Malaria prevalence and incidence in an isolated, meso-endemic area of Mozambique

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Isolated areas, such as the 2x7 km peninsula of Linga Linga in southern Mozambique, are the places where malaria might be most easily eliminated. Currently available control strategies include bed nets impregnated with pyrethroid insecticides (long-lasting insecticidal bed nets,; LLINs), artemisinin combination therapy (ACT) for treatment and rapid diagnostic tests (RDTs) for diagnosis. When these became available, they were applied on the peninsula and their effects on malaria prevalence and incidence measured over the years 2007 – 2011. Following a census of the population and mapping of 500 households, five annual all age malaria prevalence surveys were conducted. Mean prevalence varied from 16% to 65% according to the season in which the surveys were performed. Although children under one year of age had the highest incidence of fever the 5 – 9 year old age group had the highest prevalence of *Plasmodium falciparum*. They also had the highest median parasite density and the highest prevalence of gametocytes. A spatially structured generalised additive model indicated that malaria risk was greatest towards the northern end of the peninsula and that people living in houses with grass or thatch roofs had a greater risk of malaria than those living in houses with corrugated iron roofs. Malaria was diagnosed in 31% of the 4308 visits from residents attending the clinic between March 2009 to May 2011 and overall 63% of those tested were positive. Diagnosis was most accurate (80%) in children under 10 years of age. The incidence of fever was greatest in 1-4 year olds. Children with a fever were more likely to have malaria than those without a fever (χ^2 for diagnosis = 131.9 p< 0.0001, positivity among those tested χ^2 = 12.6 p = 0.0004) but bednet use did not affect the likelihood of having malaria. People living further away from the health post were less likely to attend. Incidence peaked nine weeks after rainfall (r^2= 0.34, p = 0.0002). The proportion of under ten year old resident attendees diagnosed with malaria decreased from 48% in 2009, to 35% in 2010 and 25% in 2011 (for under 1 year olds χ^2 = 10.5, p= 0.005; for 1 to 4 year olds χ^2 = 24.4, p = >0.000**,** for 5-9 year olds χ^2 = 5.92, p = 0.52). At the same time there was a shift in the peak age of cases from 1-4 year olds to 5-9 year olds. Non-residents accounted for 621 visits to the clinic and among these 34% were diagnosed with malaria and 56% (79 of 142) were confirmed. Among non-residents 84 of the diagnosed cases came from urban

areas (with low transmission) and 117 (58%) were normally resident in rural areas. In order to reduce malaria transmission in an area such as Linga Linga further measures of vector control need to be considered.

Malaria prevalence and incidence from an isolated, mesoendemic area of Mozambique

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Abstract

Isolated areas, such as the 2x7 km peninsula of Linga Linga in Mozambique, are the places where malaria might be most easily eliminated. Currently available control strategies include bed nets impregnated with pyrethroid insecticides (long-lasting insecticidal bed nets,; LLINs), artemisinin combination therapy (ACT) for treatment and rapid diagnostic tests (RDTs) for diagnosis. When these became available, they were applied on the peninsula and their effects on malaria prevalence and incidence measured over the years 2007 – 2011. Following a census of the population and mapping of 500 households, five annual all age prevalence surveys were conducted. Information on LLIN use, house construction, and animal ownership was obtained. Mean prevalence varied from 16% to 65% according to the season in which the surveys were performed. Although children under one year of age had the highest incidence of fever the 5 – 9 year old age group had the highest prevalence of *Plasmodium falciparum*. They also had the highest median parasite density and the highest prevalence of gametocytes. A spatially structured generalised additive model indicated that malaria risk was greatest towards the northern end of the peninsula and that people living in houses with grass or thatch roofs had a greater risk of malaria than those living in houses with corrugated iron roofs. Malaria was diagnosed in 31% of the 4308 visits from residents attending the clinic between March 2009 to May 2011 and overall 63% of those tested were positive. Diagnosis was most accurate in children under 10 years of age. The incidence of fever was greatest in 1-4 year olds. Children with a fever were more likely to have malaria than those without a fever (χ^2

for diagnosis = 131.9 p< 0.0001, positivity among those tested χ^2 = 12.6 p = 0.0004) but bednet use did not affect the likelihood of having malaria. People living further away from the health post were less likely to attend. Incidence peaked nine weeks after rainfall (r^2= 0.34, p = 0.0002). The proportion of under nine year old resident attendees diagnosed with malaria decreased from 48% in 2009, to 35% in 2010 and 25% in 2011 (for under 1 year olds χ^2 = 10.5, p= 0.005; for 1 to 4 year olds χ^2 = 24.4, p = >0.000, for 5-9 year olds χ^2 = 5.92, p = 0.52). At the same time there was a shift in the peak age of cases from 1-4 year olds to 5-9 year olds. Non-residents accounted for 621 visits to the clinic and among these 34% were diagnosed with malaria and 56% (79 of 142) were confirmed. Among non-residents 84 of the diagnosed cases came from urban areas (with low transmission) and 117 (58%) were normally resident in rural areas. In order to reduce malaria prevalence in an area such as Linga Linga further measures of vector control need to be considered if reductions in malaria prevalence are to be achieved.

Introduction

Malaria remains a serious problem in Mozambique. According to UNICEF it is the leading killer of children, contributing to around 33 per cent of all child deaths and overall more deaths have been attributed to it (28.8%) than to any other single cause, including HIV/AIDS [www.unicef.org/mozambique/child_survival_2933.html]. Figures like these from many areas of Africa have led to major funding initiatives directed towards its control. These have met with considerable success, much of which has occurred through widespread use of Long Lasting Insecticidal Nets (LLIN's) for prevention and the use of a highly effective drugs, the artemisinin-based combination therapies (ACT's), for treatment. These successes have prompted the idea that the disease can locally be eliminated, eventually leading to its global eradication.

If malaria can be eliminated from anywhere, it is from isolated areas such as islands and peninsulas that are surrounded by mosquito-hostile environments, be that sea, desert or uninhabited land. In such places there is much less immigration and emigration of vectors (and people) than in more connected environments (Aregawi et al., 2011, Hagmann et al., 2003, Ishengoma et al., 2013, Pinto et al., 2003, Lucas 2010, Lum et al., 2007, Sudomo et al, 2004, Teklehaimanot et al., 2010). The sandy, low altitude peninsula of Linga Linga, 500 km north of Maputo, is such an isolated area, since, apart from a 2 km stretch of uninhabited land at the narrow neck, it is surrounded by saline water making it a virtual ecological island.

In 2007, a project to determine the impact on malaria of introducing currently available control strategies, including LLINs and treatment with ACT's (artemether-lumefantrine, AL, according to national guidelines), was implemented on the peninsula. Due to the delayed acquisition of immunity the mean age of maximum prevalence may increase, although prevalence itself may not change (Smith et al., 1993). A decrease in incidence and a shift towards older age groups falling ill are, however, more sensitive measures of changes in transmission than estimates of prevalence. Incidence was therefore monitored from 2009-

2011 in a clinic established by the project whilst prevalence for the years 2007–2011 was monitored in annual all age prevalence surveys.

An understanding of risk factors for malaria can guide novel, possibly site specific, control measures to be used in places like Linga Linga. Possible risk factors were, therefore, examined. Both spatial and temporal analysis of the data was undertaken and the results are discussed in relation both to the effect that the interventions had on malaria transmission and to possible additional control techniques that might be applied on the peninsula.

Methods

Linga Linga (23°43'1.29"S, 35°24'15.04"E), which lies 6km to the east across the Morrumbene Bay opposite the district capital Morrumbene, has been described by Charlwood et al., (2013) and Thomsen et al. (2013) and appears on a number of websites. People are involved in fishing and the production of copra or the artisanal manufacture of raffia baskets, hats and bags. A number of tourist lodges, employing non-resident and local labour, have been built in recent years or are under construction. At the time of the initial survey there was no health centre on the peninsula, the nearest health centres being in the village of Coche, five km to the north of Linga Linga, or in Morrumbene itself. *Anopheles funestus* is the only malaria vector on the peninsula. During the long dry season, the mosquito may become gonotrophically discordant and individual mosquitoes may survive for long periods taking several blood meals without laying eggs. Thus, despite low numbers of mosquitoes, transmission continues (Charlwood et al., 2013).

At the start of the project all residents were censused, informed of the purpose of the study and consent to participate was obtained; houses were mapped (with Garmin e-Trex hand held global positioning system (GPS) receiver units) and numbered. House dimensions and manner of construction were noted.

Risk factors examined

Since the mosquitoes in Linga Linga may be gonotrophically discordant (Charlwood et al., 2103) they may feed where they rest (as well as rest where they feed) increasing exposure. The kind of roof that covers a house may influence the likelihood of the vector resting inside (Kirby et al., 2005). Roofs and walls were categorized on whether the material in which they were made from 'natural' materials (reed, palm leaf, grass, palm frond) or 'man-made' ones such as corrugated iron, bricks or tiles. Other possible risk factors recorded were the number of animals kept by householders, age and number of residents, bednet ownership, the duration of residency and sources of drinking and washing water (separated into 'in the house', 'from a well' or 'neighbours').

