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Molecular Characterization and Distribution of Malaria Vectors in three Agrarian Communities of Kano State, North West Nigeria

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Abstract

Malaria vector abundance has been linked to certain agricultural practices. This work examined the impact of the agricultural practice of irrigation on the composition and seasonal distribution of malaria vectors in agrarian communities of Kano state. Longitudinal data collection was done four times a year, corresponding to different transmission seasons from early rains to late rainy season, early dry season to late dry season. Indoor-biting adult mosquitoes were collected using standard pyrethrum spray collection (PSC) techniques. Female *Anopheles* mosquitoes collected from houses were morphologically identified to species level. Molecular characterisation of the members of the *Anopheles gambiae* complex was carried out using PCR technique. Two thousand four hundred fifty-two (2452) adult female *Anopheles* species were collected throughout the study period. The Large Irrigation (LIC) and Urban Irrigation Communities (UIC) had a higher mean abundance of female *Anopheles* mosquitoes across seasons. There was a significant difference in the mean adult mosquito catch across the season ($F = 113.49$, $p \leq 0.001$) and across the three communities ($F = 44.73$; $p \leq 0.001$). *Anopheles gambiae* sl. was the most encountered among the four species, with a mean abundance of 11.94±11.76, 5.39±7.45 and 2.58 ±3.41 for LIC, UIC and NIC, respectively. Molecular characterisation of *An. gambiae s.l.* by PCR showed the presence of three sibling species, *An. coluzzi, An. gambiae ss and An. arabiensis*. *An. Coluzzi was significantly more abundant across the three communities during the* wet and dry seasons. The predominance of this species has implications for malaria control. This study shows that irrigation is likely to influence mosquito breeding, thus exposing community members to a higher risk of being bitten by infected vectors. A review of the ecology of Anopheles species, especially in urban environments, is needed, considering the current abundance of malaria vectors in the urban community.

Keywords: Seasonal, Abundance, Composition, *Anopheles* species, Irrigation, Urban

Introduction

Malaria continues to be the foremost cause of death in Nigeria, with over 61 million cases and about 95,000 deaths recorded in 2019 (WHO, 2020). As of 2022, the World Health Organization (WHO) reported that Nigeria bore the most immense burden of malaria globally.

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The country alone contributed about 27% of the global malaria cases and 26% of deaths related to malaria worldwide, thus responsible for a higher number of malaria cases and mortality globally (WHO, 2022). The disease is considered to impact the growth and development of the farming population (Fink & Masiye, 2015; Rono *et al*., 2015). It is one of the major causes of food shortage in Africa due to the poor health of the populace engaged in agriculture (Ricci, 2012).

An. gambiae and *An. funestus* species are Nigeria's principal mosquito species responsible for malaria transmission *(*WHO, 2022). They pass *Plasmodium* species during blood feeding from one host to the next. The *Anopheles gambiae sensu lato* (sl**)** has been described as an efficient malaria vector in Africa (Kigadye *et al*., 2012).

Most forms of agriculture practised in Nigeria is rain dependent. In the northern part of Nigeria, rainfall duration is very short, thus limiting the duration of farming in the North. However, dams constructed for irrigation, farming, and fishing have helped boost the country's all-year-round food production. Several studies have linked irrigation schemes to an increased abundance of malaria vectors. Irrigation expands the number of potential breeding sites while also extending the time during which vectors breed much beyond their normal seasons, thereby stretching the period of malaria transmission into the dry season in the irrigated areas (Demissew *et al*., 2023; Kibret *et al*., 2014). In Nigeria, irrigation has been shown to cause an increase in the abundance of *Anopheles* species and a change in malaria pattern from seasonal to perennial, both in rural and urban areas. The resultant change from rain-fed agriculture to irrigated farming has greatly enhanced the creation of suitable aquatic habitats for the malaria vectors to breed, consequently increasing adult mosquitoes' density (Fernando, 2002; Sutherst, 2004). Amaechi *et al*. (2018), reported an increase in vector abundance in villages where irrigation farming was practised than in non-irrigated villages around the Omi reservoir irrigation area in Yagba West Local Government Area (L.G.A) of Kogi State, North Central Nigeria. A similar result was obtained in Bunkure and Gezawa irrigation sites of Kano state **(**Oguoma & Ikpeze, 2008; Yakasai *et al*., 2017), where the authors linked irrigation farming to the development of various mosquito species in irrigated ditches, bed pools, puddles, and hoof-prints.

