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The epidemiology of malaria in four districts in southern Mozambique receiving indoor residual spray as part of a cross-border initiative

Rajendra Maharaj[1,2*], Nada Abdelatif[3], Mara Maquina[4], Ishen Seocharan[3], Vishan Lakan[1], Krijn Paaijmans[4,5], Francois Maartens[6], Pedro Aide[4] and Francisco Sauté[4]

Abstract

Background Imported malaria from southern Mozambique drives low levels of disease transmission in KwaZulu-Natal, South Africa. Therefore, the South African Department of Health funded implementation of indoor residual spraying (IRS) in Mozambiquan districts identified as sources of malaria infection for border communities in KwaZulu-Natal. IRS was initiated in districts of Guija, Inharrime, Panda and Zavala. To determine impact of spraying on malaria transmission in these districts, data relating to incidence and prevalence was collected before spraying (2018) and before the second round of spraying was completed (2023). Implementation of IRS was also monitored to ensure optimal spray coverage was achieved.

Methods The study was a cross-sectional survey conducted in 6 sentinel sites in each of the four afore-mentioned districts, focusing on children 6 months to < 15 years from selected households. There was a baseline and an endline cross-sectional survey. Baseline prevalence took place during March–April 2022 whereas the endline surveys occurred during February–March 2023. One hundred and twenty children from each sentinel site were tested for malaria using rapid diagnostic tests. Monthly malaria cases were obtained from health facilities in each study district. Spray data was obtained from LSDI2 initiative who implemented IRS in the targeted districts.

Results The study showed a definite impact of IRS on malaria prevalence in the targeted districts. Prevalence for sentinel sites in Guija district indicated that the prevalence of malaria increased slightly from baseline to endline in all sentinel sites in Guija. Overall, there was no significant change in prevalence in Zavala, from baseline to endline (p-value = 0.611). Panda's overall malaria prevalence decreased from 19.20% to 10.82% (p-value < 0.001) whereas overall prevalence in Inharrime, decreased from 27.68% to 19.50% (p-value < 0.001). Malaria prevalence in children younger than 5 years decreased significantly in all four districts. In Panda there was a decrease in numbers of males and females being infected between surveys (p < 0.001), whereas for Inharrime the decrease was significant in females (p < 0.001). High coverage with IRS (> 95%) resulted in greater population protection.

Conclusion The study revealed that IRS implementation decreased malaria prevalence in Inharrime and Panda but not in Guija and Zavala. To ensure that cross-border movement of people does not result in increased malaria transmission, targeting areas identified as source of infection in travelers is paramount to reaching elimination.

Keywords Epidemiology, Indoor residual spray, Cross-border malaria, Malaria elimination

*Correspondence:
Rajendra Maharaj
rmaharaj@mrc.ac.za
Full list of author information is available at the end of the article

Background

According to the new draft strategic plan, Mozambique aims to eliminate local transmission of malaria in at least 20 districts, identified as low transmission regions, by 2030. Given porous borders with neighbouring malaria endemic countries, in-country interventions alone are not sufficient to eliminate malaria and therefore cross-border and regional collaborative efforts are needed [1, 2]. In 2015, the governments of Mozambique, South Africa, and Eswatini (MOSASWA) signed the MOSASWA Cross-border Malaria Initiative Declaration. The goal laid out in the Declaration is "to work collaboratively to accelerate from control to pre-elimination in southern Mozambique and accelerate the transition from pre-elimination to elimination of malaria in Eswatini and South-Africa, to achieve zero local transmission in Eswatini, South Africa and Maputo province by 2020 and achieve pre-elimination (*Test Positivity Rate < 5%*) status in southern Mozambique (Gaza and Inhambane Provinces) by 2025" [3]. South Africa and Mozambique have engaged in various malaria elimination efforts through MOSASWA to achieve their individual goals. These include the establishment of cross-border mobile surveillance units for the testing and treatment of mobile migrant populations.

The elimination agenda in southern Africa was being successfully implemented but in 2017, the situation changed with progress in some countries halting and reversing in other countries. Goals had to be realigned with the reality on the ground and the southernmost African countries revised their malaria elimination goal to 2030 whilst the national strategic plan of South Africa targets malaria elimination by 2028. From the lessons learnt from the resurgence of the disease, the South African malaria control programme realized that even though there were cross-border malaria control initiatives in place, there was a need to invest in controlling the disease in the source districts in neighbouring countries [4]. It was thought that through a down-stream effect there would be fewer cases being imported into the province of KwaZulu-Natal.

Considering the regional elimination strategy, the South African National Department of Health (NDOH) developed a co-financing agreement with Mozambique and allocated funding towards Indoor Residual Spraying (IRS) in southern Mozambique for the period October 2021 to September 2023. To this end, the NDOH funded the implementation of malaria control interventions in Mozambican districts that were identified by the Ministry of Health as the main source of malaria infections in people entering South Africa, especially through informal border crossings. A further objective of the funding was to determine whether the investment, in terms of IRS, made an impact on disease transmission and case numbers in Gaza and Inhambane provinces in Mozambique.

