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Malaria Burden through Routine Reporting: Relationship between Incidence and Test Positivity Rates

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Abstract. Test positivity rate (TPR)—confirmed cases per 100 suspected cases tested, and test-confirmed malaria case rate (IR)—cases per 1,000 population, are common indicators used routinely for malaria surveillance. However, few studies have explored relationships between these indicators over time and space. We studied the relationship between these indicators in children aged < 11 years presenting with suspected malaria to the outpatient departments of level IV health centers in Nagongera, Kihihi, and Walukuba in Uganda from October 2011 to June 2016. We evaluated trends in indicators over time and space, and explored associations using multivariable regression models. Overall, 65,710 participants visited the three clinics. Pairwise comparisons of TPR and IR by month showed similar trends, particularly for TPRs < 50% and during low-transmission seasons, but by village, the relationship was complex. Village mean annual TPRs remained constant, whereas IRs drastically declined with increasing distance from the health center. Villages that were furthest away from the health centers (fourth quartile for distance) had significantly lower IRs than nearby villages (first quartile), with an incidence rate ratio of 0.40 in Nagongera (95% CI: 0.23–0.63; $P = 0.001$), 0.55 in Kihihi (0.40–0.75; $P < 0.001$), and 0.25 in Walukuba (0.12–0.51; $P < 0.001$). Regression analysis results emphasized a nonlinear (cubic) relationship between TPR and IR, after accounting for month, village, season, and demographic factors. Results show that the two indicators are highly relevant for monitoring malaria burden. However, interpretation differs with TPR primarily indicating demand for malaria treatment resources and IR indicating malaria risk among health facility catchment populations.

INTRODUCTION

National strategies for malaria control and intervention planning typically rely on the existence and strengths of national health management information systems (HMIS's).[1] Strong systems that incorporate complete case notification and accurate population data provide a firm basis for monitoring the present burden and trends, and making future projections. However, in much of sub-Saharan Africa, HMIS data miss a substantial number of malaria cases from the community who either do not seek diagnosis and/or treatment through the health system, or do but are not correctly reported.[1,2] In addition, catchment population data are not readily available at the facility level.[3] As a result, determining the true burden and interpreting trends at local levels remain a challenge. Test positivity rate (TPR) defined as the proportion of tested suspected malaria cases that return a positive malaria result is an indicator generated from facility records. Suspected malaria cases are patients presenting with fever or a history of fever in the last 48 hours, without any other recognizable cause, sent to the laboratory for a malaria diagnostic test.[4] Those who have a positive test result are, therefore, diagnosed with malaria. The test positivity rate overcomes the challenges of both denominators and numerators by assuming that those seeking diagnosis and treatment with suspected malaria make a representative sentinel population, and is also recommended by the WHO as a key surveillance indicator.[5,6] Interpretation of TPR is, however, affected by the incidence or number of non-malaria fevers by potentially inflating the sentinel population (denominator for TPR).[4] When health facility catchment populations are available, however, the test-confirmed malaria care rate or incidence rate (IR), defined as the number of malaria cases per 1,000 population at risk per unit time, may provide a better indication of the burden of malaria than TPR. Whereas TPR, owing to easier accessibility, is more commonly reported than IR,[7–10] the two indicators have been complimentarily reported but with no expressed implication of one indicator on the other.[11,12] The relationship between these indicators, therefore, remains unclear, and to our knowledge, very few studies have addressed it.

Health facility–based surveillance forms the basis of malaria burden reporting in Uganda.[5,6] Data are reported through the district health services to the Ministry of Health using regular aggregated reports including suspected malaria cases; cases tested and cases confirmed by age category (by microscopy or rapid diagnostic test for malaria [mRDT]); and confirmed or presumed cases treated with antimalarial medicine; among others, and the indicators derived are used for disease burden assessment.[5,6] In addition, multiple reference centers that were selected to cover diverse transmission intensities across the country have been embedded within the HMIS to strengthen the collection of high-quality data.[13] Using the data from three of these malaria reference centers, we investigated the relationship between IR and TPR as indicators of malaria burden at the facility level, accounting for important factors in this relationship. The few previous studies that explored relationships between incidence measures have predominantly been limited to a single site—either at the village level[5] or provincial level,[14] with a general focus on a single dimension of time. Here, we evaluate the relationship between the two indicators and explore the relationship in both time and space,

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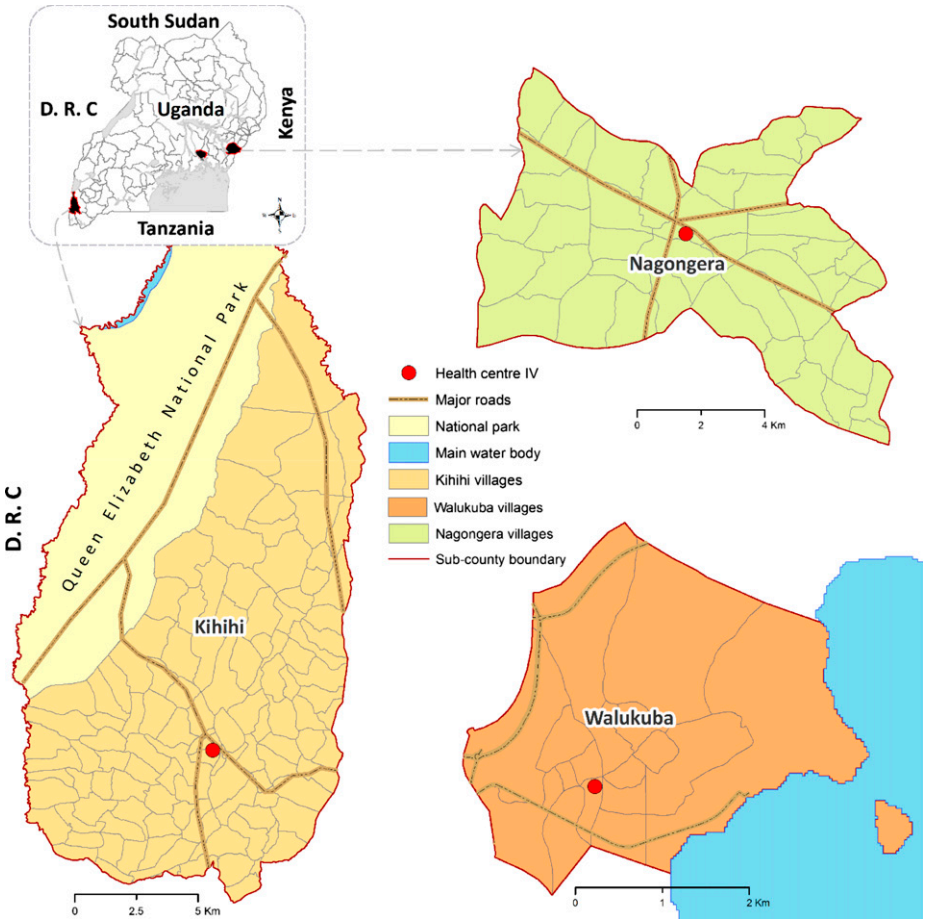
providing additional detail to our understanding of the utility and representativeness of HMIS data for understanding the changing malaria burden in endemic settings.

METHODS

Study setting. This study used routine surveillance data collected from three level IV health facilities located in different malaria transmission settings in Uganda (Figure 1). These facilities were 1) Nagongera in Tororo district with an annual entomological inoculation rate (aEIR) of 562 infective mosquito bites, 2) Walukuba in Jinja district with an aEIR of 6, and Kihihi in Kanungu district with an aEIR of 6 as per early 2000's assessments.[15] The three facilities formed part of a cohort of malaria reference centers established by the National Malaria Control Programme and led by the Uganda Malaria Surveillance Project (UMSP). Level IV health facilities typically serve a health subdistrict with an estimated population of 100,000 people and are run by a medical doctor. Each of the three sites has benefited from malaria control activities, including 1) the use of artemisinin-based combination therapies—specifically artemether–lumefantrine, which is the first-line treatment for uncomplicated malaria across the country, and 2) distribution of long-lasting insecticidal nets aiming to achieve universal coverage, conducted during September to November 2013 in Nagongera and Walukuba, and during December 2013 to February 2014 in Kihihi. In addition, Nagongera received three rounds of indoor residual spraying (IRS) with bendiocarb during December 2014 to February 2015, June to July 2015, and November to December 2015, and at least one round of IRS with pirimiphos-methyl (Actellic) during June to July 2016. The three subcounties of



Red dots reprent the site study health facilities, each being a level IV health centre for the health sub-district to which the respective sub-county (our catchment area) belongs – all shown on the inset map of Uganda in black. Kihihi (in shades of yellow) is located South west of Uganda with a 2002 estimated annual entomological innoculation rate (aEIR) of 6 infective bites per person, it is home to Queen Elizabeth national park at the border between Uganda & DRC. Walukuba (Orange area) is located in the central part of Uganda at the shores of Lake Victoria with aEIR of 6, while Nagongera (Light-green area) is located far east close to the border between Uganda and Kenya with aEIR of 562.

FIGURE 1. Location of the three sites of Nagongera (farthest east), Walukuba (central), and Kihihi (southwest) in Uganda, with the respective locations of the health facilities included. Red dots represent the site study health facilities, each being a level IV health center for the health subdistrict to which the respective subcounty (our catchment area) belongs—all shown on the inset map of Uganda in black. Kihihi (in shades of yellow) is located southwest of Uganda with a 2002 estimated annual entomological innoculation rate (aEIR) of 6 infective bites per person; it is home to Queen Elizabeth National Park at the border between Uganda and Democratic Republic of Congo. Walukuba (orange area) is located in the central part of Uganda at the shores of Lake Victoria with an aEIR of 6, whereas Nagongera (light-green area) is located far east close to the border between Uganda and Kenya with an aEIR of 562. This figure appears in color at www.ajtmh.org.

