Effectiveness of insecticide-treated bednets in malaria prevention in Haiti: a case-control study

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Summary

Background **Insecticide-treated bednets (ITNs) are effective in preventing malaria where vectors primarily bite indoors and late at night, but their effectiveness is uncertain where vectors bite outdoors and earlier in the evening. We studied the effectiveness of ITNs following a mass distribution in Haiti from May to September, 2012, where the *Anopheles albimanus* vector bites primarily outdoors and often when people are awake.**

Methods **In this case-control study, we enrolled febrile patients presenting to outpatient departments at 17 health facilities throughout Haiti from Sept 4, 2012, to Feb 27, 2014, who were tested with malaria rapid diagnostic tests (RDTs), and administered questionnaires on ITN use and other risk factors. Cases were defined by positive RDT and controls were febrile patients from the same clinic with a negative RDT. Our primary analysis retrospectively matched cases and controls by age, sex, location, and date, and used conditional logistic regression on the matched sample. A sensitivity analysis used propensity scores to match patients on ITN use propensity and analyse malaria among ITN users and non-users. Additional ITN bioefficacy and entomological data were collected.**

Findings **We enrolled 9317 patients, including 378 (4%) RDT-positive cases. 1202 (13%) patients reported ITN use. Post-hoc matching of cases and controls yielded 362 cases and 1201 matched controls, 19% (333) of whom reported consistent campaign net use. After using propensity scores to match on consistent campaign ITN use, 2298 patients, including 138 (7%) RDT-positive cases, were included: 1149 consistent campaign ITN users and 1149 non-consistent campaign ITN users. Both analyses revealed that ITNs did not significantly protect against clinical malaria (odds ratio [OR]=0·95, 95% CI 0·68–1·32, p=0·745 for case-control analysis; OR=0·95, 95% CI 0·45–1·97, p=0·884 for propensity score analysis). ITN and entomological data indicated good ITN physical integrity and bioefficacy, and no permethrin resistance among local mosquitoes.**

Interpretation **We found no evidence that mass ITN campaigns reduce clinical malaria in this observational study in Haiti; alternative malaria control strategies should be prioritised.**

Funding **The Global Fund to Fight AIDS, Tuberculosis, and Malaria, and the US-based Centers for Disease Control and Prevention (CDC).**

Introduction

Insecticide-treated bednets (ITNs) are a cornerstone of malaria prevention; multiple rigorous studies in sub-Saharan Africa have shown ITNs to be effective in preventing malaria morbidity when used consistently.[1–3] ITNs take advantage of the indoor feeding (endophagic) and indoor resting (endophilic) behaviours of some *Anopheles* mosquitoes and work by repelling and killing or decreasing the life span of mosquitoes, as well as providing a physical barrier between mosquitoes and users.[4,5] In Africa, the primary malaria vectors are from the *Anopheles gambiae* complex and the *Anopheles funestus* group, which predominantly bite indoors and when people are sleeping.[6]

Limited evidence exists on ITN effectiveness in Latin America and the Caribbean. *Anopheles albimanus*, one of the dominant malaria vectors in Latin America,[7] often bites outdoors (exophagic) and rests outdoors (exophilic).[7] Peak feeding times for *A albimanus* occur closer to sunset and generally earlier in the night than other *Anopheles* species,[8–10] although there is substantial geographic heterogeneity.[9] Findings from previous research on ITN effectiveness with *A albimanus* have been mixed. In a Guatemalan study from the early 1990s,[11] both untreated and treated nets reduced incidence of malaria (by 47% and 57%, respectively) compared with the absence of nets. A 1992 Peruvian study[12] found non-significant reductions in malaria incidence after introduction of ITNs. Nicaraguan trials[13] in 1996 reported that insecticide-treated materials significantly reduced community-level clinical malaria incidence, but only where community usage was greater than 16%.[13] Two studies[14] from Ecuador from 1989–90 and from 1991–92, respectively, found no significant difference in malaria incidence after ITN introduction. A more recent observational study from Brazil, where the predominant

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Research in context

Evidence before this study

We searched PubMed for studies on bednet effectiveness in settings outside of sub-Saharan Africa or with the *Anopheles albimanus* vector, using search terms including "ITN", "bednet", "LLIN", "*Anopheles albimanus*", and "Latin America" published between 1946 and 2016. Much of the available evidence on vector behaviour in Haiti is from older studies and describes a primarily exophagic (outdoor-biting) vector that can bite outside of sleeping hours. We found no previous studies on ITN effectiveness in Haiti, and the evidence from other Latin American settings where *A albimanus* is the primary malaria vector was mixed, with some studies showing that ITNs are significantly protective and others showing no effect.

