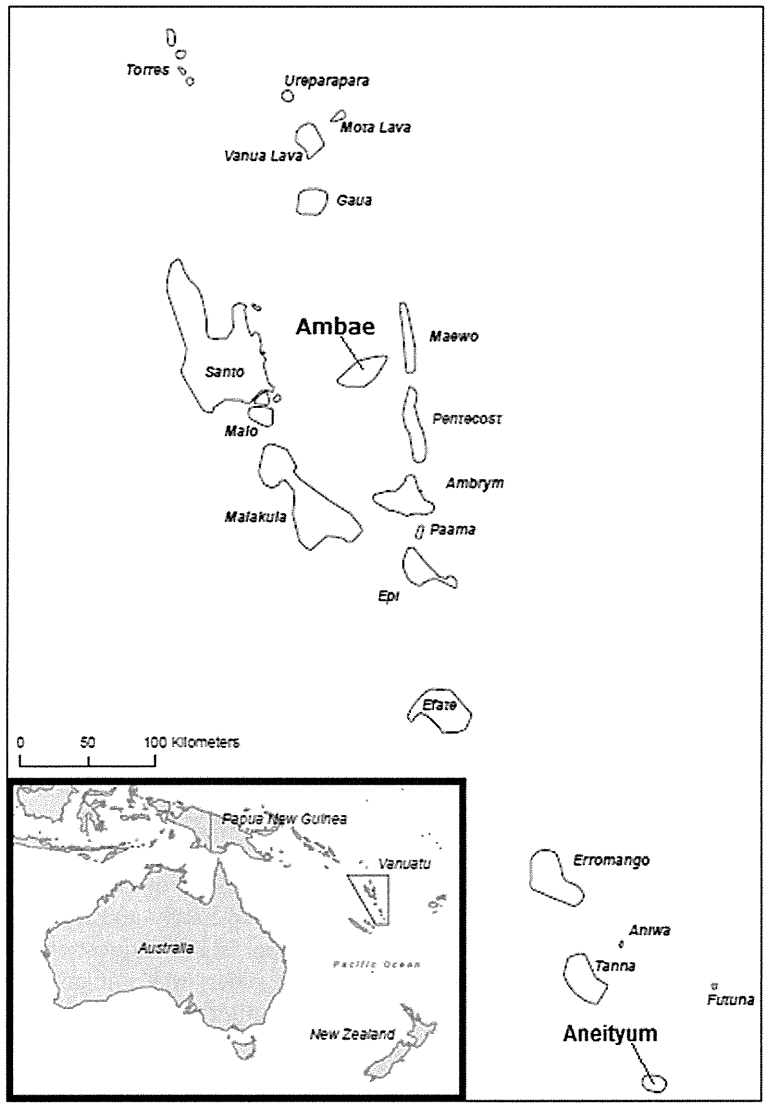

Figure 1 Locations of study areas on Vanuatu.

schoolchildren [26,27]. In addition, larvivorous fish were introduced into breeding sites of *Anopheles farauti* [26,27]. After the MDA, *P. falciparum* immediately disappeared, while *P. vivax* disappeared from 1996, with the exception of two instances of imported infections (one mixed infection in 1993 and one *P. vivax* infection in 1999) [26,27]. In 2002, a small outbreak of *P. vivax* was reported mainly among children born after 1991 [27,28]. After the age-selected MDA using chloroquine and primaquine, the outbreak quickly subsided except for a few asymptomatic infections of *P. vivax* [27].

Aneityum Island has a total land area of 159.2 sq km with a population of 915 (2009 Vanuatu National Census). Inyeug has a small airport and grass runway. Tourism is the main source of income. There are three main villages: Analgaut, Port Patrick and Unmet [26,27]. The study was conducted at the largest village, Analgaut, due to its population size and ease of access.

(ii) Data collection procedures

Data were collected on the two islands in July 2012. Residents were notified ahead of time by local leaders and health workers. Local leaders called a community meeting and explained the intent and process of the survey. At that time, community members were asked to take part in the survey and requested to arrive at the dispensary or community meeting place on scheduled dates.