Nagongera, Walukuba, and Kihihi were made up of 45, 21, and 117 villages, respectively, each identified by names as known by district and subcounty officials. All these villages were located, mapped, and uniquely identified during enumeration surveys conducted at each site over 2009/2010, and are fully described elsewhere.[16,17]

Study population. All patients aged less than 11 years, presenting with suspected malaria, who were seen at the outpatient department clinic of each facility during October 2011 to June 2016, comprised the study population. This age group includes both the age range most at risk of the effects of and most sensitive to changes in malaria transmission (< 5 years),[18,19] and the age range across which naturally acquired immunity to malaria is seen to develop (5–11 years).[20] Monthly TPR trends were similar between under-fives and those aged five to less than 11 years (over-fives). However, TPR in under-fives was generally lower in the low-transmission sites (Walukuba and Kihihi) than in over-fives, and vice versa in the high-transmission site of Nagongera. The pattern in Nagongera was consistent with findings previously reported from a high-transmission site of Aduku in Uganda.[9] Members of the study population whose clinic visit was classified as "re-attendance," referring to follow-up visits for a previously recorded episode, were excluded. All members of the study population with a new illness episode were included in temporal trend assessments, whereas those whose village of residence (VOR) was known to be within the facility–host subcounty boundaries (catchment area) were included in spatial–temporal assessments. Those whose VOR was either located outside the catchment area or unknown were excluded from the space–time evaluations (Figure 2).

Outcome measures. Outcome data for all outpatients were extracted from health facility outpatients' registers, which recorded the presence of fever, diagnostic test results, diagnosis made, and treatment prescribed, as well as demographic data, height, weight, and VOR. These data were entered from the register into an MS Access database (Microsoft Corporation, Redmond, WA) at the facility and regularly submitted to the UMSP data center, where they were checked for inconsistencies and cleaned before transfer to STATA (Stata Corporation, College Station, TX) for analysis. Detailed methods are further explained elsewhere.[10,21]

The study primary outcomes were the test-confirmed IR, defined as the number of confirmed primary malaria cases per 1,000 catchment study population of children aged < 11 years (by mRDT or microscopy), and TPR, defined as the proportion of patients (children aged < 11 years) presenting to the health facility who were suspected to have malaria and tested with a positive malaria test result (by either mRDT or microscopy). Suspected malaria was defined as patients with fever or a history of fever in the past 48 hours, without any other apparent cause. These are considered eligible for a malaria test from which positive results determine the confirmed malaria cases. Because of inaccessible population estimates of our study area from the most recent census, catchment study populations were estimated using cartographic boundaries and a gridded population surface using the ArcMap geographic information system (ESRI, Inc.), with cartographic

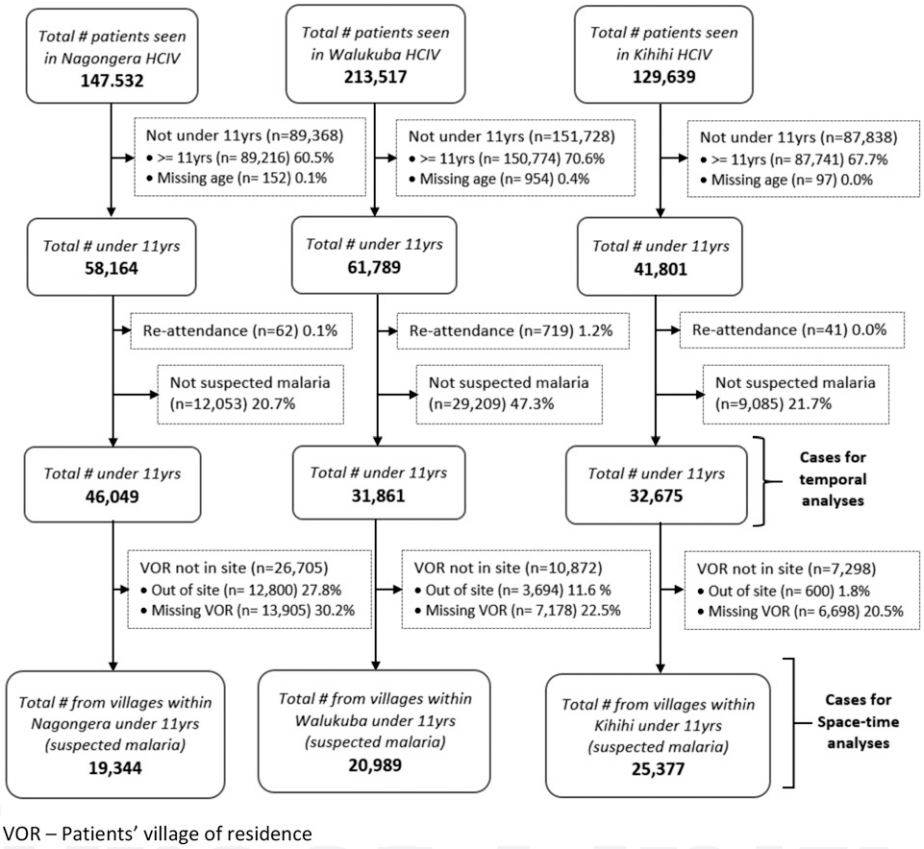


FIGURE 2. Trial profile indicating the participants included in the study and the exclusion criteria at the two levels of time and space–time evaluations. VOR = patients' village of residence.

boundaries defined as the 2006 subcounty boundary for each health facility. Subcounty total population estimates for 2010 and 2015 were obtained from the worldpop.org project data portal.[22] The population of children aged less than 11 years for each year from 2010 to 2016 was estimated from the overall estimates using population pyramids provided in the 2002 national census results, available in 5-year age bins at the district level. It was assumed that the proportion of the population aged less than 11 years was the same for all subcounties within each district, the proportion of population aged 10 years was one-fifth of the population aged 5–9 years, and the intercensal population growth rate was 3.0%, as published by the National Bureau of Statistics from the period of 2002 to 2014.[23] Per village study population estimates were then derived based on the proportion of village households enumerated per village in each subcounty.

Analysis. The two outcome indicators were summarized by month and village, and examined for temporal and spatial trends. Straight-line distance to the health facility from the VOR was estimated using the ArcGIS point distance tool (ESRI, Inc.) between the centroid point of the village and the coordinate point of the health facility. Regression analyses were conducted using STATA versions 14 and 15 (Stata Corporation).

The pairwise relationship between IR and TPR was visualized using scatter plots and approximated using locally weighted scatterplot smoothing and quadratic prediction plots. As a measure of agreement between the indicators, concordance analysis was performed through evaluation of the Bland–Altman diagrams by site, with no predetermined threshold for agreement.[24] Two of the three sites were found to be eligible for concordance evaluation based on an approximately normal distribution of the differences between TPR and IR, thereby excluding Walukuba.

Factors influencing the association between TPR and IR per village per month were explored using mixed-effects Poisson regression models, with random effects at the village and month levels, to account for clustering at both levels. Model selection was performed using Akaike's information criteria (AIC) from a list of linear, quadratic, cubic, and exponential fits. Explanatory variables considered were age (proportion of the presenting population aged 5 years and more), gender (proportion of the presenting population that was male), distance to the health facility (by quartile), and season (determined using the predominant annual patterns of rainy [March–May and September–November] and dry [rest of the year] seasons in the southern parts of Uganda).[25] Explanatory variables with $P \leq 0.05$ in the unadjusted analysis were considered for inclusion in a fully adjusted multivariable model using a step-down process and evaluated using likelihood ratio tests.

RESULTS

Characteristics of the study population. A total of 161,754 patient visits by children aged less than 11 years were recorded for the three health facilities between October 2011 and June 2016, accounting for 33% of all patient visits. Among these, 110,585 (68%) were suspected malaria cases. A monthly mean of 346, 382, and 445 suspected malaria patients aged less than 11 years were seen in Nagongera, Walukuba, and Kihihi, respectively, over the study duration (Figure 2). The respective site mean (median) age in years among these participants was 2.8 (2), 3.8 (3), and 4.2 (4), respectively. In addition, the mean age of patients less than 11 years was significantly higher for females than males, both overall and within each of the three sites ($P < 0.001$). No differences were observed in trends of TPR between participants included or excluded based on the availability of VOR data, hence the assumption of no considerable impact on our results due to the exclusion. Additional participant characteristics are detailed in Table 1 for those included in the space–time assessments.