Interventions

In 2007 the fifteen households with more than two children below ten years of age received two nets and 100 of the remaining 141 households with children a single net. In this case households with the youngest children were given priority. In 2008, two days prior to the prevalence survey, a further 500 LLINs were distributed.

In March 2009 a clinic was established in an unused cement house in a central location (Fig 1). The clinic was open from Monday to Friday in the mornings, with a resident nurse also available for emergency consultations at other times.

Prevalence surveys

The sampling selection was similar to that described in Smith et al., (1993). Following the initial census, in February 2007, residents were invited to attend an all-age baseline malaria prevalence survey. Subsequent surveys were conducted in February 2008, March 2009, April 2010 and June 2011.

Seven locations were chosen as survey field sites. Local residents were informed the day prior to the survey that it would be taking place, and invited to come to the site location to be surveyed. In addition, a survey was undertaken at the school to collect data of school-aged children who had not been previously screened. During the surveys, residents were asked if they have experienced malaria since the start of the year and where they went for treatment. In addition to these questions in the initial survey (2007), information on absence from the peninsula (duration, location, means of transport and whether they had used a net when away) was also collected. In subsequent surveys, people were asked (in the local language): 1. 'How long have you lived in your present house?' 2. 'Where did you come from?' 3. 'Do you have a mosquito bednet?' 4. 'Did you sleep under it last night?' and

5. 'Where did you obtain your net?' Thus, the parasitology datasets contained information on the individual's house number, name, age, sex, whether or not they tested positive for malaria, and whether or not they used a bednet the previous night.

In all surveys finger prick blood was used in the preparation of thick and thin blood films. Films were stained with 5% Giemsa for 20 minutes and examined at the National reference laboratory in Maputo for the presence of parasites. Slides were read twice and numbers of parasites per 500 leucocytes were counted and converted to densities per micro-litre of blood, assuming a density of 8000 leucocytes per micro-litre (Bruce-Chwatt, 1985). Parasite density per micro-litre of blood was determined according to the formula:

Density = ([P.f Count] * [8000])/ [White Blood Cell Count]

People's temperature was also taken. In surveys from 2008 onwards, a malaria Rapid diagnostic test, RDT (OptiMal ®) was given to anyone with a fever (defined as an axillary temperature of >37.5 °C). Those that tested positive by RDT were treated with AL according to national guidelines.

Incidence data

The age, sex and house number or resident status (resident or visitor) of attendees to the clinic was recorded over the period March 2009 – May 2011. Attendees were asked how long they had had their symptoms, including headache and fever and whether they had slept under a bednet the previous night.

When they were available RDTs were used to determine if patients reporting with symptoms and/or fever had malaria. At the same time (also when RDTs were not available), a blood slide was taken and subsequently read for parasite confirmation. Thick and thin blood films were prepared of diagnosed cases and subsequently read by a microscopist in Morrumbene. Parasite density was, however, not determined. In the absence of RDT's treatment was, therefore, based on clinical diagnosis, which was subsequently checked by microscopy. People with parasites confirmed by RDT, or presumptively diagnosed with malaria when RDTs were not available, were treated with AL.

Rainfall data

Daily rainfall data from the town of Maxixe, approximately 15 km from Linga Linga, kindly provided by the Rio-Sul water management project, were used to compare incidence rates with rainfall. Although it rains less on Linga Linga than it does in Maxixe, the relative difference between years is still likely to occur.

Data analysis

Data was entered into MS Excel spreadsheets and analysed with the software R (R Core Team, 2013). Summaries of the 2007 census data were produced, including the age distribution of the population and bednet ownership and use by sex. Prevalence surveys (2007-2011) were matched with the census data using the unique household ID number (additional file 1) which enabled overall annual malaria prevalence to be tabulated, and household-level malaria prevalence to be mapped using the software ArcGIS. Annual prevalence and the geometric mean parasite density by age group (<1, 1-4, 5-9, 10-19, 20-29, >29) were calculated to assess whether there was any evidence of a change in the age distribution of cases. An individual-level multiple logistic regression model was fitted to the prevalence data, with potential risk factors under consideration including age group, bednet usage and household characteristics (roof type, door type, distance to the clinic, number of people, water and sanitation access). A backwards stepwise model selection approach based on minimising the Akaike Information Criterion (AIC) was used to determine which variables to include in the final model. A generalised additive model (GAM) was then fitted to the data by adding a spatially smooth term to the final model to account for any possible residual spatial dependency in the data, and a map of this term was produced.

Summaries of the percentage of clinic attendees who were diagnosed with, or tested for malaria were calculated by age group, sex, resident status (resident or non-resident), reporting year, and bednet usage and chi-squared tests were performed in order to elucidate whether there was an association between malaria risk and these variables. The straight-line distance between households and the clinic was calculated using ArcGIS and the correlation between the number of visits per person per household and distance to the clinic was calculated to assess whether people living further away were less likely to seek treatment.

Ethics

The project received ethical clearance from the National Bioethics Committee of Mozambique (reference 123/CNBS/06) on the 2nd of August 2006.

Results

Population composition

There were 467 households recorded in the census of 2007. A further 33 houses were recorded early in 2008 giving a total of 500. The locations of the households are presented in Figure 1. The age distribution of the population is given in Table 1. Table 1 also provides data on the age of the study population and the ages of residents and non- residents attending the clinic. Of the 195 households recorded in the census of 2007 with resident children less than 15 years of age, 118 had only one child, 46 had two children, 21 had three children, nine had four and one house had five children. Five point seven percent (5.7%) of the population was between 55 and 64 years of age and 9.1% was over 65 years of age (compared to a national average of 3.5% and 2.9% respectively derived from www.theodora.com, z test $p < 0.05$).

At the start of the study only 183 (19%) people from 58 (12%) households used a bednet. Bednet use was equally divided amongst the 447 males and 528 females. Of the 410 people who completed the baseline prevalence survey in 2007, 163 (40%) had been out of Linga Linga in the previous year. Of these, 146 had left by boat, five had gone by foot and only three had travelled by car. The majority of people who reported that they had been absent from the peninsula in the previous year had only spent one or two nights away.

Prevalence and density of malaria parasites 2007-2011

An overview of the parasitology datasets, including the number of individuals per survey, and the number of individuals that matched the 2007 census data, is presented in Table 2.

Fever (axillary temperature temperature of ≥37.5°C) and malariological indices varied with age (Fig. 2). The risk of fever was at a maximum in children less than 1 year old and showed a gradual decline with age (Fig. 2a). The prevalence of *P. falciparum* parasitaemia peaked in the 5-9 year age group (Fig. 2b) but median parasite densities were highest in the 1-4 year age group (Fig. 2c). Blood stage parasites were not seen in five of the 21 gametocyte carriers identified in 2009, in seven of 14 identified in 2010 nor in eight of 21 identified in 2011. In all years the majority (67%) of gametocyte carriers were under 10 years of age, although gametocytes were seen in all age groups (Fig. 2d). The prevalence of gametocytes dropped from 39.5% (135 of 342) in *P. falciparum* positive slides before the opening of the clinic to 14.7% (33 of 224) once it had opened (χ^2 = 22.6, p = < 0.05). *Plasmodium malariae* also peaked in 5-9 year olds, but the numbers recorded were very small (Fig. 2e). Among people attending the surveys reported bednet use was lowest among 10-19 year olds (Fig. 2f). The reasons given for non-use included that the net was 'too hot'; that there were no mosquitoes; that they were ill or that they just didn't like it.

In 2007, 24.4% (11 of 45) of the malaria positive individuals were children less than five years old whilst in 2011, only 8.9% (5 of 56) of the malaria positive individuals were children less than five years old.

Overall prevalence varied from one survey to the next with a marked increase in prevalence in the 2009 survey (Fig. 3). More than 1,000mm of rain were recorded in Maxixe over the wet season of 2009 compared to the less than 400 mm recorded in 2007. More rain in 2009 may have affected prevalence. Not only was there less rain in 2007 but it fell later (the peak rain falling in the first week of April –week 14) compared to other years (which varied from week 49 to week 4). Indeed, the survey in 2007 took place during the rains whilst the other surveys were undertaken at lags of seven (2008), 13 (2009), 17 (2010) and 18 (2011) weeks after the peak week of rain.

Risk Factors.

A multiple logistic regression model was fitted to the data from the 618 surveyed people for which matching covariate data was available from the census. A significant relationship was observed between being infected with malaria and year of survey, age group, roof category, door category, number of people per household, water source category, washing water category and whether or not the surveyed person slept under a bednet on the previous night. Using a backwards, stepwise model selection approach, the final fitted model included year, age group, number of people in the house and roof category (Table 3), such that after adjusting for other risk factors, people who lived in houses having a roof made of thatch or other 'Green' material had an increased risk of having parasites than those who lived in houses with a roof of corrugated iron or other man-made material. The number of people living in the house was also a risk, as was age.

A generalised additive model (GAM), i.e. a logistic regression model with a smooth term for spatial location, was fitted to the individual-level data to determine whether there was any spatial pattern in malaria prevalence after accounting for observed risk factors (see the supplementary information). The fitted GAM indicated that there was an area of lower risk in the southeast of the study region, and an area of higher risk in the north and west of the study area (Fig. 4) after adjusting for other risk factors.

