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Malaria Prevalence and Associated Risk Factors among Batwa Indigenous People of Kanungu District in Southwestern Uganda: Does "Place" Matter?

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Abstract

Introduction: Malaria is an important disease, causing high morbidity and mortality, especially in Sub-Saharan Africa. Measuring malaria prevalence using malaria rapid diagnostic tests (mRDTs), particularly among a vulnerable population sub-group, is a vital public health step in discovering effective methods of prevention and control. This study set out to examine the association between "place" and other risk factors with malaria prevalence among Batwa Indigenous People (IP). **Methods:** An analytical cross-sectional study design was implemented. Two surveys January 2014 (n = 572) and April 2014 (n = 541) involving interviews and testing for malaria using mRDTs were conducted in 10 Batwa settlements where a total of 1113 Batwa of all ages were surveyed and tested. The data were first compiled in MS Excel and then imported and analyzed using STATA ver.14. Descriptive statistics, were generated, followed by bivariable and multivariable regression model analysis to establish associations between the predictor and outcome variables with $p \leq 0.05$ considered statistically significant. **Results:** Overall prevalence was 13.94% (n = 146). There is a significant relationship between settlement (place) and malaria prevalence AOR 11.7, 95% CI (1.38 - 98.93), p-value = 0.02. More males 16.97% (n = 84) tested positive compared to females 11.23% (n = 62) but there was no statistically significant association between gender and mRDT (p-value > 0.005). Children less than 5 years registered high prevalence and there was a significant relationship between age and mRDT (p-value ≤ 0.005). Wealth proxy indicators showed no association with prevalence p-value = 0.390. Season had no association with prevalence (p-value = 0.80). However, the proportion of the day spent in the forest/woodlands was significantly associated with malaria prevalence COR 12.83, 95% CI (1.14 - 143.73) p-value = 0.04. Low elevation was significantly associated with malaria prevalence COR 2.42, 95% CI (1.32 - 4.41), p = 0.004 but sleeping under a net and level of education did not show any association with malaria prevalence. **Conclusion:** This study highlights the importance of place in predicting malaria prevalence among Batwa Indigenous People a marginalized and remortely located sub-population. This study has shown that place matters in determining malaria prevalence. However, other factors like age, elevation and gender also contribute to malaria prevalence. Batwa have higher prevalence than the national and even non-indigenous populations in the same district. We recommend targeting hotspots intervention approach since it has proven reasonable impact on reducing malaria prevalence.

Keywords

Batwa Indigenous People, Malaria Prevalence, Place-Related Lifestyles, Season, Socio-Demographic Factors, Uganda

1. Introduction

Malaria is a preventable and curable disease [1], but continues to cause high morbidity and mortality globally, particularly in sub-Saharan Africa [2] [3]. Malaria is a life-threatening disease caused by parasites that are transmitted to people through the bites of infected female A*nopheles* mosquitoes [1]. Globally, there were an estimated 241 million malaria cases and 627,000 malaria deaths in 2020 [3]. Twenty-nine (29) countries accounted for 96% of malaria cases globally, and six (6) countries in sub-Saharan Africa including Uganda (5%), accounted for about 55% of all cases globally. Similarly, 96% of malaria deaths were in 29 countries with 6 African countries (Uganda contributing 5%) accounting for over half of all malaria deaths globally [3].

Malaria transmission in Uganda occurs throughout the year in 95% of the country, while in the remaining highland areas, transmission is unstable and epidemic-prone [4]. Malaria prevalence in Uganda has been declining over the years, from 42% in 2009 to 19% in 2015 and to 9% in 2019 due to implementation of control interventions [5] [6] [7]. The decline is attributed to use of long-lasting-insecticidal nets (LLINs), and indoor residual spraying (IRS) as the most advocated malaria prevention methods like in other malaria endemic countries [1] [8] [9] and use of chemotherapies [1]. In addition, since October 2021, the WHO recommended broad use of the RTS, S/AS01 malaria vaccine among children living in regions with moderate to high *P. falciparum* malaria transmission. The vaccine has been shown to significantly reduce malaria prevalence and deadly severe malaria among young children [1] and is due to be introduced in Uganda at the end of 2023.

Measuring the frequency of a disease (prevalence or incidence) like malaria in a population and identifying how the disease frequency may differ over time or among sub-groups is an important step in discovering potential causes of the disease and determining effective methods for prevention and care [10]. However, measuring disease frequency in populations requires the stipulation of diagnostic criteria. In clinical practice the definition of "a case" generally assumes that, for any disease, people are divided into two discrete classes—the affected and the non-affected. For epidemiological purposes the occurrence of cases of disease must be related to the population at risk giving rise to cases [11]. Dividing the population into the affected and non-affected gives rise to *prevalence* and *incidence* as the commonly used measures of disease occurrence. Prevalence represents the number of cases or infections at a given time (cross-sectional measure), while incidence represents the number of new cases arising over a specified period in the population (dynamic measure) [12] [13] [11]. **Figure 1** illustrates diagrammatically the concept of prevalence and incidence.

A higher burden of malaria prevalence has been documented to exist in the remote and isolated indigenous Ugandan communities of the Batwa IPs compared to their non-Indigenous neighbours and to regional and nationally averages [14] [15] [16] [17] [18]. Relative poverty, not owning a bed net, iron sheet roofing with openings for mosquito entry, and not avoiding mosquito bites were identified as underlying factors for the difference in risk [16]. In addition, environmental factors such as presence of bushes and stagnant water around homes, rainfall, low attitude and high temperatures favour the breeding of malaria vectors as well as parasite reproduction within them [19]. Other scholarship on malaria among Batwa has covered their healthcare seeking behaviour, experiences and barriers to care [20] [21].

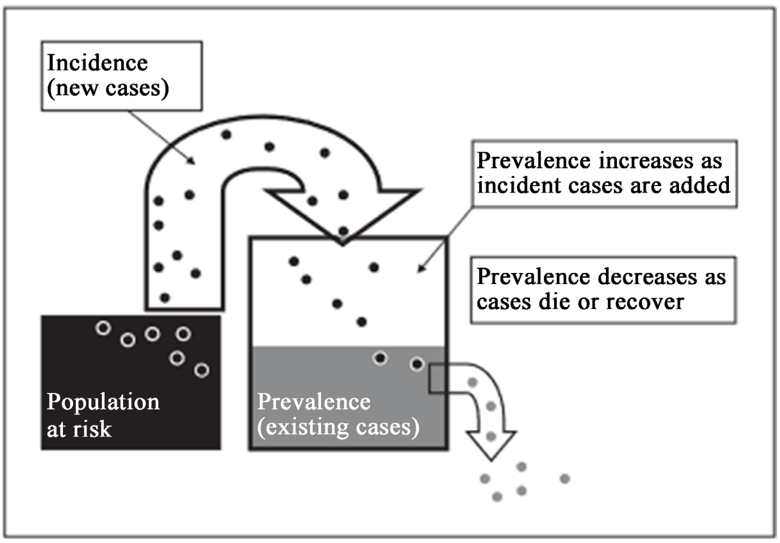

Figure 1. Relationship between incidence and prevalence (Adopted from Noordzij et al., 2010).