Added value of this study

To our knowledge, this facility-based case-control study is the first study to assess the effectiveness of ITNs in Haiti, following a 2012 mass campaign. We recruited febrile patients at 17 health-care facilities and used two different analytical methods (retrospective matching and propensity score matching) to help ensure comparability between cases and controls. The study also collected ITN and entomological data, including ITN physical integrity and bioefficacy at two timepoints as well as insecticide resistance, to aid in interpretation of results.

Implications of all the evidence

We found that consistent use of ITNs, following a mass distribution, does not appear to provide significant individual protection against clinical malaria in this case-control study in Haiti. ITN physical integrity and bioefficacy performed well after 12 and 18 months after the mass campaign, and mosquitoes were susceptible to permethrin, the insecticide used in the nets. The lack of ITN effect could be explained by the vector's predominantly exophagic behaviours and tendencies to bite before sleeping hours. Although it would be ideal to have a broader evidence base to guide vector control and malaria elimination decisions in Haiti, this case-control study provides the best evidence on mass ITN campaign effectiveness to date, and additional studies on ITN effectiveness are unlikely to be conducted in this low-transmission setting. Based on this study, wide-scale ITN distributions in Haiti are not recommended, and alternative strategies for malaria control should be prioritised.

vector, *Anopheles darlingi*, is active throughout the day, found no effect of ITNs after a mass distribution in 2012.[15]

Hispaniola is the only island in the Caribbean where malaria remains endemic, although at very low levels.[16] The vast majority of cases on the island are reported from Haiti (17 662 reported confirmed cases in 2013) compared with the Dominican Republic (496 cases in 2013).[17] *Plasmodium falciparum* is the cause of nearly all malaria cases in Haiti,[18,19] and the primary vector is *A albimanus*.

International attention and increased funding for rebuilding public health infrastructure in Haiti after the 2010 earthquake led to a renewed focus on malaria control and elimination.[20] Before 2010, published data on malaria in Haiti were limited but indicated very low transmission, with one health facility survey[21] in 1995 estimating a slide-positivity rate of 4·0% among patients with suspected malaria; a population-based study[22] in the rural Artibonite Valley in 2006 found a prevalence of 3·1% by PCR. Post-earthquake studies from areas located near the epicentre of the earthquake confirmed high proportions of malaria infection among febrile patients attending clinics (46·9% and 20·3%),[23,24] suggesting a potential increase in malaria, possibly because of improved surveillance or increased exposure to mosquito vectors after many people were left homeless.

As part of the national malaria control strategy and concern over a potential increase in malaria since the January, 2010, earthquake, a national campaign was conducted from May to September, 2012, to distribute ITNs (permethrin 2·0% treated, polyethylene monofilament) to all areas in Haiti, except in the capital Port-au-Prince, with a target of two ITNs per household. During the distribution, educational messages about ITN use were given along with pictorial brochures on how to use nets. Following this national ITN distribution, we did a case-control study to assess the effectiveness of ITNs in Haiti for preventing clinical malaria.

Methods

Study setting, design, and sample size

In this case-control study, we recruited febrile patients presenting to outpatient departments at 17 health facilities in five departments in Haiti: Artibonite, Centre, Grand'Anse, Sud, and Sud-Est. We defined cases as patients with history of fever or measured temperature of 37·5°C or higher and a positive malaria rapid diagnostic test [RDT]. We defined controls as patients with a history of fever or measured temperature of 37·5°C or higher and a negative malaria RDT. The study sample size was calculated to detect a 25% reduction in the odds of ITN use among malaria-positive patients compared with malaria-negative patients, assuming a two-sided α of 0·05, power of 0·80, an estimate of 30% controls exposed (ie, using ITNs), and a ratio of one case to three controls, resulting in 650 cases and 1950 controls required. Patients were not matched a priori on any factors, since no major imbalances were anticipated between cases and controls on relevant background variables, given the large sample size. Based on review of laboratory registers of confirmed malaria cases during the previous year, two health facilities in Sud-Est,

a department reporting higher malaria test positivity, were initially selected for the study.

Patient recruitment began on Sept 4, 2012, at the two initial facilities. Because of low enrolment of cases, 15 additional facilities in four departments (Artibonite, Centre, Grand Anse, and Sud) were added from April 29, 2013, to Aug 3, 2013, bringing the total number of facilities to 17; thereafter, patient recruitment continued at the two initial sites and three facilities in Grand Anse with the highest case yield (figure 1).