Incidence data

In the 28 months (March 2009 – May 2011) that the clinic was operational there were 4929 visits to the clinic, with 4308 (87%) of attendees residing in Linga Linga. Hence, despite its isolation 621 (13%) of the people attending were non-residents. Residents and visitors were analysed separately.

Among the residents, 31.2% (1343/4308) were clinically diagnosed with malaria and 868 (65%) of these were tested by blood slide and/or RDT, resulting in 543 (63%) who tested positive for *P. falciparum*.

Fever and malariological indices among residents attending the clinic varied with age (Fig. 5). The risk of fever was at a maximum in 1-4 year old children. As in the prevalence surveys it declined with age but in this case more slowly (Fig. 5a). Significantly more of the attendees with fever were malaria positive than those without fever (χ^2 for diagnosis = 131.9 $p< 0.0001$, positivity among those tested χ^2 = 12.6 $p = 0.0004$). Of the 586 people who had, or reported having had, a fever when attending the clinic, 348 (59.4%) were clinically diagnosed with malaria and of the 209 tested (either microscopically or with RDT), 167 (80.4%) were positive. From the 2423 people recorded attending without a history of fever, 995 (36.5%) were clinically diagnosed with malaria out of which, 659 of these patients were tested and 283 (42.9%) were positive.

Overall peak diagnosis and peak positivity occurred in the 5-9 year age group (Fig. 5b & 5c). Thus the accuracy of the diagnosis was greatest in this age age group. As in the prevalence surveys reported bednet use among residents attending the clinic was lowest among 10-19 year olds (Fig. 5e). People using a net the night before reporting ill were, however, as likely to have malaria as those who did not - of the 720 people who reported using a net that were diagnosed and tested for malaria, 450 (63%) were positive, whilst of the 148 tested who did not use a net, 93 (63%) were positive for malaria.

In the 20 – 39 and the over 40 years age groups more females than males were diagnosed or tested for malaria but the majority of these tests were negative (Fig 6).

A similar proportion (34%; 213/621) of non-residents were clinically diagnosed with malaria. Among these, 142 (67%) were tested by microscopy and/or RDT and 79 (56%) were positive. Among non-residents, 84 of the diagnosed cases came from urban areas (where transmission is low or absent) and 117 (58%) came from nearby rural areas (where autochthonous transmission is likely to occur). There was, however, no significant difference in the likelihood of urban and rural non-residents having a confirmed case of malaria (two tailed Fishers exact test p = 0.217).

Fever and malariological indices among visitors attending the clinic also varied with age in much the same way that they did among residents (Fig. 7 a-d).

The proportion of under nine year old resident attendees diagnosed with malaria decreased significantly from 48% in 2009, to 35% in 2010 and 25% in 2011 (for under 1 year olds χ^2 = 10.5 p= 0.005; for 1 to 4 year olds χ^2 = 24.4 p = >0.000, for 5-9 year olds χ^2 = 5.92 p = 0.52). At the same time there was a shift in the peak age of cases from 1-4 year olds to 5-9 year olds (Fig. 8).

In under nine year olds the incidence of malaria was seasonal and followed the rainfall (Fig. 9). The highest correlation between cases and rainfall occurred with a lag of nine weeks (Spearman correlation co-efficient between incidence and weekly rainfall = 0.34 p = 0.0002).

There was no significant clustering of cases attending the clinic, although by mapping the number of visits per household and weighting these values by number of people in the household (obtained from the census data), there was evidence that those living away from the clinic were less likely to attend (Spearman correlation co-efficient between distance to clinic and number of visits per person per household = -0.1492, p = 0.0031) (Fig. 10).

Discussion

Patterns of malariological indices observed during the prevalence surveys were similar to those reported from the Kilombero valley from 1989-1991 (Smith et al., 1993) but transmission was considerably lower. Peak prevalence of *P. falciparum* was, however, observed in the 5-9 year age group rather than the 1-4 year age group recorded in the Kilombero. The prevalence of *P. malariae* also peaked in 5-9 year olds. Despite peaking in the 1-4 year age group the median *P. falciparum* density was half that described from the Kilombero whilst clinical malaria episodes occurred in all ages of hosts in Linga Linga. This suggests that the level of clinical immunity never reaches the levels achieved by adolescents in holoendemic areas. Nevertheless, despite the lower transmission there was considerable mixing of parasites since the frequency of the *Pfcrt* CVMNK wild type gene in *P. falciparum* from Linga Linga increased from 44% to 66% within a single year (Thomsen et al., 2013). Smith et al. (1993) concluded their paper, on transmission in the Kilombero, that 'The effects of interventions such as impregnated bednets on parasite prevalence or density are likely to be minimal.' In other areas where there is moderately intense seasonal transmission, such as The Gambia, there is highly seasonal malaria morbidity but also much less seasonality in parasite prevalence (Greenwood et al., 1987, Lindsay et al., 1991). Thus even at the intensity of transmission observed in Linga Linga it is possible that effects on prevalence due to the widespread use of LLIN's are overridden by other, periodic and chaotic effects, as suggested by Kwiatkowski and Novak (1991).

In general we have no reason to suppose that isolation causes the clinical epidemiology of malaria in Linga Linga to differ from that on continental Africa. Malaria was the most common diagnosis for children under ten years of age attending the clinic. Fever peaked in the 1-4 year olds, but the proportion of attendees diagnosed with malaria was greatest in the 5-9 year olds. Diagnosis was also more accurate in children under ten years of age than in older age groups, most of whom were women. It is likely that these were mothers or carers of sick children who also asked to be tested for malaria when they bought their sick child to the clinic.

Together the interventions appeared to have a major impact on incidence and morbidity. Among children below ten years of age the proportion diagnosed with malaria, decreased by almost a half during the time that the clinic was open. At the same time there was a shift in the peak age of incidence towards older age groups. Although not statistically significant the possible peak shift from 1-4 to 5-9 year olds in prevalence rates in sequential prevalence surveys may also be due the interventions (Smith et al., 2001, Ishengoma et al, 2013). Although these changes may have been partly due to the use of nets these had been available for more than a year prior to the opening of the clinic and it may be that the clinic itself was having an impact. Treatment with ACT significantly reduces infectiousness of individual patients with uncomplicated falciparum malaria compared to previous first line treatments. Rapid treatment of cases before gametocytaemia is well developed may enhance the impact of ACT on transmission (Okell et al., 2011). The drop in the prevalence of gametocytes from surveys undertaken before the opening of the clinic to that observed (by the same two microscopists) once it was in operation may, therefore, have been due to the more widespread use of ACT's and this may have reduced transmission.

Reducing risk factors may also reduce transmission. We were able to identify a variety of risk factors, some of which can perhaps be reduced. For example, living in a house with a thatched roof was associated with an enhanced malaria risk. *Anopheles funestus* may be more likely to rest inside houses that have thatch, rather than iron, roofs. Should the mosquito, due to the lack of suitable oviposition sites, have an extended gonotrophic cycle, as postulated by Charlwood et al., (2013), then it may feed where it rests (rather than merely rests where it feeds). Hence occupants of thatched roofed houses may be at greater risk of transmission than those in iron roofed ones (Kirby et al., 2008, Mmbando et al., 2011, Tami et al., 2012). Although it produces a shift, as e.e. cummings would say, from a 'world of born' to a 'world of made', the replacement of thatch with tin roofs would probably reduce transmission in Linga Linga and similar areas.

The number of inhabitants in a house and their age were also risk factors. Greater numbers of mosquito are attracted to houses as the number of occupants increase (Charlwood et al., 2013). Should infected mosquitoes be more likely to take interrupted feeds on different hosts (Anderson et al., 2000) then, even if the numbers of mosquito per inhabitant remain the same, the risk of transmission will be greater. Having individual bedrooms would make it more difficult for the mosquito to take such interrupted feeds on multiple hosts.

In Linga Linga the risk of being parasite positive was higher towards the northern end of the peninsular. The northern end of the peninsula is more sheltered and less exposed to wind and has a higher exposure to anophelines (Charlwood et al., 2013) than the southern end of the peninsula. The use of LLINs should particularly be encouraged (and monitored) among the inhabitants of the northern end of the peninsular. Incidence was seasonal, with a peak of cases nine weeks after peak rainfall. This should enable health authorities to plan drug supplies to ensure that the clinic has an adequate supply of drugs for such times.

The increasing number of people who did not have an associated house number in the years following the census indicates that, despite its isolation, there was a considerable movement of people into, and perhaps out of, Linga Linga. Among non-residents, 58% of attendees at the clinic came from areas where active transmission was likely to have occurred and so they may have been importing malaria into the area, whilst 42% came from urban areas where transmission is low or absent and they may have acquired their malaria on the peninsula. Thus, not only do areas like Linga Linga pose a threat to non-immunes (from the cities) but importation of malaria is also a continuing possibility. Importation of malaria will pose problems for future elimination projects in isolated areas like Linga Linga.

Should people arrive without nets and should there not be a system that enables them to obtain them, then the risk of transmission will be maintained. Visitors, including those from urban areas, attending the clinic were, however, as likely as residents to have slept under a bednet before attending. Since people rarely travel with their own net this implies that, despite the low numbers of nets distributed, there were sufficient nets available for guests to be provided with one.