While a body of literature exists with malaria prevalence and associated factors in Uganda e.g., [7] [9] [21]-[28], this literature does not bring out malaria prevalence and associated risk factors of minority indigenous populations like the Batwa. To this end, [29] observed that indigenous health in Africa is not widely researched and that publications in this area are sparse. Like in Uganda, in Brazil the indigenous peoples of the Amazon are highly exposed to *Plasmodium* infection, but despite their vulnerability to malaria epidemics, they have been subject to very few epidemiologic studies, thus precluding any possible generalization at the regional level [30]. Further to this knowledge gap among indigenous people, little is known about the extent to which *place* is responsible for malaria prevalence. To the best of our knowledge, no previous study has used *place or geography* and geospatial techniques to understand malaria prevalence among Batwa. Yet, *place* is vital in explaining disease patterns and health outcomes [31] [32] [33] [34] and developing place-specific targeted interventions. Cummins *et al.* 2007 stressed the role of place in shaping health and health inequalities and how some of the relevant empirical research has relied on conventional conceptions of space and place and focused on isolating the "independent" contribution of place-level and individual-level factors. Understanding the arrangement of health services and the location and nature of environmental exposures, is crucial in assessing the interrelations inherent in many health-related risk exposures [31].

This paper set out to examine the association between "place" and other risk factors with malaria prevalence among Batwa Indigenous People (IP). Using quantitative and geospatial methods, this study focused on examining the role of *place* and place-related lifestyles of Batwa and how they are associated with malaria prevalence as a health outcome. More specifically we sought to answer the following questions: 1) How is malaria prevalence spatially distributed? 2) Is there an association between Batwa place/*place*-related lifestyle characteristics and malaria prevalence? 3) Is there an association between elevation and malaria prevalence? 4) Is there an association between season (*i.e.*, dry and rainy season) and malaria prevalence? 5) Is there an association between Batwa socio-demographic characteristics (gender, age, education, wealth, use of ITNs) and malaria prevalence?

2. Methods and Materials

An analytical cross-sectional study design was implemented. Analytical cross-sectional studies provide estimates of disease prevalence in a population and aim to assess associations between different parameters [35] [36]. Two cross-sectional surveys were conducted in January, 27th to 31st and April, 20th to 28th 2014 under Indigenous Health Adaptation to Climate Change (IHACC) program (www.ihacc.ca). The individual survey questionnaires had sections on social demographics, place related lifestyle characteristics and relevant malaria variables. Quantitative data related to the above variables was extracted from this IHACC database for further analysis and to answer the research questions for this study.

2.1. Study Area, Population and Sample Size

The study area is Kanungu district in Southwestern Uganda located 29˚50'E and 0˚45'S of the Equator, covers an area of 1297.5 sq. km. and it borders Rukungiri district to the east, the Democratic Republic of Congo (DRC) in the west, Kabale and Kisoro districts on the south. Like much of Uganda, Kanungu typically experiences two rainy seasons—March to May and August to October—with an annual rainfall of 1000 - 1500 mm. The district is largely a rural area of rolling hills located at an average elevation of 1310 m above sea level [37] [38].

This study investigated malaria prevalence among Batwa indigenous people living in 10 settlements (**Figure 2**): Buhoma, Byumba, Bikuto, Karehe, Kebiremu, Kihembe, Kitahurira, Mukongoro, Rulangara and Kitariro, all in Kanungu district of South western Uganda. The district is largely rural and is characterized

Figure 2. Map of study area showing Batwa settlements.

by meso-endemic malaria transmission with intermittent epidemics of disease [17] [26]. Batwa communities are small with 25 - 200 people, with total Batwa population ranging between 500 and 700 [38].

The Batwa were traditional hunter-gatherers from Uganda, Burundi, Rwanda, and Eastern Democratic Republic of Congo [29] [39] [40]. In Kanungu district they originally lived in the Bwindi Impenetrable Forest as hunter-gatherers till 1991 when they were removed by government to establish a national park to protect mountain gorillas. They now live in the 10 settlements and engage in small subsistence cultivation, tourism activities and offering paid labour for food and money [39] [41]. Batwa settlements are generally characterized by poorer conditions with less access to adequate shelter, safe water and sanitation infrastructure compared to their non-indigenous neighbours [22] [42].

In terms of sample size, because the target population of the Batwa in Kanungu district is small (n = 500 - 700), IHACC surveys conducted a full census where all the Batwa were invited to participate including children. For children under 12 years, responses were solicited from family adult members especially parents or guardians.

2.2. Data Collection Procedures and Tools

A census covering all Batwa was carried out administering face-to-face survey questionnaires and taking mRDTs by surveyors and a community nurse respectively (**Figure 3**). In both surveys of January and April 2014, 130 and 133 households were covered, capturing Batwa of all ages viz. January 2014 (n = 572) and April 2014 (n = 541). January and April represent two different seasons in the area, namely January the dry and April rainy seasons respectively [43]. Prior to the first survey in January a group of surveyors was trained and the survey questionnaire pre-tested to ensure consistency and flow of the questions.

2.3. Survey Questionnaire

The survey questionnaire was designed to capture: the settlement of residence, socio-demographic characteristics of the participants (gender, age, highest education attained, and whether they slept under an insecticidal treated net (ITN) the previous night to the survey). The questionnaire also covered participants' 10 lifestyle characteristics that are place-related and deemed to have bearing on exposure to malaria vectors (these included the proportion of the day spent around the home, animal sheds, fields/cropland/pasture, forest, by the river/lake, and proportion of the night spent around the home, animal sheds, fields/cropland/pasture, forest/woodland and by the lake/river. Survey questionnaire also captured information on household ownership of goods viz. radio, cellphone and animals that were to be used to compute a wealth index. The survey data were extracted from the main IHACC dataset and compiled in Microsoft Excel tables following a Codes Book for all questions and expected response.

2.4. Measuring Malaria Prevalence Using mRDT

We used SD BIOLINE Malaria Ag P.f. test guidelines, a one-step rapid, qualitative detection of HRP-II (Histidine-rich protein II) specific to *P. falciparum* in human blood sample. Finger-prick blood samples were collected from all participants performed by a community nurse in each survey team (**Figure 3**). Whoever tested positive would be immediately referred to Bwindi Community Hospital for treatment. Verbal consent was obtained from each individual and for children it was granted by the child's parent or guardian.

Figure 3. A community nurse taking a blood sample (above) and (below) applying the blood to a malaria rapid diagnostic test (mRDT) during the survey (Photo credit: D. Namanya).