The objective of this investigation was to determine the impact of implementing indoor residual spraying in districts that had no sustained vector control intervention. This was achieved by comparing the epidemiological variables of the selected districts in southern Mozambique before and after the implementation of indoor residual spraying with an effective insecticide.

Methods

Selected sentinel sites

The 24 sentinel sites included in the study are all located in the provinces of Gaza and Inhambane. Six sentinel sites were selected from Guija district in Gaza province, and 18 in Inhambane province with six sentinel sites from each of the districts of Inharrime, Panda and Zavala. This selection was based on their high malaria incidence, determined by the proportion of annual cases in 2021. These districts also benefited from IRS as part of the co-funding from South Africa.

Study design

The study was a cross-sectional survey, in which the identification and selection of individuals eligible for the study were carried out in the households selected in the provinces of Gaza and Inhambane. The data collection activities in the field were conducted by fieldworkers between the months of October 2021 and March 2023. In these districts, the monitoring of the incidence of malaria was carried out using epidemiological data.

Study population

The study population consisted of children aged 6 months to < 15 years of age, residing in the households selected. During the endline survey, the children tested during the baseline survey were not purposely targeted as many children would have aged beyond 15 years and others would have moved away from the sentinel sites.

Malaria incidence data

Monthly malaria cases (per age group and gender) were obtained from the health facilities in each study district. Data was included for November 2018 to April 2023 and was obtained from the national malaria surveillance system. Incidence data was calculated using the district yearly population as denominator and assuming 3% yearly population increase.

Malaria prevalence data

The baseline survey took place over the period: March–April 2022 whereas the endline surveys occurred during February–March 2023. In each district, 6 teams of fieldworkers visited households to recruit study participants. Each team worked in one sentinel site. At each sentinel site, at least 120 households were randomly selected using the bottle method. A bottle was placed in the center of the neighbourhood on a flat surface and rotated clockwise. The team walked in the direction to which the top of the bottle pointed, and every 3rd house was visited. When there were no more houses, the procedure (bottle rotation) was repeated, until enough participants were enrolled.

In each household, only one participant from each age group (0– < 2 years, 2– < 5 years, 5– < 15 years) was selected to participate in the study. After selecting the participant(s), the workers administered the informed consent form(s) explaining the details and study procedures to the adult caregiver and asking them if their children could participate in the study. If they did agree to participate, written informed consent form(s) were signed by the caregiver, and each participant was identified with a study number. An electronic questionnaire was completed using a tablet device, where demographic and clinical information such as axillary temperature, fever in the last 24 h and in the last 30 days, history of anti-malarial treatment in the last 30 days, malaria control measures, malaria rapid diagnostic test (RDT) result and the sample identification number were recorded. At the end of each day of recruitment, the supervisors of each district checked and sent all the data to the Centro de Investigação em Saúde de Manhiça (CISM) database.

Rapid diagnostic tests

Malaria testing was done through qualitative rapid diagnostic tests (SD Bioline Malaria AgPf), which identifies *Plasmodium falciparum* HRP2 antigens. A capillary blood sample was collected from each participant, through a finger prick (on the left hand), which was placed in the test hole with 4 drops of the buffer. The results were recorded 15 minutes later. The test was considered 'positive' when the control and the process band were stained, 'negative' when only the internal control was stained, and 'invalid' when the internal control did not become visible. For invalid results, the test was repeated.

Sample size calculations

The district specific sample size was determined to allow detection of a significant change at the 5% significance level, assuming a reduction in *P. falciparum* prevalence of at least 5% after intervention. Initially, the minimum required sample size per district was determined to be 384. Considering cluster sampling (children selected within households) with a design effect of 1.5, the adjusted sample size became 576. To account for 20% expected attrition or refusal, the final sample size was corrected to 720 children. Given the objective of district-level inference, 720 children were recruited in each of the four districts, resulting in an overall total sample size of 2880.

For each district, four sentinel sites were selected. At each of these 24 sentinel sites, cross-sectional parasite surveys were performed on a random sample of 120 individuals $\geq$ 6 months and <15 years of age. Sentinel sites were each divided into localities from which participants were selected to ensure as much geographical spread as feasible.

Indoor residual spraying campaign

Spraying is typically implemented before the rainy season which is from December to March. IRS activities commenced in September and finished in February the following year. Houses in the four study districts in Gaza and Inhambane provinces were blanket sprayed by trained spray operators. All houses in these districts were eligible to be spayed since a spray coverage of 100% was targeted. The insecticide used was determined by the national malaria control programme after evaluating entomological surveillance and insecticide resistance data. Fludora®Fusion (Envu) was sprayed onto the inner walls of houses, starting in September 2021 and ending in February of 2022. The second spray round was from September 2022 to February 2023, covering the peak transmission period. A spray coverage of > 95% was targeted to ensure that the populations in each district was optimally protected. Coverage was calculated using the following formula:

$$Coverage(\%) = \left(Number\ of\ houses\ sprayed \,/ Number\ of\ houses\ targeted\right) \times 100.$$

This calculation provides a clear measure of the proportion of targeted houses that were sprayed, allowing us to assess the effectiveness and reach of the IRS campaigns. Targeted structures included all structures except those were food was stored and prepared, granaries, structures without roofs and structures in a state of disrepair.