Knowledge, attitudes and practices (KAP) survey

The team conducted a cross-sectional survey (knowledge, attitudes and practices) on the two islands. A questionnaire (see Additional file 1) translated into Bislama was

administered by local survey assistants fluent in local languages and known to the community and who had been hired and trained to interview respondents. Responses for very young children were provided by an accompanying parent or adult household representative. A questionnaire was structured to capture some of the HBM constructs (perceived severity, benefits and self-efficacy) and action (ITN use the previous night). Potential answers (reasons) for non-use such as absence of mosquitoes/rain, low risk of infection (not being afraid of contracting malaria), excessive heat, inconvenience of hanging nets, nets in poor condition and a lack of nets in the home were provided in a questionnaire.

Interviews (FGDs, KIIs and IDIs)

For data triangulation, focus group discussions (FGDs), key informant interviews (KIIs) and in-depth interviews (IDIs) were used. A researcher with local facilitators, conducted all discussion and interviews on both islands. Focus group discussants were recruited by local facilitators. Key informants such as *kastomary* chiefs (customary chiefs), teachers, religious leaders, health committee members, health care workers, and shop sellers were purposefully selected. In-depth interviewees were recruited through convenience sampling to ensure a breadth and depth of insights.

Semi-structured interview questions (see Additional file 2) were pilot tested on a few informants to review the answers and assess the relevance to the different HBM constructs. All FGDs, KIIs and IDIs were recorded using notes and a digital device in the presence of local facilitators. The questions were asked in English by a researcher, and then translated into Bislama by local facilitators. The participants' answers were directly translated from local language to English by local facilitators. Where this was not necessary, English was used as a common language. Each interview transcript was shared and discussed among local facilitators and key informants to explain results and obtain feedback.

(iii) Data management and analysis

Analysis of the KAP survey instrument (statistical methods)

Differences between the two islands were analysed using standard Chi-square tests for categorical variables. T-tests and Wilcoxon tests were used to compare continuous measures between islands. All statistical analyses were performed using R version 2.15.1 (CRAN 2012).

Analysis of interviews

The HBM was used as the theoretical framework, where six main HBM constructs (i.e., severity of malaria, susceptibility to malaria, benefits of ITN use, barriers to ITN use, cues to ITN use and self-efficacy) served as the pre-existing categories. The theory-based analysis style (a deductive approach) was applied [29,30]. First, a categorization matrix was developed [30]. Each interview transcript was read multiple times to identify meaning units, which were condensed, coded and assigned to the pre-existing HBM categories in a matrix [30,31].

Parallel analysis in a mixed methods study

This study employed parallel analysis in a mixed methods study [32,33]. Data collection and analysis were carried out separately and the findings were not compared or consolidated until the interpretation stage [33]. Qualitative and quantitative results were used to complement each other [29,33].

Ethical considerations

This research was approved by the Vanuatu Ministry of Health, and the Institutional Review Board of State University of New York, Binghamton (#1578-10). Written and verbal consent was obtained before starting the survey and interviews. All respondents were assured that their responses would remain confidential.

Results

KAP survey

The result from the KAP survey instrument used on Ambae and Aneityum islands are presented in Table 1. A total of 91 of nearly 200 residents participated in the survey in the village of Lolovoli on Ambae Island, compared to 354 of nearly 400 residents of Analgaut village on Aneityum Island. Educational attainment of adults (aged 18 or older) was similar in both islands. The vast majority of adults had completed only primary education, with half completing secondary school (modifying factors). About half of the residents of Ambae, but only one quarter of Aneityum (48.8 *vs* 25.3%, P <0.0001), reported having been diagnosed with malaria by a health professional in the past (modifying factors). Significantly more children under the age of five on Ambae reported having had malaria in the past than on Aneityum (42.9 *vs* 7.9%).

No significant difference was found between the two islands in terms of questions which might be applicable to the HBM constructs (perceived severity, benefits, and self-efficacy) and action. ITN use the previous night on Aneityum (73.0%) was higher than that on Ambae (68.2%) although not significant. Nearly all survey respondents claimed to be afraid of malaria (severity). Half stated that malaria was a deadly disease (severity). Seventy-nine per cent of survey respondents reported that they considered ITNs to be an effective means of malaria prevention (the malaria-prevention benefits). 93% of respondents reported liking to sleep under an ITN (self-efficacy).