2.5. Geographic Data Collection

Batwa households were mapped using handheld Global Positioning System (GPS) Garmin Etrex H2.8 to capture their geographic locations and elevation. The mapping was done by the principal investigator and one surveyor who was trained in using the GPS machine and familiar with all the Batwa settlements. The geographic locations of all 10 Batwa settlements were obtained from IHACC. Other geographic layers e.g., shapefiles of administrative boundaries, road network, digital elevation model were obtained from relevant agencies like Uganda Bureau of Statistics (UBOS) and Uganda National Roads Authority.

2.6. Data Analysis

Data analysis involved quantitative statistical analysis and geospatial analysis. First, tables extracted in MS Excel containing all the 9 variables of interest were used to produce descriptive statistics on malaria prevalence, socio-demographic and place-related lifestyle characteristics of the participants (**Table 1**). Secondly, the Excel tables were exported to STATA SE 14 for further quantitative analysis. At univariate analysis, the distribution of respondents by the different variables was determined and summarized in frequencies and proportions. At bivariate analysis, the association between malaria prevalence and each of the independent variables was assessed by ordinary logistic regression using STATA SE 14 to generate Crude Odds Ratios (CORs), their 95% confidence intervals (CI) and p-values (p). Multi-variable analysis was applied to all variables that were significantly associated with malaria prevalence at bivariate analysis where a cutoff point of $p \leq 0.2$ was considered for factors taken to multivariable level. All statistical tests were two-sided, 95% CIs were used and p-values of ≤ 0.05 were considered statistically significant. All the results from the analysis are presented in tables, bar graphs and pie charts. To compute an average wealth index, information on household ownership of radio, cellphone and animal (s) was analysed using principal components analysis (PCA) and then classified into an index of 1 - 3 *i.e.*, Least Poor, Poor and Poorest.

3. Ethics Approvals

Approval for this study was granted by Mbarara University of Science and Technology Research Ethics Committee (MUST-REC) reference no. 29/04-18, Uganda National Council of Science and Technology (UNCST) reference HS2460 and McGill University research ethics board reference A11-M120-13B under the IHACC project. Participants provided verbal informed consent and for those aged under 18 years verbal informed consent for the study was obtained from their parents or guardians. Before the consent was obtained trained surveyors provided information regarding the study objectives and goals to the participants, gave contacts in case participants had questions, and informed participants that they could leave the study any time, and that non-participation would not deny them any involvement in other research activities. For the

Table 1. Socio-demographics and other variables.

Variable	Variable categories	Total n. participants	Percentage
Settlement	Bikuto	75	13.91
	Buhoma	31	5.75
	Byumba	67	12.43
	Karehe	55	10.20
	Kebiremu	58	10.76
	Kihembe	74	13.73
	Kitahurira	56	10.39
	Kitariro	72	13.36
	Mukongoro	44	8.16
	Rurangara	7	1.30
Gender	Male	245	46.05
	Female	287	53.95
Age Category	0 - 5	18	3.37
	6 - 12	113	21.16
	13 - 18	114	21.35
	20 - 30	102	19.10
	31 - 40	77	14.42
	41 - 50	43	8.05
	51+	67	12.55
Highest education attained	No formal schooling (cannot read or write)	232	43.36
	No formal schooling (can read or write)	5	0.93
	Nursery school	232	43.36
	Primary incomplete	48	8.97
	Primary complete	2	0.37
	Secondary complete	2	0.37
	Higher education of any form	3	0.56
	No response	11	2.06
Slept under ITN	No	126	58.88
	Yes	77	35.98
	Unsure	2	0.93
	No answer	9	4.21
Elevation/Altitude (M above sea level)	Low	132	24.49
	Medium	160	29.68
	High	247	45.83
Wealth class	Poorest	184	35.05
	Poor	160	30.48
	Least poor	181	34.48

mRDTs, whoever tested positive would be immediately referred to Bwindi Community Hospital for treatment.

4. Results

4.1. Socio-Demographic Characteristics

A total of 1113 individuals from the 10 Batwa settlements in Kanungu district participated in the two cross-sectional surveys viz. in January n = 572 and April n = 541. In January 46.06% (n = 245) were male while 53.95% (n = 287) were female. The response rate was 98.4% (563/572) in January and 92.6% (501/541) in April respectively.

In terms of age categories, the majority of the participants were below 30 years 64.98% (n = 347) while those aged 31 years and above accounted for 35.02% n = (187). Results regarding education attainment show that Batwa are distributed into two dominant categories *i.e.*, those who cannot read and write 43.36% (n = 232) and those who attained equivalent of nursery education 43.36 (n = 232). Less than 2% (n = 7) have either completed primary, secondary or higher education of any form (**Table 1**).

The survey also captured information on whether participants "slept under a net" or "slept under ITN" the night prior to the survey. Results show that majority of the Batwa did not sleep under a net 76.26% (n = 408) and 58.8% (n = 126) did not sleep under ITN Only 21.31% (n = 114) and 35.98% (n = 77) of respondents reported sleeping under a net and an ITN respectively (**Table 1**).

4.2. Malaria Prevalence

Out of the 1,029 individuals tested using mRDT, 13.94% (n = 146) tested positive, while 84.34% (n = 883) tested negative and 1.72% (n = 18) declined the test. This translated to the overall cross-sectional malaria prevalence of 13.94% (**Figure 4** & **Figure 5** and **Table 2**). There is a significant association between settlement (Kebiremu) and malaria prevalence (mRDT) (p-value = 0.002) (**Table 5**).

In terms of gender, more males 16.97% (n = 84) tested positive compared to females 11.23% (n = 62). Findings show that there was no association between gender and malaria prevalence (p-value = 0.39) 95% CI (**Table 2**). This therefore suggests that gender may not explain the variation in malaria prevalence.

Children aged less than or equal to two years 5.42% (n = 57), 3 years 3.80% (n = 40) and those aged between greater than or equal to 4 years less than or equal to 5 years 3.42% (n = 36) were more likely to test positive compared to individuals aged greater or equal to 6 years 1.52% (n = 16) (**Table 3**). Results show there is a significant association between age and mRDT (p-value ≤ 0.005) at 95% CI. This implies that variations in prevalence can be explained by differences in age.