Elsewhere malaria appears to be close to elimination in a number of islands in which ACTs and bednets, (Battarai et al., 2007) and/or indoor residual spraying of insecticides (Teklehaimanot et al., 2010) combined with active surveillance of cases (Lucas, 2010, Lum et al., 2007) have been used, although the caveat to this is that resurgence is always possible (Hadji et al., 2013). Resistance to pyrethroids in *An. funestus* is widespread (being detected from South Africa to Mozambique and Malawi). The mosquito from the village of Furvela, 8km, from Linga Linga, was resistant to the insecticide when tested in 2009 (Charlwood, J.D. and Kampango, A., unpublished data). It is, therefore, likely that the mosquito in Linga Linga was also resistant to the insecticide used on the nets in the present study. Given the endophilic nature of *An. funestus* indoor residual spraying (IRS) has been successful against this vector in the past. IRS is expensive (30$ per house), however, and time limited.

Even with an effective insecticide resistance will eventually develop and reliance on conventional control measures (including LLIN's, ACT's and IRS) may therefore eventually lead to rebounds in transmission as selection against these measures (in the mosquito or the parasite) starts becoming effective (Hadji et al., 2013). Hence, despite a proven effectiveness of IRS, and because of its cost, additional alternative control measures are likely to be needed, even to maintain present gains. Such measures should be simple, easy to apply on a do-it-yourself basis, and should be long lasting in their effect and not based on insecticides. In addition, or as an alternative, to replacing a thatch roof with one of tin, applying old mosquito netting over the openings where mosquitoes enter houses would be useful (Kampango et al., 2013). The technique does not dramatically reduce airflow or illumination but reduces mosquito entry. It can be done on a DIY basis and once in place does not need the householder to do anything to maintain protection.

We have also previously shown that exposure to vectors in Linga Linga is greatest close to the temporary pond, some 800m from the clinic. Larviciding this pond and the limited number of known breeding sites at the start of the rainy season would also be an obvious thing to do (Keiser et al., 2005). Preventively treating children under nine years of age, the most at risk group, at this time may also be useful (Aponte et al., 2009).

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1

Map of Linga Linga showing the distribution of houses recorded in the census of 2007 according to roof type.

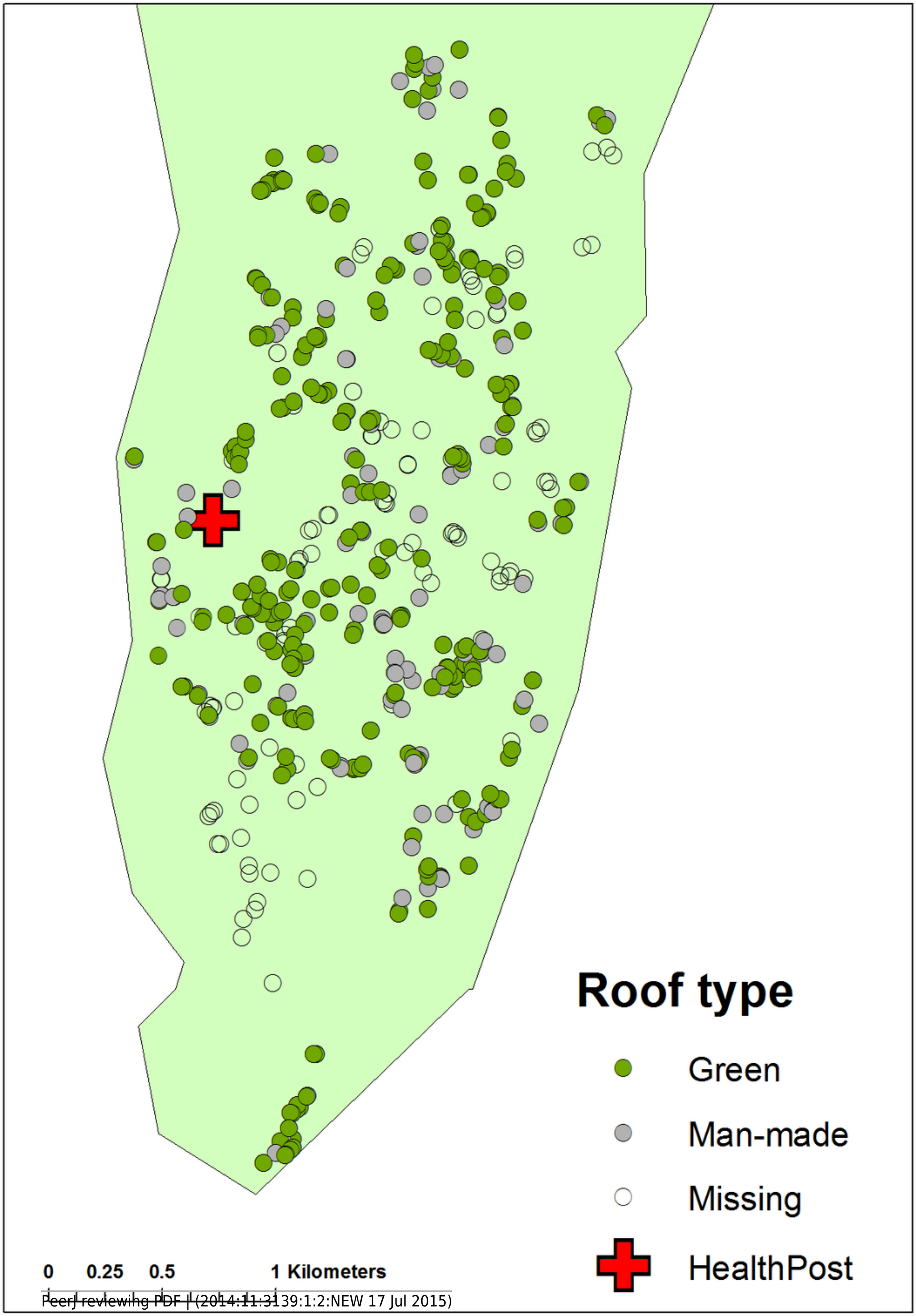

Roof type
Green
Man-made
Missing
HealthPost
0 0.25 0.5 1 Kilometers

Table 1 (on next page)

Number of people recorded in the 2007 census, number of slides taken during prevalence surveys and attendance at the clinic (residents and visitors) by age group, Linga Linga, Mozambique

		Prevalence	Incidence					
Age in years	Census 2007	Number of slides	Resident attendance	Number diagnosed	% positive	Visitor attendance	Number diagnosed	% positive
< 1	43	49	342	52	74	25	5	100
1-4	66	182	968	223	83	93	31	80
5-9	119	442	588	159	80	28	28	100
10-19	227	457	466	108	70	65	65	75
20-39	248	182	2435	484	54	237	237	68
> 40	266	386	2051	317	48	170	170	53

Table 2 (on next page)

Summary of data sets from the prevalence surveys 2007-2011, Linga Linga peninsular, Mozambique.

	Raw data				Matched data		
Year	Number of individuals	% with house number	Number of Houses	% Positive	Number of individuals	Number of Houses	% Positive
2007	411	91%	229	16%	308	179	15%
2008	345	59%	158	34%	191	136	24%
2009	435	68%	183	65%	285	160	67%
2010	398	56%	137	29%	220	127	27%
2011	282	48%	103	44%	131	99	44%
Total	1871	66%	230	38%	1135	332	35%

Figure 2(on next page)

Age dependence and malariological indices, Linga Linga, Mozambique

Prevalence surveys – a) fever, b) prevalence *P. falciparum*, c) median *P. falciparum* density, d) prevalence of *P. falciparum* gametocytes, e) *P. malariae*, f) used net