Table 1 Knowledge, attitudes and practices (KAP) survey results

HBM constructs	Questions	All	Ambae	Aneityum	P
	N	445	91	354	
	Under 5 years old	53	6	47	
	5 to 17 years old	215	34	181	
	18 to 30 years old	69	15	54	
	Over 30 years old	108	36	72	
	Age range	0-75	0-75	0-74	
	Female	221	49	172	
Action	Used last night	72.0%	68.2%	73.0%	0.44
Modifying factors					
Educational attainment (aged 18 or older)	None	0.6%	0.0%	0.8%	0.22
	Primary	41.2%	45.1%	39.7%	
	Secondary	48.6%	47.1%	49.2%	
	Unknown	9.6%	7.8%	10.3%	
Malaria history	I had malaria	30.1%	48.8%	25.3%	<.0001
By age	Under 5 years old	13.3%	42.9%	7.9%	
	5 to 17 years old	20.1%	35.7%	17.5%	
	18 to 30 years old	32.8%	50.0%	27.7%	
	Over 30 years old	54.2%	60.0%	51.4%	
Individual beliefs					
Perceived severity	I am afraid of malaria (disease)	98.7%	100%	98.4%	1
	Malaria is deadly	55.1%	55.3%	55.1%	1
Perceived benefits	Malaria-prevention	79.4%	78.8%	79.6%	1
Self-efficacy	I am willing to sleep under a net	92.8%	95.3%	92.2%	0.4

The reasons given for a lack of ITN use are shown in Figure 2. Although the malaria history of respondents under the age of five suggested indigenous malaria transmission on Ambae during recent years (Table 1), low malaria risk perception was the most common barrier to compliance (Figure 2). On Aneityum, perceived low mosquito density with excessive heat acted as barriers to ITN use during the dry season (Figure 2). On both islands, a lack of nets in the home, poor condition and the inconvenience of hanging were common barriers (Figure 2).

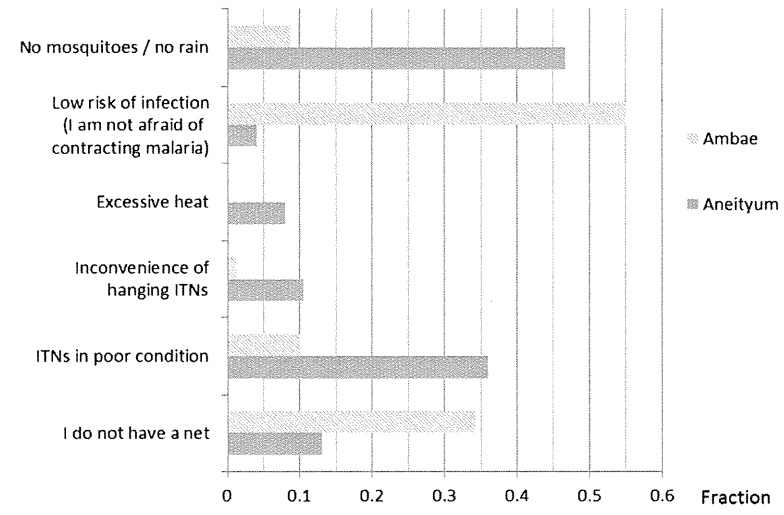

Figure 2 Reasons for non-use of insecticide-treated bed nets based on knowledge, attitudes and practices (KAP) survey.

Interviews

A total of 35 residents of Lolovoli village on Ambae Island took part in five FGDs, seven KIIs and eight IDIs, while a total of 57 residents of Analgaut on Aneityum took part in six FGDs, ten KIIs and 17 IDIs on Aneityum (Table 2). All results are presented in Figure 3.

Action

Many participants on both islands reported using ITNs. A few key informants on both islands stated that they had screens on windows. A few households on both islands reported not owning an ITN. Some participants on both islands noted that their ITNs had multiple holes. A few participants on both islands noted that they used their ITNs despite having multiple holes. A few participants on both islands reported alternative use.

"A few people recycled old nets. Pieces were used for various purposes." (Male FGD, Ambae)

"Five years ago, I saw a child using nets for fishing. Now, health committee encourages people not to use nets for farming or fishing." (Female KII, Ambae)

"I saw some children using nets for fishing this year." (Male KII, Aneityum)

A male key informant on Aneityum expressed his concern over the ecological and safe disposal methods for used and worn-out ITNs.

