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A CROSS-SECTIONAL SURVEY ON THE IMPACT OF MALARIA INTERVENTION MEASURES ON PREVALENCE AND VECTORIAL INFECTION RATES IN PARTS OF IMO STATE, NIGERIA

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ABSTRACT: *There is a dearth of information on the Impact of malarial intervention measures on vectorial infection rates in the study area. This study therefore investigated the Impact of malaria intervention measures on prevalence and vectorial infection rates in two Local Government Areas of Imo State, Nigeria. The study employed a cross-sectional design involving 735 participants aged 5 years to >60 years, division of study subjects into four groups and was conducted between July 2023 and May 2024. Participants' blood was collected and processed using Rapid Diagnostic Tests (RDTs). Indoor resting malaria vectors collected by pyrethrum knockdown (PKD) were assessed for parasitological/entomological indices with standard methods. Malarial intervention compliance was monitored and impact was assessed by comparing results from different intervention measures/cohorts. Overall, pre-intervention malaria prevalence (26.12%) was five times significantly higher than the intervention prevalence result of 9.05% ($P < 0.05$). All intervention measures Insecticide treated bed nets (ITNs), Indoor residual spray (IRS) and prophylactic drugs (drugs)—reduced malaria prevalence significantly ($P < 0.05$). Malaria vectors in non-intervention cohorts (NICs) were 76.87%, three times higher than those caught in intervention cohorts (ICs), 23.12%. NICs had a higher composition of malarial vector density and sporozoite infection rates (3.25%) and the differences between ICs (ITN 2.04%, Drug 0.65%, IRS 2.32%) were insignificant ($P > 0.05$). Species from NICs, Anopheles gambiae (44.81%) and An. funestus (32.05%), were higher than those from ICs, Anopheles gambiae (14.84%) and An. funestus (3.44%). NICs had more parous mosquitoes, ICs had comparable sporozoite rates (1.36% vs 2.06%), Entomological Inoculation Rate (0.099 vs 0.0331) and infectivity rates (1.05% vs 0.59%). In conclusion, this study suggests that malaria management efforts should involve an integrated strategy that revolves around proper environmental sanitation and human behavioral patterns.*

KEYWORDS: Malaria infection, Intervention measures, Impact assessment, Malaria transmission, Transmitting vectors.

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INTRODUCTION

Malaria, a vector-borne/neglected tropical disease, is the leading cause of mortality and morbidity in Nigeria. Transmission occurs in every part of the country. Globally, an estimated 3.2 billion people are at risk of developing malaria each year (WHO, 2015). In 2020, there were an estimated 241 million cases worldwide (WHO, 2022). According to an estimate, 76% of Nigeria's population lives in regions with significant malaria-related fatality in 2019 (USAID, 2021). Due to unforeseen costs for treatment management and prevention, there is now a higher level of poverty in affected areas (Awosolu *et al.* 2021).

Nigeria has the highest burden of this disease in Africa (WHO, 2005a). It has been associated with a major negative economic impact on places where it is widespread, with the economic impact in Africa being estimated at 1.2 billion US dollars every year. The economic burden includes costs of health care, decreased productivity due to brain damage from cerebral malaria/cerebral plasmodiasis, poor work attendance due to sickness leading to working days lost, days lost in education and lost investment and tourism (Greenwood *et al.* 2005). About half the world's population (3.3 billion) is at risk of contracting malaria (WHO, 2013a), and approximately 75% of the cases occur in Africa, with the remainder occurring in Southeast Asia, the western Pacific and the Americas. In 2010, there were about 219 million malaria cases and about 660,000 malaria deaths (WHO, 2013a). Approximately 90% of all malaria deaths occurred in the World Health Organization (WHO) African Region, mostly among children under five years of age (WHO, 2013a). In addition, malaria is said to kill one African (whether child or adult) every 15 seconds and roughly 300,000 Nigerian children annually (Salako, 2002). Furthermore, as a major cause of ill health in Africa, malaria is responsible for over 10% of the overall African disease burden. People who live below the poverty line, children under five years of age (22% of population) and pregnant women (20% of the population) are the most vulnerable to malaria disease (Guillet *et al.,* 2001), even where some degree of acquired immunity in areas of intense transmission (stable malaria) for most adult population is offered. Children are the main victims of malaria, particularly in Africa (Bechem *et al.,* 1999). Current WHO initiatives in malaria control, such as Roll Back Malaria (RBM), emphasized the use of Insecticide Treated Nets (ITNs) as one of the key strategies for malaria prevention and control in sub-Saharan Africa (Jones, 2000).

According to the World Health Organization (WHO) Global Malaria Program, three important interventions, including diagnosis and treatment of malaria cases with effective medications, insecticide-treated nets (ITNs) distribution and indoor residual spraying (IRS), are imperative for the control and prevention of residents from malaria (Keating *et al.*, 2011; Kleinschmidt *et al*., 2009; WHO, 2006). The IRS, as a highly cost-effective intervention (Kleinschmidt *et al*., 2009) to control malaria on a large scale (Kaufman et al., 2012), is coating the interior of homes, comprising all walls, roofs and other surfaces, and domestic animal shelters with chemical insecticides (Kaufman *et al*., 2012; WHO, 2006). Multiple studies have shown the effectiveness and efficacy of IRS in reducing malaria vectors and preventing transmission of infection in the nations where it was performed (Steinhard *et al*., 2013; Keating *et al.*, 2011; Kleinschmidt *et al*., 2009; WHO, 2006). In 2010, the IRS protected 185 million people (6% of the world population) at risk for malaria infection.