Trends in indicators of malaria. Figure 3 shows the trends in TPR and IR stratified by site over the study duration, on a monthly time scale, suggesting that the two show similar

TABLE 1

Characteristics of study population and distribution of suspected and clinically confirmed malaria cases for the duration of October 2011 through June 2016, evaluated in the space–time analysis for this study

Site	Nagongera, *N* (%)	Walukuba, *N* (%)	Kihihi, *N* (%)
Participants	19,344	20,989	25,377
Gender			
Male	9,407 (49%)	9,367 (45%)	12,003 (47%)
Female	9,937 (51%)	11,622 (55%)	13,371 (53%)
Age (years), mean (SD)			
All	2.79 (2.61)	3.78 (3.01)	4.17 (3.02)
Male	2.62 (2.49)	3.54 (2.93)	3.98 (2.93)
Female	2.94 (2.71)	3.97 (3.07)	4.34 (3.09)
Testing rates			
Diagnostic tests with recorded results (proportion of all participants who were tested)			
Tested and results recorded	18,655 (96%)	19,140 (91%)	25,048 (99%)
Testing rates by the diagnostic method (proportion of all participants tested by the method)			
Microscopy	17,701 (95%)	19,073 (99%)	25,043 (99%)
Rapid diagnostic test	954 (5%)	67 (0%)	5 (0%)
TPR	15,295 (38%)	7,910 (24%)	15,212 (47%)
TPR by gender, i.e., proportion of tested blood slides that were positive for malaria			
Male	3,479 (38%)	2,120 (25%)	5,699 (48%)
Female	3,637 (38%)	2,506 (24%)	6,107 (46%)
Malaria diagnosis			
Malaria diagnosed with a confirmatory test (proportion of all malaria diagnoses made that had a confirmatory test)			
Confirmed positive	7,604 (97%)	4,554 (97%)	11,856 (99%)

TPR = test positivity rate.

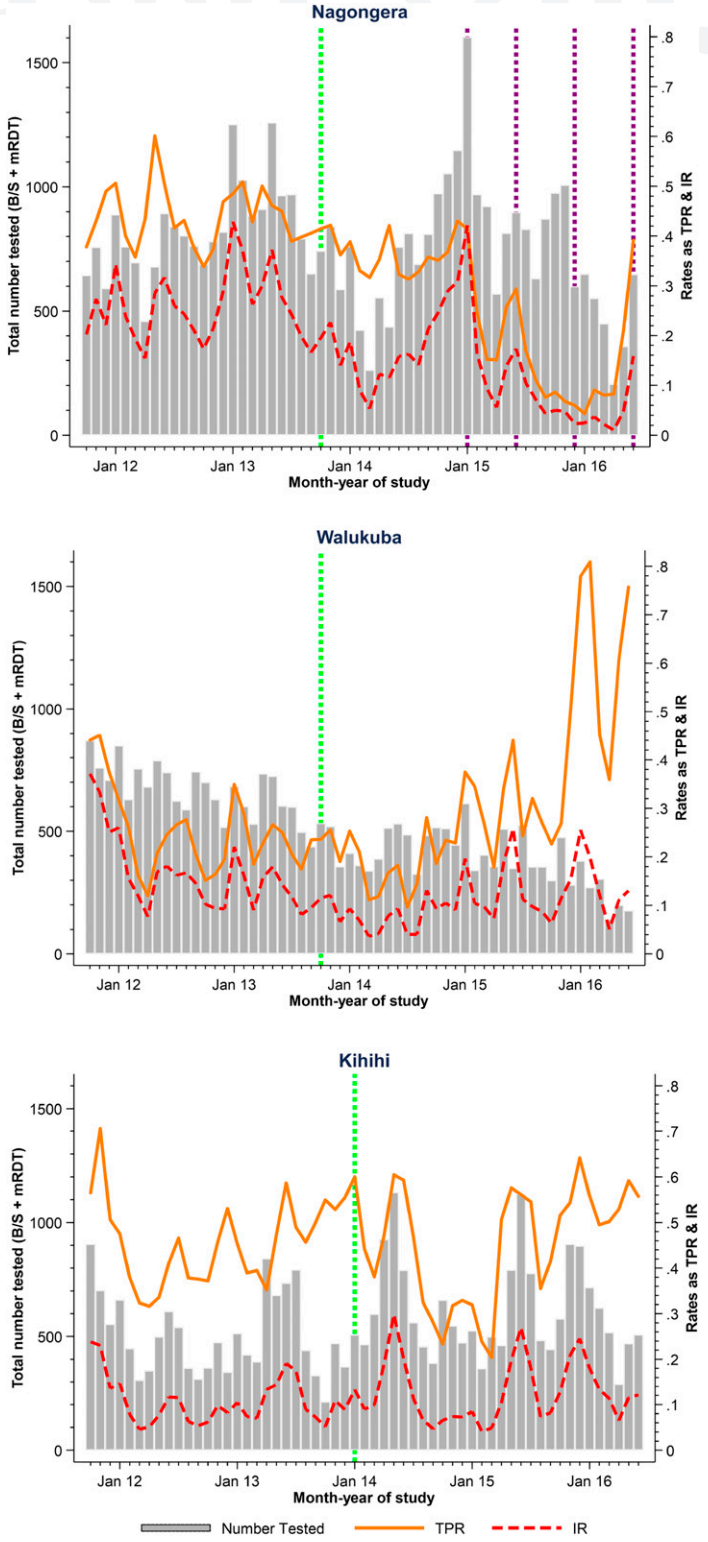


FIGURE 3. Trends in incidence and test positivity rates (TPRs) over time (monthly), by site. In Figure 3, the solid orange line represents TPR, whereas the dashed red line represents incidence rate, both overlaid on bar plots of total number of children < 11 years tested for malaria per month. A scale of monthly numbers tested is shown on the left *y* axis, whereas that for the two incidence measures (indicators) is on the right *y* axis. The green dotted line represents the time of a universal long-lasting insecticidal-treated net distribution campaign in each site, whereas the purple dotted lines represent the timing of indoor residual spraying in Nagongera. This figure appears in color at www.ajtmh.org.

trends. In addition, there is evidence of good agreement between these indicators through concordance analysis using Bland–Altman's diagrams shown in Supplemental Figures 1 and 2 in the Supplemental Information, for the two sites that fulfilled Bland–Altman's criteria of approximately normally distributed differences.

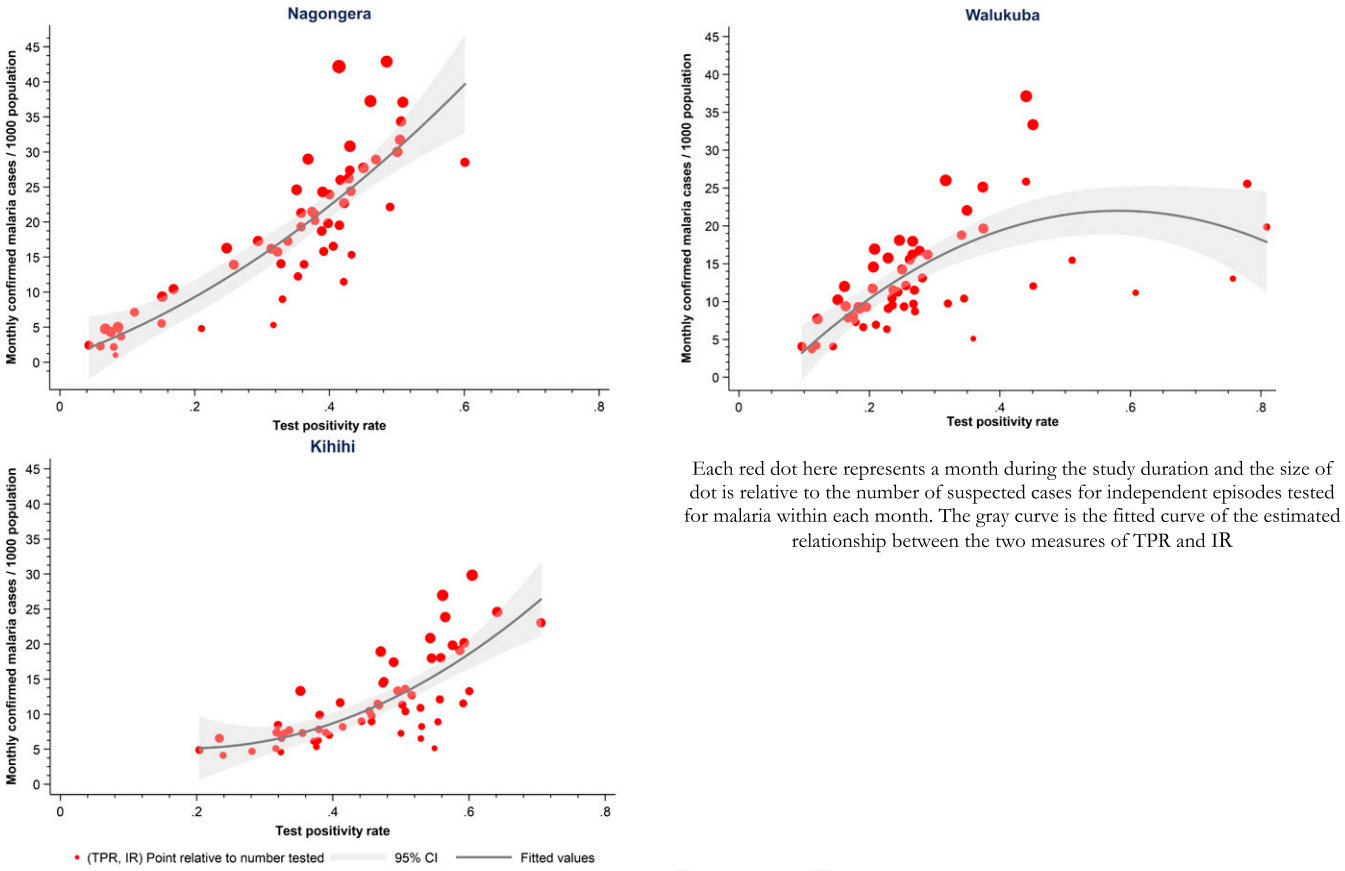
Figure 4 shows the relationship between TPR and IR per month, suggesting a tendency for months with low case