A community sensitisation campaign to increase health facility use for fever treatment was conducted during the study period.

Written informed consent was obtained from patients enrolling in the study. Patients younger than 18 years required consent from a parent or guardian; written assent was also obtained from patients aged 7–17 years. The study protocol was approved by the National Bioethics Committee of Haiti and the Institutional Review Board at the US Centers for Disease Control and Prevention (CDC) in Atlanta, GA, USA.

Procedures

Patients presenting to outpatient departments at study sites were systematically screened by study staff for fever (defined as axillary temperature ≥37·5°C) or history of fever during the past 2 days. Eligible patients were given a brief questionnaire about their illness and previous treatment history, ownership and use of bednets, other malaria risk factors, knowledge of malaria, and household assets and characteristics. Finger prick samples of blood were taken for a malaria RDT and dried blood spots were also stored for PCR analysis. RDT results were read by study staff and given to clinicians to aid in case management at the facility.

To explore potential confounders to ITN effectiveness, we carried out a series of entomological and ITN assessments.

ITN physical integrity was assessed on a convenience sample of 30 ITNs from each department through visual inspection and categorisation of holes using the proportional hole index (PHI) developed by WHO.[25] Up to 30 campaign ITNs from each department where the study was active at 12 and 18 months after distribution were collected, mounted on a frame in Port-au-Prince, and inspected.

Bioefficacy of ITNs after 12 and 18 months was assessed at CDC in Atlanta (GA, USA) through cone bioassays[25] on swatches cut from each side and the roof of collected nets. Five swatches per net were cut and 20 mosquitoes exposed to each swatch for a total of 100 mosquitoes exposed per net. Collected ITNs were replaced with new ITNs.

Insecticide content of ITNs was measured using gas chromatography on the sample of the same ITNs collected.[25]

Insecticide resistance testing was done for permethrin, using the CDC bottle bioassay[26] on mosquitoes reared from field-collected larvae in each department where the study took place. Mosquitoes were exposed to 21·5 µg of permethrin per bottle for up to 120 min; control mosquitoes were field-reared mosquitoes exposed to acetone-impregnated bottles.

Pyrethrum spray catches[25] were conducted in the two initial study sites to evaluate differences in mosquito densities in homes with and without ITNs but very few *Anopheles* (only ten in total) were collected, possibly because of the timing of collections, which occurred later in the morning due to logistical constraints.

For the RDT diagnosis, CareStart (HRP2; AccessBio, Somerset, New Jersey, USA) *P falciparum* malaria RDTs were performed using finger-prick blood and read in the field according to the manufacturer's instructions, and results were communicated to patients and health facility clinicians. PCR analysis of filter paper blood spots for RDT-positive patients and a sample of RDT-negative

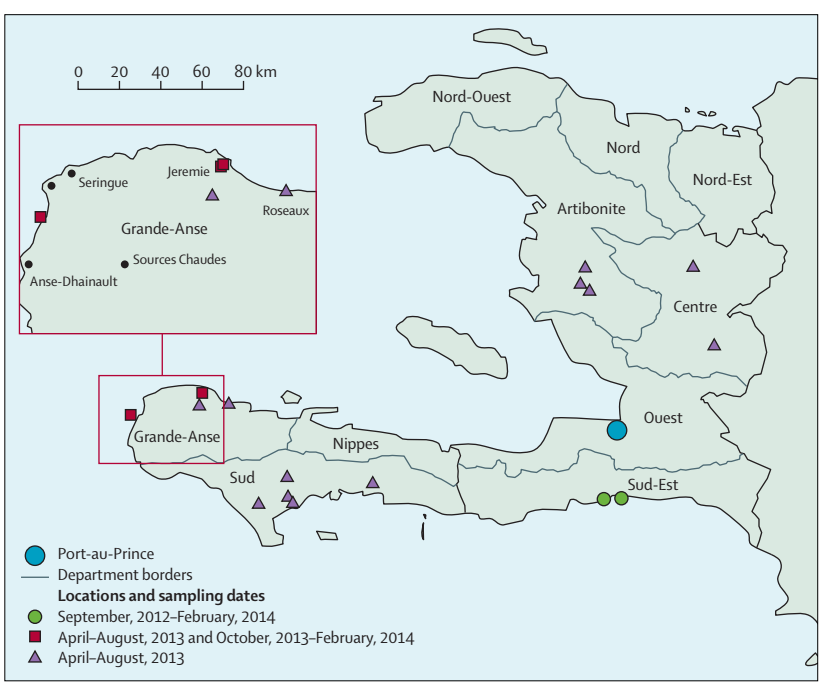


Figure 1: **Map of study locations in Haiti (and timing of enrolment)**

	Unmatched		Matched	
	Unexposed*	Exposed	Unexposed*	Exposed
Consistent campaign ITN use before illness†	8115	1202 (12·9%)	1149	1149
Slept under campaign net the previous night	7987	1330 (14·3%)	1274	1274
Slept under any bednet the previous night	6135	3178 (34·1%)	2758	2758
Owns any bednet	3843	5474 (58·8%)	3039	3039