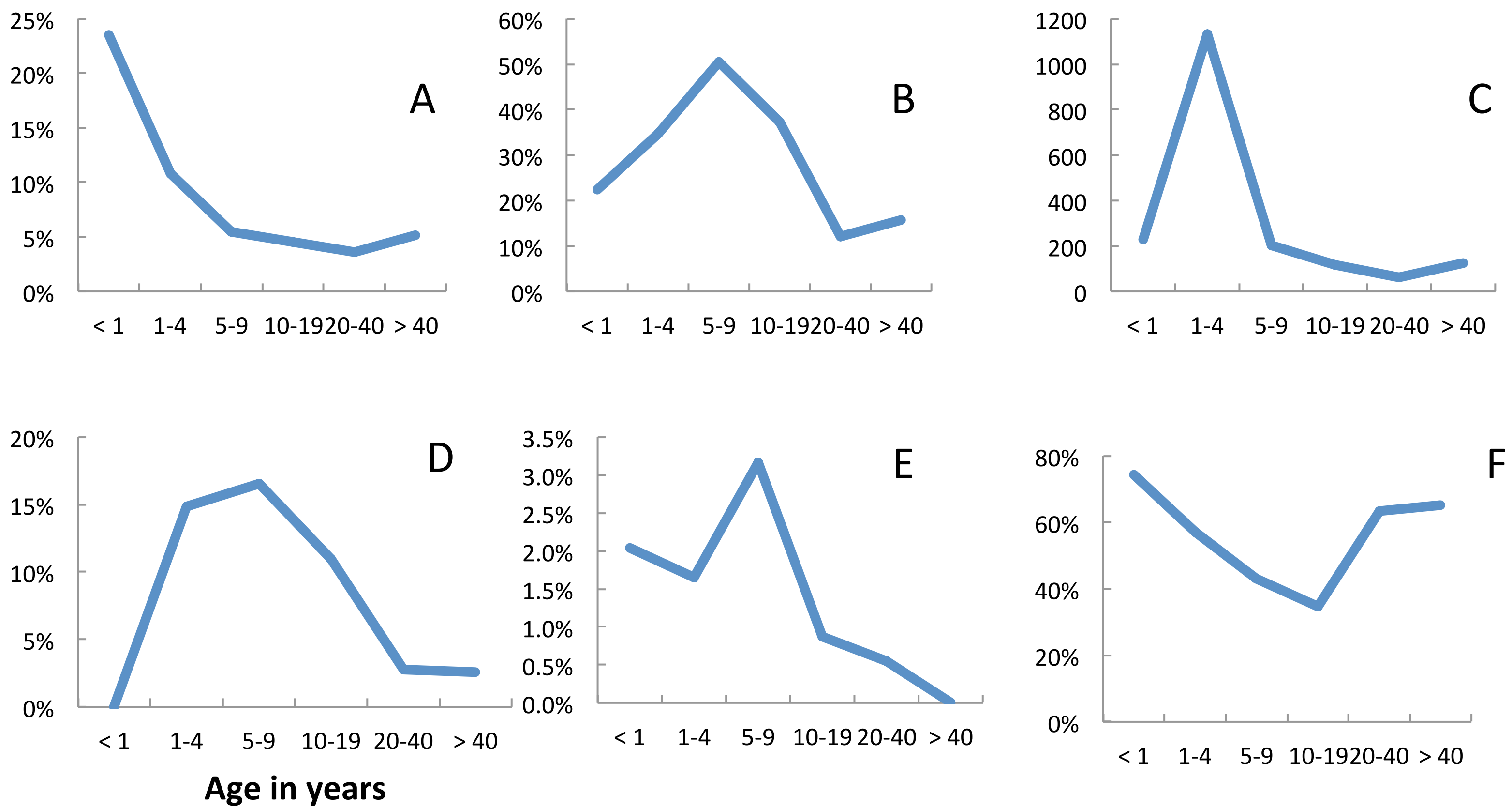

A
25%
20%
15%
10%
5%
0%
< 1
1-4
5-9
10-19
20-40
> 40
B
60%
50%
40%
30%
20%
10%
0%
C
1200
1000
800
600
400
200
0
D
E
3.5%
3.0%
2.5%
2.0%
1.5%
1.0%
0.5%
0.0%
F
80%
60%
40%
20%
Age in years

Figure 3(on next page)

Prevalence and rainfall, Linga Linga, Mozambique

Annual prevalence by age group and rainfall (measured in Maxixe), Linga Linga, Mozambique

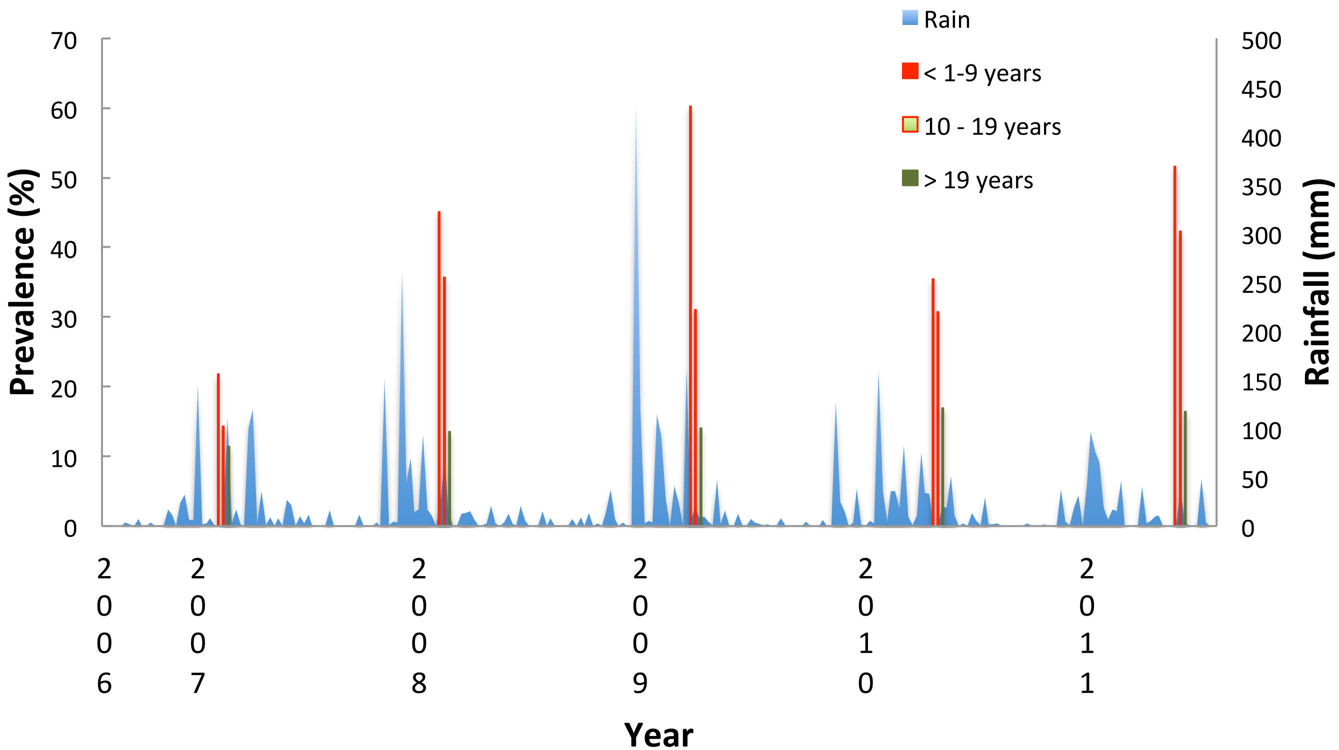

Rain
< 1-9 years
10 - 19 years
> 19 years
Prevalence (%)
Rainfall (mm)
Year
70
60
50
40
30
20
10
0
500
450
400
350
300
250
200
150
100
50
0
2006
2007
2008
2009
2010
2011

Table 3 (on next page)

Individual and household characteristics by malaria status

Summaries of individual and household characteristics by malaria status and adjusted oddratios obtained from fitting a multiple logistic regression model to the data from malaria prevalence surveys 2007-2011, Linga Linga peninsular, Mozambique

	Malaria test result							
	Positive		**Negative**		**Total**	**OR**	**95% CI**	**p-value**
	N	**(%)**	**N**	**(%)**				
Year								
2007	45	(15%)	263	(85%)	308			
2008	46	(24%)	145	(76%)	191	1.91	1.10- 3.29	0.0206
2009	190	(67%)	94	(33%)	284	11.4	7.29-18.07	<0.0001
2010	60	(27%)	160	(73%)	220	3.05	1.88 -5.00	<0.0001
2011	57	(44%)	74	(56%)	131	4.97	2.88-8.63	<0.0001
Sex								
Female	226	(32%)	472	(68%)	698			
Male	167	(40%)	255	(60%)	422			
Missing	5	(36%)	9	(64%)	14			
Age group								
< 1	12	(27%)	32	(73%)	44			
1-4	39	(44%)	49	(56%)	88	2.71	1.04- 7.50	0.0472
5-9	108	(45%)	133	(55%)	241	3.31	1.40- 8.44	0.0086
10-15	106	(39%)	167	(61%)	273	2.22	0.94 -5.64	0.0783
16-25	21	(21%)	78	(79%)	99	0.76	0.28 -2.14	0.5879
>25	83	(27%)	223	(73%)	306	1.18	(0.50 3.00)	0.7119
NA	29	(35%)	54	(65%)	83			
Used net								
No	124	(30%)	289	(70%)	413			
Yes	143	(38%)	229	(62%)	372			
NA	131	(38%)	218	(62%)	349			
Nº people								
1	46	(26%)	134	(74%)	180			
2	137	(37%)	231	(63%)	368	1.43	0.86-2.39	0.1744
3	120	(43%)	160	(57%)	280	1.85	1.09-3.17	0.0236
>3	95	(31%)	211	(69%)	306	0.93	(0.55 1.61)	0.7987
Nº bedrooms								
1	314	(34%)	600	(66%)	914			
2	70	(38%)	113	(62%)	183			
3	14	(38%)	23	(62%)	37			
Own animals								
Yes	248	(35%)	468	(65%)	716			
No	150	(36%)	268	(64%)	418			
Wall category								
Other	53	(39%)	83	(61%)	136			
'Green'	331	(35%)	621	(65%)	952	0.52	0.289-0.898	0.0115
NA	14	(30%)	32	(70%)	46			
Roof category								

	Malaria test result							
	Positive		Negative		Total	OR	95% CI	p-value
	N	**(%)**	**N**	**(%)**				
Other	93	(30%)	222	(70%)	315			
'Green'	293	(37%)	501	(63%)	794	2.16	(1.41-3.38)	0.0005
NA	12	(48%)	13	(52%)	25			
Water source category								
House	62	(31%)	138	(69%)	200			
Neighbouring	84	(28%)	214	(72%)	298			
Well	252	(40%)	384	(60%)	636			
Washing category								
House	70	(27%)	188	(73%)	258			
Neighbouring	75	(30%)	175	(70%)	250			
Well	253	(40%)	373	(60%)	626			

4

4

Spatial pattern in malaria prevalence

Spatial pattern in malaria prevalence, after accounting for observed risk factors, determined by a Generalised Additive Model (GAM), fitted to the individual-level data. (For details see the supplementary information).