Data collection

Training

All data collectors were given training to ensure that all new what data was required and were familiar with the data collecting tools.

Data collection and validation

Data audits were conducted each day of the prevalence survey to prevent incomplete data sheets and missing data.

Regulatory requirements

Only data approved by the ethics committee (Ministry of Health, Mozambique) were collected, according to the mandate of the Ministry of Health.

Use of technology

The study programmed the data collection tool using the REDCap web interface and linked it to the REDCap mobile app on the tablet device that the field teams operated. The data was transferred daily to the REDCap server and the data team inspected and validated the data for quality and correctness with regular feedback to the field team.

Statistical analysis

To determine whether there was a statistically significant difference in malaria prevalence from baseline to endline, the Chi-squared test was used. The malaria incidence rate (per 1000 people at risk per district) was presented as both means and medians. Univariate regression with Newey-West standard errors was used to determine whether there was a statistically significant difference between the baseline malaria incidence rate and endline malaria incidence rate, for participants under 5 years old and for participants 5 years or older, up to three lags. This variance estimator allows the error structure to be possibly heteroskedastic and autocorrelated. To adjust for confounders, multivariate logistic regression was used to determine whether there effects of age and gender on malaria prevalence, as well as the effects of IRS. All analyses were stratified by district. For the number of new malaria cases from January 2018 to June 2023, the Mann–Kendall trend test was used to determine whether there is a trend detected from month to month. A p-value < 0.05 is used to determine statistical significance.

Results

The results of the baseline and endline prevalence surveys show a definite impact of IRS on malaria prevalence in two of the four targeted districts.

Parasite prevalence survey

The prevalence for the sentinel sites in Guija district of Gaza Province (Table 1) showed that the prevalence of malaria increased slightly from baseline to post-spraying in all the sentinel sites in Guija, Gaza, ranging from 0.79% to 1.63% at endline, except for Chivongoene, which decreased slightly at baseline from 0.85% to 0.81% at endline. The overall malaria prevalence in Guija at baseline was 0.97% and 0.80% at endline, an overall increase, but it was not statistically significant (p-value = 0.728).

In the Inhambane province (Table 2), the overall prevalence increased in Zavala, from 18.33% at baseline to 19.38% post spraying (not statistically significant, p-value = 0.611). Panda's overall malaria prevalence decreased from 19.20% to 10.82% post spraying (statistically significant difference, p-value < 0.001) whereas the overall prevalence in Inharrime, decreased from 27.68% to 19.50% at endline (statistically significant difference, p-value < 0.001). Five of the 18 sentinel sites in Inhambane recorded endline prevalences of greater than 30%.

In Zavala, the prevalence in most of the sentinel sites decreased except for in Nhangave, which increased from 11.45% to 13.60% post spraying (Table 2). Similarly, in Panda, a decrease in the prevalence was seen from baseline to post spraying in all of the sentinel sites except for in Inhassume, which increased from 2.48% to 4.96%. Five of the six sentinel sites in Inharrime saw a big decrease in prevalence from baseline to post spraying, whereas malaria prevalence in Nhamuesse increased from 26.06% to 37.90% post spraying.

The results of the baseline and endline surveys are outlined in Table 3. The number of RDT positive for malaria is given in Table 3 which also shows the percentage of negative RDTs per district. There was a small percentage

Table 1 Prevalence for the sentinel sites in Guija, Gaza Province

District	Sentinel site	Baseline number surveyed (n)	Baseline prevalence (%)	Endline number surveyed (n)	Endline Prevalence (%)	P-value
Guija	25 de Setembro	116	5.17	125	0.00	0.010
	7 de April	102	0.00	127	0.79	0.369
	Tomanine	137	0.00	123	1.63	0.134
	Nhampuguene	139	0.00	127	0.79	0.295
	Chivongoene	117	0.85	124	0.81	0.967
	Canicado	110	0.00	123	0.81	0.343
	Overall	721	0.97	749	0.80	0.728