Afrane *et al*. (2012) discovered a high larval survival rate and increased abundance of adult *Anopheles* mosquitoes in vegetable farms under small-scale irrigation in urban Ghana. Hawaria *et al*. (2020) reported the diversity of the breeding habitats of mosquitoes in irrigated areas to be twice those found in non-irrigated regions, showing that irrigation farming has the likelihood of increasing *Anopheles* vector breeding sites. Another study in Ethiopia linked the increase in malaria incidence to small-scale irrigation farming systems. Malaria incidence was found to be almost all year round and higher in communities living in the villages where irrigation farming was practised (Kibret *et al*., 2014).

Urban agriculture has become widespread within the Sub-Saharan African region during the last decade, spreading into the outskirts and centres of many towns and cities. A study in Benin reported higher *Anopheles* biting and sporozoite rates all year round in urban houses close to irrigated farms than those far from it. The authors attributed this to the creation of suitable breeding grounds in the farms for mosquito propagation (Yadouléton *et al*., 2010)

The present study examined the composition and seasonal distribution of malaria mosquito vectors in agrarian communities of Kano state with varying ecological microclimates. It aimed to aid the development of malaria control interventions suitable for specific localities and efficiently reduce the disease burden in the study communities and the state.

Materials and Method

Study Design

The survey was a two-year longitudinal study carried out between 2018 to 2019. The survey was undertaken in two phases, during the early and late dry seasons and early and late rainy seasons corresponding to the low and peak malaria transmission season in the State, respectively (Yakudima & Adamu, 2017); thus, data were collected four times a year. The survey was carried out in three agrarian communities based on the agricultural practices in the community. The study sites were designated as follows: a Large-scale

Irrigation Community (LIC), a rainfed Non-Irrigation Community (NIC), and an Urban irrigation community (UIC) to allow for comparison in vector densities and species composition and malaria entomological indices driving incessant malaria in the different agrarian communities.

Study Area

Kano is located at latitude 12^0.0'0.43°N and longitude $8^0$31'0.19"E of the equator. The State is located in West Africa's Sudan savannah zone. Kano is one of the country's most irrigated states, with over 3 million hectares of cultivable land (Abakpa *et al*., 2013). The State boasts six irrigation schemes and over twenty earth dams, making the state the most irrigated state in Nigeria (Ibrahim *et al*., 2014). Kano State is historically a commercial and agricultural state. Urban agriculture and livestock farming are also major activities in the state. The state has a short wet season between May and October, while the cold and dry season starts in November through April. A 25°C– 40°C temperature range and relative humidity of 47.43 % have been reported (Yahaya *et al*., 2014).

Inclusion/Exclusion Criteria

Three communities were chosen for the study based on the type of agriculture practised, whether irrigation or rain-fed agriculture, and based on the rural or urban nature of the community**.**

Adult mosquito collection

Mosquitoes that rest and bite indoors were collected from houses using pyrethrum (insecticide) knockdown collection techniques (PSC) (Service, 1993) from thirty study houses in each community four times a year corresponding to Early rainy/wet season (May), late rainy/wet season (October), early dry season (November) and late dry season (March). Mosquitoes were collected for two consecutive years between 2018 and 2019. Samples collected were moved to the laboratory, sorted, counted, and morphologically identified to species.

Morphological identification

Anopheles species were morphologically separated and distinguished from *Culex* and other mosquito species using features on the mosquitoes' maxillary palps. The female *Anopheles* species were identified morphologically using the keys developed by Gillett & Smith (1972) and Gillies & Coetzee (1987). Each species identified was counted, and the number was recorded. *Anopheles gambiae* s.l was then preserved individually in well-labeled Eppendorf tubes containing silica gel. This was then taken to the Nigeria Medical Research Institute, Yaba, Lagos State laboratory for further analysis.