Modifying factors

A few participants on both islands noted that they did not know how to read and write. All participants were aware that mosquito bites are associated with malaria. Some participants on both islands stated that they had financial difficulty in paying school expenses for several children. *Kastom* (traditional) medicine was used on both islands. A few adults from both islands who reported having had malaria in the past stated that they used ITNs. Some participants on both islands reported forming proactive health behaviours.

"Some children use nets. They are used to sleeping under a net." (Female KII, Ambae)

"I use a net every day. I am used to sleeping under a net, because I try to prevent malaria." (Male IDI, Ambae)

"I use a net. Everyone uses a net. We feel strange, if we do not use nets." (Female IDI, Aneityum)

Participants on Ambae were reported difficulties in consistently using ITNs, while participants on Aneityum reported being highly motivated to use them. On Ambae, many participants stated that the disappearance of malaria reduced the perceived need for sleeping under ITNs. In this context, some male discussants and key informants expressed difficulties in sustaining malaria control efforts in the village.

"It is very difficult to sustain efforts, because of the absence of malaria. Some people do not use nets. After the net distribution, malaria is not endemic." (Male FGD, Ambae)

"Malaria is disappearing. Some people no longer need nets. Raising awareness is necessary, but a community radio has been broken. Additional funding will be needed to repair a radio. This area faces critical shortage of nurses. The dispensary and hospital are too far. This is a completely neglected area. We need more funds." (Male KII, Ambae)

In contrast, an effort to promote healthy life and to eliminate malaria on Aneityum motivated many individuals to use ITNs. Most people reportedly engaged in elimination efforts.

"We are happy to live on this island. We try to maintain a healthy environment. We sleep under a net and keep our village clean." (Female FGD, Aneityum)

"Malaria depopulated this island in the past. Now we should increase the population. Many people use nets to live a healthier life." (Male KII, Aneityum)

Perceived threat of malaria (perceived severity of and susceptibility to malaria)

Participants on Ambae were likely to state that some villagers did not use ITNs because they were not concerned

Table 2 Demographics of interviews

	Ambae		
	N	**Age (years old)**	**Female**
FGD	20 (5 groups)	13-44	65.0%
KII	7	28-64	20.0%
IDI	8	20-52	40.0%
ALL	35	13-64	54.3%
	Aneityum		
	N	**Age (years old)**	**Female**
FGD	30 (6 groups)	16-31	66.7%
KII	10	21-65	50.0%
IDI	17	16-67	52.9%
ALL	57	16-67	59.6%

FGD: Focus Group Discussion.
KII: Key Informant Interview.
IDI: In-Depth Interview.