Table 2 The baseline prevalence at the sentinel sites in Inhambane Province

District	Sentinel Site	Baseline number surveyed (n)	Baseline Prevalence (%)	Endline number surveyed (n)	Endline Prevalence (%)	P-value
Zavala	Ticongolo	145	8.28	124	0.81	0.004
	Dombe	134	4.48	122	4.1	0.881
	Nhangave	166	11.45	125	13.6	0.581
	Chinhanga	90	43.33	121	39.67	0.593
	Chipole	80	51.25	123	48.78	0.731
	Mavila	105	14.29	123	9.76	0.291
	Overall	720	18.33	738	19.38	0.611
Panda	Inhassume	121	2.48	121	4.96	0.308
	Mubique	120	48.33	126	36.51	0.061
	Jacubecua	143	8.39	121	0.83	0.005
	Mwatimamba	140	15.71	131	10.69	0.223
	Chivalo	122	24.59	129	9.3	0.001
	Mawayela	125	18.4	130	2.31	< 0.001
	Overall	771	19.2	758	10.82	< 0.001
Inharrime	Chacane	100	19	126	6.35	0.004
	Chelengo	149	18.12	134	2.99	< 0.001
	Mafassane	130	40.77	128	31.25	0.111
	Nhamuesse	188	26.06	124	37.9	0.027
	Ocula	124	38.71	129	31.78	0.249
	Sihane	104	24.04	123	7.32	< 0.001
	Overall	795	27.68	764	19.5	< 0.001

Table 3 Malaria results for the districts in Gaza and Inhambane provinces

District		Baseline number surveyed (n)	Baseline (%)	Endline number surveyed (n)	Endline (%)	P-value
Gaza						
Guija	Overall	727	–	751	–	–
	Positive	7	0.96	6	0.80	
	Negative	714	98.21	743	98.93	0.483
	Invalid	4	0.55	2	0.27	
	Not done	2	0.28			
Inhambane						
Zavala	Overall	723	–	738	–	–
	Positive	132	18.26	143	19.38	0.235
	Negative	588	81.33	595	80.62	
	Invalid	3	0.41			
	Not done					
Panda	Overall	773	–	758	–	–
	Positive	148	19.15	82	10.82	< 0.001
	Negative	623	80.6	676	89.18	
	Invalid	2	0.26			
	Not done					
Inharrime	Overall	797	–	767	–	–
	Positive	221	27.73	149	19.43	< 0.001
	Negative	574	72.02	615	80.18	
	Invalid			3	0.39	
	Not done	2	0.25			

of RDTs that were invalid either through incorrect use or error in the RDT cartridge.

Guija saw a slight decrease in the number of people who tested positive from 0.96% to 0.8%. The number of people who tested positive for malaria in Zavala increased slightly between baseline and endline (18.3% to 19.4%), whereas the number positive cases decreased in Panda (19.2% to 10.8%) and Inharrime (27.7% to 19.4%). The difference in the number of people positive for malaria from baseline to endline was statistically significant in Panda and Inharrime.

Malaria is influenced by a number of variables including age and gender of individuals. Table 4 highlights the prevalence data according to age, i.e., children under the age of 5 years and children greater than and equal to 5 years of age.

The malaria prevalence in children younger than 5 years old decreased in all four districts. That difference was statistically significant in Panda (p-value = 0.009) and Inharrime (p-value = 0.003). The prevalence increased in those 5 years and older in Guija (p-value = 0.645) and Zavala (p-value = 0.160) from baseline to post-spraying, whereas in Panda (p-value = 0.001) and Inharrime (p-value = 0.014), the prevalence decreased from baseline to post-spraying.

Malaria infectivity may also be influenced by the gender of an infected individual. In Guija and Zavala, there was no relationship between the number of males and females reported positive between the baseline and the endline surveys (Table 5). In Panda on the other hand, there was a statistically significant decrease in the numbers of males and females being infected between the baseline and endline surveys, whereas for Inharrime the decrease was statistically significant in females.

Incidence data for the targeted districts

In order to look at long term trends in malaria transmission, the incidence data for children less than 5 years old and those greater than 5 was analysed from January 2018 to June 2023 (Fig. 1).

In children younger than 5, Inharrime had the largest number of cases between 2018 and 2023 followed by Zavala. Guija had a low number of malaria cases until 2020 when the number of cases increased then dropped again to lower numbers towards the end of 2021. Panda saw a decreasing trend between 2018 and 2023, except for a slight increase mid-2022. Kendall's τ for Inharrime, Panda and Zavala were − 0.22, − 0.60 and − 0.52, respectively (all statistically significant), whereas Guija was 0.01 (p-value = 0.864), indicating no trend over the years.

Table 4 Prevalence data according to age

District	Age	Baseline number surveyed (n)	Baseline prevalence (%)	Endline number surveyed (n)	Endline prevalence (%)	P-value
Guija	< 5 years old	231	1.30	298	0.34	0.207
	5 years or more	496	0.81	453	1.10	0.645
Zavala	< 5 years old	302	16.89	347	14.99	0.509
	5 years or more	421	19.24	391	23.27	0.160
Panda	< 5 years old	250	14.40	317	7.57	0.009
	5 years or more	523	21.41	441	13.15	0.001
Inharrime	< 5 years old	317	26.50	338	16.86	0.003
	5 years or more	480	28.54	429	21.45	0.014