Identification of Sibling Species of *Anopheles gambiae* s.l Complex by PCR

The molecular identification and characterisation of members of the *An gambiae* complex was done using the multiplex PCR technique as described by Fanello *et al.* (2002). The wings and legs of morphologically identified *Anopheles gambiae* s.l. were separated from the main body of the mosquito and placed in separate PCR tubes. This was then grinded in 50µl of deionised water and rinsed in 20ul of the same deionised water. The mixture was incubated at 94°C for 10 minutes and centrifuged for one minute at 13 rpm to obtain DNA in the supernatant. 112.5 µl of reaction mixture was prepared using 4 µl of master mix. The master mix contained DNA polymerase reaction buffer, 7.5 ml $MgCl_2$ and 1 ml of dNTPs, 6.15 ul of deionised distilled water and 0.5 ul sets of species-specific primers for *Anopheles gambiae* complexes and universal primer for the amplification of several targets in a single PCR experiment of each *Anopheles* complex (Kabbale *et al*., 2016). The PCR process included an initial cycle of denaturation at 95°C for 2 minutes, 30 cycles of denaturation at 95^0C for 30 seconds, annealing at 55^0C for 30 seconds, an extension step at 72^0C for 30 seconds and a final extension at 72^0C for 7 minutes using a Hybaid TM thermal cycler. The amplified DNA was separated on the 2.5 % agarose gel electrophoresis stained with ethidium bromide at 80 volts for 50 minutes and visualised on an ultraviolent (UV) transilluminator (gel documentation system). The size of the bands of the separated DNA was compared with positive

controls from where the sibling species of the target samples were identified. Primers create fragments 390bp for *An. gambiae* and 315bp for *An. arabiensis*. Species of *An. gambiae* and *An. coluzzii* were further identified by PCR restriction fragment length polymorphism (RFLP) based analysis as described by Fanello *et al*. (2002). The restriction enzyme, Hha I, was used to digest the PCR product positive for *An. gambiae* ss. The Digest mix consisted of 0.6 ul buffer, 0.2 ul restriction enzyme, and 1 ul distilled water. Digest mix was added to each tube, and 10 ul of PCR product was identified as *An. gambiae* ss. The incubation comprised an initial digestion cycle at 37^0C for 8 hours and enzyme deactivation at 65^oC for 20 minutes. The samples were then run on 1.5% agarose gel stained with Ethidium bromide. Primers create fragments of 367 bp for *Anopheles coluzzii* and 257 and 110bp for *Anopheles gambiae*.

Data analysis

The data obtained in the study were encoded and analysed for *Anopheles* species abundance and composition using one-way ANOVA. Statistical tests were considered statistically significant at a p-value of < 0.05.

Results

Seasonal Abundance of Malaria Vectors in Agrarian Communities of Kano State

A summary of the relative abundance of female *Anopheles* species sampled by PSC during the study period is presented in Table 1. A total of 2452 adult female *Anopheles s*pecies were collected throughout the study period. The Large Irrigation Community (LIC) had a significantly higher mean abundance of female *Anopheles* mosquitoes during the late wet seasons (LWS) I and II (11.70±8.46, 10.70±7.81 respectively), while the lowest was during the early dry season II (2.53±1.46). The trend was similar in the Urban Irrigation Community (UIC), where mean *Anopheles* collection was significantly higher during the LWS I and II 7.80±5.86 and 6.83±4.45 respectively) but lowest during the Late dry season I and II (0.77±0.77 and 0.53±0.63 respectively). The Non-Irrigation Community (NIC) recorded the lowest mean Anopheline abundance throughout the study period, with the highest mean abundance during the LWS I (3.23±4.42) and lowest during LDS II (0.00). On the whole, there was a significant difference in the mean adult mosquito catch across the season (F =113.49, p≤0.001) and across the three communities (F = 44.73; p ≤0.001).

Table 1: Mean Seasonal Abundance of Female *Anopheles* species Sampled by Pyrethrum Spray Catches (PSC)

	LIC	UIC	NIC
Season	Mean no of female *Anopheles* spp	Mean no of female *Anopheles* spp	Mean no of female *Anopheles* spp
LWS I	11.70±8.46^{a*}	7.80±5.86^{a*}	3.23±4.42^{a*}
EWS I	6.53±5.24^{b}	1.37±1.19^{b}	0.77±0.63^{cd}
LWS II	10.70±7.81^{a*}	6.83±4.45^{a*}	2.40±2.80^{ab}
EWS II	7.43±5.39^{b}	1.83±1.62^{b}	1.27±0.58^{c}
LDS I	2.70±2.18^{c}	0.77±0.77^{b}	0.07±0.25^{d}
EDS I	3.30±2.33^{c}	0.80±0.76^{b}	0.77±1.41^{cd}
LDS II	3.10±1.49^{c}	0.53±0.63^{b}	0.00±0.00^{d}
EDS II	2.53±1.46^{c}	1.80±1.27^{b}	1.80±1.09^{bc}
LS	≤0.001*		
Season x Community	≤0.001*		

* significance at p≤ 0.05

Means with different superscripts across columns are statistically significant.