detection rates to also have low TPRs for all three sites. Given the strong seasonal trends in transmission, this confirmed TPR as a reasonable indicator for transmission intensity. However, when plotting TPR and IR by village, this relationship was lost as seen in Supplemental Figure 3 in the Supplemental Information. This suggests that factors other than intensity of transmission were influencing the number of malaria cases from each village that attend the health center for diagnosis and/or treatment. This was also confirmed through the spatial distribution of IR compared with that of TPR by village shown in Figure 5. Here, the highest IR is seen to cluster closer to the health facility and less so further away, whereas TPR has no distinguishable spatial variation. Moreover, results in Figure 6 showed that there was no change in TPR with distance, whereas in Figure 7, IR was considerably reduced with increasing distance from the health facility in all three settings. The number of patients tested for malaria was not as highly varied by month as it was by village, although Walukuba recorded steady decline across the study duration and Nagongera recorded the highest mean (arithmetic) monthly number of patients tested for malaria, as seen in Figure 3. Kihihi recorded the highest mean number of patients tested for malaria, by village, compared with the other sites, and Walukuba, the lowest for the same. The mean TPR per month ranged from 28.9% to 45.7%, whereas the village mean TPR per month ranged from 23.9% to 47.1% excluding outliers. In addition, the mean IR ranged from 11.8 to 18.6 cases per 1,000 children per month, whereas the village mean IR ranged from 21.8 to 40.3 cases per 1,000 children per month.

Association between the TPR and IR. Univariable analysis of the association between IR and explanatory variables revealed significant associations with the proportion of the presenting population aged more than 5 years, distance from the health facility, and TPR in all three settings; and for season in Nagongera (see Supplemental Information). Model selection revealed a cubic fit for TPR as best in all settings, based on AIC, compared with linear, quadratic, and exponential fits (see Supplemental Information).

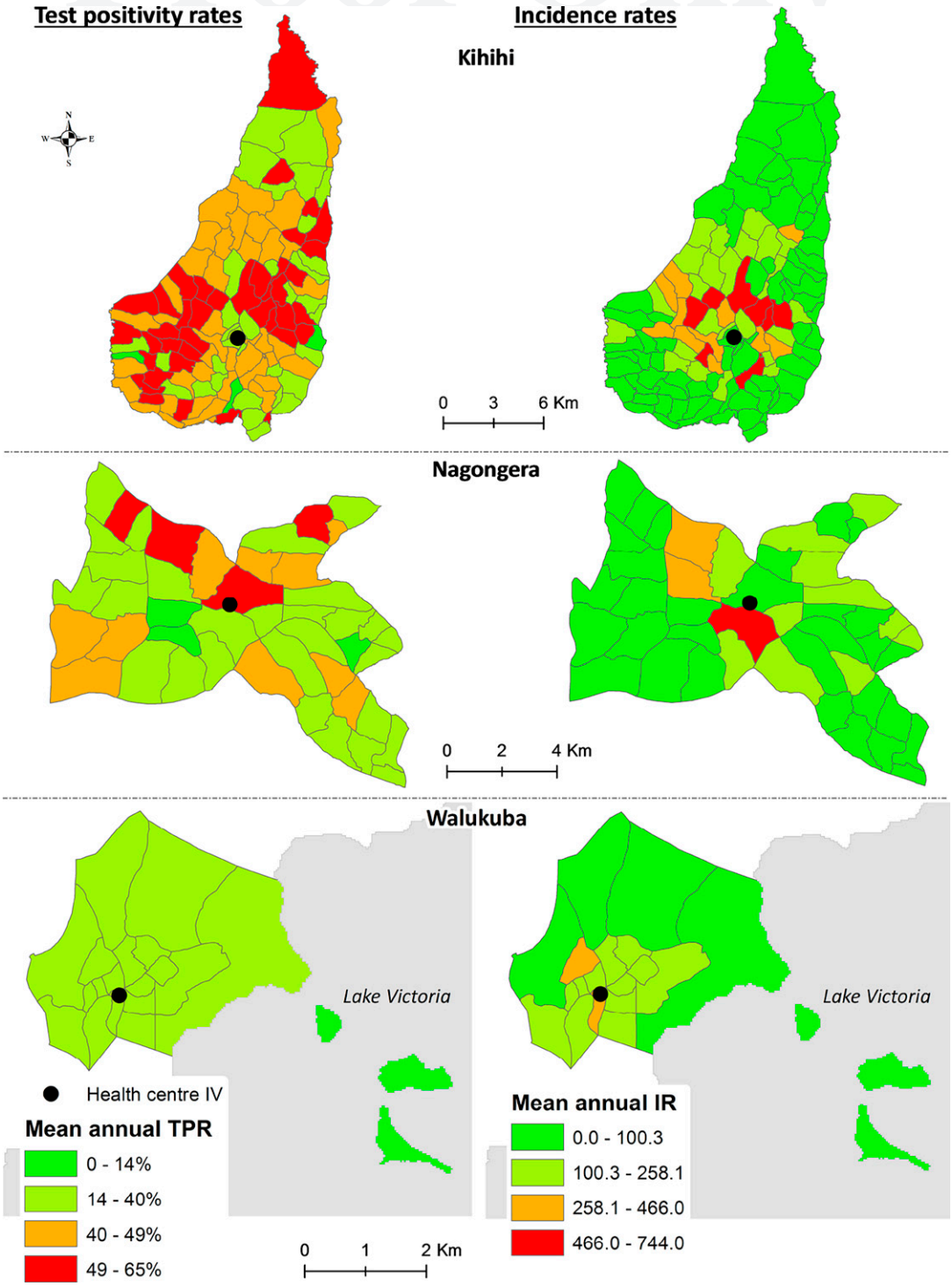
In the final multivariable models, IR was significantly lower in villages further from the health facility (Table 2). Associations with seasonal and population factors also varied by site. Incidence rate was significantly lower during the rainy season in Nagongera only, whereas in Kihihi and Walukuba, when an increasing proportion presenting were aged more than 5 years, IR stayed borderline significantly associated with age (Table 2).

Comparison of village and temporal random effects suggested considerably more unexplained heterogeneity between villages than over time (month). Average variability between villages was lowest in Kihihi and highest in Nagongera, although this relationship was reversed when examining unexplained temporal variation, with Nagongera being lowest.



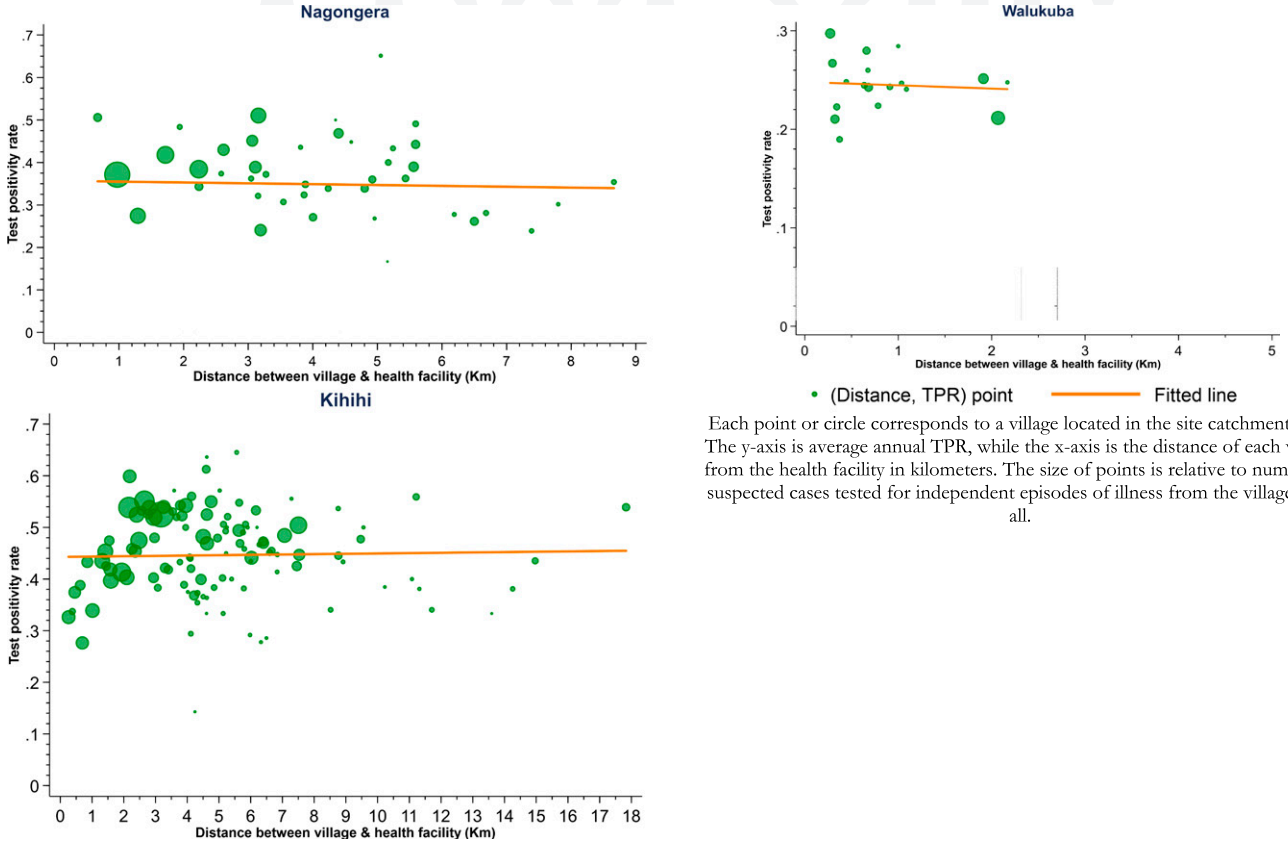
Each red dot here represents a month during the study duration and the size of dot is relative to the number of suspected cases for independent episodes tested for malaria within each month. The gray curve is the fitted curve of the estimated relationship between the two measures of TPR and IR

FIGURE 4. Plots of test positivity rate (TPR) against test-confirmed malaria case rate with points as months and point sizes accounting for the number tested for malaria by month. Each red dot here represents a month during the study duration, and the size of dots is relative to the number of suspected cases for independent episodes tested for malaria within each month. The gray curve is the fitted curve of the estimated relationship between the two measures of TPR and incidence rate. This figure appears in color at www.ajtmh.org.