*Unexposed means no campaign net use or non-consistent campaign net use, not sleeping under campaign net the previous night, not sleeping under any bednet the previous night, and not owning a net. †Defined as patient reporting sleeping under a campaign ITN "always" or 14 nights in the 2 weeks before illness onset.

Table 1: **Various measures of bednet ownership and use in complete (n=9317) and propensity score-matched datasets**

	Unmatched sample			Matched sample		
	Negative RDT result (n=8939)	Positive RDT result (n=378)	Total (n=9137)	Negative RDT result (n=1201)	Positive RDT result n=362)	Total (n=1563)
Age						
<5 years	3409 (38·1%)	30 (7·9%)	3439 (36·9%)	115 (9·6%)	30 (8·3%)	145 (9·3%)
5–9 years	1024 (11·5%)	28 (7·4%)	1052 (11·3%)	86 (7·2%)	27 (7·5%)	113 (7·3%)
10–19 years	1120 (12·5%)	93 (24·6%)	1213 (13·0%)	227 (18·9%)	81 (22·4%)	308 (19·7%)
≥20 years	3386 (37·9%)	227 (60·1%)	3613 (38·8%)	773 (64·4%)	224 (61·9%)	997 (63·8%)
Season						
9/2012–2/2013	1137 (12·7%)	167 (44·2%)	1304 (14·0%)	472 (39·3%)	155 (42·8%)	627 (40·1%)
3/2013–4/2013	508 (5·7%)	36 (9·5%)	544 (5·8%)	138 (11·5%)	36 (9·9%)	174 (11·1%)
5/2013–10/2013	5682 (63·6%)	114 (30·2%	5796 (62·2%)	403 (33·6%)	113 (31·1%)	516 (33·0%)
11/2013–2/2014	1612 (18·0%)	61 (16·1%)	1673 (18·0%)	188 (15·7%)	58 (16·0%)	246 (15·7%)
Male sex	3894 (43·6%)	93 (51·0%)	4087 (43·9%)	644 (46·4%)	182 (49·7%)	826 (47·2%)
Electricity at home	4430 (49·6%)	181 (48·0%)	4611 (49·5%)	592 (49·3%)	175 (48·5%)	767 (49·1%)
Asset ownership						
Radio	5767 (64·6%)	239 (63·6%)	6006 (64·5%)	771 (64·2%)	234 (65·0%)	1005 (64·4%)
Television	2973 (33·3%)	111 (29·5%)	3084 (33·1%)	352 (29·3%)	108 (30·0%)	460 (29·5%)
Mobile phone	7492 (83·9%)	324 (85·9%)	7816 (84·0%)	1031 (85·9%)	309 (85·6%)	1340 (85·8%)
Owns any bednet	5253 (58·8%)	223 (58·5%)	1202 (58·8%)	690 (57·5%)	215 (59·4%)	905 (57·9%)
Campaign net use previous night	1247 (14·0%)	83 (22·0%)	1330 (14·3%)	246 (20·5%)	80 (22·1%)	326 (20·1%)
Consistent campaign net use	1132 (12·7%)	70 (18·5%)	1202 (12·9%)	236 (19·7%)	67 (18·5%)	303 (19·4%)
Use of indoor insect spray (eg, RAID, DOOM)	1446 (16·2%)	36 (9·6%)	1482 (15·9%)	157 (13·1%)	33 (9·1%)	190 (12·2%)
Knowledge that malaria is caused by mosquitos	7230 (80·9%)	317 (84·1%)	7547 (81·1%)	1021 (85·0%)	302 (83·7%)	1323 (84·7%)
Knowledge of ways to avoid malaria	7054 (79·0%)	302 (80·1%)	7356 (79·0%)	968 (80·6%)	289 (80·0%)	1257 (80·4%)
Education level of head of household						
None	1998 (24·7%)	117 (34·7%)	2115 (25·1%)	328 (30·1%)	111 (34·4%)	439 (31·1%)
Primary	2782 (34·4%)	127 (37·7%)	2909 (34·6%)	404 (37·1%)	122 (37·8%)	526 (37·2%)
Secondary	2947 (36·4%)	83 (24·6%)	3030 (36·0%)	321 (29·5%)	82 (25·4%)	403 (28·5%)
Higher	354 (4·4%)	9 (2·7%)	363 (4·3%)	37 (3·4%)	8 (2·5%)	45 (3·2%)

Data are n (%).