Similarly, IRS has been shown to significantly disrupt malaria transmission, eliminate malaria vectors and reduce malaria incidence (Pluess *et al*., 2010; Mabaso *et al*., 2004; Curtis, 2000). Today, universal coverage with long lasting insecticide-treated nets (LLINs) or

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IRS is actively promoted as the primary prevention strategy under the WHO-endorsed malaria control and elimination plan (WHO, 2015). Several previous studies have documented a high prevalence of malaria throughout Nigeria (Onyiri, 2015; Nmadu et al., 2015; Noland *et al*., 2014; Oche & Aminu, 2012; Gajida *et al*., 2010; Ibekwe *et al*., 2009). Since the adoption of intervention measures, few parasitological studies have reassessed the impacts of these single interventions on malaria. These results notwithstanding, entomological assessments of *Anopheles* (are lacking) need to be fully undertaken to complement parasitological studies. Our study has identified in these proposed areas differential use of these intervention measures. There is a need to clarify and confirm the activity/impact of these practices, as false positive results may impede the search for new and workable controls.

MATERIALS AND METHODS

Study Area

The study was conducted in Four (4) selected communities, each from two Local Government Areas (Obowo and Owerri North) in Imo state Nigeria from July 2023 to May 2024 (Figure 1: Obowo LGA Latitude $5^{o}10^{1}$N-$5^{o}5^{1}$N and Longitude $6^{o}35^{1}$ E-$7^{o}28^{1}$ E; Owerri North Latitude $5°15^{1}$ N-$5°34^{1}$ N and Longitude $7°15^{1}$E-$7^{o}30^{1}$ E). The study area has been detailed (Amaechi et al., 2024; Iwunze & Amaechi, 2021; Egejuru et al., 2016). In brief, features contributing to perennial transmission in the areas are mainly due to population movement and favorable environmental factors that enhance insect breeding. The malaria vectors (Anopheles species) are very potent, with a high anthropophilic index and frequent man-biting habits. Above all, there could be a presence of varying degrees of resistance to insecticides and drugs (Okere, 2024).

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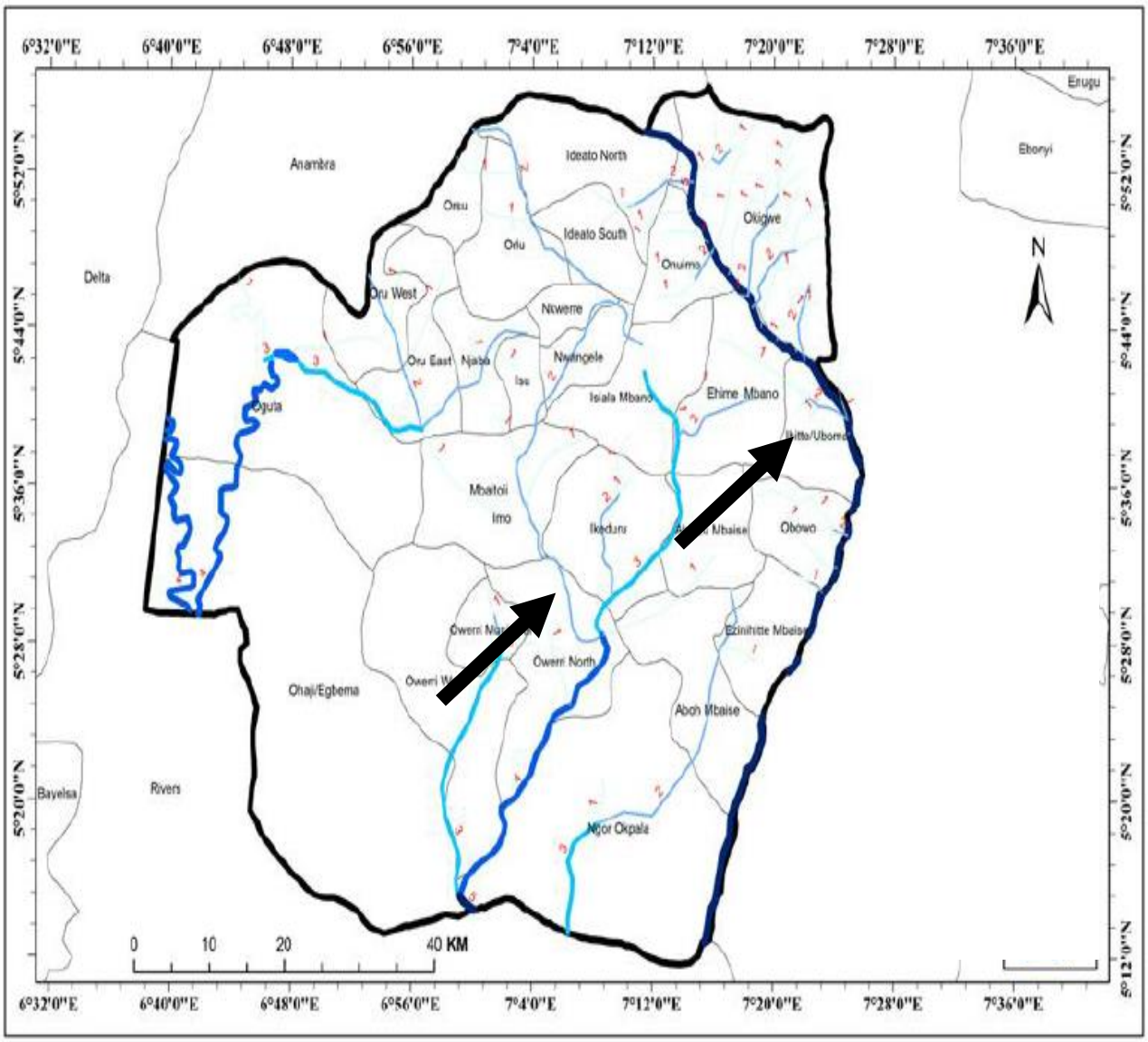

Fig. 1: Map of Imo State showing the study sites

Source: *wikipedia*

Study population

Participants for the study were drawn from Urban and Rural dwellers of Imo State aged 5 years and above. Selected study areas were Obowo L.G.A. (rural) and Owerri North L.G.A. (Urban) due to varied ecology. The survey involved 4 villages from Urban and 4 villages from rural areas based on the inclusion criteria. The study included 1,600 participants (200 each from the 4 rural villages and 4 urban villages).