Table 5 Prevalence data according to gender

District	Gender	Baseline number surveyed (n)	Baseline prevalence (%)	Endline number surveyed (n)	Endline prevalence (%)	P-value
Guija	Male	351	0.85	335	0.60	0.700
	Female	376	1.06	416	0.96	0.888
Zavala	Male	351	20.23	346	19.36	0.773
	Female	372	16.40	392	19.39	0.282
Panda	Male	371	20.49	352	13.07	0.008
	Female	402	17.91	406	8.87	0.000
Inharrime	Male	391	27.88	398	22.11	0.061
	Female	406	27.59	369	16.53	0.000

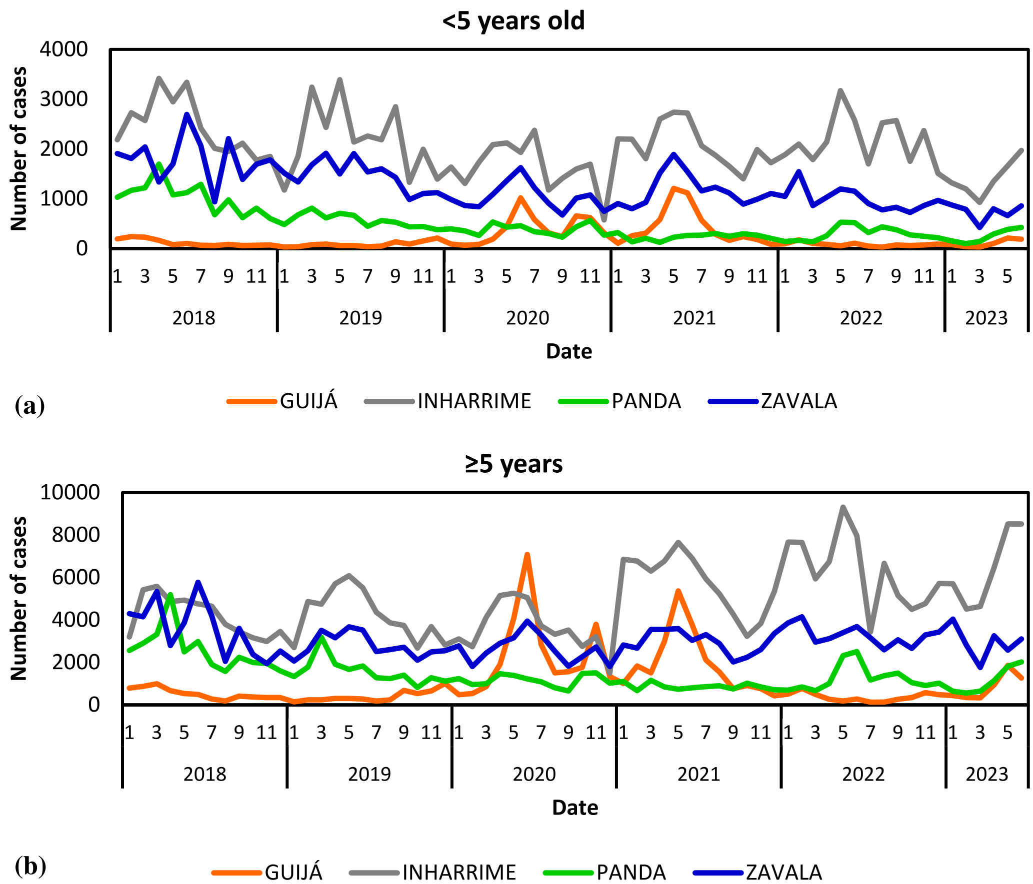

Fig. 1 Malaria morbidity in (**a**) children < 5 years old and (**b**) children ≥ 5 years old in the 4 districts

The number of new malaria cases across the four districts fluctuated from 2018 to 2023 in children 5 years and older. The number of cases increased and remained the highest after 2021. Guija experienced two large peaks in new malaria cases mid-2020 and again in mid-2021. Panda followed a similar decreasing trend for this age group across the years in new malaria cases except for an increase in mid-2022. Kendall's τ for Inharrime was 0.27 (p-value = 0.001), indicating a statistically significant increasing trend. Panda had a decreasing trend over the years (τ = − 0.45), which was statistically significant (p-value < 0.001). Both Guija (τ = 0.05, p-value = 0.554) and Zavala (τ = − 0.01, p-value = 0.947) had no evidence of a trend between 2018 and 2023.

The median number of new malaria cases in children younger than 5 and those 5 years and older increased in Guija (Table 6) from baseline to endline, whereas in Zavala, Panda and Inharrime, the median number of new malaria cases decreased in both age groups. There was a statistically significant difference in the median number of new cases between baseline and endline in Guija for children 5 years and older and in Inharrime in children under the age of 5 years.

The median number of new malaria cases was higher from 2019–2021 in Zavala (Table 7) followed by Inharrime for females and lowest in Guija and Panda (statistically significant difference, p-value < 0.001). For males, the highest median number of new malaria cases was highest in Zavala and Inharrime and lowest in Guija and Panda (statistically significant difference in the districts, p-value < 0.001).

Data on indoor residual spraying

The percentage coverage with the insecticide Fludora®Fusion achieved over the 2021/2022 and 2022/2023 seasons is shown in Table 8. Although > 95% spray coverage was achieved over both spray seasons, overall, a higher coverage was achieved in the second spray season.