Three sites of Kihihi, Nagongera and Walukuba shown side by side to depict a comparison between the spatial distribution of village mean annual TPR (left) against IR (right). Village boundaries for each site are represented with the gray lines, while the respective site study health facility location is represented by the black dot. A single legend per indicator is placed at the bottom on respective sides.

FIGURE 5. Comparison of the spatial distribution of incidence rate (IR) and test positivity rate (TPR) based on the village-level annual mean of each indicator in the three sites of Kihihi, Nagongera, and Walukuba. Three sites of Kihihi, Nagongera, and Walukuba shown side by side to depict a comparison between the spatial distribution of the village mean annual TPR (left) against IR (right). Village boundaries for each site are represented with the gray lines, whereas the respective site study health facility location is represented by the black dot. A single legend per indicator is placed at the bottom on respective sides. This figure appears in color at www.ajtmh.org.



Each point or circle corresponds to a village located in the site catchment area. The y-axis is average annual TPR, while the x-axis is the distance of each village from the health facility in kilometers. The size of points is relative to number of suspected cases tested for independent episodes of illness from the village over all.

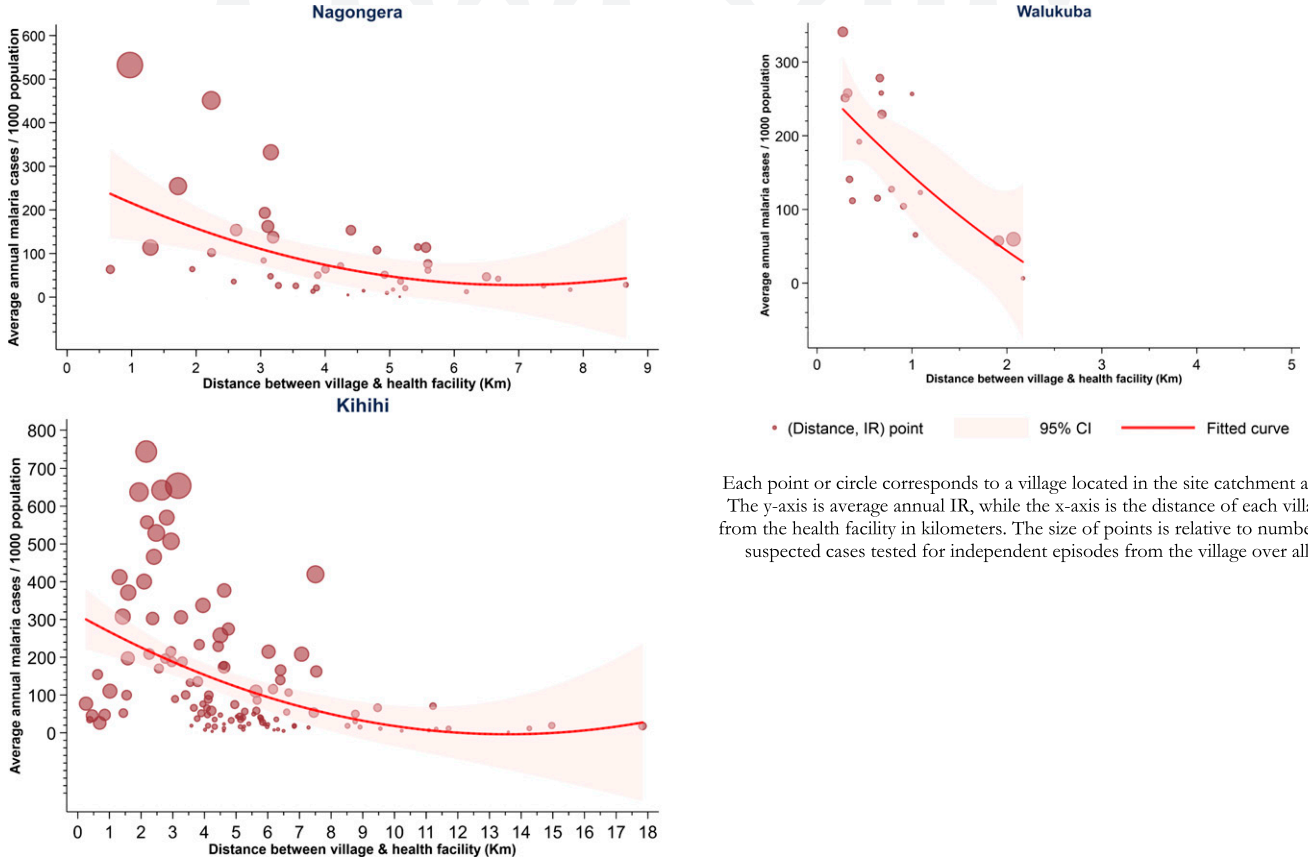
FIGURE 6. Plot of the distance between the village and health facility against the average annual test positivity rate (TPR), by site. Each point or circle corresponds to a village located in the site catchment area. The *y* axis is the average annual TPR, whereas the *x* axis is the distance of each village from the health facility in kilometers. The size of points is relative to the number of suspected cases tested for independent episodes of illness from the village overall. This figure appears in color at www.ajtmh.org.

DISCUSSION

This study took advantage of enhanced malaria surveillance, conducted at three health facilities in varied malaria transmission settings in Uganda. We investigated the relationship between two standard indicators of malaria burden: TPR and IR. Findings showed that these indicators strongly agreed over time (month) in all three settings, most notably during periods of low transmission. However, TPR remained unchanged, whereas IR was drastically reduced with increased distance from the health facility. This further points to TPR as an appropriate proxy of transmission intensity regardless of the setting, but suggested that despite enhanced reporting, IR is strongly influenced by facility accessibility and/or use by the catchment populations using distance to the health facility as a proxy.[26] We demonstrated here that after accounting for transmission via TPR, some variability in IR remained unexplained both between villages and months. This pointed to important systemic differences in access to diagnosis and treatment within health facility catchments.

Trends in and concordance between these indicators over time (month) showed patterns suggestive of stronger agreement during low-transmission seasons. This was particularly evident in Nagongera during the period of intense IRS activity. This suggests that assessment of the trends over time in the two measures together may serve as a more sensitive marker of drastic changes in transmission within low-transmission settings and, thus, jointly provide an important surveillance tool. Consistent with other studies, including one in western Uganda,[27] we demonstrated that the relationship between TPR and IR was nonlinear, with both indicators increasing steadily until TPR reached around 50%. This suggested that either an inherent saturation point in TPR or that dynamics in treatment-seeking and care practices masked any further increase in TPR. It also reiterated the presence of malaria heterogeneity within each site.

The three-level mixed-effects Poisson model results from this study revealed that after controlling for the transmission intensity (via TPR), IR remained significantly influenced by the age of the patients, distance to the health facility from the VOR, and climatic conditions represented by a wet or dry season of the year. Studies have previously reported the influence of village or "area of residence" and age on TPR[27] as well as season[25] on malaria through routine reporting. Consequently, age, distance, village, and time (month) or season (rain versus dry) were important factors in the assessment of malaria burden through routine health facility–based surveillance. Concerning age, the small but strongly significant association of increased IRs with age in Kihihi and Walukuba but not Nagongera could be explained by Nagongera having significantly lower mean age, coupled with its historically significantly higher parasite prevalence in children than the other two sites.[17] Distance to the health facility, the increase of which was associated with a significant decline in IR, is known to influence the care-seeking



Each point or circle corresponds to a village located in the site catchment area. The y-axis is average annual IR, while the x-axis is the distance of each village from the health facility in kilometers. The size of points is relative to number of suspected cases tested for independent episodes from the village over all.

FIGURE 7. Plot of the distance between the village and health facility against the average annual incidence rate (IR), by site. Each point or circle corresponds to a village located in the site catchment area. The *y* axis is the average annual IR, whereas the *x* axis is the distance of each village from the health facility in kilometers. The size of points is relative to the number of suspected cases tested for independent episodes from the village overall. This figure appears in color at www.ajtmh.org.

behavior[28] for reasons such as cost[29] and, thus, may be considered a proxy for accessibility.[30] There were significant associations between distance and IR in all three settings. These highlight a strong influence of access to health facility and factors associated with access such as the use of drug shops as the first action, reported to be anywhere between 25% in patients of all ages[31] and 62.7% in children aged less than 5 years,[32] in parts of Uganda. Accessibility may also provide an explanation for the reduced IR observed in Nagongera during the rainy season, given that floods, intense farming activity, and inaccessible roads are common in this area during this time. It should be noted that distance did not explain all between-village variation, suggesting that other factors play an important role in health facility accessibility and use.