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Sample Size Determination

The total sample size for the survey was 1,600 Participants, 200 from each of the study areas. The sample size of 1,600 participants was derived from the table for a minimum sample size estimate for a population survey with a 95% confidence interval using Lemeshow *et al.'s* (1990) formula:

$$n = \frac{Z^2[p(1-p)]}{d^2}$$

where

n = sample size,

Z = level of significance (1.96 at 95%),

p = the estimated proportion of the factor to be studied (0.187 or 18.7%),

d = sampling error that can be tolerated (0.05 or 5%).

With the formula, the minimum sample size was 200

Data Collection

Data collection involved blood collection, malaria vectors collection and questionnaire administration.

Diagnosis of malaria

Diagnosis of malaria was conducted among consenting residents located within the study areas. Diagnosis was done using HRP2-based Paracheck P^R (Orchid Biomedical System, Goa, India). Malaria rapid diagnostic test (RDT) specific for *P. falciparum* malaria detection. Study participants were apparently healthy individuals without any symptoms of malaria residing in the communities where intervention measures were provided and mosquitoes were previously collected. Participants gave oral and written informed consent after the study goals were made known to them. For minors, consent was sought and obtained from their parents or guardians. About 5 µL of blood was collected from a finger prick and dropped in the sample area of a labeled Paracheck $PF^{(R).}$ RDT kit using the sample applicator. Then six drops of clearing buffer solution (300 µL) were added in the wicking area and the test was allowed to run for 15 minutes. A malaria-positive test was indicated by the presence of two visible lines, one on the test line and another on the control line. A negative sample test showed only a control line, while an invalid sample test either showed a single test line or none at all (NPC, 2012). Malaria parasite intensity was determined by counting malaria parasites alongside leukocytes (Ukaga & Nwoke, 2007).

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Experimental Design and Distribution of Intervention Measures

The study subjects were divided into 4 groups

Group 1 were given Malaria drugs

Group 2 were given ITNs

Group 3 was given Insecticides.

Group 4 served as Control group

Four (4) villages were sampled from rural and urban areas.

Those from Rural (Obowo) include

Group 1 Avutu (200 participants)

Group 2 Umuarama (200 participants)

Group 3 Ehume (200 participants)

Group 4 Amuzi (200 participants)

Those in Urban area (Owerri North) were

Group 1 Orji (200 participants)

Group 2 Akwakuma (200 participants)

Group 3 Amakohia (200 participants)

Group 4 Works Layout (200 participants)

Distribution of Single intervention measures

For ease of study, study participants were grouped into three. Study participants from group one were given 196 pieces of ITNs as an intervention measure. Participants from Avutu received 113 pieces of nets (83 hung on Doors/Windows and 31 hung on the bed). Also participants from Orji received 83 pieces of nets (51 hanged on Doors/Windows and 32 hanged on the bed)

For antimalarial drugs, Group 2 participants were administered 140 pieces of two different malaria drugs. Those from Umuariam received 83 pieces of Antimalarial drugs while Akwakuma received 57 pieces of Antimalarial drugs. In all, 154 IRS were distributed to study participants from group 3, of which Ehume received 93 while Amakohia received 61. Amuzi and the works layout (Group 4) served as control.

Selection/Training 0f Personnels

The six (6) personnel recruited for the study included two (2) Nurses, two (2) Medical Laboratory Scientist and two (2) Health personnels from each LGA. They were given two (2) weeks of training on the research topic and objectives. Their presence and contributions were

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invaluable. Drug admission, data capturing, IRS usage and perception and questionnaire administration for the study and protocol/procedure were evaluated and adopted.

Intervention Sources, Distribution and observational study

Sources of malaria intervention

Two different types of Malaria Drugs (Combisunate and Loatherm) were given to the study participants, which were purchased from a licensed pharmacy store. Long Lasting Insecticide Treated Nets used comprised two colors (white and blue) and different shapes and sizes. The IRS was a locally made sniper rifle that was purchased from the market.

Baseline Observational Study

Surveys on demographic informations and status before intervention and Abuse of malaria intervention were assessed

Observational Phase

In this phase, post-intervention measures were assessed for the efficacy/impact of intervention measures.

Compliance and Monitoring of the Intervention Measures

A village committee, which consisted of youth leaders and other opinion leaders, was constituted in intervention and control villages to monitor proper use and compliance with the intervention measures. This committee were in turn monitored by the research team

Malaria Vectors Collection, Preservation and Identification

Twenty-five (25) households (10 mud and 15 brick) were selected from each study area (due to the uneven spread of households and status of users). It was to represent homes in the area. Anthropophilic (indoor resting) mosquitoes were collected using pyrethrum spray catches twice monthly during the hours of 7.00 am and 10.00 am (Mboera *et al.*, 2006), modified by Amaechi (2009). The proportion of indoor mosquitoes was sampled by covering the floor with a white sheet of 5 m x 5 m, each edge held to the wall by masking tape. The rooms were sprayed with pyrethrin insecticide and then left for 10 minutes with every opening (doors and windows) shut. Mosquitoes were collected thereafter with forceps and emptied into a dish lined with normal saline and transported to the Entomology laboratory of Imo State University Owerri for identification and processing. Upon collection, Anophelines were sorted from other mosquitoes and identified to species based on morphological features (Gilles & Coetzee, 1987; Gillies & De-Mellion, 1968).

Dissection and Parity Rate Determination

After removing the wings and legs, the female mosquitoes were placed ventrally on a slide where dissection took place. The mosquitoes were pierced through the thorax with the right-hand needle while a small cut was made between the sixth and seventh sternites. The second needle was used to extract the ovaries. Blood-fed females were dissected to determine parity by observing the degree of ovarian tracheoles. Determination of the Entomological Inoculation Rate (EIR) necessitated two other measurements: the sporozoite rate and the

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human biting rate. The abdominal and ovary dissection was conducted following the standard of WHO (1975). Ovaries with coiled tracheal skeins were considered nulliparous, while those with stretched-out tracheoles were taken to be parous, as described by WHO (1975).