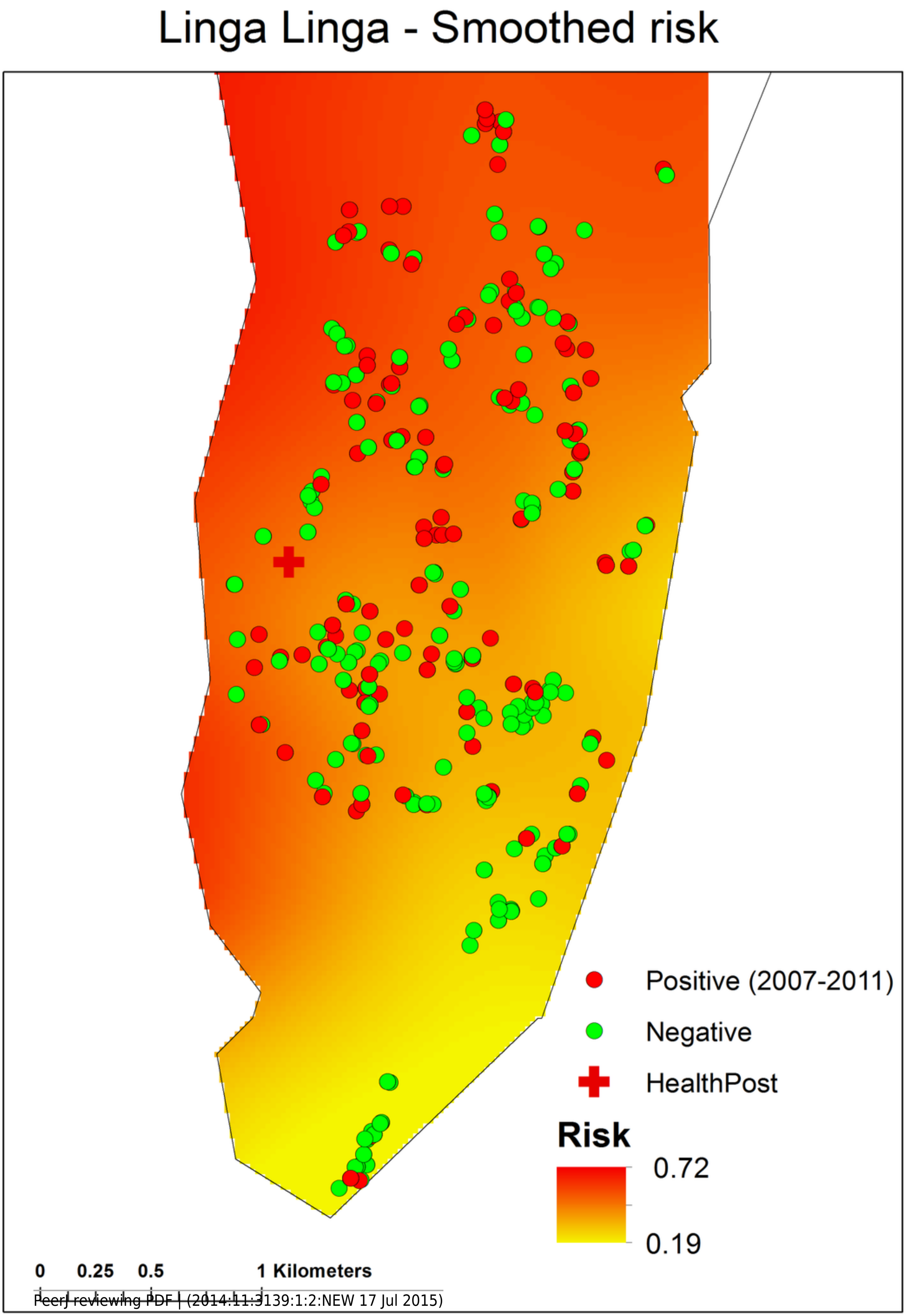

Linga Linga - Smoothed risk
Positive (2007-2011)
Negative
HealthPost
Risk
0.72
0.19
0 0.25 0.5 1 Kilometers

Figure 5 (on next page)

Malaria incidence among residents, Linga Linga, Mozambique

Incidence among residents – a) fever, b) diagnosed *P. falciparum*, c) confirmed *P. falciparum*, d) proportion used net

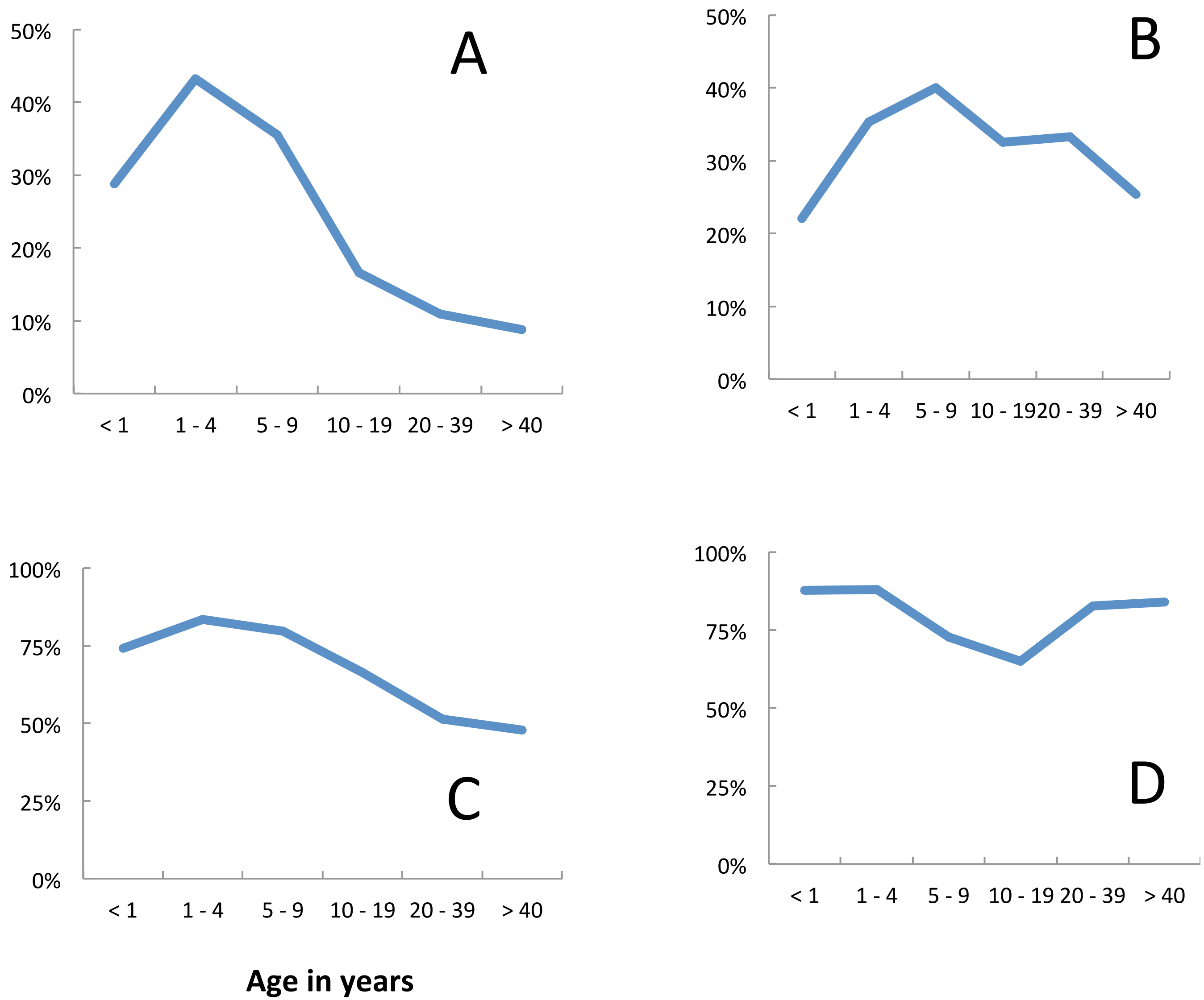

A
50%
40%
30%
20%
10%
0%
< 1
1 - 4
5 - 9
10 - 19
20 - 39
> 40
B
50%
40%
30%
20%
10%
0%
< 1
1 - 4
5 - 9
10 - 19
20 - 39
> 40
C
100%
75%
50%
25%
0%
< 1
1 - 4
5 - 9
10 - 19
20 - 39
> 40
D
100%
75%
50%
25%
0%
< 1
1 - 4
5 - 9
10 - 19
20 - 39
> 40
Age in years

