.

Cases tended to be older than controls (Table 1) but were similar in distribution of gender. Though the overall ratio of controls to cases was 4.2:1, control ascertainment resulted in a heterogeneity of the ratio of controls to cases between residential wards (Figure 1). Six residential wards did not contain any cases. Amongst the 123 controls tested, 0/123(0%) were stool culture positive for S. Typhi, therefore no further action was taken, however, 3/123(2.4%) were PCR positive (95% CI:0.8-6.9%).

Variable selection reduced the 97 initial variables to 14 (Table 2, Supplement 3). The 125 cases and 514 controls were reduced to 122 and 507, respectively, due to missing data in the final variable set. Out of the 14 final variables selected in the model, 8 were directly related to water exposures.

Logistic regression identified several significant risk factors for typhoid in children (Table 2). Factors suggestion environmental exposure included cooking and cleaning with river water (OR 4.6 [CI:1.7-12.5]) and water from an open dug well (OR 2.4 [CI:1.1-5.1]), having more than one drinking water source (OR 3.2 [CI:1.6-6.2]), and being from a household growing crops (OR 1.8 [CI:1.1-3.0]). Conversely, availability of soap to wash hands after the toilet (OR 0.6 [CI:0.4-0.98]), was protective. Risk factors suggesting the importance of social interaction patterns were identified, including spending the day at school or in child care (OR 2.7 [CI:1.4-5.3]) and having one or more household members admitted to the hospital with febrile illness in

the last four weeks (OR 8.9 [CI:1.9-41.2]. Seeking care for severe illness at QECH was selected for in the model, adjusting for differential case-ascertainment through the hospital between cases and controls. Estimates of attributable risk are summarized in Table 2. The highest attributable risk percentage was spending the day at school or daycare (51.3%), followed by growing crops by the household (17.4%). Attributable risk percentages were lower, and similar, for cooking and cleaning with river water (10.3%) and water from an open dug well (8.3%).

There was no significant spatial correlation of residuals from the analysis (Supplement 3), indicating that the variables in the questionnaire and/or spatially matching on residential ward sufficiently accounted for unexplained spatial variation in risk.

Discussion

This study provides detailed insight into the risk factors for pediatric typhoid in an urban African setting. Our findings point to complex and varied risks for typhoid in Blantyre, including water sources, household indicators of sanitation and hygiene, and social interaction patterns such as school attendance.

In multivariate analysis, cooking and cleaning with river water was the principal environmental exposure identified in the study. Cooking and cleaning with water from an open dug well was additionally identified as a risk factor. No sources of drinking water were associated with typhoid, contrasting with other studies that implicate drinking water sources as risk factors [18,19,21,22]. Potential explanations include that communities are aware of the risks associated with drinking unclean water, but less aware of the risks of indirect exposure, such as through pans or other items that may come into contact with food. Alternatively, people may prioritize safe water for drinking, but cannot afford to purchase or transport the volume of safe

water needed for use in other household tasks. It is estimated that less than 5% of the population is connected to the sewage network, with the majority of the population utilizing pit latrines[30]. Open dug wells and nearby rivers used for cooking and cleaning water may become contaminated with runoff from pit latrines, particularly during rain events, providing a plausible epidemiological link.

Our findings indicate that individuals are at a higher risk for typhoid when using multiple drinking water sources. Previous work examining water access in urban Malawi identified limited access hours, tariffs, low water pressure, and too few water kiosks as structural barriers to adequate potable water for household activities [31]. These water access challenges are likely to influence the number and type of water sources used, and may necessitate the use of unsafe sources. In other studies, distance, access, and behavioral factors have been found to influence decisions around accessing potable water [32–34].

We also identify risk factors where exposure could occur through either interaction with contaminated environments, infected individuals, or both. Having household members hospitalized for febrile illness was identified as a risk factor; as was attending school or other day care. In the context of schools, however, it is uncertain whether the key exposure is direct contact with a contaminated environment[35], food handlers contaminating meals [35,36], or transmission routes such as contact with infectious children. The presence of soap in the household was found to be protective, consistent with findings in other locations [15,19,20,23,24], further supporting a tool that interrupts exposure.

Coming from a household that grows crops is a risk factor for typhoid in Blantyre, consistent with the experience in Santiago, Chile, where irrigation of crops with wastewater was a driver of typhoid transmission [12]. Neither irrigation with human nor animal waste was found

to be a significant risk, however fecal contamination of food crops still may be possible in Blantyre through runoff from latrines, or irrigation with fecally contaminated river water.