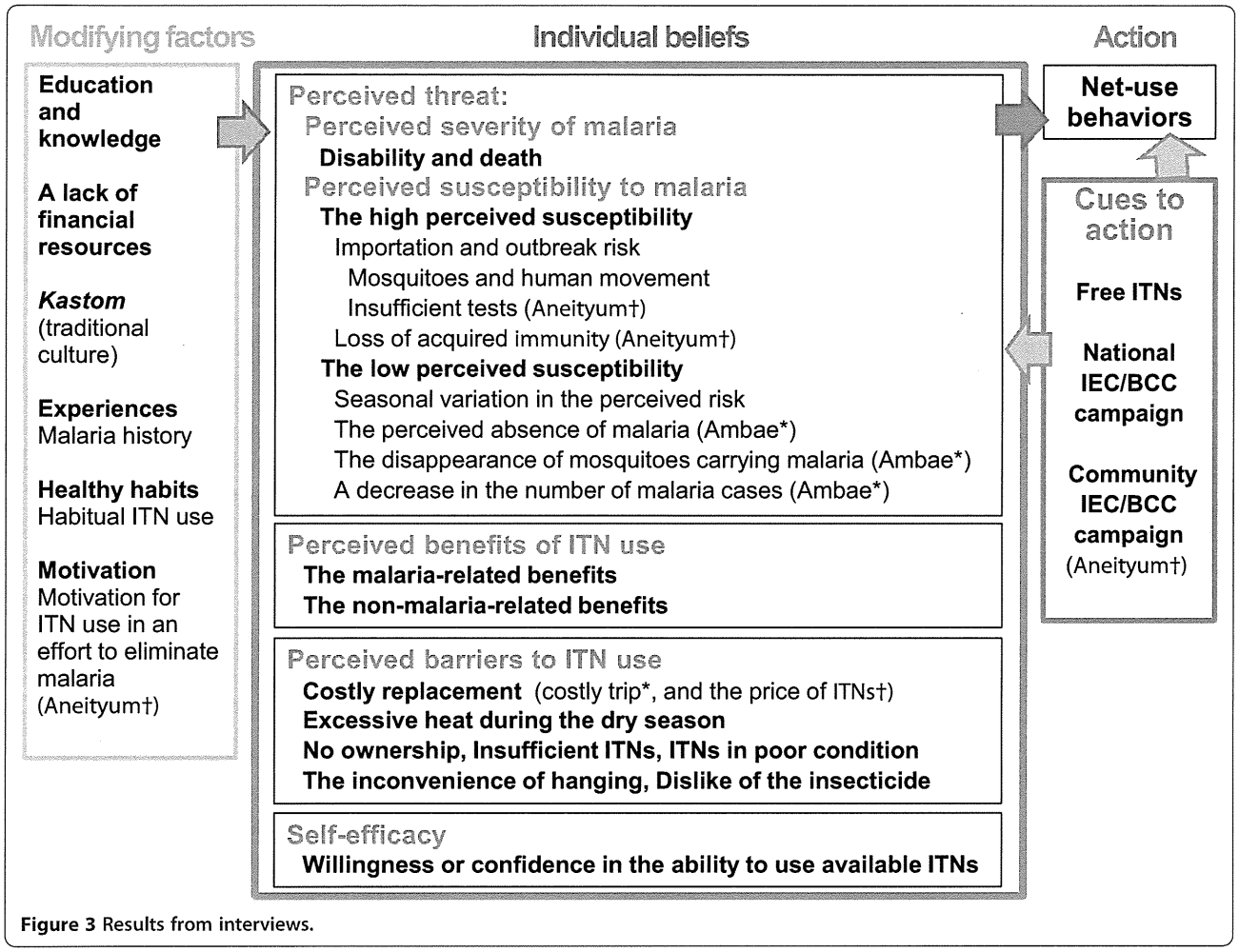

Figure 3 Results from interviews.

about the possibility of contracting malaria, while participants on Aneityum stated that using ITNs was necessary out of fear that accidental importation could cause an outbreak of malaria on the island.

Malaria was not recognized as a matter of community concern requiring community action on Ambae. A few youth interviewees stated that visitors from the malarious villages would drive parasite importation, while many participants believed that they were not at risk for malaria infection. Beliefs about the disappearance of malaria transmitting mosquitoes and the reduction in malaria cases were cited as major reasons for decreased concerns of risk.

"Last year, Lolowai hospital malaria team came and killed mosquito larvae. Now we are not bothered by malaria-infected mosquitoes. All 14 patients with fever had negative RDT results in this year. Men were not willing to come to the aid post at the onset of fever. No new case of filariasis, dengue fever, or malaria has occurred. But some people keep on using nets, because they are afraid of malaria, and they are used to sleeping under a net." (Female KII, Ambae)

"We live in a relatively cold climate on the island. The cold weather reduces mosquito populations. Other villages have more mosquitoes. People catch malaria at South Ambae or North Ambae, but no one has malaria in this village, because anopheline mosquitoes are not found in this village. So some people do not use nets. I am usually bitten 20 times per day by mosquitoes such as Culex and Aedes. A few cases of malaria have been reported in recent years. Malaria is not a serious problem in this village. But malaria remains a serious problem in Sakao." (Male IDI, Ambae)

In contrast, malaria was widely perceived as a serious disease and recognized as a matter of community concern requiring community action on Aneityum. Except for a few participants who did not perceive themselves to be at risk for malaria infection, most participants on Aneityum believed malaria posed a very real threat.

"We want live in a healthy environment. We fear that imported malaria will increase. Because only one person takes blood on this island." (Female KII, Aneityum)

"I am afraid of malaria. Malaria will come back again. So, I think I need a net." (Male IDI, Aneityum)

Young people were viewed as more susceptible to malaria due to loss of acquired immunity by some participants on Aneityum.