Table 2: **Selected characteristics of unmatched and retrospectively matched samples**

patients and gas chromatography for chloroquine detection in 24 RDT-positive/PCR-negative samples were carried out in Atlanta (GA, USA) using standard laboratory methods (appendix).

Statistical analysis

ITNs were defined as campaign ITNs if participants reported receiving them from the campaign and provided a date of receipt within 4 months of the campaign period. No other ITNs were widely available or distributed in Haiti before this time. The main predictor of interest was consistent use of campaign ITN, defined as using a campaign ITN 14 out of 14 nights in the 2 weeks preceding illness onset. We also assessed alternative definitions of bednet use, including use of a campaign ITN or any net the previous night. We defined cases of clinical malaria as patients with a positive RDT. We compared samples with both RDT and PCR results to assess potential bias from low-density infections below the limit of detection for high-performing RDTs.

We retrospectively matched up to four controls per case, using exact matching on age group, sex, location (health facility and commune of residence), and date of presentation (within 14 days), and analysed the matched patients using conditional logistic regression.[27] Each of the four matching variables was significantly related to the exposure (consistent campaign net use) and to the outcome (RDT positivity) and thus constituted an important confounder to match on (data not shown). Covariates were included in a multivariable model if their p value was below 0·20 in univariable analysis, and model selection was based on the lowest Akaike Information Criterion.[28]

As an alternative analytic approach to control for confounders related to bednet use, we used propensity scores, which can reduce bias in observational studies more effectively than other statistical approaches.[29] We used logistic regression to estimate each patient's propensity of consistent campaign ITN use. After restricting matches to the same geographical area (section communale), the lowest official and standardly recognised administrative unit in Haiti, patients were matched one-to-one using nearest-neighbour matching with a caliper of 10%.[30] Nearest neighbor matching resulted in good balance in the sample, with standardised differences of less than 10% on all included variables[31] (appendix). A logistic regression model was fit on the matched sample with clinical malaria as the outcome and consistent campaign net use as the predictor. Matching based on the other predictor variables—campaign ITN used last night, any net used last night, and ownership of any net—yielded larger matched datasets, as frequencies of these variables were higher (table 1).

We calculated standard descriptive statistics for entomological and ITN integrity measures, and compared distributions of continuous variables using the Wilcoxon rank-sum test. All analyses were carried out using SAS version 9.3 (SAS Institute Inc, Cary, NC, USA).

Role of the funding source
CDC investigators were involved in study design, data analysis, interpretation, and preparation of the report.

Results

We recruited a total of 9317 patients with a valid RDT result, including 378 (4%) RDT-positive cases, across 17 health facilities in five departments in Haiti.[30] In the full sample, 1202 (13%) patients reported consistent campaign ITN use and 1330 (14%) reported sleeping under a campaign ITN the previous night (table 1). Post-hoc matching of cases and controls yielded 362 cases and 1201 matched controls, 19% (333) of whom reported consistent campaign net use (table 2). Consistent campaign ITN use was not associated with clinical malaria in either univariable conditional logistic regression analysis (odds ratio [OR]=0·94, 95% CI 0·68–1·31, p=0·614) or multivariable analysis (OR=0·95, 0·68–1·32, p=0·745), adjusting for use of insect spray indoors and rudimentary roofing material, the latter of which seemed to remain a risk factor for malaria (OR=1·78, 1·03–3·07, p=0·038; table 3). Variables indicating where patients spent the majority of their time between sunset and going to sleep (eg, indoors, outdoors) and for how much time they spent outside after sunset seemed to not be associated with clinical malaria (table 3). Multivariable conditional logistic regression models found no association between alternative definitions of ownership or use of bednets and malaria (appendix).

After using propensity scores to match on consistent campaign ITN use, 2298 patients, including 138 (7%) RDT-positive cases, were included: 1149 consistent campaign ITN users and 1149 non-consistent campaign ITN users (table 1). Logistic regression on the propensity score-matched sample found no association between consistent campaign ITN use and clinical malaria (table 4). Alternative measures of bednet use, including sleeping under a campaign net or any net the previous night, were also not related to clinical malaria.