When the spray cover at each sentinel site was interrogated, it was found that coverage in 2021 in Dombe and Xinhangue was lower than the expected > 90%. In 2022, spray coverage was suboptimal in Dombe but high

Table 6 Incidence of malaria in the four districts by age

District		Mean baseline incidence (SD)	Median baseline incidence (range)	Mean endline incidence (SD)	Median endline incidence (range)	P-value
Guija	Total	452 (228.42)	396 (163–955)	973 (689.43)	779 (367–2075)	0.047
	< 5 years old	83 (36.78)	78 (31–177)	109 (75.00)	91 (33–209)	0.578
	≥ 5 years	369 (197.39)	313 (132–778)	864 (617.63)	688 (334–1866)	0.043
Zavala	Total	4272 (670)	4149 (3365–5693)	3656 (913.50)	3775 (2183–4910)	0.4
	< 5 years old	992 (225.80)	938 (724–1543)	735 (170.25)	796 (423–875)	0.231
	≥ 5 years	3280 (465.32)	3225 (2586–4150)	2921 (757.00)	2951 (1760–4035)	0.365
Panda	Total	1560 (725.32)	1297 (808–3031)	1386 (777.02)	1113 (658–2443)	0.874
	< 5 years old	302 (138.64)	267 (134–530)	250 (135.96)	224 (101–425)	0.879
	≥ 5 years	1258 (593.41)	1037 (674–2507)	1136 (642.87)	893 (557–2018)	0.873
Inharrime	Total	8467 (2010)	8305 (5133–12,483)	7796 (2140)	7414 (5563–10,491)	0.487
	< 5 years old	2175 (482.21)	2120 (1510–3173)	1404 (364.95)	1336 (925–1969)	0.022
	≥ 5 years	6292 (1692.84)	6300 (3430–9310)	6393 (1798.42)	6029 (4514–8525)	0.678

in Nhangave where additional structures than those targeted were sprayed. The sentinel sites in Zavala recorded discordant spray coverages.

From Table 8 a high coverage of structures was achieved over both rounds of spraying. In 2021 the coverage was in the high nineties (> 97.5%), however in 2022 the coverage was even higher (98.8%). The high spray coverage translated into high numbers of population protected but in 2021 only 91% of the population in Panda were protected by IRS. However, in 2022 when the spray coverage increased in Panda so did the population protected. In 2021 in Zavala the spray coverage was 96.2% the population protected was still 99%. This may indicate that the average number of individuals living in a structure was slightly greater than the other districts. Therefore, it is apparent that when spray coverage is high the number of people protected by IRS is high.

Influence of socio-demographic variables on malaria transmission

Univariate and multivariate logistic regression was conducted for each district and included sociodemographic variables, symptoms, control measures and access to health services (Supplementary Tables 1–4).

In Guija, being five years and older, a female and having a caregiver or parent who was a student increased the likelihood of having a positive malaria result compared to children who were less than 5 years, a male or having a caregiver or parent who was a domestic worker/farmer. Having a fever within the last 24 h was significantly associated with a decrease in having a positive malaria test result compared to those who did not (Supplementary Table 1).

In Zavala, being five years or older was significantly associated with an increase in having a positive malaria result compared to children under the age of 5 years old. No gender differences were found. Having a caregiver or parent who had secondary or higher education decreased significantly the likelihood of having a positive malaria result compared to having a caregiver or parent who had a primary school education. Similarly for having a caregiver or parent who was a trader or in the services industry. Having a house made of cement is significantly associated with having less of a likelihood of having a positive malaria result compared to having a house made of other materials (Supplementary Table 2).

In Inharrime, there was no significant association found between age and gender and having a positive malaria test. Having a caregiver or parent who had a secondary education or higher was significantly associated with being less likely to have a positive malaria result compared to caregivers or parents with a primary school education (Supplementary Table 3).

In Panda, being five years and older was significantly associated with having more of a likelihood of having a positive malaria result compared to children less than five years old. Being female decreased the likelihood, significantly, of having a positive malaria result compared to males. No other sociodemographic indicators were found to have an effect (Supplementary Table 4).

Discussion

Malaria transmission in southern Mozambique, like in many other regions with similar climates and ecological conditions, is primarily facilitated by *Anopheles gambiae* and *Anopheles funestus*, which are efficient vectors of the malaria parasite [5]. Factors such as socio-economic status, housing conditions, access to healthcare, and preventive measures like the use of insecticide-treated bed nets and IRS can significantly impact the risk of malaria

Table 7 Incidence of malaria by gender from 2019 to 2021

Variable		Guija		Zavala		Panda		Inharrime		P-value
		Mean cases (SD)	Median cases (range)	Mean cases (SD)	Median cases (range)	Mean cases (SD)	Median cases (range)	Mean cases (SD)	Median cases (range)	
Gender	Females	25.64 (45.32)	7 (1–342)	30.81 (31.61)	22.5 (1–225)	12.47 (17.18)	7 (1–137)	23.42 (24.90)	17.5 (1–139)	< 0.001
	Males	28.00 (50.33)	8 (1–378)	29.31 (29.71)	19 (1–171)	12.04 (17.67)	6 (1–147)	20.49 (22.01)	14 (1–150)	< 0.001