Calculation of attributable risk has enabled us to estimate frequency of exposure to these risk factors in the population. Spending the day in school or daycare was associated with the highest attributable risk, highlighting the importance of this common exposure among children in our study and associated challenges with WASH in schools[37]. A small percentage of cases and controls reported cooking and cleaning with river water/water from an open dug and thus these factors were associated with lower estimated attributable risks, however such behaviors are commonly described in qualitative and observational research in Malawi [38,39]. There is therefore a possibility of under-reporting these types of exposures, and further research on quantifying these patterns would be useful.The study has some limitations; the extended incubation period of typhoid necessitated a 2-3 week window for assessing potential exposures, and recall bias cannot be excluded. Controls were recruited throughout the study period, and not matched over time, limiting our ability to control for seasonality. We focused on young children with the goal of capturing household-related risk factors, assuming younger children move around the city less than adults and are therefore less likely to become exposed outside of the household. Regardless, the potential for differential risk factors for older children and adults may limit the generalizability of these findings to older age groups. We assessed WASH risk factors through a questionnaire, rather than by direct observation in or transect walks around participant households. Lastly, by basing our study on sentinel surveillance of patients presenting to QECH, we have selected for more severe disease, and have not captured minimally symptomatic or sub-clinical typhoid, which may be associated with differential risk factors.

We provide new insights into risk factors for typhoid in an urban African context, challenging the dogma that transmission of *S.* Typhi can be interrupted solely by the provision of safe drinking water. Instead, we highlight the importance of usage of water for purposes other than drinking, of hand hygiene, and of preschool/daycare attendance in the transmission of typhoid in this setting. Future work should confirm our findings by direct assessment of *S.* Typhi in the environment. Developing novel tools for the identification of *S.* Typhi in the environment will help to identify transmission routes rapidly, and without in-depth risk factor analyses for each epidemic or endemic location.

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Conflicts of Interest

Authors declare no conflicts of interest.

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Table 1. Baseline characteristics of cases and controls enrolled in the study

Characteristic	**Cases (n=125)**		**Controls (n=514)**		**p value**
Age (years)	n	%	n	(%)	< 0.005
≤ 2	28	(22)	185	(37)	
3-5	35	(28)	209	(40)	
6-8	61	(49)	120	(23)	
Gender					0.38
Male	61	(49)	294	(51)	
Female	64	(51)	278	(49)	

Table 2. Estimated odds ratios for univariate models and selected multivariate model. Numeric variables are scaled for presentation of estimates, thus odds ratios are presented as increased risk per 1 standard deviation increase in the value.

Variable	Cases (122)	Controls (507)	Unadjusted OR	p value	Adjusted OR (95% CI)	p value	Attributable Risk (%)	Adjusted OR (95% CI)
Seeking care at QECH if child is severely ill, no. (%)	118 (97)	370 (73)	10.9 (4.0, 30.2)	<0.001	14.1 (4.7, 41.8)	<0.001	–	
One or more household members admitted to hospital for febrile illness in last four weeks, no. (%)	9 (7)	7 (1)	5.7 (2.1, 15.6)	<0.001	8.9 (1.9, 41.2)	0.006	–	
Cooking and cleaning with river water in the previous three weeks, no. (%)	15 (12)	16 (3)	4.3 (2.1, 9.0)	<0.001	4.6 (1.7, 12.5)	0.002	10.3	
More than one drinking water sources used last three weeks, no. (%)	28 (23)	38 (7)	3.7 (2.2, 6.3)	<0.001	3.2 (1.6, 6.2)	<0.001	15.4	
Child spends the day at school, preschool, nursery or any other daycare, no. (%)	99 (81)	312 (62)	2.7 (1.7, 4.4)	<0.001	2.7 (1.4, 5.3)	0.005	51.3	
Cooking and cleaning using water from an open dug well in the previous three weeks, no. (%)	20 (16)	35 (7)	2.6 (1.5, 4.8)	0.001	2.4 (1.1, 5.1)	0.020	8.3	
Family grows crops, no. (%)	47 (38)	137 (27)	1.7 (1.1, 2.6)	0.127	1.8 (1.1, 3.0)	0.027	17.4	
Age (years), median (range)	5 (0− 8)	3 (0− 8)	1.7 (1.4, 2.1)	<0.001	1.4 (1.0, 1.8)	0.053	–	
Distance to from household to primary water source (meters), median (range)	78 (1− 738)	52 (0− 748)	1.2 (1.0, 1.5)	0.013	1.2 (1.0, 1.6)	0.118	–	
Number of days water is stored, median (range)	2 (1− 7)	2 (1− 20)	0.74 (0.6, 0.96)	0.024	0.8 (0.6, 1.0)	0.054	–	
Experienced water shortage in the house or surrounding area in the past two weeks, no. (%)	38 (31)	172 (31)	1.0 (0.7, 1.6)	0.897	0.6 (0.3, 1.0)	0.056	–	
Soap available to wash hands after the toilet in the previous three weeks, no. (%)	70 (57)	360 (71)	0.5 (0.4, 0.8)	0.002	0.6 (0.4, 0. 98)	0.042	–	
Stores drinking water in drum, no. (%)	0 (0)	20 (4)	2.6 e−07 (0, inf)	0.977	1.2 e −7 (0, inf)	0.984	–	
Used stream or river water for drinking in the last three weeks, no. (%)	0 (0)	4 (1)	7.2 e−07 (0, inf)	0.984	1.1 e −8 (0, inf)	0.992	–	