Among the 2695 samples with both RDT and PCR results, there was very good agreement between the two tests (κ=0·897), indicating that it was unlikely our study missed a large proportion of low-density malaria infections (table 5). The RDT-negative/PCR-positive samples were predominantly samples with low-density parasitaemia; of these 37 samples, 28 had an equivalent parasite density of less than 100 parasites per µL, based on PCR cycle time values, and two tested positive only for *Plasmodium vivax* by species-specific nested rRNA PCR analysis. 21 (87%) of the 24 RDT-positive and PCR-negative samples were tested with gas chromatography, and nine (38%) of these indicated presence of chloroquine in the blood, supporting the hypothesis that some of this subset of patients had taken chloroquine before coming to the health facility. Only five of these nine patients reported previous chloroquine use during the health facility interview, and among all patients with RDT and PCR results, only 81 (3%) of 2693 reported chloroquine use before their facility visit.

Assessment of physical integrity, bioefficacy, and insecticide content showed that campaign ITNs performed within WHO recommended standards. Using the proportional hole index cutoff of 300 (equivalent to a combined hole area of 1000 cm²) to define a failed net,[32] 16% of sampled campaign ITNs were considered to be failed after 12 months and 13% (from two study sites) after 18 months (figure 2).

Bioefficacy testing using cone bioassays on campaign ITNs collected from all five study departments after 12 months of use found an average of 97·9% mosquito knockdown after 60 min and mean 24-h mortality of

	Univariable models (n=1563)		Multivariable model (n=1563)	
	Odds ratio (95% CI)	p value	Adjusted odds ratio (95% CI)	p value
Consistent campaign net use	0·94 (0·68–1·31)	0·614	0·95 (0·68–1·32)	0·745
Rudimentary* roofing material	1·83 (1·06–3·16)	0·029	1·78 (1·03–3·07)	0·038
Use of insect spray indoors	0·69 (0·46–1·03)	0·069	0·70 (0·46–1·05)	0·083
Has electricity	0·95 (0·73–1·23)	0·672	..	..
Sought care elsewhere before facility	0·67 (0·41–1·10)	0·113	..	..
Took antimalarial drugs before facility	0·97 (0·58–1·62)	0·903	..	..
Time spent outside after sunset, minutes	1·00 (1·00–1·00)	0·956	..	..
Knowledge that malaria caused by mosquitoes	0·67 (0·61–1·23)	0·413	..	..
Education level of respondent or caregiver				
None	(Reference)		..	..
Primary	0·80 (0·44–1·47)	0·481	..	..
Some secondary	0·92 (0·66–1·27)	0·594	..	..
Completed secondary or higher	0·76 (0·43–1·36)	0·358	..	..

Univariable models are shown for predictors included in the multivariable model and for selected additional predictors. *Includes natural (thatched straw, leaves, or sod) or rudimentary (mat, wood planks) roofing material, versus finished material (metal, wood planks, tile, cement or concrete, shingles), as reported by patient or caregiver.

Table 3: **Predictors of malaria from conditional logistic regression models using retrospective case-control matching**

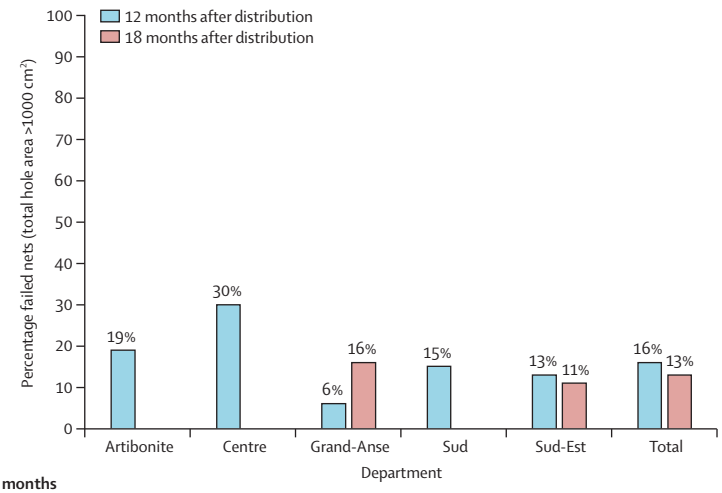
	Odds ratio (95% CI)	p value
Consistent campaign net use before illness	0·95 (0·45–1·97)	0·884
Slept under campaign net last night	1·00 (0·83–1·21)	0·971
Slept under any bednet last night	1·17 (0·80–1·70)	0·423
Owns any bednet	1·21 (0·85–1·71)	0·286