Table 8 Spray coverage and population protected in the 4 districts over two spray round 2021/22 and 2022/23

District	Year	Houses found	Houses sprayed	% coverage (%)	Population targeted	Population protected	% Protected (%)
Guija	2021	27,352	27,056	98.9	92,218	90,701	98.0
	2022	29,476	29,160	98.9	92,892	91,896	99.0
Inharrime	2021	37,328	36,894	98.8	116,977	114,530	98.0
	2022	38,229	37,902	99.1	119,733	118,709	99.0
Panda	2021	13,891	13,402	96.5	41,734	37,886	91.0
	2022	13,179	13,051	99.0	45,768	45,323	99.0
Zavala	2021	56,335	54,189	96.2	158,950	157,360	99.0
	2022	57,902	57,037	98.5	160,571	158,172	99.0

transmission [6]. This study was designed to provide information and actionable data on the effectiveness of IRS in four districts in Gaza and Inhambane provinces. The aim of this study was to determine whether or not IRS could be effective in currently untargeted areas in Gaza and Inhambane provinces.

IRS was successfully rolled out in all districts, achieving over 95% coverage in terms of number of households sprayed. The population protected by achieving a high coverage also resulted in more than 90% of the population protected in most of the districts. The impact of IRS was successful in reducing the prevalence of malaria in Panda and Inharrime, but the district of Zavala recorded a 1% increase in cases after the two rounds of spraying. There was no change in Guija. The incidence of malaria fluctuated from 2018 to 2023 across all four districts in children 5 years and older. The increase in the number of cases after 2021 may be attributed to the incorrect diagnosis of covid patients as malaria patients as demonstrated by [7]. This was reinforced by the downward trend seen in three of the four districts. There is no statistically significant difference between the baseline and endline prevalence data. In Guija, the incidence of malaria in children < 5 years of age and in those older than 5 years increased over the two rounds of spraying but in the districts of Inhambane province, the number of malaria cases decreased in both age groups. Malaria affected females far more than it did the male population. This difference in age and gender is borne out by [8] where it is stated that children under 5 years of age and pregnant women are the most vulnerable population.

Although the same intervention measures were implemented in Gaza and Inhambane provinces, the impact of IRS varied in the sentinel sites within the selected districts. In Guija there was no increase in the number of malaria cases. In the Inhambane districts, there were significant decreases in Panda and Inharrime. However, there is cause for concern in Inhambane since 33% of the sentinel sites in this province registered prevalences of greater than 30% two years after IRS was implemented. With regards to the interaction between age and malaria test positivity, fewer children under five were found to be infected after two rounds of spraying with an effective insecticide. Although there was a slight increase in malaria cases in the > 5-year age group in Guija and Zavala, Panda and Inharrime recorded significant increases in the same age group. The number of malaria cases according to gender was also influenced by the implementation of malaria control measures. All districts apart from Zavala recorded decreases in malaria prevalence, especially in Panda and Inharrime where the decreases were significant. There were also decreased prevalence in males in all districts, but it was most marked in Panda.

Incidence history shows that there was a decrease in the malaria incidence in the targeted four districts prior to 2021 but this changed during and after the covid-19 pandemic. This trend was seen across the districts when considering the age distribution of reported malaria cases. In Guija there was a significant increase in morbidity in children ≥ 5 years however a significant decrease was seen in the < 5-year population. When conducting a gender influenced analysis, it was found that incidence among the genders increased in Inharrime and Zavala and decreased in Guija and Panda.

The data generated by this study is similar to that reported by the Lubombo Spatial Development Initiative [9] and subsequently by the MoSaSwa (Mozambique, South Africa, Swaziland) initiative [3]. Historically, Mozambique has not implemented a widespread, coordinated IRS programme due to financial and logistical challenges, and had also prioritized its limited resources for the higher transmission and more populated areas in the northern part of the country. As a result of this, South Africa's efforts to eliminate malaria is significantly challenged by the large number of imported malaria cases, especially from neighbouring Mozambique

[10]. However, the southern region of Mozambique, especially Maputo City and Province, had historically implemented activities aimed at reducing the burden of malaria in the area, and consequently lowered the number of importations into its neighbouring countries namely that of South Africa and eSwatini.