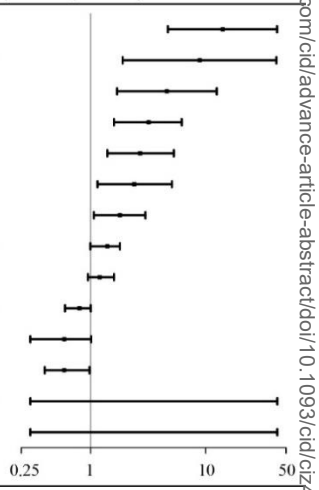

Figure 1. Location of Blantyre within the country of Malawi (inset), and the Blantyre study boundaries. Enumeration areas are represented by the smaller polygons, while residential wards are indicated in the larger, shaded by the ratio of controls to cases. Households of cases (red) and controls (black) are plotted as points, with precise locations masked by randomization

Figure 2. Consort chart for cases and controls in the study.

Table 1

Variable	Cases (122)	Controls (507)	Unadjusted OR	p value	Adjusted OR (95% CI)	p value	Attributable Risk (%)	Adjusted OR (95% CI)
Seeking care at QECH if child is severely ill, no. (%)	118 (97)	370 (73)	10.9 (4.0, 30.2)	<0.001	14.1 (4.7, 41.8)	<0.001	–	
One or more household members admitted to hospital for febrile illness in last four weeks, no. (%)	9 (7)	7 (1)	5.7 (2.1, 15.6)	<0.001	8.9 (1.9, 41.2)	0.006	–	
Cooking and cleaning with river water in the previous three weeks, no. (%)	15 (12)	16 (3)	4.3 (2.1, 9.0)	<0.001	4.6 (1.7, 12.5)	0.002	10.3	
More than one drinking water sources used last three weeks, no. (%)	28 (23)	38 (7)	3.7 (2.2, 6.3)	<0.001	3.2 (1.6, 6.2)	<0.001	15.4	
Child spends the day at school, preschool, nursery or any other daycare, no. (%)	99 (81)	312 (62)	2.7 (1.7, 4.4)	<0.001	2.7 (1.4, 5.3)	0.005	51.3	
Cooking and cleaning using water from an open dug well in the previous three weeks, no. (%)	20 (16)	35 (7)	2.6 (1.5, 4.8)	0.001	2.4 (1.1, 5.1)	0.020	8.3	
Family grows crops, no. (%)	47 (38)	137 (27)	1.7 (1.1, 2.6)	0.127	1.8 (1.1, 3.0)	0.027	17.4	
Age (years), median (range)	5 (0− 8)	3 (0− 8)	1.7 (1.4, 2.1)	<0.001	1.4 (1.0, 1.8)	0.053	–	
Distance to from household to primary water source (meters), median (range)	78 (1− 738)	52 (0− 748)	1.2 (1.0, 1.5)	0.013	1.2 (1.0, 1.6)	0.118	–	
Number of days water is stored, median (range)	2 (1− 7)	2 (1− 20)	0.74 (0.6, 0.96)	0.024	0.8 (0.6, 1.0)	0.054	–	
Experienced water shortage in the house or surrounding area in the past two weeks, no. (%)	38 (31)	172 (31)	1.0 (0.7, 1.6)	0.897	0.6 (0.3, 1.0)	0.056	–	
Soap available to wash hands after the toilet in the previous three weeks, no. (%)	70 (57)	360 (71)	0.5 (0.4, 0.8)	0.002	0.6 (0.4, 0. 98)	0.042	–	
Stores drinking water in drum, no. (%)	0 (0)	20 (4)	2.6 e−07 (0, inf)	0.977	1.2 e −7 (0, inf)	0.984	–	
Used stream or river water for drinking in the last three weeks, no. (%)	0 (0)	4 (1)	7.2 e−07 (0, inf)	0.984	1.1 e −8 (0, inf)	0.992	–	