Table 4: **Odds ratios from propensity score-matched models of key bednet predictors on clinical malaria**

	PCR negative (n=2350)	PCR positive (n=345)	Total (n=2695)
RDT negative	2326	37	2363
RDT positive	24	308	332

Table 5: **Comparison of RDT and PCR results**

See Online for appendix



	Artibonite	Centre	Grand-Anse	Sud	Sud-Est	Total
12 months post-distribution						
N	31	27	32	26	30	146
Median area (cm², IQR)	48 (2·5–558)	202 (30–998)	31 (7–100·5)	3 (0–292)	107 (5–238)	34 (8–533)
18 months post-distribution						
N	..	..	19	..	28	47
Median area (cm², IQR)			64 (3–741)		35 (8–533)	36 (4–708)

***Figure 2*: Percentage of failed nets (total hole area >1000 cm²) and hole areas from bednet integrity evaluations at 12 and 18 months post-distribution**

72·1%, compliant with WHO-recommended functional ITN thresholds of either 95% knockdown *or* 80% mortality (appendix);[25] at 18 months, ITNs from two departments demonstrated 98·4% knock-down and 68.3% mortality. Analysis of permethrin content indicated a mean of 26·5 g/kg (95% CI 26·2–26·8) among ITNs collected at baseline, mean of 23·8 g/kg (23·0–24·5) for 12-month nets, and mean of 15·8 g/kg (15·1–16·5) for 18-month-old ITNs, all within the WHO-specified range of 15–25 g/kg for permethrin; all nets at baseline and 12 months had permethrin content above 15 g/kg and 36 (77%) of 47 were above this minimum threshold at 18 months.

Lastly, to assess the presence of insecticide resistance in study areas, more than 700 mosquitoes reared from larvae collected in each of the five study departments underwent testing by CDC bottle bioassay. Results indicated no evidence of permethrin resistance, with mortality rates of 99–100% among mosquitoes exposed to permethrin and no mortality among control mosquitoes.

Discussion

We found no evidence that consistently used ITNs, following a mass distribution, reduced clinical malaria in this case-control study at 17 health facilities in five departments in Haiti. Bednet and entomological data indicated that nets were performing as intended, with good physical integrity, insecticide availability, and bioefficacy after 12 and 18 months of use. Although there was some variability in mosquito mortality rates after exposure to nets collected from study participants across departments, knockdown measurements, rather than mortality, are typically used to assess efficacy of permethrin-treated bednets, and these were consistently high across departments. No resistance of mosquitoes to permethrin was observed. Thus compromised ITN integrity, insecticide content, or vector resistance did not explain the lack of effect. Reasons for the lack of association between ITN usage and malaria transmission in Haiti are not entirely clear and a single case-control study may not be definitive. But one likely explanation for a lack of protective effect may be vector behaviour. *A albimanus* in Haiti tends to bite outdoors and, at least in some locations, at times when people are not likely to be under nets.[8,9,33,34] More broadly, the effectiveness of ITNs against malaria in areas where *A albimanus* is the primary vector might depend on this vector's predominant biting and resting locations (indoors *vs* outdoors) and preferred biting times. A study from areas of Nicaragua where *A albimanus* is dominant and primarily bites indoors and late at night, found that ITNs were effective in reducing malaria in study clusters with rates of ITN use above 16%; however, a similar study in areas of Peru where *A albimanus* had greater outdoor and earlier biting rates found that ITNs did not significantly reduce malaria.[12,13] An alternative explanation is that an effect exists, but it was not detected by our study. Cases and controls were matched at the section communale level, but there is possibly heterogeneity in transmission within these administrative areas, which would weaken the power of the study.