TABLE 2

Mixed-effects Poisson model results (adjusted) assessing association between incidence rate and TPR in Nagongera, Kihihi, and Walukuba controlling for age, distance to the health facility in all sites, gender, and season in Nagongera and Kihihi as fixed effects and including random effects of village of residence and month of study year

		Nagongera		Kihihi		Walukuba	
		Fixed effects					
Exposure		IRR (95% CI)	*P*-value	IRR (95% CI)	*P*-value	IRR (95% CI)	*P*-value
Case positivity	Cubic TPR term	1.00 (1.00–1.00)	< 0.001	1.00 (1.00–1.00)	< 0.001	1.00 (1.00–1.00)	< 0.001
	Quadratic TPR term	0.98 (0.98–0.98)	< 0.001	0.98 (0.98–0.98)	< 0.001	0.98 (0.98–0.98)	< 0.001
	Linear TPR term	1.11 (1.09–1.13)	< 0.001	1.18 (1.17–1.19)	< 0.001	1.02 (1.00–1.05)	0.111
Age	Increasing proportion of ≥ 5 years	1.00 (0.97–1.02)	0.727	1.01 (1.00–1.02)	< 0.043	1.03 (1.00–1.06)	0.068
Distance to health facility	1st quartile	1	Reference	1	Reference	1	Reference
	2nd quartile	0.48 (0.28–0.83)	0.009	1.06 (0.76–1.49)	0.723	0.70 (0.31–1.60)	0.395
	3rd quartile	0.38 (0.22–0.67)	0.001	0.68 (0.49–0.94)	0.018	0.88 (0.43–1.79)	0.716
	4th quartile	0.40 (0.23–0.68)	0.001	0.55 (0.40–0.75)	< 0.001	0.25 (0.12–0.51)	< 0.001
Season	Dry/sunny	1	Reference	1	Reference	1	Reference
	Wet/rain	0.86 (0.80–0.91)	< 0.001	N/A	N/A	N/A	N/A
Random effects	Village (standard error)	0.3"75 (0.088)		0.305 (0.045)		0.325 (0.114)	
	Month (standard error)	0.075 (0.009)		0.104 (0.008)		0.105 (0.014)	

IRR = Incidence rate ratio; TPR = test positivity rate.

There were a number of limitations that should be acknowledged. Incomplete records represent a major challenge when interpreting health facility data. However, an evaluation of the greatest source of missing data (VOR) suggested no systematic differences between the two populations. Distance to the health facility could only be measured as a straight-line distance between the health facility and the centroid of the village as more detailed information was unavailable within the confines of routine passive surveillance. In addition, given possible repeated measures on the individual and household levels, there could be clustering at those levels that remained unaccounted for in this analysis. Nevertheless, repeated measures within the same episode of malaria were minimized using visit classification. Last, there are several lower level facilities within the same catchment of each of our study facilities that may influence the results observed; however, these data were not available at the same level of quality for inclusion in this study. Nevertheless, although lower level facilities absorb many malaria cases in their proximity, they too see patients from much farther away in a relatively similar form to the higher level facilities (such as our study facilities) for myriad reasons, which may undermine their influence on results in this study.

CONCLUSION

Strong nonlinear relationships between the two indicators of TPR and IR emphasize their distinct relevance to monitor malaria; however, caution is necessary in their interpretation. Given the strong impact of the distance of patients' residence from the health facility, a good proxy for the care accessibility, on IR and none on TPR, burden estimates in the assumed health facility catchment differs from one indicator to the other. The influence of access to health facility on IR depicts it as a good indicator for malaria burden in the health facility catchment, whereas the absence of the same effect of access on TPR suggests TPR as a good indicator for resource planning within the health facility system. More information is needed, however, on how well IR reflects the true burden on well-characterized catchments.

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Availability of data and materials: The dataset used and/or analyzed during the present study are available from the corresponding author on reasonable request.

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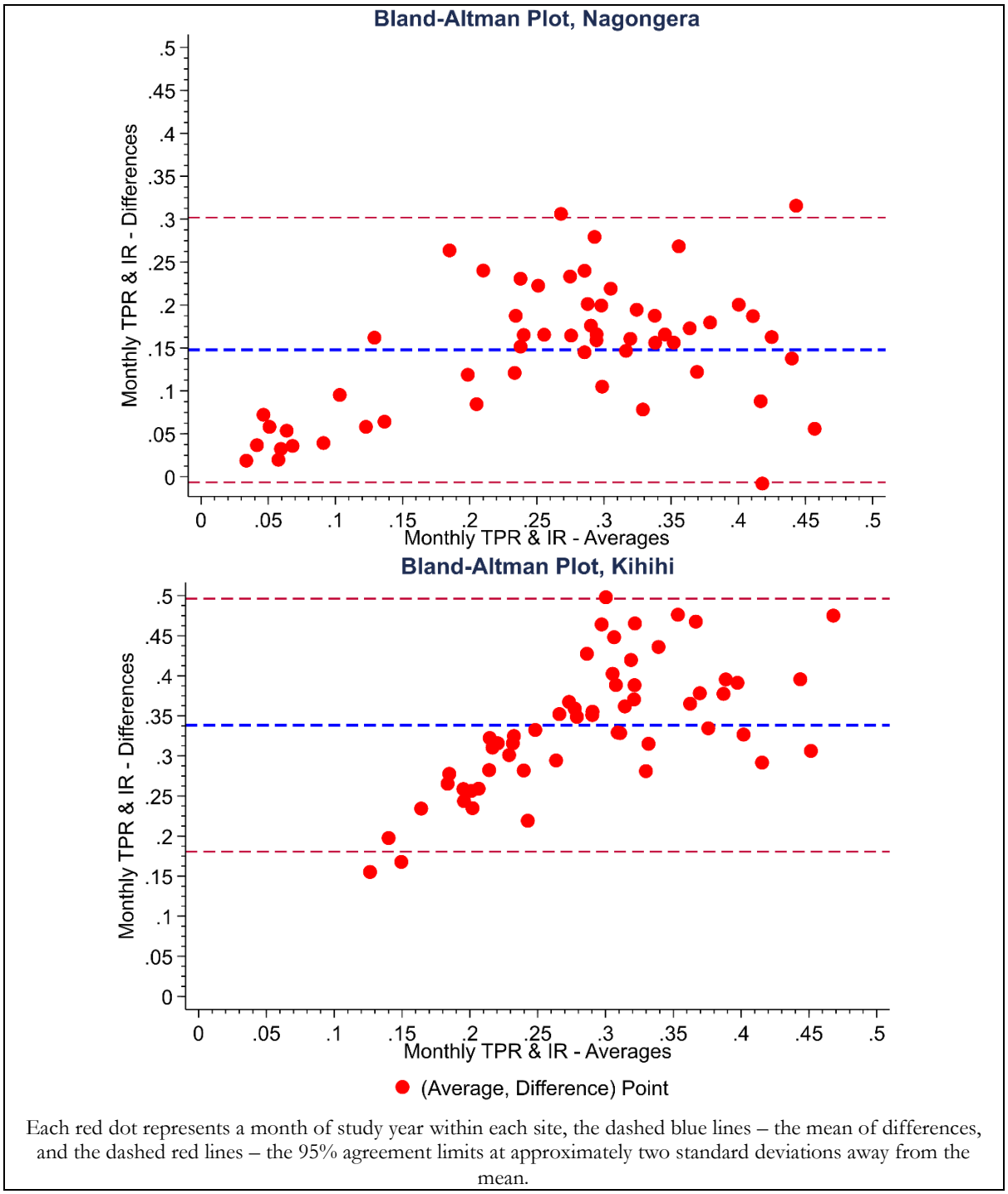
Proof Only

Concordance analysis

Concordance analysis results are presented by month in Figure 1 and by village in Figure 2 below. The included two (Nagongera and Kihihi) of three sites are they that met the 'normal distribution of differences' criteria required in Bland-Altman's method, whereas Walukuba did not qualify and was therefore, excluded.