After the termination of the LSDI in 2011, an increase in the burden of malaria in all three countries were noted [9]. The number of malaria cases recorded in 2011 was compared with the baseline conducted for the MoSaSwa initiative in 2014 which revealed that the gains achieved by the LSDI have been eroded subsequent to project termination. This suggests that IRS is needed to maintain a low malaria incidence, as it may recur if IRS is halted [11]. While it may not be the tool to get to elimination, it certainly helped accelerating to zero. Now it needs to be combined with other tools and used in an integrated way [12]. Although the covid-19 pandemic may have influenced the number of malaria cases reported due to the similarity of symptoms, it was found that in South Africa there was no statistically significant increase in the number of cases reported during the pandemic compared to the average of the five malaria seasons prior to the pandemic [13]. Globally as well, the World Health Organization (WHO) had predicted dire consequences for malaria control during and immediately after the pandemic, but this scenario did not materialize.

The results of this study have shown that IRS implementation did decrease the prevalence and incidence of malaria in the Panda and Inharrime. Apart from effective vector control in the source districts in Gaza and Inhambane, KwaZulu-Natal needs to successfully halt local malaria transmission and prevent the re-introduction of malaria. Greater emphasis needs to be placed on detecting and treating malaria carriers at both formal and informal border crossings with transmission blocking anti-malarials [14]. The results of this study lends credence to the findings of the LSDI in that a greater impact can be made on the malaria burden in the region if the strategy of cross-border malaria control is implemented together with an effective monitoring an evaluation system [3, 9].

Some factors that may have affected the impact of IRS on malaria cases are the feeding and resting behaviours of vector mosquito [15]. In certain districts *Anopheles arabiensis* was the main vector whilst in others it was *An. funestus*. Since these vectors have different feeding and resting habits, it may have influenced their interaction with the insecticide sprayed inside the houses. Furthermore, the house construction type would have influenced the insecticide on the sprayed surfaces since mud is more conducive to residual insecticides, other surface such as paint would be less so [16, 17]. However, the slight increase in malaria cases in Zavala between the baseline and end line surveys suggest that these are factors in this district that may have minimized the impact of IRS. This could also signify the early development of resistance to the insecticide used. Entomological variables need to be monitored to understand the effect that the use of chemicals has on malaria transmission. Entomological correlates such as feeding and resting behaviours, vector density, dominant vectors, sporozoite rates need to be established.

Inadequate housing with poor mosquito-proofing and limited access to healthcare services contribute to higher vulnerability to malaria in some communities [18]. Other technologies such as the distribution of insecticide-treated bed nets (ITNs), and larval control initiatives targeting mosquito breeding sites should be considered as supplementary measures to IRS [19]. Additionally, prompt diagnosis and treatment of malaria cases with effective anti-malarial drugs are crucial for reducing parasite transmission. Overall, malaria transmission in southern Mozambique is complex and influenced by a combination of ecological, socio-economic, and behavioral factors. Efforts to control malaria require a multifaceted approach addressing both vector control and access to effective healthcare services. Nevertheless, it has been shown that the use of IRS in the districts of Gaza and Inhambane has an impact on the epidemiology of malaria and does influence entomological variables.

Gains achieved through the sustained use of IRS must be maintained since these gains can be eroded in a few months when preventative and curative processes are discontinued. In areas, where there is a paucity of resources, novel interventions need to be implemented and it was shown in studies within the southern African region that focal spraying can be as effective as blanket spraying [20]. Intercountry collaboration is a necessity if the goal of regional elimination is to be achieved. In-country initiatives need to be supplemented by regional initiatives.

Conclusions

The investment case grant for malaria control in southern Mozambique is an adjunct to country pre-elimination and elimination efforts. The funding resulted in the implementation of IRS in areas where resources were not available. The positive impact of two rounds of IRS was an overall decrease in malaria prevalence and incidence in two of the targeted districts. However, due to interrupted funding for the implementation of IRS in the targeted districts a third round of IRS was not implemented in these four districts and the consequences of stopping IRS was shown by [11]. Efforts to control malaria require a coordinated and sustainable approach to prevent disease transmission.

Abbreviations

CISM	Centro de Investigação em Saúde de Manhiça
MoSaSwa	Mozambique, South Africa, Swaziland
NDoH	National Department of Health
IRS	Indoor residual spraying
RDT	Rapid diagnostic test
REDCap	Research electronic data capture

Supplementary Information

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Supplementary Material 1

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Author contributions
RM, PA, FS were responsible for conceptualization of the study. IS and FM were responsible for data curation. Formal analysis was conducted by NA. data collection was overseen by VL, MM, KP. The original draft was prepared by RM and all authors reviewed the manuscript.

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Availability of data and materials
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Prior to research commencing, approval for the study was obtained from the Ethics Review Board of the Mozambiquan Ministry of Health.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Author details
[1]Malaria Research Group, South African Medical Research Council, Durban, South Africa. [2]Institute for Sustainable Malaria Control, University of Pretoria, Pretoria, South Africa. [3]Biostatistics Research Unit, South African Medical Research Council, Durban, South Africa. [4]Centro de Investigação Em Saúde de Manhiça, Fundação Manhiça, Maputo, Mozambique. [5]Center for Evolution and Medicine, School of Life Sciences, Arizona State University, Tempe, AZ, USA. [6]Lubombo Spatial Development Initiative 2, Johannesburg, South Africa.

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