Figure 6 (on next page)

Number of people attending the Linga Linga clinic (2009-2011) reporting symptoms of malaria by sex, age group and positivity

Number of people attending the Linga Linga clinic (2009-2011) reporting symptoms of malaria by sex, age group and positivity

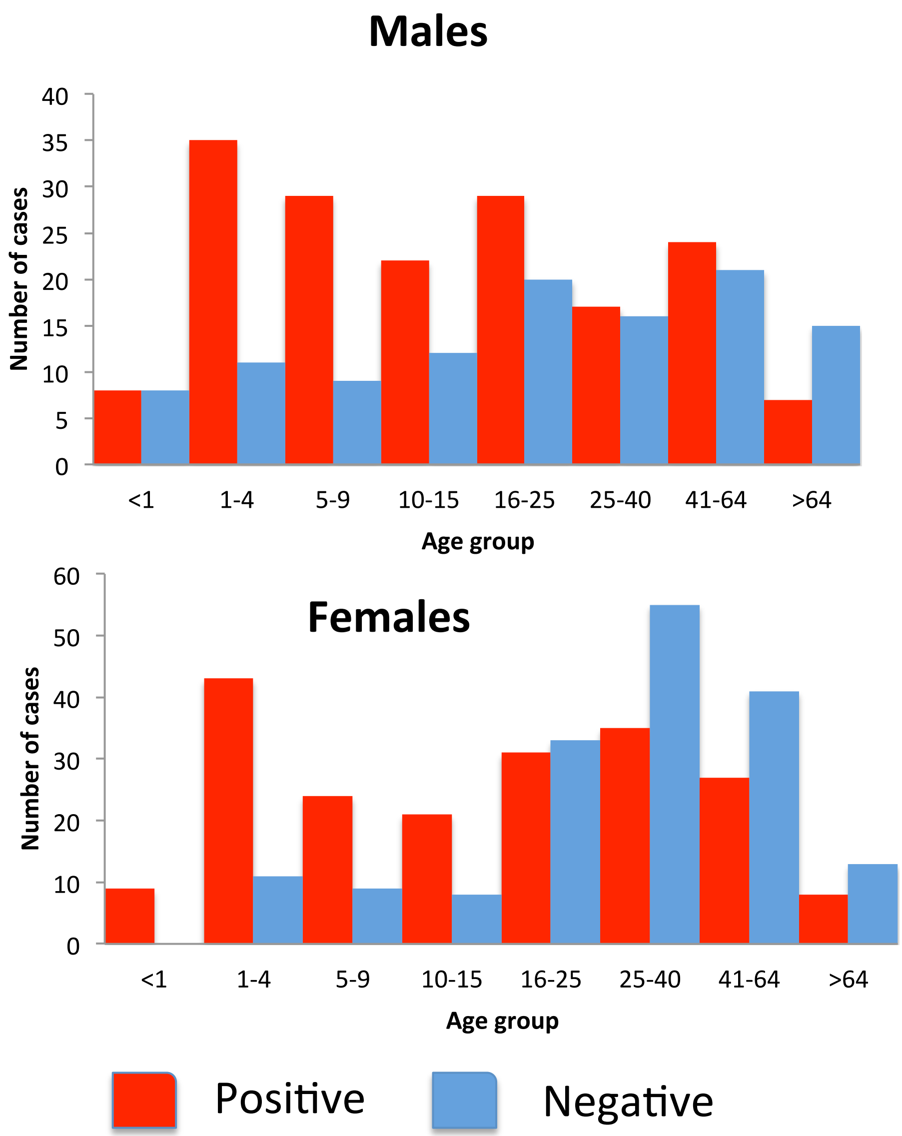

Males
Number of cases
40
35
30
25
20
15
10
5
0
<1
1-4
5-9
10-15
16-25
25-40
41-64
>64
Age group
Females
Number of cases
60
50
40
30
20
10
0
<1
1-4
5-9
10-15
16-25
25-40
41-64
>64
Age group
Positive
Negative

Figure 7(on next page)

Age dependence of fever and malariological indices: Incidence among visitors, Linga Linga, Mozambique

Age dependence of fever and malariological indices: Incidence among visitors – a) fever, b) diagnosed *P. falciparum*, c) confirmed *P. falciparum*, d) proportion used net

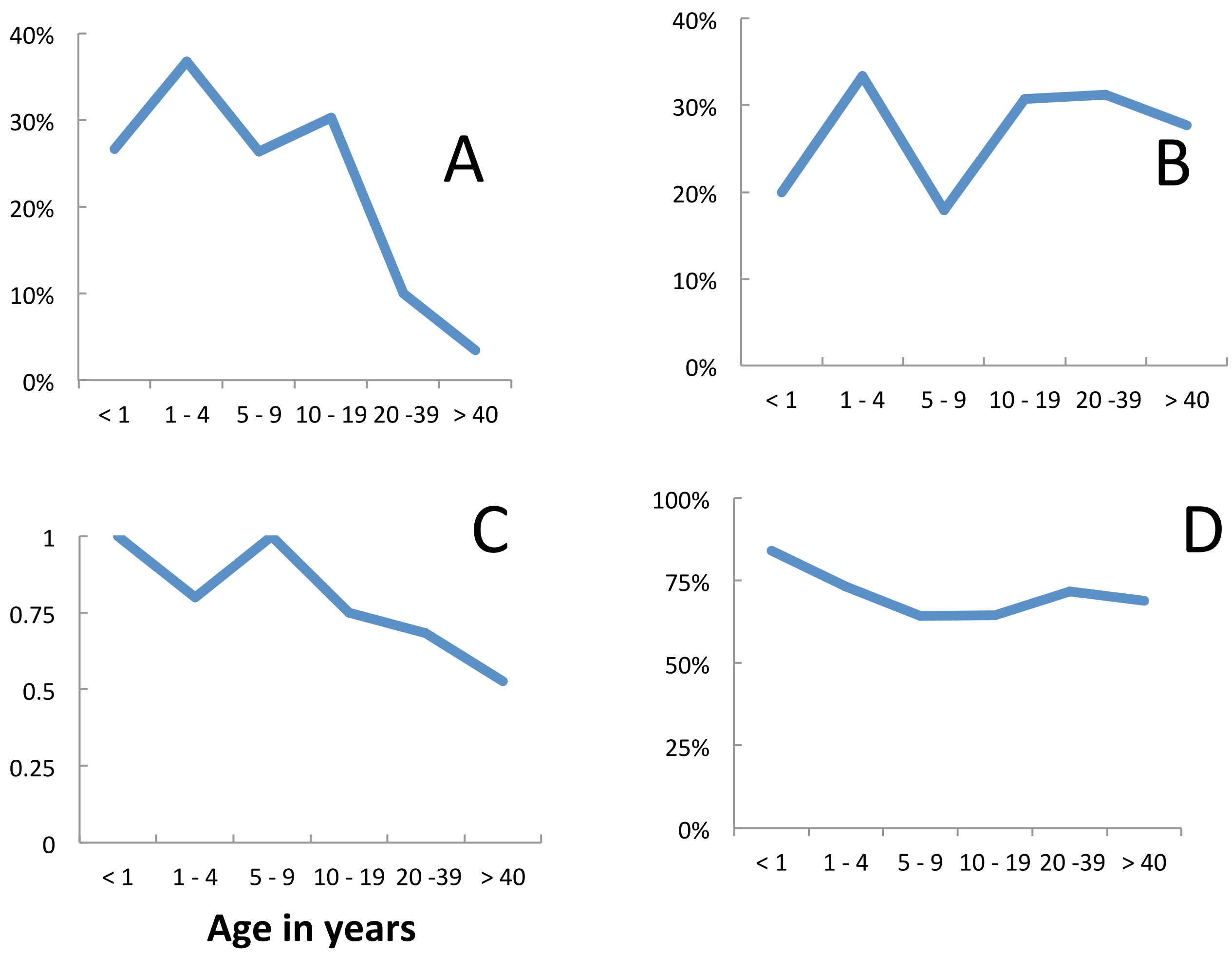

40%
30%
20%
10%
0%
< 1
1 - 4
5 - 9
10 - 19
20 -39
> 40
A
B
1
0.75
0.5
0.25
0
C
100%
75%
50%
25%
D
Age in years

Table 4(on next page)

Seasonality in incidence of diagnosed malaria among resident children below 10 years of age, Linga Linga, Mozambique.