Among those who tested positive using our wealth index showed that the most frequently owned assets of the participants were a radio 5.33% (n = 54), cellphone (36.03%) and animals (32.08%). There is no association between wealth and mRDT using our wealth proxy indicators of "own radio, own cellphone, own

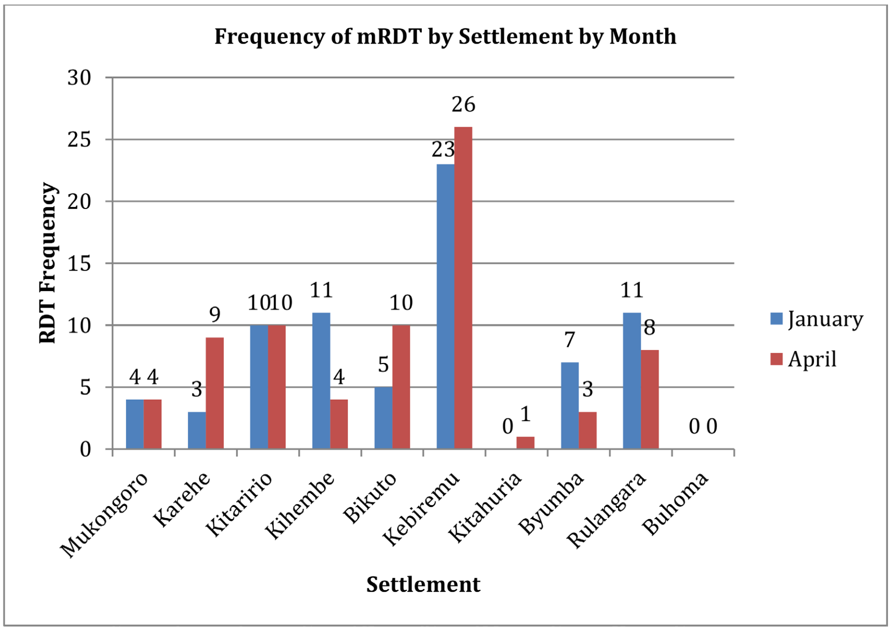

Figure 4. Batwa malaria cases by settlement and month.

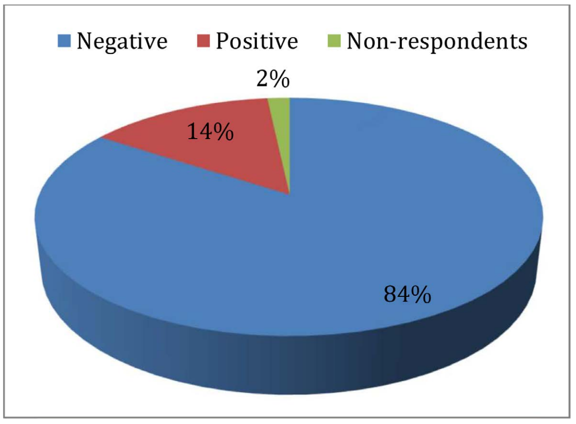

Figure 5. Overall malaria prevalence.

Table 2. Shows the relationship between RDTs and gender.

RDT	GENDER		Total
	Male	Female	
Negative	403	480	883
	81.41	86.96	84.34
Positive	84	62	146
	16.97	11.23	13.94
Non-respondents	8	10	18
	1.62	1.81	1.72
Total	495	552	1047
	100	100	100.00

Table 3. Shows the association between mRDTs and age among respondents.

RDT	AGE CATEGORY				Total
	≤2 years	3 years	≥4 and ≤5 years	≥6 years	
Negative	205	172	318	190	885
	19.49	16.35	30.23	18.06	84.13
Positive	57	40	36	16	149
	5.42	3.80	3.42	1.52	14.16
Non-respondents	1	2	10	5	18
	0.10	0.19	0.95	0.48	1.71
Total	263	214	364	211	1052
	25.00	20.34	34.60	20.06	100.00

animals", p-value = 0.05.

4.3. Spatial Distribution of Malaria Cases

Generally, Kebiremu settlement had the highest number of malaria cases in both cross-sectional surveys in January (n = 23) and April (n = 26). Kitariro follows with January (n = 10) and April (n = 10). Rulangara settlement comes third in terms of malaria cases with January (n = 11) and April (n = 08). Kitahurira registered no case in January and only 1 case in April, while Buhoma registered no case in both January and April (Table 4, Figure 4).

4.4. Spatial Malaria Prevalence

Prevalence represents the number of cases or infections at a given time (cross-sectional measure), as a percentage of the population at risk [12] [13]. Our analysis showed that malaria prevalence in some Batwa settlements was higher than 14.02% the overall among the Batwa revealed by this study. Kebiremu settlement with 35.38% and 36.11% in January and April respectively, had the highest malaria prevalence. Rulangara settlement follows with 21.57% and 18.6% in January and April respectively. Karehe and Kitariro settlements also experienced high malaria prevalence of more than 10% in January and more than 20% in April. (Figure 6)

4.5. Batwas' Place-Related Life-Style Characteristics

Results from analysis of place-related lifestyle characteristic (*i.e.*, how much time of the day or night is spent in certain places) are presented in Table 5. According Table 5 variable 1) the participants frequently spent less than a day around the home 39.21% (n = 209), followed by those who spent all day 29.46% (n = 157) and followed by those who spent about half the day 19.51% (n = 104). The rest of the variable categories have ≤ 6%.

Regarding variable (2) proportion of the day spent in or around the animal

Table 4. Malaria prevalence distribution by settlement.

Settlement	mRDT			Total
	Negative	Positive	Non-respondent	
Mukongoro	137	8	1	146
	12.89	0.75	0.09	13.73
Karehe	43	12	0	55
	4.05	1.13	0.00	5.17
Kitariro	101	20	3	124
	9.50	1.88	0.28	11.67
Kihembe	105	15	0	120
	9.97	1.41	0.00	11.38
Bikuto	106	15	0	121
	9.97	1.41	0.00	11.38
Kebiremu	84	49	4	137
	7.90	4.61	0.38	12.89
Kitahuria	95	1	5	101
	8.94	0.09	0.47	9.50
Byumba	137	10	3	150
	12.89	0.94	0.28	14.11
Rulangara	75	19	0	94
	7.06	1.79	0.00	8.84
Buhoma	13	0	2	15
	1.22	0.00	0.19	1.41
Total	896	149	18	1063
	84.29	14.02	1.69	100.00

sheds over 95% (n = 498) of Batwa fell under the variable category of "None", leaving other variable categories with less than 2%. Another lifestyle variable (3) was proportion of the day spent in the fields/cropland/pasture, where half of the participants 50.67% (n = 256) responded "None" and, 28.30% (n = 148) spent about half a day.

According to results for variable (4) proportion of day time in the forest or woodlands, 83.69% (n = 436) of the total participants responded "None" leaving only 14.59% (n = 76) that spent less than half a day and the rest of the variable categories with < 1%. Majority of participants 68.39% (n = 354) did not spend any time by the river or lake (variable 5), with only 30.46% (n = 159) spending less than half a day.