"Older people would notice the signs of malaria. But younger people would not notice. Their bodies do not learn how to deal with malaria. We used to have malaria here, but we do not have malaria now. I do not know what will happen in the future." (Female KII, Aneityum)

Community members on Aneityum were very concerned that both humans and mosquitoes constituted and continuing threat to maintaining malaria elimination.

"A big swamp has been managed by committee members. Most people use nets every day." (Female KII, Aneiyum)

"The only problem on this island is that malaria-infected people stay without being tested." (Male KII, Aneityum)

"Malaria-carrying mosquitoes will kill us. I use a net." (Female IDI, Aneityum)

"Because Aneityum is a place of quarantine in Vanuatu, many yachts visit this island. Some foreigners walk around the village without the testing for a few weeks. Local boat passengers are not screened. I am worried that I may have contracted malaria in Tanna, because we (fishers) travel back and forth. All people entering Aneityum are not screened. Air passengers have been screened, but cruise passengers have not been screened this year. An airplane and a ship are problematic." (Male IDI, Aneityum)

Perceived benefits of ITN use

The most cited benefit of ITN use on both islands was the prevention of malaria. The protection from mosquito bites was the other most commonly cited benefit on both islands.

"Malaria-infected anopheline mosquitoes are not found in this village, however people still use nets, because people protect themselves from mosquito bites. I am bitten all the time." (Female KII, Ambae)

"I appreciate the benefits of the insecticide, because the treated nets provide very good protection from being bitten by mosquitoes. I use a net every day." (Male KII, Aneityum)

Protection against other diseases (lymphatic filariasis on Ambae, and scabies on Aneityum) as well as against pests (cockroaches, fleas, flies, and head lice on Aneityum), and keeping warm in cold weather on Ambae were very occasionally reported as the non-malaria-related benefits of ITN use. A few key informants on Aneityum noted that mass use of ITNs eliminated scabies.

Perceived barriers to ITN use and maintenance

On both islands, the most commonly reported barrier to ITN use was that the ITNs were uncomfortable to sleep under during very hot conditions. Many participants reported that they slept without ITNs during the dry season when temperatures are very hot and winds quite weak.

"When the weather is hot during the dry season, we do not use nets." (Male KII, Ambae)

"If people feel uncomfortable during the dry season, people enjoy sleeping outside and use mosquito coils. If we do not see mosquitoes outside, we do not use coils. During the rainy season, we use nets." (Female KII, Aneityum)

Dislike of the insecticide was identified as barriers on both islands by a limited number of participants.

"I do not use a net, because I use kastom medicine. My grandmother is a kastom healer. But my family members go to the dispensary at the onset of fever. My mother encourages me to go to the dispensary, but I do not want to go." (Female IDI, Ambae)

"I do not like the chemical smell. Babies suck a net. But I use a net, because I know it works for protection." (Female KII, Aneityum)

A female discussant on Aneityum noted that she had never slept under an ITN, because she believed that ITNs caused suffocation. A few participants on both islands reported the inconvenience of hanging rectangular ITNs and a preference for conical ITNs.

High costs were reported as barriers to replacement of old and worn out ITNs. Distance and accessibility to ITN distribution points (the cost of travelling to and from hospital in North Ambae) reportedly acted as hurdles to obtaining new ITNs on Ambae. A key informant noted that Lolowai hospital did not allow the aid post to deliver ITNs. The price of ITNs (almost US$5) on Aneityum was noted among the majority of female participants. The issue of user charges was initially raised within the female

FGDs. A few discussants and interviewees reported having no intention to purchase in spite of their beliefs about their chances of getting malaria and the malaria-prevention benefits of ITN use. A female interviewee on Aneityum who reportedly did not buy an ITN for her baby stated that she would be willing to get free ITNs. Another female interviewee on Aneityum noted that she sold a portion of her crops or woven baskets to purchase a new ITN.

Self-efficacy and cues to action

Most participants on both islands stated that they were confident in their ability to use available ITNs. Receipt of a free ITN was commonly seen as beneficial on both islands.