Enumeration of the proportion and distribution of *Plasmodium* sporozoites and EIR of the vectors (*Anopheles*), indoor resting mosquitoes collected by permethrin knockdown were sorted and only *Anopheles spp* were identified using standard methods and evaluated for entomological inoculation rate (EIR). Dissection of the salivary glands for sporozoites was carried out according to the techniques of WHO (2002). Entomological Inoculation Rates (EIR) and the sporozoite rate were determined using the WHO (2013b) method.

SR=No of mosquitoes with sporozoites / No of dissected mosquitoes

EIR = HBR X SR/100

Intervention Impact Assessment

Determining the impact of intervention measures on malaria control

Malaria prevalence was assessed from different groups. Blood collection was reported by Ukaga and Nwoke (2009). The result gotten from different groups used were recorded and compared with the initial result gotten before intervention

Comparison of the efficacies of different intervention measures

The blood collected was used to check their malaria status. The results obtained were compared among different groups with different intervention measures and analyzed statistically.

Data Analysis

Chi-Square was used to test for the significant difference in the species composition. Student's T test was used to test the significant difference between IRD and HBR of the vectors in rainy and dry seasons. Anova was used to test the significant difference between IRD and MBR in the study areas.

RESULTS

Pre- and Intervention overall results of malaria prevalence (Figure 2) showed that pre-results (26.12%) were five(5) times significantly higher than intervention results (9.05%, $P<0.05$). Also, all the intervention measures significantly lowered malaria prevalence in the various communities ($P<0.05$). Overall Adult mosquitoes composition and relative abundance (Table 1) revealed that the Month of July recorded the highest mosquito species (27.69%), followed by the month of June (19.85%), while December was the least (9.55%). Table 2 summarizes the comparison of malaria vector densities and sporozoite infection rates between households with/without single intervention. The result reveals that the Non-Intervention Cohort (NIC) recorded the highest proportion of mosquitoes with 4 (3.25%), followed by S 2 (2.32%) ($P < 0.05$). Two (2.04%) of 147 mosquitoes recovered from households using ITN had sporozoites, while the least isolation of 1 (0.65%) was recorded in households using only

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drugs (P < 0.05). Summary of relationship between malaria prevalence and intervention measures (Table 3) Participants from NICs (17.4%) had significantly higher malaria prevalence than those from ICs (ITN 4.59%, Drug 5.71%, IRS 7.14%) (P < 0.05). However, the differences between the prevalences for ICs were insignificant (P > 0.05). Sex-wise, males had a higher prevalence (5.85%) than females (3.67%). The trend was reflected in Control (3.40% vs 2.31%), ITN (0.95% vs 0.27%), and IRS (0.95% vs 0.54%). But in drug the case was different, where both the males and females had equal malaria prevalence (0.54% vs. 0.54%). People in the 21-30 age group had the highest malaria prevalence (2.72%), while 0-10 years had the least (0.54%). In terms of location, Rural (5.98%) had the highest malaria prevalence compared to Urban (3.53%). The trend was reflected in Rural having the highest and Urban the least in ITN (0.68% vs. 0.54%), Drug (0.68% vs. 0.40%), IRS (0.95% vs. 0.54%) and Control (3.67% vs. 2.04%) (P < 0.05). Farmers/Traders (4.62%) had the highest prevalence, while health workers/civil servants (0.95%) had the least. The trend of prevalence was the same, with Farmers the highest prevalence, while health workers/civil servants had the least in ITN (0.54% vs 0.13%), Drug (0.54% vs 0.13%), IRS (0.68% vs. 0.13%) and Control (2.85% vs 0.54%) (P <0.05). Table 4 summarized the monthly malaria species caught in the study area. From the table, malaria species caught on non-intervention cohort (76.87%) was three times higher than (23.12%) caught from intervention cohorts. On non-intervention cohort, Amuzi community had the highest malaria species *An. gambaie* (33.19%), *An. funestus* (22.61%), while Works layout had the least *An. gambaie* (25.10%), *An. funestus* (19.08%). The month of March (40.24%) had the highest number of malaria species caught while December (12.65%) had the least. Similarly on Intervention measures, Umuariam community had the highest number of malaria species caught *An. gambaie* (13.79%), *An. funestus* (7.58%), while Akwakuma had the least *An. gambaie* (7.58%), *An. funestus* (4.82%). Furthermore, the month of July (33.10%) had the highest number of malaria species caught, while October (14.48%) had the least (P <0.05). Table 5 presents overall malaria species and transmission indices. Malaria species from non-intervention cohorts *An. gambaie* (44.81%), *An. funestus* (32.05%) were higher compared to *An. gambaie* (14.84%), *An. funestus* (3.44%) species caught from intervention cohorts. The month of July *An. gambaie* (20.68%), *An. funestus* (12.41%) had the highest in intervention cohorts, while October *An. gambaie* (3.44%), *An. funestus* (3.44%) was the least. On non-intervention cohorts, the month of March *An. gambaie* (22.82%), *An. funestus* (17.42%) had the highest number of species in intervention cohorts while December *An. gambaie* (7.05%), *An. funestus* (8.29%) was the least. Parity status showed that (335) malaria vectors from non-intervention cohorts were gravid while (147) were not gravid. Also in intervention cohorts, (95) were gravid, while 50 were not gravid. In terms of blood meal, 342 were blood-fed, while 140 were not blood-fed for non-intervention cohorts, whereas in intervention cohorts, 97 were blood-fed, while 48 were not blood-fed. Furthermore, in non-intervention cohorts there were 2 infections and 2 infective mosquitoes, while in intervention cohorts there were 2 infections and 1 infective mosquito, respectively. However, in sporozoite rate, intervention cohorts (2.06) were higher compared to (0.58) from non-intervention cohorts. EIR from non-intervention cohorts (0.0991) was higher compared to (0.0331) from intervention cohorts (P < 0.05).