Variable (6) covered the proportion of the night spent in different places. According to the results; over 92% (n = 494) of the participants spent all night

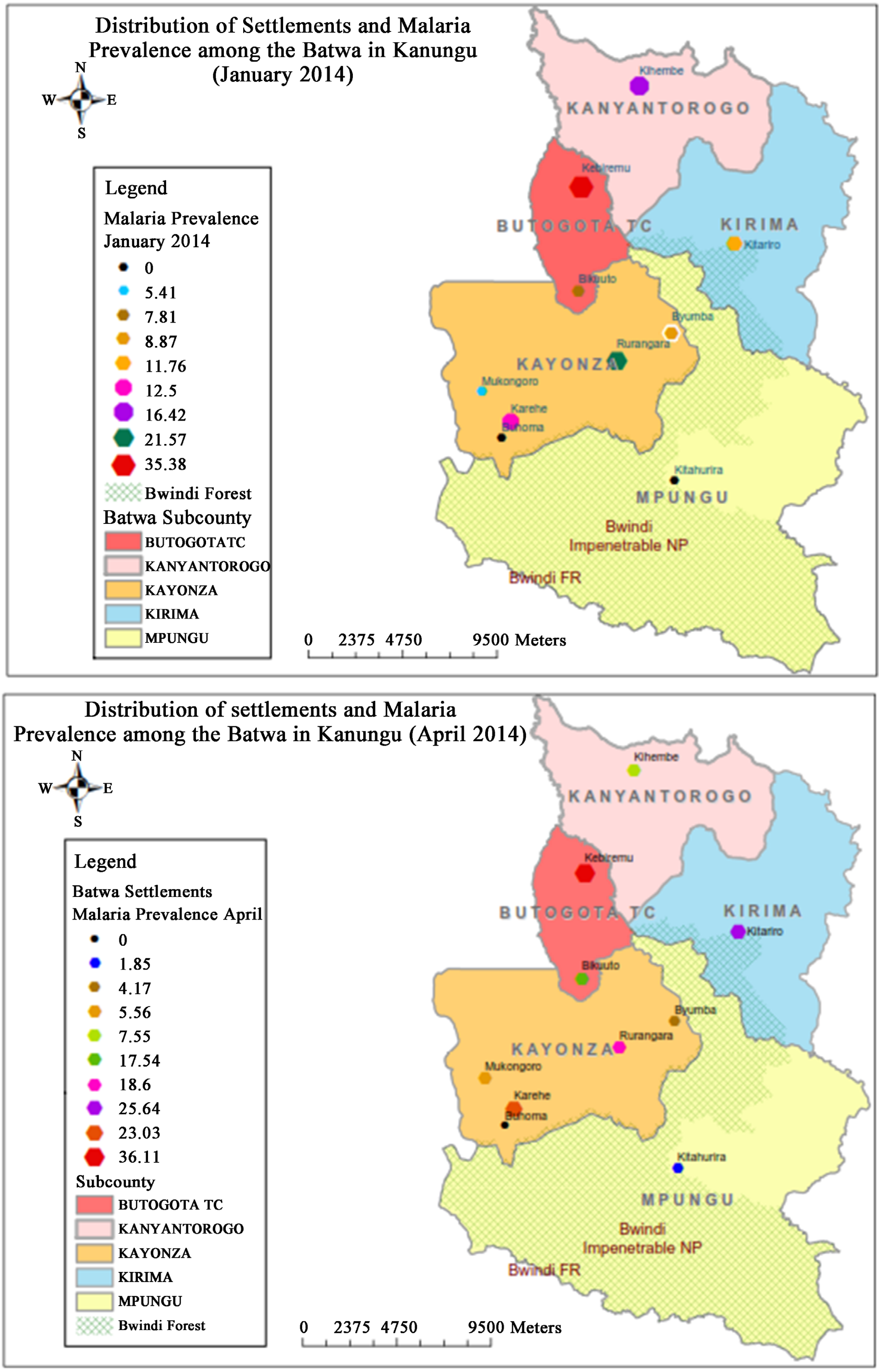

Figure 6. Malaria prevalence by settlement in January and April 2014.

Table 5. Batwas' place-related lifestyle characteristics.

Variable	Variable categories	Frequency	Percentage
1) Proportion of the day spent in/around the home	None	23	4.32
	Less than half the day	209	39.21
	About half the day	104	19.51
	More than half the day	35	6.57
	All day	157	29.46
	No response	5	0.94
2) Proportion of the day spent in/around animal sheds	None	498	95.95
	Less than half the day	9	1.73
	About half the day	2	0.39
	More than half the day	4	0.77
	No response	6	1.16
3) Proportion of the day spent in the fields/cropland/pasture	None	265	50.67
	Less than half the day	53	10.13
	About half the day	148	28.30
	More than half the day	49	9.37
	All day	2	0.38
	Unsure	1	0.19
	No response	5	0.96
4) Proportion of the day spent in the forest/woodland	None	436	83.69
	Less than half the day	76	14.59
	About half the day	3	0.58
	More than half the day	1	0.19
	No response	5	0.96
5) Proportion of the day spent on/by the river or lake	None	357	68.39
	Less than half the day	159	30.46
	About half the day	1	0.19
	No response	5	0.96
6) Proportion of the night spent in/around the home	None	14	2.63
	Less than half the night	7	1.31
	About half the night	1	0.19
	More than half the night	13	2.44
	All night	494	92.68
	No response	4	0.75

Continued

7) Proportion of the night spent in/around animal sheds	None	496	99.00
	Less than half the night	1	0.20
	No response	4	0.80
8) Proportion of the night spent in the fields/cropland/pasture	None	492	98.20
	Less than half the night	3	0.60
	All night	2	0.40
	No response	4	0.80
9) Proportion of the night spent in the forest/woodland	None	495	99.20
	No response	4	0.80
10) Proportion of the night spent on/by the river or lake?	None	488	97.60
	Less than half the night	8	1.60
	No response	4	0.80

in/around the home. Almost every participant (99%) reported they did not spend any time in/around animal sheds (variable 7). Other variables related to proportion of night spent in the fields/cropland/pasture (variable 8) or forest/woodland (variable 9) and by the river/lake (variable 10). The results show that for all the three variables 8, 9 and 10, almost all participants 98.20% (n = 492), 99.20% (n = 495) and 97.60% (n = 488) fell under the response "None" variable category.

4.6. Malaria Prevalence and Seasons

A key question of this study was to find out whether there are differences in malaria in between the dry and wet (rainy) seasons. Findings show that malaria prevalence as measured by positive mRDT was 14% (n = 74) in January which is a dry season and 15% (n = 75) in April which is a wet (rainy) season **Figure 7**.

The test of equality of proportions was used to compare malaria prevalence in January (dry season) and April (rainy season). The results show that the difference between malaria prevalence in January 15, 95% CI (0.12 - 0.20) and April Mean 13, 95% CI: (0.10 - 0.16) was not statistically significant (p = 0.80) (**Table 6**).