Keys large Scale Irrigation Community; UIC=Urban Irrigation Community; NIC= Non Irrigation Community; LWS=Late Wet Season; EWS= Early Wet Season; LDS=Late Dry Season; EDS=Early Dry Season.

Composition of *Anopheles* species Sampled in the three communities

Table 2 shows the species composition of *Anopheles* species sampled by pyrethrum Spray Catch (PSC) during the study period. Four *Anopheles* species were sampled throughout the study period. These include *An. gambiae sl., An. funestus, An. coustani* and *An. pharoensis*. All four *Anopheles* species were sampled in the large irrigation community. The urban irrigation community recorded only two species, *An. gambiae s.l and An. funestus* while the non-irrigation community recorded 100% *An. gambiae* s.l.

An. gambiae sl. was the most encountered species with a mean abundance of 11.94±11.76,

5.39±7.45 and 2.58 ±3.41 for LIC, UIC and NIC, respectively. This was statistical significance across the three communities (F_2= 64.29; p-value ≤ 0.001). LWS had the highest mean *An. gambiae s*l *collection* (14.14±12.99), while LDS had the lowest mean collection (2.39 ±3.14). There was a significant difference in the mean abundance of *An. gambiae* sl across seasons (F= 58.14; p-value ≤ 0.001). The interaction between location and season was also highly significant for *An. gambiae* sl. (F= 7.4; p-value ≤ 0.001). The other three species formed only a small percentage of the total Anopheline collected, and their composition did not differ significantly across seasons and the three communities.

Table 2: Composition of *Anopheles* species across the three communities and season

	Species			
Treatment	*An. gambiae*	*An. funestus*	*An. coustani*	*An. pharoensis*
UIC	5.39[b]	0.03[a]	0.02[a]	0.00a
LIC	11.94[c]	0.03[a]	0.01[a]	0.02a
NIC	2.58[a]	0.00[a]	0.00[a]	0.00a
Location	***	ns	ns	ns
LWS	14.14[c]	0.04[b]	0.01[a]	0.02[a]
EWS	6.39[b]	0.01[ab]	0.00[a]	0.00[a]
LDS	2.39[a]	0.00[a]	0.00[a]	0.00[a]
EDS	3.62[a]	0.02[ab]	0.02[a]	0.00[a]
Season	***	ns	ns	ns
Interactions				
Location x Season	***	ns	ns	ns

***P <0.001,**P <0.01, *P <0.05; ns=Non-significant. Columns and rows followed by the same letter(s) are not significantly different at a 5% significance level.

Keys: UIC = Urban Irrigation Community, LIC=Large Scale Irrigation Community, NIC= Non-Irrigation Community, LWS=Late Wet Season, EWS= Early Wet Season, LDS=Late Dry Season, EDS=Early Dry Season.

Distribution of Sibling Species of *Anopheles gambiae s.l*

The result of the PCR assay to distinguish the molecular forms of *An. gambiae s.l* is shown in Figure 1. Molecular characterisation of *An. gambiae s.l.* by Polymerase Chain Reaction showed the presence of three sibling species: *An. coluzzi, An. gambiae ss and An. arabiensis*. 438 female *Anopheles gambiae sl.* were assayed during the wet season across the three communities, of which 326 (74.4%) were *An. coluzzi*, 103 (23.52%) were *An. gambiae* and 9 (2.06%) were *An. arabiensis*. The trend was similar during the dry season, where *An. colluzi* (n=171) was also the most predominant species, followed by *An. gambiae* (n=51) and *An. arabiensis* (n=9) having the least occurrence out of the two hundred and twenty-seven female *An. gambiae* sl collected.