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Table 1: Adult mosquitoes composition and relative abundance

Communities	Species	Rainy season				Dry season		Total
		May	June		July	Octo	Nov	
		Dec						
Avutu	*An. gambiae*	13(10.92)	16(12.59)	29(15.34)	11(16.17)	11(12.64)	06(9.09)	86(13.01)
	An. funestus	09(7.56)	08(6.29)	11(5.82)	09(13.23)	08(9.19)	06(9.09)	51(7.77)
	Others	15(12.60)	13(10.23)	18(9.52)	06(8,82)	11(12.64)	08(12.11)	71(10.82)
Umuariam	*An. gambiae*	12(10.08)	09(7.08)	13(6.87)	06(8.82)	07(8.04)	09(13.63)	56(8.53)
	An. funestus	07(5.88)	06(4.72)	11(5.82)	07(10.29)	05(5.74)	03(4.54)	39(5.94)
	Others	14(11.76)	16(12.59)	24(12.69)	08(11.76)	13(14.94)	07(10.60)	82(12.5)
Ehume	*An. gambiae*	06(5.04)	09(7.08)	11(5.82)	03(4.41)	03(3.44)	04(6.06)	36(5.48)
	An. funestus	05(4.20)	08(6.29)	06(3.17)	01(1.47)	02(2.29)	02(3.03)	24(3.65)
	Others	11(9.24)	09(7.08)	18(9.52)	05(7.35)	06(6.89)	03(4.54)	52(7.92)
Amuzi	*An. gambiae*	11(9.24)	08(6.29)	18(9.52)	05(7.35)	03(3.44)	04(6.06)	49(7.46)
	An. funestus	08(6.72)	13(10.23)	16(8.46)	03(4.41)	09(10.34)	08(12.11)	57(8.68)
	Others	08(6.72)	12(9.44)	14(7.40)	04(5.88)	09(10.34)	06(9.09)	53(8.07)
`	**Sub total**	**119(50.0)**	**127(52.26)**	**189(55.75)**	**68(52.71)**	**87(55.06)**	**66(56.41)**	**656(53.59)**
Orji	*An. gambiae*	11(9.24)	09(7.75)	13(8.66)	04(6.55)	07(9.85)	06(11.76)	50(8.80)
	An. funestus	11(9.24)	07(6.03)	11(7.33)	05(8.19)	03(4.22)	02(1.70)	39(6.86)
	Others	13(10.92)	14(12.06)	18(12.00)	07(11.47)	11(15.49)	09(7.69)	72(12.67)
Akwakuma	*An. gambiae*	11(9.24)	13(11.20)	17(11.33)	06(9.83)	09(12.67)	04(3.41)	60(10.56)
	An. funestus	13(10.92)	18(15.51)	15(10.00)	11(18.03)	08(11.26)	07(5.98)	72(12.67)
	Others	06(5.04)	09(7.75)	17(11.33)	02(3.27)	04(5.63)	03(2.56)	41(7.21)
Amakohia	*An. gambiae*	08(6.72)	07(6.03)	13(8.66)	04(6.55)	04(5.63)	02(1.70)	38(6.69)
	An. funestus	04(3.36)	06(5.17)	09(6.00)	05(8.19)	02(2.81)	03(2.56)	29(5.10)
	Others	11(9.24)	08(6.89)	11(7.33)	04(6.55)	08(11.26)	06(11.76)	48(8.45)
Works layout	*An. gambiae*	14(11.76)	09(7.75)	11(7.33)	05(8.19)	06(8.45)	04(3.41)	49(8.62)
	An. funestus	04(3.36)	09(7.75)	07(4.66)	6(9.83)	03(4.22)	02(1.70)	31(5.45)
	Others	13(10.92)	07(6.03)	08(5.33)	02(3.27)	06(8.45)	03(2.56)	39(6.86)
	Sub total	**119(50.0)**	**116(47.73)**	**150(44.24)**	**61(47.28)**	**71(44.93)**	**51(43.58)**	**568(46.40)**
	Total	**238**(19.44)	**243**(19.85)	**339(27.69)**	**129**(10.53)	**158**(12.90)	**117(9.55)**	**1,224(100.00)**

Key:: *Others *Aedes* species *Culex* species

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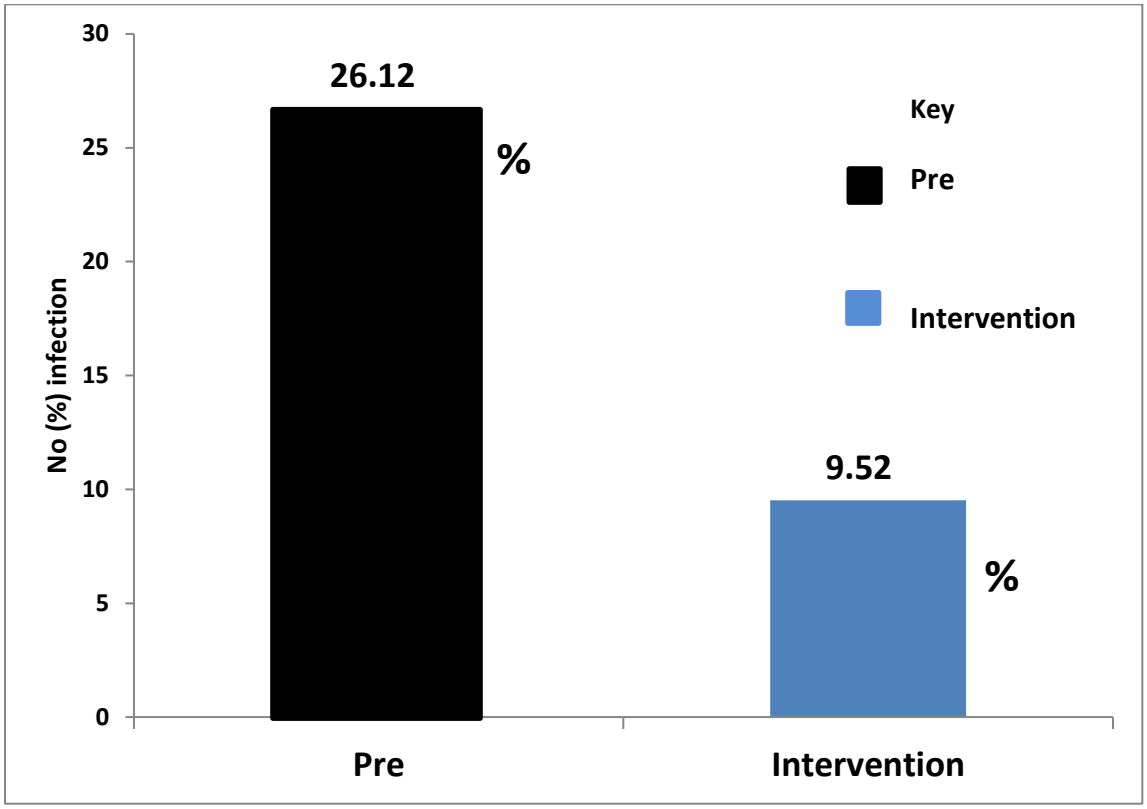