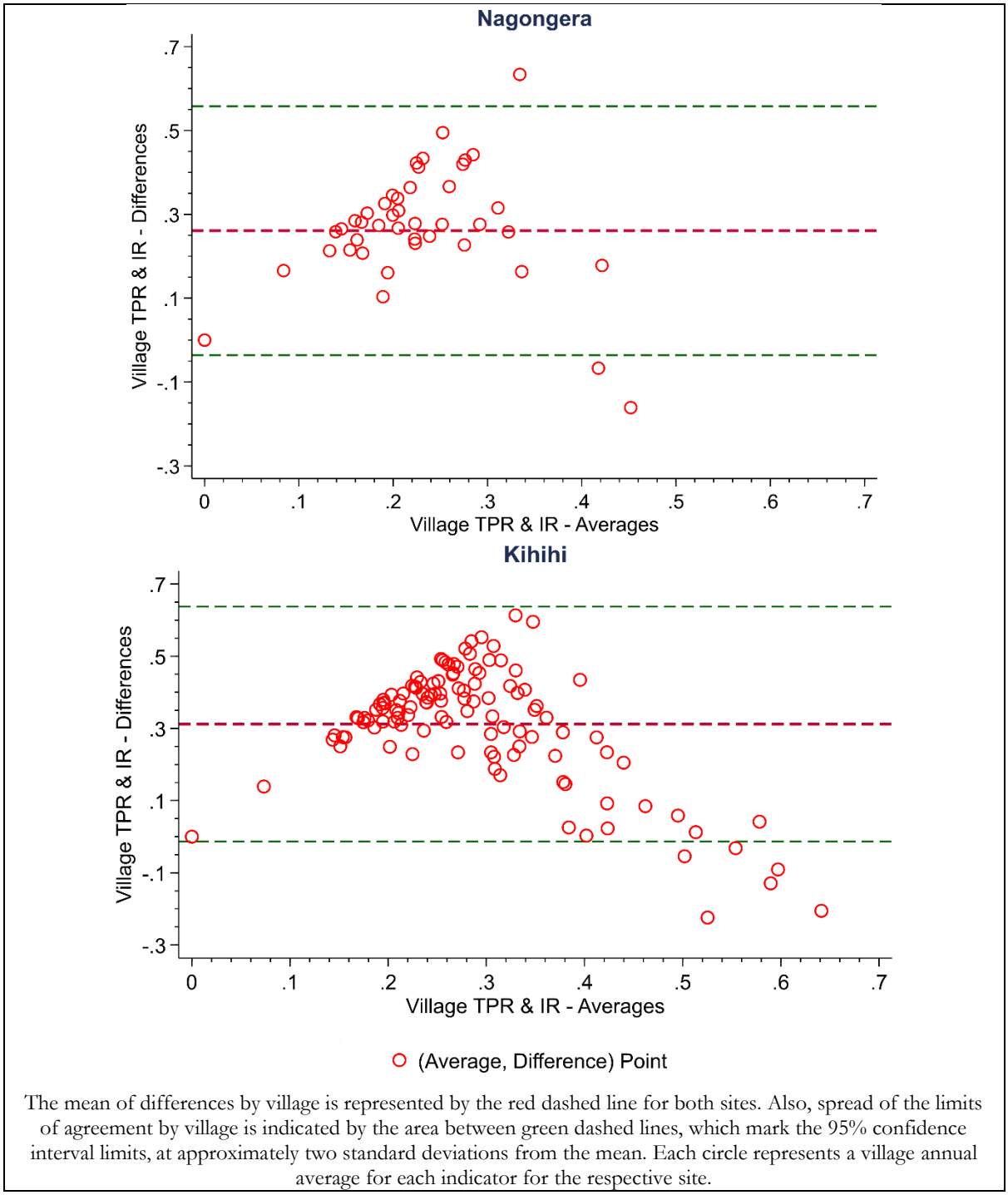
Figure 1. Bland-Altman diagram for Nagongera and Kihihi, assessing incidence estimates of TPR and IR at the level of time (month).



Each red dot represents a month of study year within each site, the dashed blue lines – the mean of differences, and the dashed red lines – the 95% agreement limits at approximately two standard deviations away from the mean.

The mean of differences for these monthly assessments (Figure 1) was much lower in Nagongera than Kihihi, being 0.148 and 0.338 respectively, a higher than two-fold and significant difference ($p<0.001$) with the means represented by the blue dashed line. However, the spread of limits of agreement was nearly the same for both sites i.e. 0.154 and 0.158 respectively, indicated by the dark-red dashed lines. Thus, difference between TPR and IR per month was less than 0.08 at both sites within 95% confidence bounds.

Figure 2. Bland-Altman diagram for Nagongera and Kihihi, assessing TPR against IR at by village, stratified by year of study.



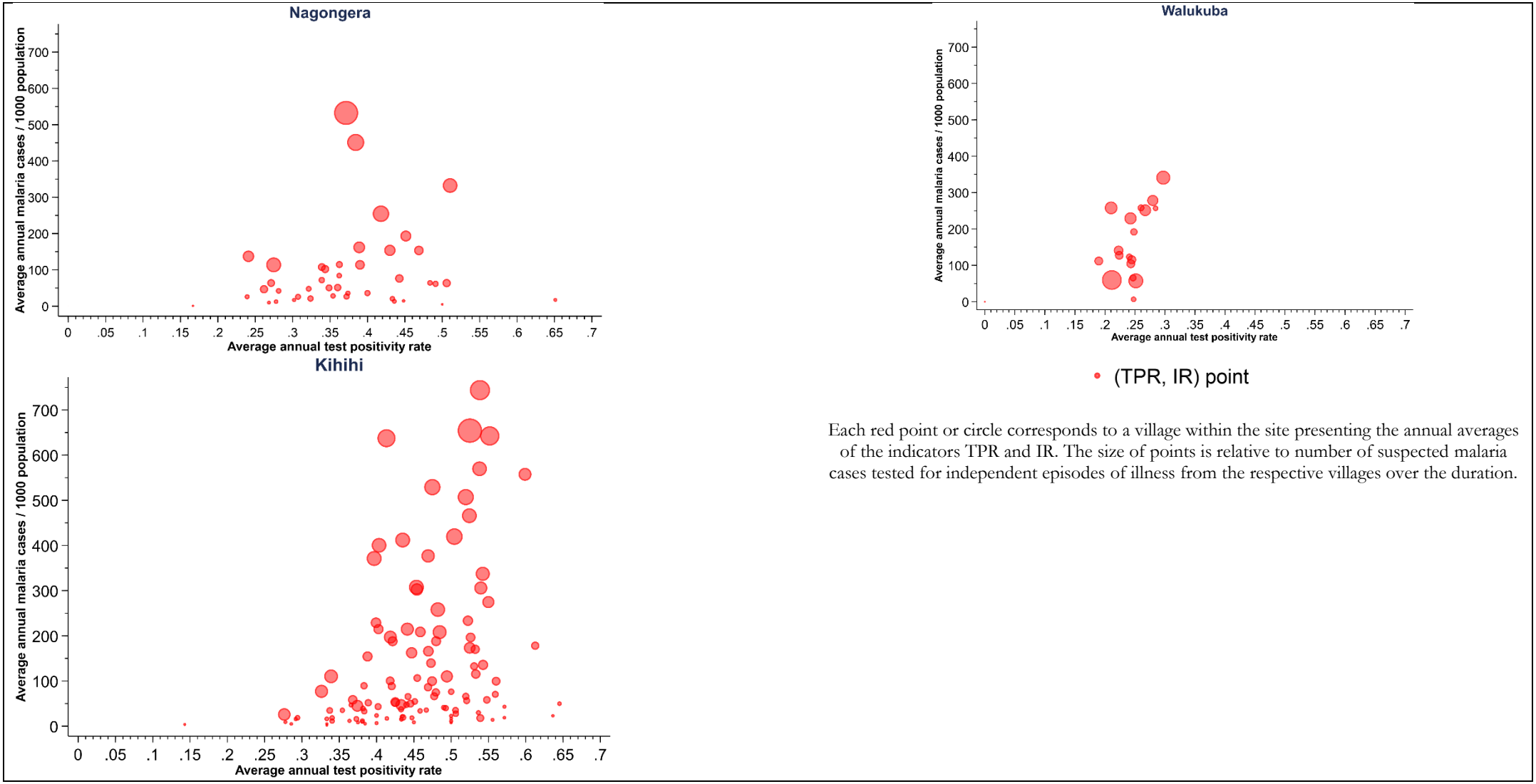
The mean of differences by village is represented by the red dashed line for both sites. Also, spread of the limits of agreement by village is indicated by the area between green dashed lines, which mark the 95% confidence interval limits, at approximately two standard deviations from the mean. Each circle represents a village annual average for each indicator for the respective site.

Concordance results (shown in Figures 1 and 2) revealed higher mean of differences between TPR and IR in Kihihi, 0.33 than Nagongera, 0.17 within 95% CI, suggesting a similarly large average difference between the sites. Furthermore, differences between TPR and IR by village were limited to 0.16 in either site and greater than differences by month that were limited to 0.08 within 95% CI, pointing to greater heterogeneity between villages than months. Consistency in the differences between TPR and IR for either site on the two dimensions of month and village, provides further evidence in support of agreement between these indicators [1] regardless of transmission setting. By village, TPR was on average 30% higher than IR for both sites and there was an apparent relationship between variability in the two indicators and the quantity of each, with smaller differences observed at lower quantities in each of the indicators and greater differences as well as uncertainty when these are larger, that implies that there is greater agreement at lower transmission levels.

Relationship between TPR and IR

The relationship between the two indicators of TPR and IR by village, was explored using the mean annual value of each indicator, for each village. Here, unlike the case of the same examination by month presented in Figure 4 in the paper, the relationship is unclear as seen in Figure 3 below

Figure 3. Scatter plot of village-level average annual test positivity rate against average annual incidence rate, by site. Point sizes account for number tested for malaria by village



Each red point or circle corresponds to a village within the site presenting the annual averages of the indicators TPR and IR. The size of points is relative to number of suspected malaria cases tested for independent episodes of illness from the respective villages over the duration.

Univariable analysis

For each of the sites, explanatory variables including: sex or gender as 10% increments in the proportion of males among the study participants; distance to the health facility first determined in kilometres and then transformed to site specific quartiles; and, season determined using the predominant annual patterns of rain (March-May and September-November) and dry (rest of the year) seasons in the southern parts of Uganda, [2] were evaluated. For each site, age as 5% increments in the proportion of children 5 to under 11 years of age, was considered a default variable for inclusion, given that the existence of significant association between age with risk of infection is well known. [3, 4] Results from the univariate analysis are presented in Tables 1, 2, and 3 for Nagongera, Kihihi, and Walukuba respectively.

Table 1. Mixed effects Poisson model results (crude) assessing associations in Nagongera between IR and TPR, age, gender, distance to health facility, and season as fixed effects; and, including random effects of village of residence and month of study year.