An. colluzzi was significantly more abundant across the three communities (n = 56 and 155 during the wet and dry seasons, respectively) and across the seasons than *An. gambiae* and *An. arabiensis*. *An. arabiensis* had the least abundance across the communities *and* was not found in the non-irrigation community for the wet and dry seasons.

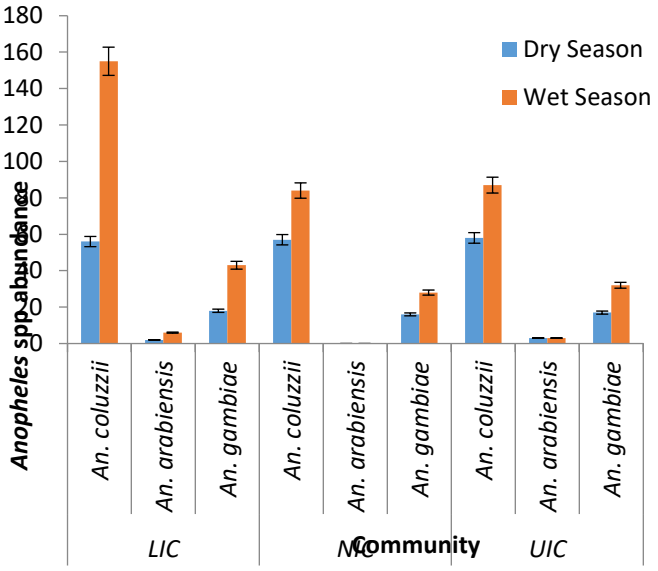

Figure 3 Distribution of Sibling Species of *An. gambiae* sl.

C = Urban Irrigation Community, LIC=Large Scale Irrigation Community, NIC= Non Irrigation Community

Discussion

The research findings show that malaria vectors were more abundant during the late wet season (LWS) and early wet season (EWS)

compared to the early and late dry season in the three agrarian communities under study (Table 1). Previous studies have shown that *Anopheles* abundance and diversity vary with season (Wang *et al*., 2011; Churcher *et al*., 2017). This is because of the availability of suitable breeding sites during the wet season. The present work is in tandem with the results obtained by other researchers in Northern Nigeria who reported higher density of *Anopheles* species sampled in various study locations during the wet season compared to the dry months (Oringanje *et al*., 2011; Lamidi *et al*., 2017; Bashir *et al*., 2018) thus establishing a positive relationship between mosquito abundance and rainfall (Olayemi *et al*., 2011; Umar *et al*., 2015; Amaechi *et al*, 2018). This explains why malaria intensity has been reported to be high immediately after the wet season (NPC & ICF, 2019). However, the findings of this work differed from the results obtained by other researchers (Basher *et al*., 2014; Okorie *et al*., 2015; Tahereh *et al*., 2021). The reported low density of malaria vectors during the wet season by these workers was attributed to heavy rainfall, which washes away mosquito larvae breeding sites, thereby reducing the abundance of adult mosquitoes during the wet season.

The communities where irrigation was practised had higher *Anopheles* species abundance than the non-irrigation community. The LIC, however, had higher *Anopheles* density all year round than the urban Irrigation community (UIC). This study establishes an all-year abundance of malaria vectors in the two communities where irrigation is practised. Kibret *et al*. (2014) opined that the high density of *Anopheles* mosquitoes throughout the year could be attributed to the persistence of water for mosquito proliferation because of the practice of irrigation, which is likely to prolong the duration of malaria transmission into the dry season.

Therefore, this study shows that irrigation is likely to influence the breeding of mosquitoes all year round, thus increasing the risk of being exposed to the bite of an infected mosquito. This could also make malaria transmission continuous in irrigated areas (Klinkenberg *et al*., 2005). This finding agreed with Hawaria *et al.* (2020), who found a two-fold increase in mosquito density in communities where irrigation farming is practised compared to communities where farming activity depends on rain. The work of other researchers in Nigeria also corroborates with the present findings. For instance, Amaechi *et al*. (2018) reported increased vector abundance in villages where irrigation farming was practised more than in non-irrigated villages around a reservoir in Kogi State. A similar result was obtained in the Kano state's Bunkure and Gezawa irrigation sites **(**Oguoma & Ikpeze, 2008; Yakasai *et al*., 2017).