Figure 2: Pre and Intervention result of malaria prevalence in the study area

Table 2: Comparison of Malaria vector densities and sporozoite rate between households with single intervention and Non intervention households

Groups	Communities/status	Species	Malaria interventions (%)							
			ITN		Drug		IRS		NIC	
			No ex	No (%) inf	No ex	No (%) inf	No ex	No (%) inf	No ex	No (%) inf
1	Avutu (Rural)	*An. gambaie*	51	0(0.00)	-	-	-	-	-	-
		An. funestus	28	1(50.00)	-	-	-	-	-	-
	Orji (Urban)	*An. gambaie*	41	1(50.00)	-	-	-	-	-	-
		An. funestus	27	0(0.00)	-	-	-	-	-	-
2	Umuariam (Rural)	*An. gambaie*	-	-	38	1(100.00)	-	-	-	-
		An. funestus	-	-	23	0(0.00)	-	-	-	-
	Akwakuma (Urban)	*An. gambaie*	-	-	42	0(0.00)	-	-	-	-
		An. funestus	-	-	49	0(0.00)	-	-	-	-
3	Ehume (Rural)	*An. gambaie*	-	-	-	-	26	2(100.00)	-	-

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		An. fnestus	-	-	-	-	18	0(0.00)	-	-
	Amakohia (Urban)	***An. gambaie***	-	-	-	-	23	0(0.00)	-	-
		An. funestus	-	-	-	-	19	0(0.00)	-	-
4	Amuzi (Rural)	***An. gambaie***	-	-	-	-	-	-	32	2(50.00)
		An. funestus	-	-	-	-	-	-	39	1(50.00)
	Works Layout (Urban)	***An. gambaie***	-	-	-	-	-	-	31	1(50.00)
		An. funestus	-	-	-	-	-	-	22	0(0.00)
		Total	**147**	**2(2.04)**	**152**	**1(0.65)**	**86**	**2(2.32)**	**124**	**4(3.25)**

Key:
ITN: Insecticide Treated Net
IRS: Indoor Residual Spray
NIC: Non Intervention Cohort

Table 3: Summary of the relationship between malaria prevalence and intervention measures

Variables	**No Exam**	**No (%) inf**	**Intervention measures**							
			ITN		**Drug**		**IRS**		**Control**	
			No Exam	**No (%) inf**	**No Exam**	**No (%) inf**	**No Exam**	**No (%) inf**	**No Exam**	**No (%) inf**
Sex										
Male	309	43(5.85)	86	7(0.95)	57	4(0.54)	64	7(0.95)	102	25(3.40)
Female	426	27(3.67)	110	2(0.27)	83	4(0.54)	90	4(0.54)	143	17(2.31)
Age										
0-10	80	4(0.54)	21	0(0.00)	18	1(0.13)	9	0(0.00)	32	03(0.40)
11-20	94	11(1.49)	34	2(0.27)	15	1(0.13)	18	1(0.13)	27	07(0.95)
21-30	162	20(2.72)	40	3(0.40)	29	3(0.40)	44	3(0.40)	49	11(1.49)
31-40	156	17(2.31)	39	2(0.27)	28	1(0.13)	35	4(0.54)	54	10(1.36)
41-50	128	11(1.49)	30	1(0.13)	26	1(0.13)	29	2(0.27)	43	07(0.95)
>51	115	07(0.95)	32	1(0.13)	24	1(0.13)	19	1(0.13)	40	04(0.54)
Location										
Rural	427	44(5.98)	113	5(0.68)	83	5(0.68)	93	7(0.95)	138	27(3.67)
Urban	308	26(3.54)	43	4(0.54)	57	3(0.40)	61	4(0.54)	107	15(2.04)
Occupation										
Farmers/Traders	305	34(4.62)	81	4(0.54)	58	4(0.54)	67	5(0.68)	99	21(2.85)
Health workers/ Civil servants	104	07(0.95)	27	1(0.13)	20	1(0.13)	21	1(0.13)	36	04(0.54)
Students	188	15(2.04)	57	2(0.27)	30	2(0.27)	37	3(0.40)	64	08(1.02)
Others	138	14(1.90)	31	2(0.27)	32	1(0.13)	29	2(0.27)	46	09(1.22)

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Total	**735**	**70(9.52)**	**196**	**9(4.59)**	**140**	**8(5.71)**	**154**	**11(7.14)**	**245**	**42(17.14)**

Key:
ITN: Insecticide Treated Net
IRS: Indoor Residual Spray
NIC: Non Intervention Cohort