4.7. Bivariate Analysis of Socio-Demographic Characteristics and Malaria Prevalence

A bivariate model was constructed to assess the association between participants' socio-demographic characteristic and malaria prevalence. The results of the bivariate model analysis are presented in **Table 7**. Using the Batwa settlement of residence (place) as a predictor variable, only living in Kebiremu 11% was significantly associated with malaria prevalence (COR 10.9, 95% CI: (2.40 - 48.80), p-value = 0.002). All other factors were not significantly associated with malaria

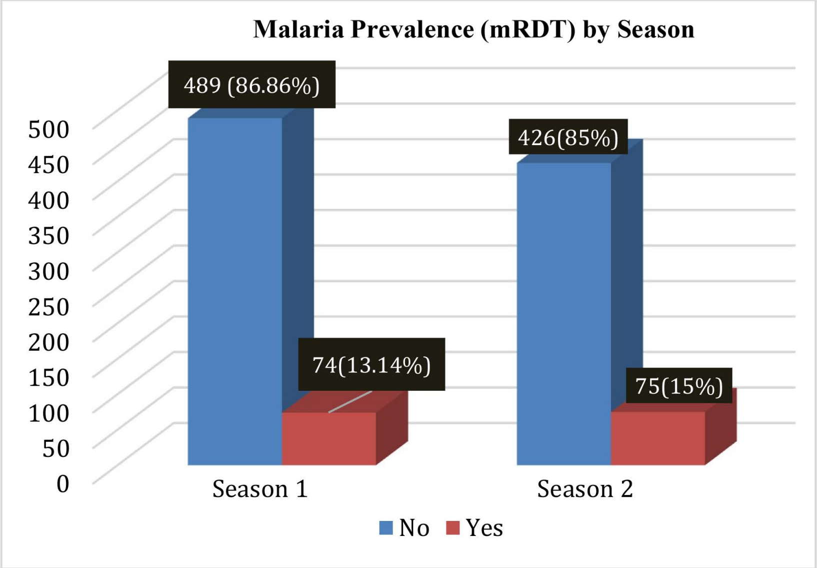

Figure 7. Malaria prevalence in January and April 2014.

Table 6. Test of equality between malaria prevalence in rainy season and dry season.

Variable	Mean (95% CI)	Standard error	p-value
Prevalence in season 1	15 (0.12 - 0.20)	1.6	
Prevalence in season 2	13 (0.10 - 0.16)	1.4	
Difference	0.018 (−0.02 - 0.06)	0.021	
Ho: difference = 0		0.021	0.39
Ha: Ha: diff < 0			0.80
Ha: diff > 0			0.19

prevalence. However, low elevation (COR: 2.42, 95% CI (1.32-4.41), p-value = 0.004) was significantly associated with malaria prevalence.

4.8. Bivariate Analysis Place-Related Lifestyle Characteristics and Malaria Prevalence

Results from the association of participants' place-related characteristics and malaria prevalence indicated that only spending about half the day in the forest or woodland was significantly associated with malaria prevalence at COR 12.83, 95% CI (1.14 - 143.73), p = 0.004 (Table 8). Similarly, low elevation was significantly associated with malaria prevalence COR 2.42, 95% CI (1.32 - 4.41), p = 0.004 (Table 7) But sleeping under a net and level of education did not show any association with malaria prevalence (Table 7).

4.9. Multivariable Analysis of Batwas' Socio-Demographic and Place-Related Life-Style Characteristics with Malaria Prevalence

Five predictor variables were considered for the final multivariable regression

Table 7. Association of Batwas' socio-demographic characteristics and malaria prevalence at bivariate level.

Variable	Variable categories	Code	Freq (%)	COR (95% CI)	p-value
Settlement (n = 1111)	Kitahurira	1	56 (10.39)	ref	
	Bikuto	6	75 (13.91)	3.7 (0.77 - 18.00)	0.101
	Buhoma	7	31 (5.75)	4.0 (0.70 - 23.20)	0.122
	Byumba	8	67 (12.43)	3.7 (0.76 - 18.30)	0.106
	Karehe	9	55 (10.2)	4.7 (0.95 - 23.20)	0.058
	Kebiremu	10	58 (10.76)	10.9 (2.40 - 48.80)	0.002
	Kihembe	11	74 (13.73)	3.2 (0.62 - 16.80)	0.162
	Kitariro	13	72 (13.36)	4.3 (0.91 - 20.75)	0.065
	Mukongoro	14	44 (8.16)	2.1 (0.33 - 13.02)	0.435
	Rurangara	15	7 (1.30)	-	-
Gender (n = 1094)	Male	1	245 (46.05)	ref	
	Female	2	287 (53.95)	0.80 (0.48 - 1.33)	0.39
Age Category (n = 1113)	20 - 30	1	102 (19.1)	ref	
	0 - 5	4	18 (3.37)	0.93 (0.19 - 4.54)	0.92
	6 - 12	2	113 (21.16)	1.16 (0.51 - 2.61)	0.72
	13 - 18	3	114 (21.35)	0.81 (0.34 - 1.92)	0.63
	31 - 40	5	77 (14.42)	1.36 (0.58 - 3.2)	0.47
	41 - 50	6	43 (8.05)	1.48 (0.54 - 4.07)	0.44
	51+	7	67 (12.55)	1.34 (0.54 - 3.3)	0.65
Highest education attained (n = 1097)	No formal schooling	1	237 (45.23)	ref	
	Nursery school/ Primary	3	280 (53.44)	0.77 (0.46 - 1.29)	0.32
	Secondary	5	04 (0.76)	1.95 (0.19 - 19.31)	0.57
	Higher education	7	03 (0.57)	-	-
Slept under a net (n = 1099)	Yes	1	114 (21.84)	ref	
	No	2	408 (78.16)	1.19 (0.63 - 2.28)	0.58
Slept under ITN (n = 423)	Yes	1	77 (37.93)	ref	
	No	2	126 (62.07)	2.24 (0.86 - 5.86)	0.09
Elevation/Altitude	High	1	247 (45.83)	ref	
	Medium	2	160 (29.68)	1.33 (0.71 - 2.49)	0.38
	Low	4	132 (24.49)	2.42 (1.32 - 4.41)	0.004
Wealth	Least poor	1	181 (34.48)	ref	
	Poor	2	160 (30.48)	1.05 (0.56 - 1.96)	0.87
	Poorest	3	184 (35.05)	1.03 (0.56 - 1.87)	0.93

Table 8. Association of Batwas' place-related lifestyle characteristics and malaria prevalence at bivariate level.