Two rural communities (LIC and NIC) and an urban community (UIC) constituted the study communities. The high density of *Anopheles* species in UIC confirms the findings of other studies that malaria vectors are now adapted to urban environments (Oyewole & Awolola, 2006; Adeleke *et al*., 2008; Townroe & Callaghan, 2014; Surendran *et al*., 2019). The high density of malaria vectors in UIC in the present study could be linked to irrigation farming, which creates suitable grounds for the immature forms of the vector to thrive.

Four species of *Anopheles* mosquitoes were collected from the three communities throughout the study. These are *An. gambiae, An. coustani, An. pharoensis* and *An. funestus. An. gambiae* sl. was the primary vector species encountered throughout the two-year duration of the study. This contrasts with Bamou *et al*. (2021) and other African workers who reported a high diversity of the anopheline fauna. The dominance of *An. gambiae* sl. is, however, consistent with other workers in Nigeria and across the African continent where *Anopheles gambiae* sl. contributes over ninety per cent of the total malaria transmission (Bunza *et al*. (2010); Oluwasogo *et al*., 2016; Yohanna *et al*., 2019).

Anopheles gambiae sensu lato members are known to be the principal malaria vectors in sub-Saharan Africa due to their indoor feeding and resting tendencies (Bamou *et al*., 2021; Sinka *et al.*, 2010). Thus, the abundance of these species may have severe implications for malaria control in the State. This study, however, differed from the result of another study in the Kadawa irrigation area of Kano, where *An. funestus* was reported to be the primary malaria vector (Bashir *et al*., 2018). This shows that malaria vectors may differ considerably

from one locality to another depending on differences in climatic and ecological factors (Appawu *et al*., 2004). Therefore, it is important to have a comprehensive understanding of peculiar factors affecting malaria vector abundance in a locality to develop area-specific control measures that will suit each locality and efficiently lessen the burden of malaria in the state.

The occurrence of three sibling species: *An.gambiae s.s*. and *An. coluzzii* and *An. Arabiensis* was established in the present study. All three sibling species have been incriminated as effective malaria vectors (Awolola *et al*., 2002) and have been reported to co-exist in a sympatric relationship (Akpan *et al*., 2018). *An*. *coluzzii* was the most predominant of the three sibling species across the season (Table 2). In tandem with the results of the present study, Oguoma & Ikpeze (2008) in Kano, Oduola *et al*. (2016) in Kwara and Aju-Ameh *et al*. (2016) in Benue State who separately found *An. coluzzii* to be the dominant of the *An. gambiae* complex across the season in their various studies. *An. colluzii* is known to breed in the dry season and arid areas (Simard *et al*., 2009). The predominance of this species during the wet and dry seasons in this study shows changing ecology and adaptation to varying environmental conditions. It is, therefore, essential to have an in-depth understanding of the forms of *Anopheles* species that are prevalent in a locality for efficient use and disbursement of vector control measures.

Conclusion

This study found that *Anopheles* species were more abundant during wet months than dry ones. Malaria vector density was higher in the communities where irrigation farming is practised (LIC and UIC) than in the communities that depend on rain for farming (NIC) through the dry and wet seasons. Vector density was also higher in the urban irrigated community than in the non-irrigated community. The present work confirms the adaptation of malaria vectors to the urban community due to suitable breeding conditions created through irrigated urban farming. It can be concluded that irrigation farming contributes largely to *Anopheles* mosquito abundance in the three communities studied. The study showed the co-existence of the three sibling species of *Anopheles gambiae* complex in the study communities. *Anopheles coluzzii* was the most dominant malaria vector in the three agrarian communities, irrespective of the farming type practised during the wet and dry seasons. Identifying which form is predominant in a locality, especially considering the diverse ecological microclimate of the study communities, will assist in making the correct decision on the form of control method to employ in each locality.

Study Limitation

The frequency of mosquito collection was quarterly, not monthly. Moreover, only indoor *Anopheles* mosquitoes were sampled; the outdoor biting density was not considered. This might bias the result presented.

Ethical Approval

This study received ethical clearance (AKTH/MAC/SUB/12A/P-3/VI/1805) from the Aminu Kano Teaching Hospital Research Ethics Committee. Consent for participation in this study was obtained from the village heads of the study communities. Verbal and written informed consent to participate in the study was obtained from the heads of households. All participants were informed about the study objectives.

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