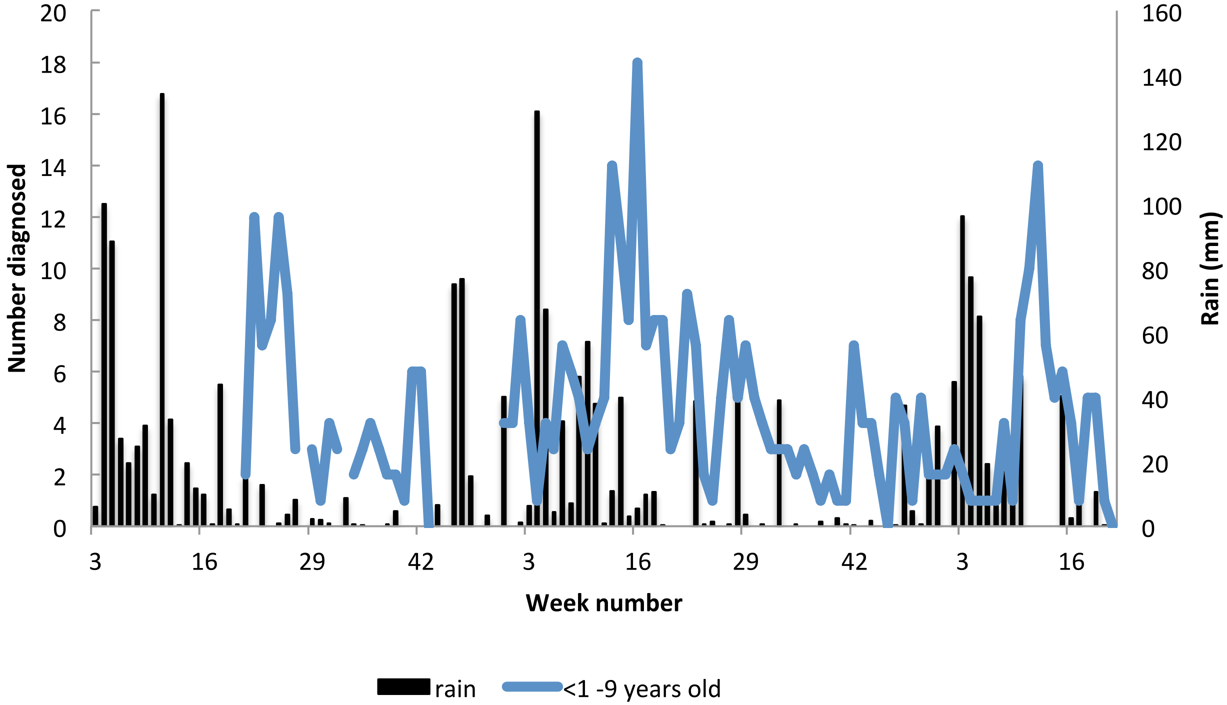
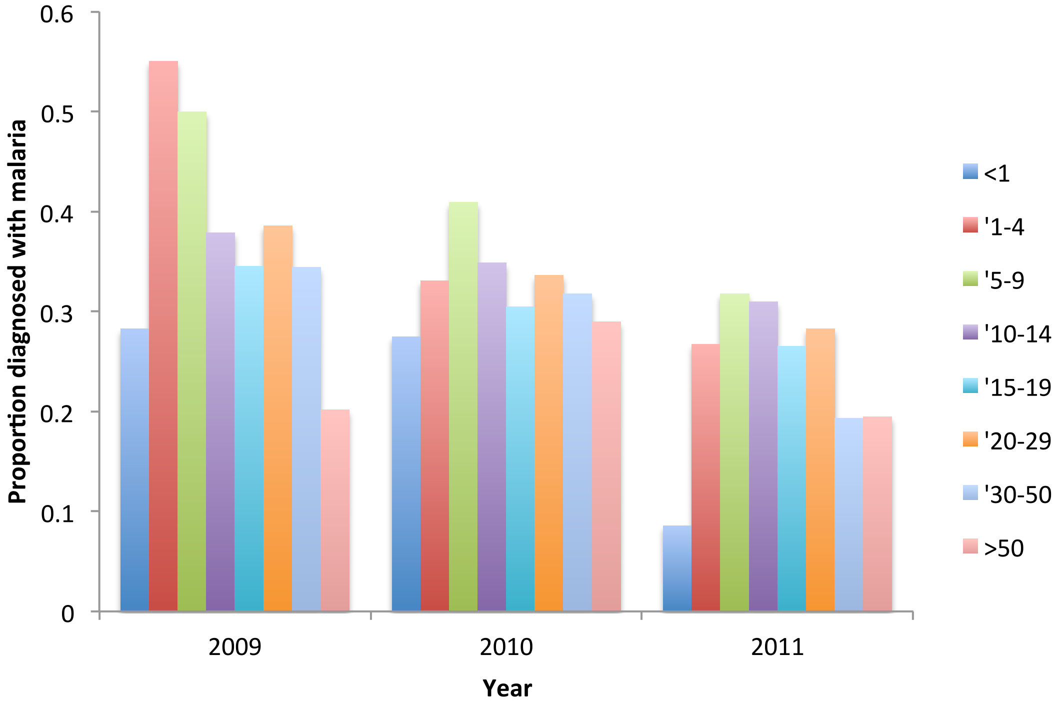

20
18
16
14
12
10
8
6
4
2
0
Number diagnosed
160
140
120
100
80
60
40
20
0
Rain (mm)
3
16
29
42
3
16
29
42
3
16
Week number
rain
<1 -9 years old

Figure 8(on next page)

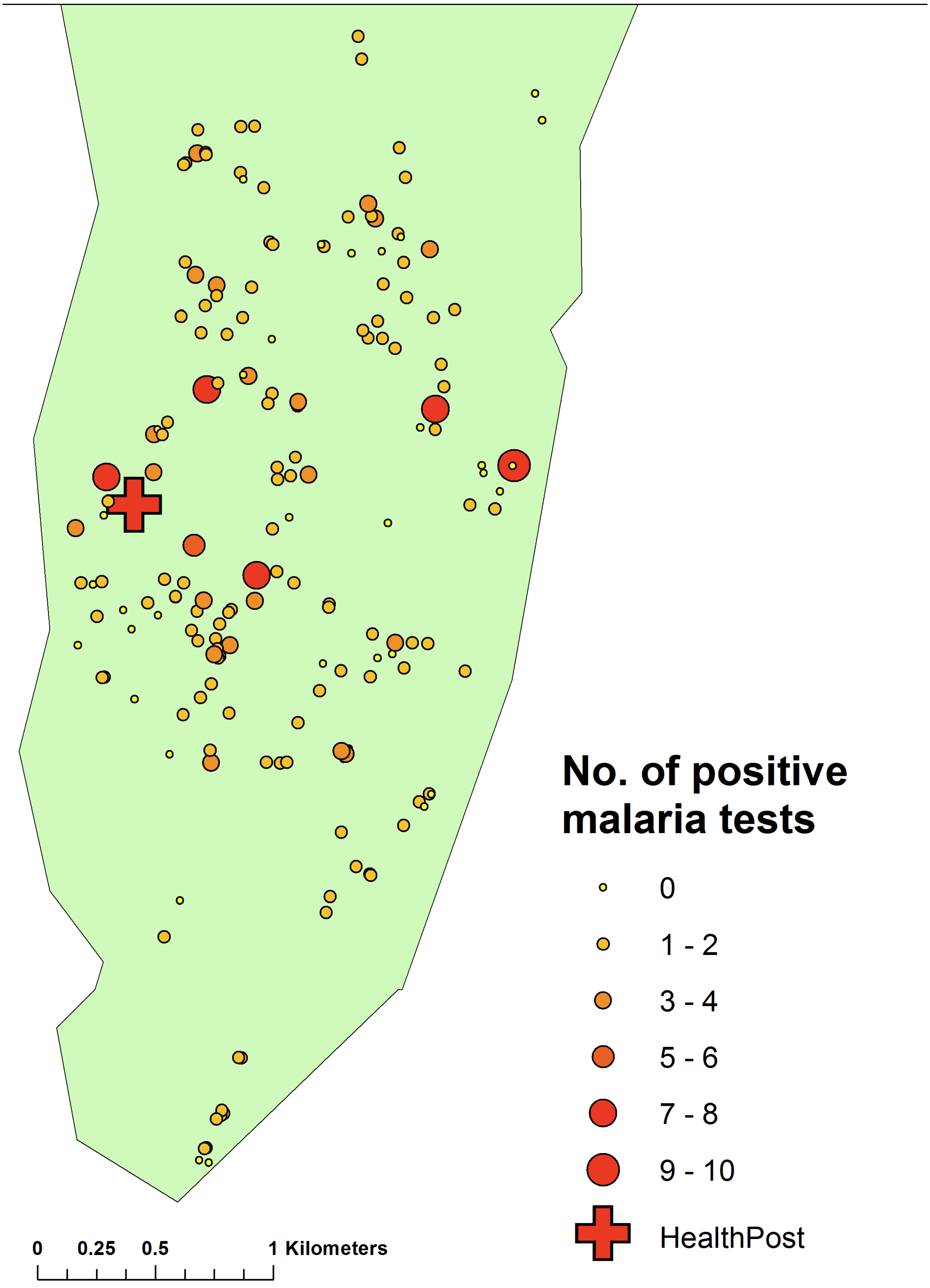
Proportion of resident attendees at the clinic diagnosed with malaria by year and age group, Linga Linga, Mozambique.



0.6
0.5
0.4
0.3
0.2
0.1
0
Proportion diagnosed with malaria
2009
2010
2011
Year
<1
'1-4
'5-9
'10-14
'15-19
'20-29
'30-50
>50

Table 5(on next page)

Map of the number of cases of malaria diagnosed at the clinic by household, Linga Linga, Mozambique


No. of positive malaria tests
0
1 - 2
3 - 4
5 - 6
7 - 8
9 - 10
HealthPost
0 0.25 0.5 1 Kilometers