Exposure		Un-adjusted			
		Fixed effects		Random effects	
		IRR (95% CI)	p-value	Village (Std. Err.)	Month (Std. Err.)
Case positivity	TPR	1.14 (1.13-1.15)	<0.001	0.759 (0.175)	0.172 (0.016)
Age	Increasing proportion of >=5yrs	0.97 (0.94-1.00)	0.042	0.748 (0.173)	0.398 (0.030)
Gender	Increasing proportion of Males	1.01 (0.98-1.03)	0.595	0.721 (0.167)	0.395 (0.030)
Distance to health facility	1st Quartile	*1*	*Reference*	0.548 (0.128)	0.398 (0.030)
	2nd Quartile	0.45 (0.23-0.86)	0.016		
	3rd Quartile	0.34 (0.17-0.65)	0.001		
	4th Quartile	0.33 (0.17-0.62)	0.001		
Season	Dry / Sunny	*1*	*Reference*	0.743 (0.172)	0.387 (0.029)
	Wet / Rain	0.83 (0.75-0.91)	<0.001		

Table 2. Mixed effects Poisson model results (crude) assessing associations in Kihihi between IR and TPR, age, and distance to health facility as fixed effects; and, including random effects of village of residence and month of study year.

Exposure		Un-adjusted			
		Fixed effects		Random effects	
		IRR (95% CI)	p-value	Village (Std. Err.)	Month (Std. Err.)
Case positivity	TPR	1.12 (1.11-1.13)	<0.001	0.545 (0.079)	0.225 (0.013)
Age	Increasing proportion of >=5yrs	1.06 (1.04-1.07)	<0.001	0.534 (0.079)	0.428 (0.021)
Gender	Increasing proportion of Males	1.01 (0.99-1.02)	0.298	0.565 (0.082)	0.442 (0.022)
Distance to health facility	1st Quartile	*1*	*Reference*	0.459 (0.067)	0.442 (0.022)
	2nd Quartile	1.16 (0.78-1.74)	0.467		
	3rd Quartile	0.66 (0.45-0.98)	0.041		
	4th Quartile	0.49 (0.33-0.72)	<0.001		
Season	Dry / Sunny	*1*	*Reference*	0.565 (0.082)	0.442 (0.022)
	Wet / Rain	0.93 (0.88-0.99)	0.034		

Table 3. Mixed effects Poisson model results (crude) assessing associations in Walukuba between IR and TPR, age, and distance to health facility as fixed effects; and, including random effects of village of residence and month of study year.

Exposure		Un-adjusted			
		Fixed effects		Random effects	
		IRR (95% CI)	p-value	Village (Std. Err.)	Month (Std. Err.)
Case positivity	TPR	1.14 (1.12-1.15)	<0.001	0.671 (0.230)	0.285 (0.027)
Age	Increasing proportion of >=5yrs	1.09 (1.05-1.13)	<0.001	0.594 (0.205)	0.481 (0.041)
Gender	Increasing proportion of Males	1.03 (0.99-1.08)	0.088	0.645 (0.222)	0.494 (0.042)
Distance to health facility	1st Quartile	*1*	*Reference*	0.320 (0.115)	0.495 (0.042)
	2nd Quartile	0.72 (0.32-1.62)	0.423		
	3rd Quartile	0.84 (0.45-0.98)	0.623		
	4th Quartile	0.25 (0.33-0.72)	<0.001		
Season	Dry / Sunny	*1*	*Reference*	0.646 (0.222)	0.494 (0.042)
	Wet / Rain	0.92 (0.81-1.04)	0.202		

Model selection

The best model fit was selected using the Akaike's information criteria where the model with the lowest value is considered better than others with higher values. This model can be considered as the model with maximum precision using all the important covariates accounted for. In this study, four models were considered including the linear, the quadratic, the exponential and the cubic. Results for each of these models considered are presented in Table 4 below, indicating that the cubic was preferable.

Table 4. Akaike's information criteria values for the models each compared to the linear model to determine significant improvement of the linear model to fit the relationship between TPR and IR

Site	Model			
	Linear	Quadratic	Exponential *	Cubic
Nagongera	5847.68	5363.82	5650.67	**5317.82**
Kihihi	13298.93	11878.39	12399.11	**11857.46**
Walukuba	3828.50	3510.93	3710.12	**3452.89**

******The exponential model considered here was one that included a linear term of TPR given it was better than model that was purely exponential and excluded a linear term*

Multi-variable analysis

The cubic fit of the model, as compared to the linear, quadratic, and exponential models was selected as best based on AIC (Table 4). This fitted relationship from the multi-variable model was presented as a predicted plot using values of all covariates in the model, fixed at their mean values in each of the three sites (Figure 4).

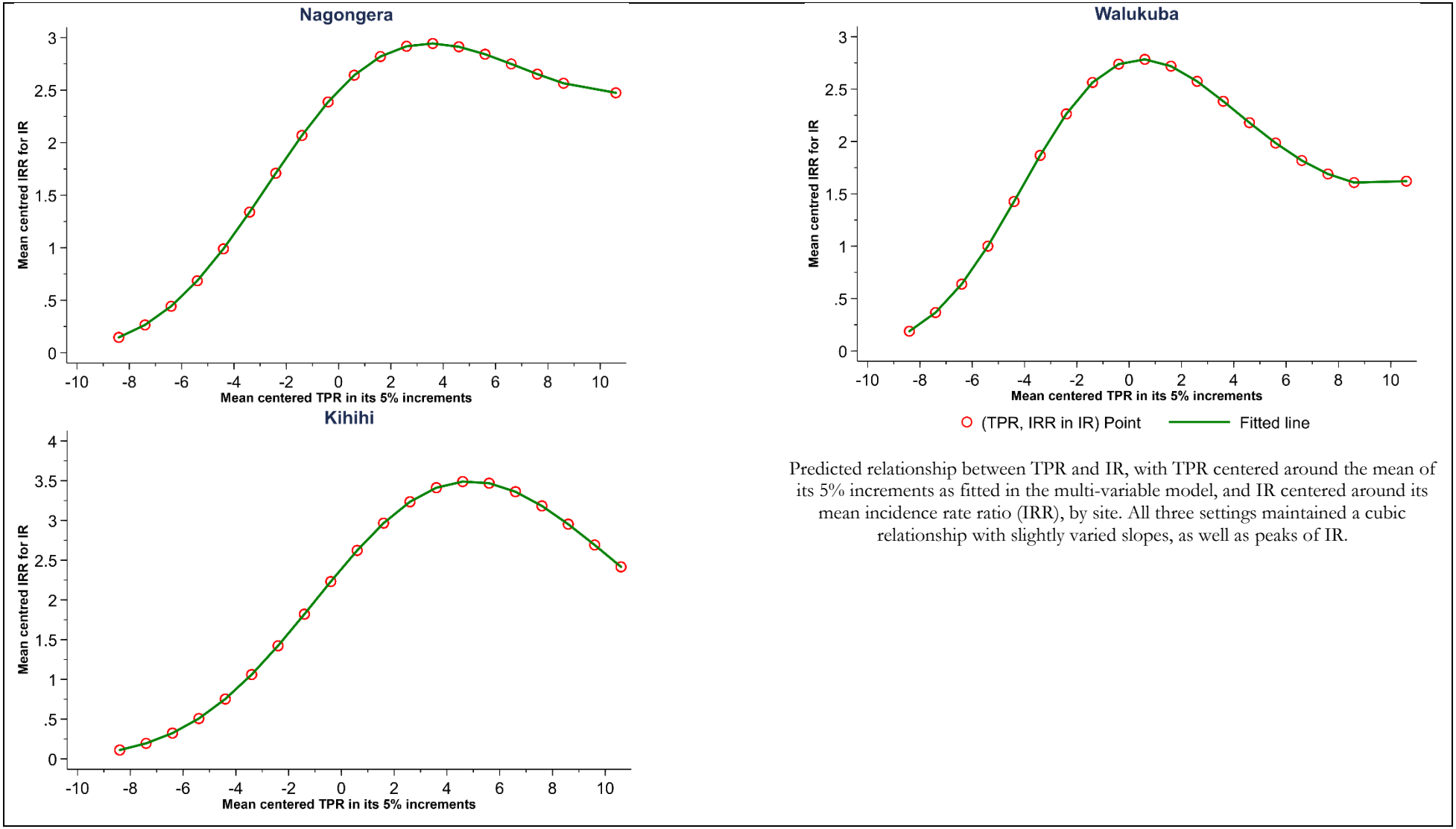
This relationship takes on the form of

$$y = ax^3 + bx^2 + cx + \beta$$

Where y = village IR per month, x = village TPR per month, and a, b, & c are coefficients, while β is an error term.

The same relationship between TPR and IR was sustained at all three settings with one exception in Walukuba where the linear term does not hold a significant effect. In all three settings, the fitted relationships between TPR and IR suggested that observed IR were highest when TPR was above the site mean, although the nature of the relationship had slight variations by site: in Nagongera, fitted IR peaked at 25% above the mean of TPR, whilst in Walukuba this was at 10% above and in Kihihi at 50% above mean of TPR (Figure 4).

Figure 4. Prediction plots for the relationship (cubic) between TPR and IR from the multi-variable mixed effects model for the sites of Nagongera, Walukuba, and Kihihi.



Predicted relationship between TPR and IR, with TPR centered around the mean of its 5% increments as fitted in the multi-variable model, and IR centered around its mean incidence rate ratio (IRR), by site. All three settings maintained a cubic relationship with slightly varied slopes, as well as peaks of IR.

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