Variable	Variable categories	Freq (%)	COR (95% CI)	p-value
Proportion of the day spent in/around the home	None	23 (4.36)	ref	
	Less than half the day	209 (39.58)	0.84 (0.23 - 3.04)	0.79
	About half the day	104 (19.70)	0.98 (0.25 - 3.78)	0.98
	More than half the day	35 (6.63)	0.86 (0.17 - 4.25)	0.85
	All day	157 (29.73)	1.28 (0.35 - 4.64)	0.70
Proportion of the day spent in/around animal sheds	None	498 (97.08)	ref	
	Less than half the day	9 (1.75)	-	-
	About half the day	2 (0.39)	6.67 (0.41 - 108)	0.18
	More than half the day	4 (0.78)	-	-
Proportion of the day spent in the fields/cropland/pasture	None	265 (51.26)	ref	
	Less than half the day	53 (10.25)	0.86 (0.34 - 2.17)	0.75
	About half the day	148 (28.63)	0.96 (0.52 - 1.74)	0.89
	More than half the day	49 (9.48)	0.73 (0.27 - 1.97)	0.54
	All day	2 (0.39)	-	-
Proportion of the day spent in the forest/woodland	None	436 (84.5)	ref	
	Less than half the day	76 (14.73)	0.56 (0.23 - 1.34)	0.19
	About half the day	3 (0.58)	12.83 (1.14 - 143.73)	0.04
	More than half the day	1 (0.19)	-	-
Proportion of the day spent on/by the river or lake	None	357 (69.05)	ref	
	Less than half the day	159 (30.75)	0.69 (0.38 - 1.26)	0.23
	About half the day	1 (0.19)	-	-
Proportion of the night spent in/around the home	None	14 (2.65)	ref	
	Less than half the night	7 (1.32)	2.17 (0.11 - 40.81)	0.60
	About half the night	1 (0.19)	-	-
	More than half the night	13 (2.46)	3.90 (0.35 - 43.36)	0.27
	All night	494 (93.38)	1.93 (0.25 - 15.05)	0.53
Proportion of the night spent on/by the river or lake	None	488 (98.39)	ref	
	Less than half the night	8 (1.61)	0.95 (0.11 - 7.83)	0.96

model (Table 9). After adjusting for settlement, slept under ITN, proportion of the day spent in the forest/woodland and proportion of the day spent on/by the river, results show that, living in Kebiremu was significantly associated with malaria prevalence AOR 11.7, 95% CI (1.38 - 98.93), p = 0.02.

5. Discussion

Findings of this study on prevalence highlight important insights into malaria

Table 9. Association of Batwas' place-related lifestyle characteristics and malaria prevalence at multivariable level.

Variable	Variable categories	Freq (%)	COR (95% CI)	p-value	AOR (95% CI)	p-value
Settlement	Kitahurira	56 (10.39)	1		ref	
	Bikuto	75 (13.91)	3.7 (0.77 - 18.00)	0.101	6.2 (0.65 - 58.90)	0.11
	Buhoma	31 (5.75)	4.0 (0.70 - 23.20)	0.122	-	-
	Byumba	67 (12.43)	3.7 (0.76 - 18.30)	0.106	2.1 (0.11 - 37.05)	0.61
	Karehe	55 (10.2)	4.7 (0.95 - 23.20)	0.058	-	-
	Kebiremu	58 (10.76)	10.9 (2.40 - 48.80)	0.002	11.7 (1.38 - 98.93)	0.02
	Kihembe	74 (13.73)	3.2 (0.62 - 16.80	0.162	-	-
	Kitariro	72 (13.36)	4.3 (0.91 - 20.75)	0.065	7.8 (0.79 - 76.52)	0.08
	Mukongoro	44 (8.16)	2.1 (0.33 - 13.02)	0.435	13.8 (0.82 - 232.25)	0.07
	Rurangara	7 (1.30)	-	-	-	-
Slept under ITN	Yes	77 (37.93)	1		ref	
	No	126 (62.07)	2.24 (0.86 - 5.86)	0.09	2.45 (0.72 - 8.40)	0.15
Proportion of the day spent in the forest/woodland	None	436 (84.5)	1		ref	
	Less than half the day	76 (14.73)	0.56 (0.23 - 1.34)	0.19	0.31 (0.03 - 3.30)	0.33
	About half the day	3 (0.58)	12.83 (1.14 - 143.73)	0.04	-	-
	More than half the day	1 (0.19)	-	-		
Proportion of the day spend on/by the river	None	357 (69.05)	1		1	
	Less than half the day	159 (30.75)	0.69 (0.38 - 1.26)	0.23	1.05 (0.31 - 3.52)	0.93
	About half the day	1 (0.19)	-	-	-	-

burden and underlying factors among Batwa IPs of Kanungu district Southwestern Uganda. More importantly, this study used a multilevel analysis combining Batwa place-related lifestyle variables together with socio-demographic and geographic variables to underpin the association of place and malaria prevalence. While many studies and surveys have been conducted at national and sub national levels [5] [6] [7] [9] [25] [26] [44] [45], even within the Batwa [16] [22] [43], none to the best of our knowledge has explicitly interrogated the role of place in the distribution of malaria prevalence among the Batwa. With a response rate of over 90% this paper attempts to fill this knowledge gap.

The overall malaria prevalence among Batwa stood at 13.94% as established by positive mRDTs. This is higher than the national malaria prevalence of 9% and 1% for Kigezi region [25], and [16] who found out that Batwa had a prevalence of 9.35% malaria parisitaemia shortly after a free ITN distribution campaign. However, the malaria prevalence results of Batwa in this study are comparable with previous studies that put it at 12% for the southwestern region and consistent with very high and stable transmission of malaria in most of Uganda [46]. Batwa malaria prevalence of 14.02% is low compared to 46.6% in southwest Nigeria [47], 29.2% in Democratic Republic of Congo (RDC) [19] and 22% peaking at 44% in northwestern Ethiopia [48]. Some of the underlying risk factors for this high prevalence among Batwa have been linked to relative poverty, not owning a bed net, iron sheet roofing with openings for mosquito entry, not avoiding mosquito bites, malnutrition, stagnant water and bushy environment around homesteads [16] [17] [23] [48]. Relatedly, [43] strongly suggested that indigenous Batwa in Kanungu are a population at heightened risk of malaria exposure and infection. After adjusting for age, infection status, elevation, and clustering by villages, [43] found that the Batwa had two-fold higher odds of being malaria seropositive compared with the Bakiga (OR = 2.08, 95% CI = 1.51 - 2.88). In line with this study, it was discovered that majority of Batwa 76.6% (n = 408) did not sleep under any net, and 58% did not sleep under an ITN the night prior to the survey. This means that mosquito net usage stood at 23.4% for any net and 42% for ITNs, compared to national 60% and Kigezi region 55% ITN usage [25]. This finding suggests high exposure to mosquito bites due to less protection from use of nets which may partly be responsible for the high malaria prevalence among Batwa. Such high malaria prevalence rates warrant sharpened and focused interventions to achieve the goal of malaria elimination [49].

The bivariate analysis, using Batwa settlement (place) as predictor variable revealed that living in Kebiremu was significantly associated with malaria prevalence (COR10.9, 95% CI: (2.40 - 48.80), p = 0.002). The multivariable model after adjusting for other predictor variables (slept under net, proportion of day spent in forest/woodland and proportion of day spent by the river) further significantly associated Kebiremu with malaria prevalence AOR 11.7, 95% CI (1.38 - 98.93), p = 0.02. This means that there is a significant relationship between place and malaria prevalence. The finding indicates that someone living in Kebiremu was about 11 times more likely to suffer from malaria compared to other settlements. These findings bring out the importance of "place" to health status that became increasingly clear in the last decades of the twentieth century because places in which people work and live have an enormous impact on their health [50].