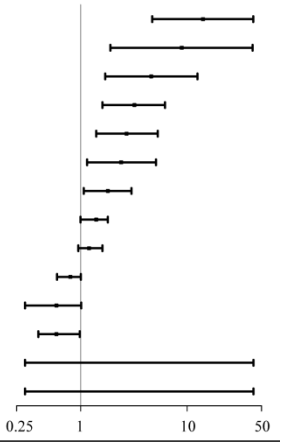

Figure 1

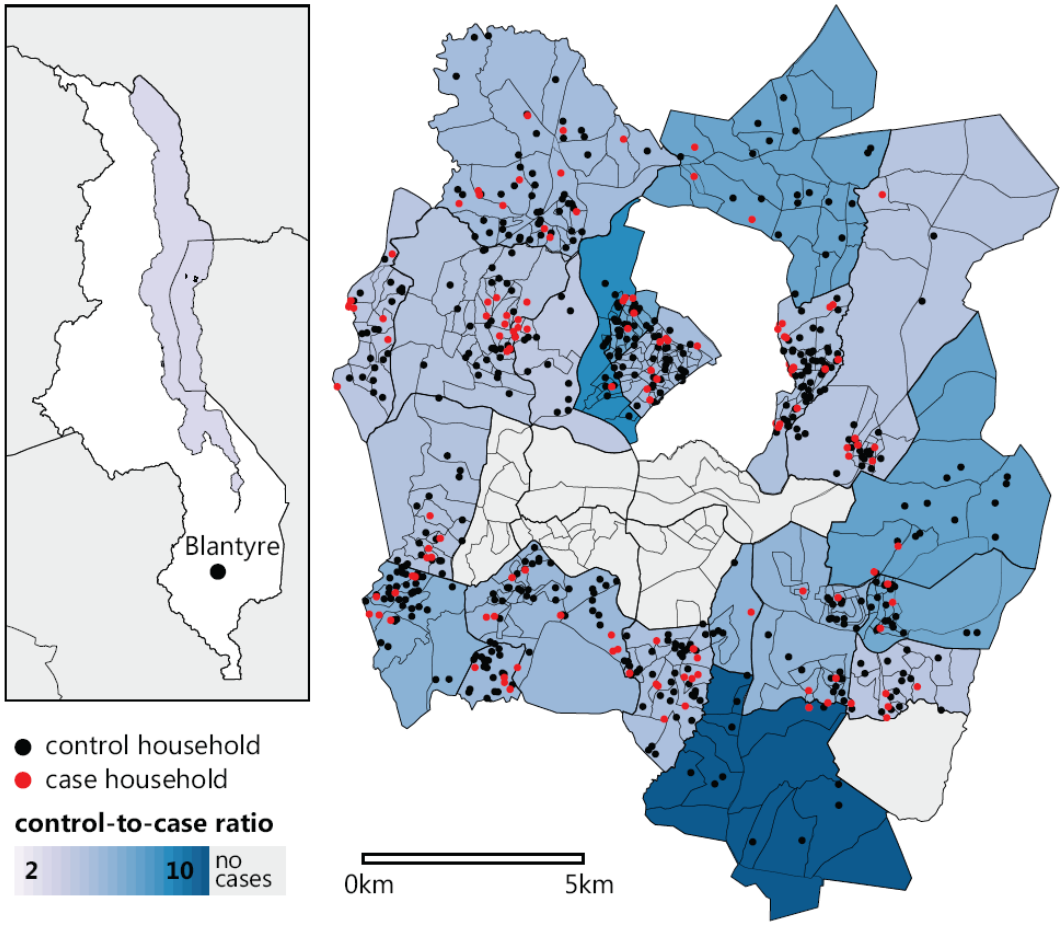

Figure 2