In Haiti, studies in the north have suggested early outdoor biting,[8,10] whereas a study in the south suggested later (middle-of-the-night) biting occurring equally indoors and outdoors.[35] These findings might lead one to expect a more evident protective effect of ITNs in the south, where most sites for this study were located, compared with the north; we nevertheless observed no such protective effect of ITNs. More broadly, there is increasing recognition that vector control strategies in Latin America need to encompass methods that go beyond ITNs, especially in areas where *A albimanus* or other primarily exophagic vectors predominate.[36]

Our study indicated that indoor spraying with cans of non-residual insect sprays, which are primarily pyrethroid-based, was protective (OR=0·70), although this effect was only marginally significant in both univariable (p=0·069) and multivariable analyses (p=0·083). This finding suggests that indoor biting potentially has a role in malaria transmission in Haiti, even if the vector is predominantly exophagic. It is possible that insecticides, including those used in indoor residual spraying that reduced malaria cases in the 1960s[37] might be more effective than ITNs, which presumably provide protection primarily during the later hours when household residents are sleeping under them. Even if a population of vectors is primarily

exophilic, individual mosquitoes can still occasionally feed indoors, with concomitant exposure to insecticides. Thus, vector control interventions targeted indoors might still have some effect in settings with exophagic vectors, but interventions, such as ITNs, that work only during times when people are asleep under them might be less effective where the vector also bites earlier.

Our study also found rudimentary roofing material to be a risk factor for clinical malaria. This finding is consistent with other studies that have found that housing characteristics, including roofing material, are a risk factor for malaria because more porous housing materials, including thatched roofs, open eaves and windows, provide more conducive places for mosquitoes to rest and enter homes than do less porous ones, including more modern housing materials, such as tile, cement, or tin roofs and closed eaves and windows.[38]

This study has several important limitations. The observational (non-experimental) case-control study design is not as strong for determining associations and causality as randomised trials, quasi-experimental designs, or other types of observational studies, such as cohort studies.[39] However, randomised trials for interventions, such as ITNs, that have been shown to be effective in other settings, and are included in a country's national policy, would potentially raise ethical questions; more importantly, randomised trials, as well as cohort studies are not practical in low-transmission settings such as Haiti. Prospective cohort studies could provide stronger causal conclusions but are often impractical because large sample sizes are required for rare diseases. Case-control studies are appropriate for relatively rare diseases, such as malaria in very low-transmission settings like Haiti.[27] Further, previous studies have successfully used case-control designs in Malawi and Afghanistan,[40,41] and case-control designs have been recommended to assess ITN effectiveness outside study settings.[42] To mitigate bias, we attempted to control for measured potential confounders in two ways: using propensity score-matched samples and retrospectively matching cases and controls, with both methods showing very similar results across various definitions of ITN use. Nonetheless, despite collecting data on a large number of covariates, an unmeasured confounder could be introducing bias into the estimates. Also, because the matching methods try to increase the interval validity of the study, possibly some bias is introduced as the external validity is diminished.

Another limitation that has been raised about health facility-based case-control studies is attendance bias, whereby sick people at facilities might be more likely to come from households owning or using ITNs, thus underestimating the true effectiveness of ITNs.[43] This study took place following a universal ITN distribution campaign, probably minimising inequities in net ownership among households. Additionally, community health workers affiliated with study facilities conducted community sensitisation campaigns to increase case-seeking for fever at health facilities, where malaria treatment was free.

We did not achieve our target sample size, despite expanding from two facilities to 17, and extending the study time frame. This limitation illustrates the difficulty of conducting such studies in settings with very low and variable malaria transmission. However, ITNs were not significantly protective in our setting: the odds ratio for malaria with our primary exposure variable was close to one, along with the odds ratios from alternative definitions of ITN use, including a sensitivity analysis we conducted of near-consistent users (12–14 of 14 nights prior to illness onset) versus non or infrequent users (0–2 of 14 nights), which should have maximised our likelihood of finding an effect.

Despite these limitations, this study provides the best available evidence on the effectiveness of ITNs following the 2012 mass distribution campaign. The study strongly suggests that the campaign did not appear to be effective for the prevention of clinical malaria and can help inform investments in malaria control as Haiti moves towards elimination. Other methods such as drug-based interventions to target the parasite reservoir, including targeted mass drug administration, which hold promise in low-transmission settings,[44] in the context of enhanced surveillance and an effective vector control strategy,[45] as well as emerging strategies to address outdoor biting[46] might be more effective. Widescale generalised community ITNs distribution was not supported by our findings. Given limited resources for malaria control in Haiti, alternative strategies should be prioritised.

Contributors

MC conceived of the idea for the study, and LCS, LS, and SPK contributed to the study design. YSJ, JFL, JF, LCS, JSA, EN, SJ, MC, KM, and BW oversaw the surveyor training and helped supervise data collection. DI and ED led the collection of entomological and ITN-related data. CH carried out polymerase chain reaction on relevant samples, and JWB oversaw the laboratory analysis for the study. RW oversaw the statistical analysis for the study. LCS drafted the initial version of the manuscript. All authors have reviewed and approve the final version of the report.

Declaration of interests

We declare no competing interests.

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