To further interrogate the importance of place, this study identified Batwa place-related lifestyle characteristic (*i.e.*, how much time of the day or night is spent in certain places) and critically examined the association between them and malaria prevalence. This analysis was intended to show how and where one is at a given time of the day or night may lead to relative exposure to malaria vectors. Out of the 10 variables (**Table 2**), our findings indicated that only spending "about half the day in the forest or woodland" was significantly associated with malaria prevalence at COR 12.83, 95% CI (1.14 - 143.73), p = 0.04. This suggests that spending time in the forests and woodland increases the exposure and likelihood of catching malaria about twelve times. This finding is supported by studies that have shown *Anopheles* have changed behaviour from indoor to outdoor and resting and biting tendencies [4]. To this end, [51] stated that physical environment, and people's proximity and exposure to vectors or parasites including microbiological and parasitological factors, are clearly essential for transmission of infection and constitute necessary and immediate risk factors. However, poor diet and relative undernourishment [17] [52] and environment conditions as well as bites of mosquitoes are also perceived to be underlying causes of malaria [52]. It has also been reported that the main reason for increased malaria trends may be the change in *Anopheles* behaviour favouring outdoor biting and resting tendence or due to the insecticide resistant vector species or drug resistant *Plasmodium* parasites [4] [48].

The results for gender showed a significant relationship between gender and mRDT (p-value ≤ 0.005). Surprisingly, the results from this study indicated that malaria infections were more prevalent among males compared to females. This is consistent with other studies in Ethiopia [48], Uganda [16], Kenya [53], although this finding counters the longstanding vulnerability of females (especially pregnant women) and children usually leading to higher prevalence rates among these sub groups [1] [3] [17]. Contrary to this finding, [18] in a study of hospital admissions in the same study area found that gender for malaria cases was relatively evenly distributed, with 53.1% being female. It is likely that the higher malaria cases in males are a result of stakeholders focusing interventions like ITN distribution and health education on women and children, unfavorably leaving out males. Past studies have also attributed high malaria prevalence in males to outdoor activities in the evenings and night that increase exposure and transmission [48] [53].

Age is a crucial underlying risk factor that affects malaria infections. Results of this study revealed a significant relationship between age and mRDT (p-value ≤ 0.005) at COR95% CI. This implies that variations in prevalence can be explained by differences in age. Like [19] our study showed that individual variables like lower age, low household wealth index and gender significantly increased the odds of having a positive mRDT.

According to this study low elevation showed significant association with malaria prevalence COR95% CI 2.42 (1.32 - 4.41) p-value = 0.004. This finding is consistent with [43] and [19] that characterized higher elevations with low risk of malaria infection and vice versa. Noteworthy in this study, is that the descriptive statistics also generally showed low lying settlements like Kebiremu (1100 M above sea level, a.s.l.) and Kihembe (1172 M a.s.l.) to have high malaria prevalence compared to those located at high elevation e.g., Kitahurira (1652 M a.s.l.). This finding suggests that even within a small area difference in elevation may have a bearing on prevalence. However, this physical association of malaria and environment is increasingly being distorted by climate change with malaria vectors moving to high elevation ranges [18] [54] [55] [56].

This research also explored the association of season (*i.e.*, dry and wet/rainy) and malaria prevalence. Our results showed that there is no association between season and malaria prevalence among Batwa. This is inconsistent with [44] who established malaria seasonal peaks in February-March and June-July in south-western Uganda and [45] April-May and September-November in Northern Uganda. Relatedly, [57] and [58] also established a significant positive correlation between malaria prevalence and monthly rainfall in India. For Kanungu district where this study was conducted rainfall is bimodal with peaks in April and October [43] and therefore it was expected that malaria prevalence would be higher in April (rainy season) compared to January (dry season).

The findings of this study of the Batwa Indigenous Population are comparable with other indigenous populations in other countries. Generally, indigenous populations experience higher burden of disease, for example because of high malaria exposure and epidemics among indigenous people of the Amazon in Brazil [30] relatively high estimated burden of Acute Gastrointestinal Illness (AGI) and relatively low health care seeking behaviours in some indigenous communities in Arctic Canada compared to national estimates [58] [59]. Also, like the Batwa, the San of Namibia are faced with poverty and serious health challenges, with malaria, HIV/AIDS, gastro-intestinal illness, teenage pregnancies and alcohol abuse as key health concerns [60] [61]. United Nations declared the rights of all indigenous peoples to their traditional medicines, maintaining their health practices, and equal rights to the enjoyment of the highest attainable of physical and mental health [62]. Most indigenous people are located in remorte and hard to reach areas rendering their access to healthcare services inadequate. Lithe Bawa in Kanungu district, indigenous peoples' health status is severely affected by their living conditions, income levels, employment rates, access to safe water, sanitation, health services and food availability [62].

This study had some limitations; Firstly, operationally the use of mRDT is not as efficient in detecting malaria parisitaemia as microscopy or polymerase chain reaction (PCR) [63] [64] [65]. Therefore this may have affected the mRDT positivity or negativity rates (due to some false positives and negatives) thereby having a bearing on the prevalence [59] [60]. We believe that this error is not significant and also think it is covered under the 5% CI. Secondly, although we attempted a full census of all the Batwa IPs, the number was relatively small: approximately 500 for each of the seasons and only in Kanungu district. To overcome this we pooled both surveys in some analyses to achieve prevalence reflective across seasons and to account for sporadic nature of infections and risk factors as suggested by [16]. Future studies may cover all Batwa in all districts in Uganda.

6. Conclusions

This study highlights the importance of place in predicting malaria prevalence among Batwa Indigenous People a marginalized and remortely located sub-population. This study has shown that place matters in determining malaria prevalence. However, other factors like age, elevation and gender also contribute to malaria prevalence.

The results of this study revealed higher Batwa malaria prevalence than national and even non-indigenous population in the same district. It is therefore crucial for government and other stakeholders engaged in malaria control to develop targeted malaria prevention and control interventions. We recommend targeting hotspots intervention approach since it has proven reasonable impact on reducing malaria prevalence [66].

Data Sharing Statement

Datasets used in this manuscript are available from the corresponding author upon reasonable request.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Abbreviations

AOR	Adjusted Odds Ratio;
a. s. l.	above sea level;
BCH	Bwindi Community Hospital;
BDP	Batwa Development Programme;
CI	Confidence Interval;
COR	Crude Odds Ratio;
IHACC	Indigenous Health Adaptation to Climate Change;
IPs	Indigenous People (s);
mRDT	malaria Rapid Diagnostic Test;
PCR	polymerase chain reaction.