Table 4: Monthly malaria species caught in the study Area

Villages	**Species**	**Months, Numbers and % Collected**									
		Intervention measures					**No intervention measures**				
		J	**A**	**S**	**O**	**Total**	**D**	**J**	**F**	**M**	**Total**
Avutu *	*An. gambaie*	05(10.41)	05(10.86)	04(13.33)	02(9.52)	16(11.03)	-	-	-	-	-
	An. funestus	06(12.50)	04(8.69)	03(10.00)	0(0.00)	13(8.96)	-	-	-	-	-
Umuariam **	*An. gambaie*	07(14.58)	06(13.04)	03(10.00)	04(19.04)	20(13.79)	-	-	-	-	-
	An. funestus	04(8.33)	04(8.69)	03(10.00)	0(0.00)	11(7.58)	-	-	-	-	-
Ehume ***	*An. gambaie*	03(6.25)	05(10.86)	06(20.00)	3(13.28)	17(11.72)	-	-	-	-	-
	An. funestus	01(2.08)	02(4.34)	02(6.66)	1(4.76)	6(4.13)	-	-	-	-	-
Amuzi ****	*An. gambaie*	-	-	-	-	-	21(34.42)	38(40.00)	43(32.57)	58(29.89)	160(33.19)
	An. funestus	-	-	-	-	-	18(29.50)	21(22.10)	29(21.96)	41(21.13)	109(22.61)
Orji *	*An. gambaie*	5(10.41)	6(13.04)	3(10.00)	03(14.28)	17(11.72)	-	-	-	-	-
	An. fnestus	2(4.16)	4(8.69)	2(6.66)	01(4.76)	09(6.20)	-	-	-	-	-
Akwakuma **	*An. gambaie*	5(10.41)	4(8.69)	0(0.00)	02(9.52)	11(7.58)	-	-	-	-	-
	An. funestus	3(6.25)	2(4.34)	2(6.66)	0(0.00)	07(4.82)	-	-	-	-	-
Amakohia ***	*An. gambaie*	5(10.41)	3(6.52)	2(6.66)	02(9.52)	12(8.27)	-	-	-	-	-
	An. funestus	2(4.16)	1(2.17)	0(0.00)	03(14.28)	06(4.13)	-	-	-	-	-
Workslayout ****	*An. gambaie*	-	-	-	-	-	13(21.33)	17(17.89)	39(29.54)	52(26.80)	121(25.10)
	An. funestus	-	-	-	-	-	09(14.75)	19(20.00)	21(15.90)	43(32.16)	92(19.08)
	Total	**48(33.10)**	**46(31.72)**	**30(13.79)**	**21(14.48)**	**145(23.12)**	**61(12.65)**	**95(19.70)**	**132(27.38)**	**194(40.24)**	**482(76.87)**

Key:
J= July A= August S= September O= October D= December J= January F= February M= M

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Key:
Intervention cohorts **Non- Intervention cohorts = ****
*** = ITN**
**** = Drug**

Comments:
Malaria vector all months = 627
Malaria vector all months for Intervention cohorts = 145(23.12)
Malaria vector all months for Non-Intervention cohorts = 482(76.87)

Table 5: Overall malaria species and transmission indices

Classification of data	Intervention measures					No intervention measures				
	J	**A**	**S**	**O**	**Total**	**D**	**J**	**F**	**M**	**Total**
Malaria vectors (%)										
An. gambaie	30(20.68)	29(20.00)	18(12.41)	16(11.03)	93(14.83)	34(7.05)	55(11.41)	82(17.01)	110(22.82)	281(44.81)
An. funestus	18(12.41)	17(11.72)	12(8.27)	05(3.44)	52(8.29)	27(5.60)	40(8.29)	50(10.37)	84(17.42)	201(32.05)
Parity										
Gravid	30	33	18	14	95	42	63	94	136	335
not gravid	18	13	12	07	50	19	32	38	58	147
Blood meal										
Blood fed	35	39	19	13	97	48	62	85	147	342
Not fed	13	16	11	08	48	13	33	47	47	140
Infection status										
Infection	01	0	01	0	02	0	0	0	2	2
Infective	01	0	0	0	01	0	0	0	2	2
Rate										
Sporozoite rate	2.85	0.00	5.26	0.00	2.06	0.00	0.00	0.00	1.36	0.58
EIR	0.0165	0.0000	0.0163	0.0000	0.0331	0.0000	0.0000	0.0000	0.0999	0.0991

Key:
J-M = Months of the Year
Intervention (July-October)
Non-Intervention (December-March
EIR Entomological Inoculation Rate

J= July A= August S= September O= October D= December J= January F= February M= March

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DISCUSSION

The study was conducted almost 15 years after integrated efforts to contain malaria were launched in Nigeria (Carter Center, 2010). In this study, 26.12% vs. 9.05% of the participants tested positive for *Plasmodium falciparum* infection pre- and post-intervention, results demonstrating that malaria is still widespread in the investigated study areas of Imo State, Nigeria. The pre-intervention result is higher than the geographical average of 16% reported by the Severe Malaria Observatory (2020) in Southern parts of Nigeria. However, according to recent malaria risk maps, the frequency of malaria in Nigeria ranged from less than 20% in certain places to over 70% in the Southern parts of Nigeria (Onyiri, 2015). Neither microscopy nor reverse transcriptase PCR (qRT-PCR) was used to confirm parasitemia or determine gametocyte prevalence, which could be a latent reservoir for malaria transmission by the local transmitting vectors. It is known that HRP2-based RDTs like the Paracheck P^R to overestimate malaria prevalence; PCR methods perform better to compute submicroscopic parasitemia or gametocytemia that is hardly detected by RDT or microscopy (Mwiringa et al 2014). Probably the rate of malaria would have remained at or surpassed the present rates of 26.42% vs. 9.05%. Thus, the reported malaria prevalence rate. This could constitute a threat to malaria elimination efforts in the study area. Prevalence of more asymptomatic malaria with gametocyte carriers will constitute an obstacle aimed at breaking the chain that links malaria vectors through interventions. This premised the recommendations of drugs (artemisinin combination therapy, ACT). However, it will be necessary to map the prevalence of glucose-6-phosphate dehydrogenase deficiency (G6PDd) in the study areas and assess genotype, especially AA, as these increase risk factors and might limit the suitability of drug interventions. The overall prevalence recorded is lower than previous studies in Kano and Oyo States, Nigeria (Awosolu et al., 2021). The variation could be linked to differences in climatic and environmental conditions. The environment of these study areas was observed to be bushy, with the presence of gutters and potholes filled with stagnant water and refuse dumps. In most cases these were close to households. All these environmental characteristics have been found to be associated with mosquito abundance. Housing features, viz., holes in walls, uncovered roof space, and unscreened windows, and living close to breeding areas encouraged mosquito presence in the study area, as has been reported by Ngadjea et al. (2020) in Cameroon. Regular sanitation, especially around households, is advocated in malaria control.

Two malaria mosquito species (*Anopheles gambiae* and *Anopheles funestus*) were found to forage in the cohorts. The most common was *An. gambiae sl* (34.88%), which is consistent with previous findings (Amaechi et al., 2011; 2019) in Imo and Ebonyi States, (Okwa et al., 2006) in Lagos, Nigeria and elsewhere (Mboera et al., 2002, 2006) in Tanzania. Its higher presence in intervention households is proof of high resistance and “omnipresent” status in Nigeria together with endophilic and endophagic habits. The second abundant species *An. funestus* (27.94%), co-breed with *An. gambiae*. Its proportion points to its being exophagic but endophilic, with implications for control. Availability of these positive *Anopheles* mosquitoes in the study communities ensures transmission of malaria parasites, probably in other endemic places of Imo State. To measure malaria transmission, a better knowledge of vectors is needed. To achieve this requires entomological assessment of collected *Anopheles* species. The presence of malaria sporozoites remains an essential component to understanding transmission dynamics. Although the presence of these vectors is crucial to malaria transmission, transmission does not depend on the vectors only. Malaria parasites

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gametocyte reservoir in human population is important, especially the asymptomatic malaria infection reservoir

The results of malaria vector densities and sporozoite rates (ITN 2.04%, Drug 1.65%, IRS 2.32% and NIC 3.25%), malaria prevalence and intervention measures (ITN 4.59%, Drug 5.71%, IRS 7.14% and NIC 17.4%), and monthly malaria vector composition (intervention cohorts 12.12% vs. 76.87% non-intervention cohorts),, which differed significantly, are indicative of the fact that malaria interventions had effects on prevalence against malaria infection. However, the prevalence of malaria varied significantly among different intervention measures and remained comparable in efficacies between the different intervention measures.

Transmission control of malaria adopted in this study focused on the elimination of a reservoir of parasites via drugs and reducing human-vector contact. Among these measures, ITN reduced the incidence of infection by inhibiting mosquito entrance to houses, as has been reported elsewhere (Maxwell et al 1999). Drugs act by clearing existing parasitemia to a level below the fever threshold and stopping new infection (Cairns et al., 2008; Menedez et al., 2007). The IRS principle is to protect users against vector bites by killing the blood-fed female that rests on the walls after feeding and also protect users against vector bites by diverting them from entering a sprayed house with excito-repellency repellency (WHO, 2006; Hamisse et al., 2012). Recalcitrant female mosquitoes that enter will rest on sprayed surfaces and pick up a lethal dose of insecticide. This will prevent parasite transmission to others and only a few will survive the proximity of 12 days needed for sporozoite maturation for parasite transmission (Curtis et al., 2006). IRS reduces malaria transmission by reducing mosquito longevity and abundance and also household-level protection (Pluess et al., 2010). There is no scientific evidence, to our knowledge, to prove that one method is better than the other. Despite all the benefits of these interventions, our analysis suggested a consistently better efficacy for combinations against single interventions. Since the IRS requires trained personnel for applications while Drugs and ITN require monitoring of compliance, that might not always be done effectively.

The study used different intervention measures to introduce similar attractiveness or repellency by the vectors. This could indicate that the slightly higher prevalence by the males proves that malaria susceptibility is not gender-based (Gilles & Warrell, 1993). The prevalence could be due to any other reasons, including by chance. The slow acquisition of malaria immunity plus the effect of intervention measures could explain why prevalence varied among different age groups, occupational class and locations among intervention users. In this study, entomological indices of malarial transmission (EIR, PR and SPR) are well established, thus confirming endemicity. Intensity of malaria parasite transmission is usually expressed as EIR and in Africa it is highly variable, ranging from less than 1 to 1,000 infective bites per person per year (Beier et al., 1999). The comparable sporozoite rates for intervention and non-intervention cohorts (2.06% vs. 0.58%) are similar to those reported elsewhere (Aju-Ameh et al., 2006; Msugh-Tur et al., 2014; Messebo et al., 2013). However, it was lower than those reported by Omulu et al. (2015) and Olayemi and Ande (2008). This could indicate malaria vectorial systems being more complex than expressed. Thus, pointing out that infective females that are compromised with *Plasmodium* parasites can put the inhabitants of the study areas at risk of malaria disease. Despite the disparity in the vector densities. Status of blood-fed and parity of those mosquitoes: there was an insignificant difference in the infectivity status for intervention and non-intervention cohorts. It is

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important to note that the abundance/density of mosquitoes in an area depends on the complex interaction of several factors, such as rainfall, the rivers, water level and availability of suitable larval breeding habitats. EIR, which is a product of SPR and BR, describes the intensity of malarial transmission (WHO, 1975). The extracted EIR was 0.0331 vs. 0.0991 infective bites/person/year. That is, a person in the study area might get one infective bite approximately every 3 months in either cohort. This could show that the prevalence of malaria is influenced by weather variables.

Conclusively, despite the introduction of malaria intervention measures, malaria cases and transmitting vectors are still recorded. Poor drainage systems, dirty environments and poor housing conditions in these areas could be contributing factors to the malarial vector densities and the prevalence of malaria. There is therefore a need to adopt an integrated approach consisting of a combination of vector intervention measures, proper environmental sanitation, parasite epidemiology and human behavioral patterns.

Declarations:

Ethical considerations

Ethical approval for the study was obtained from the Postgraduate Board of the Department of Zoology, the Imo State University Owerri ethics committee and the Imo State Ministry of Health. Consent was sought and obtained from the village heads. Also, Informed consent was obtained from the participants. Participants were fully informed of their right to withdraw without feeling constrained. Information confidentiality was ensured throughout the study.

Authors' Contributions

AAA and JII conceptualized the study. AAA, JII and OEM designed the study. OEM, APA, OOG, NJU and WAC participated in sample and data collection. JII and OEM performed the data analysis. AAA and JII interpreted the data and prepared the manuscript. All authors approved the submission.

Conflict of Interest

The authors say they have no competing interests.

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