> *"In the past, nets were not easily available for every person. Now nets are distributed free of charge. Everyone has a net. That is why people keep and use." (Male KII, Ambae)*

> *"In the past, rich people could afford to buy nets, but now everybody has a treated net. That is good." (Female KII, Aneityum)*

A few key informants on Ambae stated that high community coverage would contribute to a reduction in malaria transmission.

All participants on both islands recognized the sources of malaria information (cues) such as service delivery points (the dispensary on Aneityum and hospital on Ambae), health staff, the provincial malaria team, public notice, a community meeting, family meeting, health talk, church activities, school activities and Radio Vanuatu. Some male discussants and key informants on Ambae reported that they suffered lack of funds to sustain government-led efforts against a disappearing disease, while most participants on Aneityum intended to utilize available resources to prevent the re-introduction of malaria. Community-based elimination activities such as surveillance, vector control measures and awareness campaigns reportedly encouraged consistent ITN use on Aneityum.

KAP survey and interviews

Results from the KAP survey (Figure 2) and interviews (Figure 3) showed that the HBM constructs which negatively influenced ITN use were the low perceived susceptibility to malaria (a reduction in malaria risk) and barriers to ITN use (Table 3). The low perceived susceptibility had more impact on Ambae than Aneityum (Table 3). Results from interviews (FGDs, KIIs and IDIs) revealed the motivation and healthy habits were less influenced by the low perceived susceptibility and perceived barriers.

This study indicated that three determinants of ITN use influenced net-use behaviours: (1) malaria risk, (2) intervention services (tools and services), and (3) personal factors (modifying factors and self-efficacy) (Table 4). These factors were not independent, but rather were interlinked. Seasonal variation in the perceived risk of malaria influenced utilization of and compliance to malaria interventions and attitudes. Interventions influenced the knowledge, attitudes and practices. A lack of resources such as money, time and knowledge occasionally acted as a brake, while motivational beliefs sustained intervention and malaria risk.

Discussion

Malaria risk perception (mainly due to perceived susceptibility), free ITNs (cues to action), community-based intervention services (cues to action) and personal factors (modifying factors) encouraged individuals on Aneityum to maintain the use of ITNs, in spite of material and psychological costs. On Ambae, the low perceived susceptibility along with material and psychological costs was associated with reduced compliance to ITNs.

Table 3 Determinants of non-use

Low perceived susceptibility					**Perceived barriers**				
Beliefs	**Islands**		**QL**	**QT**	**Beliefs**	**Islands**		**QL**	**QT**
Dry season	Am	An	QL	QT	Excessive heat	Am	An	QL	
							An		QT
Perceived low mosquito density	Am	An	QL	QT	Dislike of the insecticide	Am	An	QL	
Low risk of infection	Am	An	QL	QT	Inconvenience of hanging nets	Am	An	QL	QT
Perceived absence of malaria	Am		OL		Nets in poor condition	Am	An	QL	QT
Disappearance of mosquitoes carrying malaria	Am		QL		A lack of nets in the home	Am	An	QL	QT
					Costly services				
					Time and cost to access	Am	An	QL	
Reductions in the numbers of malaria cases	Am		QL		User charge		An	QL	

QL: Qualitative results (FGDs, KIIs and IDIs), QT: Quantitative results (KAP survey).
Am: Ambae, An: Aneityum.

Table 4 Determinants of access and use

Determinants	Beliefs or factors	HBM constructs	Islands	
Malaria risk				
Beliefs or factors associated with ITN access and use	Health consequences (death)	Severity	Am	An
	Social consequences (depopulation)	Severity		An
	Loss of acquired immunity	Susceptibility		An
	Human movement	Susceptibility	Am	An
	Insufficient screening	Susceptibility		An
	Hot, wet (rainy) season	Susceptibility	Am	An
	High mosquito density	Susceptibility	Am	An
	The potential risks of malaria infections	Susceptibility	Am	An
Beliefs or factors associated with non-use	Cold, dry season	Susceptibility	Am	An
	Low mosquito density	Susceptibility	Am	An
	Low risk of infection	Susceptibility	Am	An
	The disappearance of malaria-infected anopheline mosquitoes	Susceptibility	Am	
	The reduction in malaria incidence	Susceptibility	Am	
Intervention services (tools and services)				
Beliefs or factors associated with ITN access and use	Free mass distribution (catch-up)	Cues	Am	An
	National campaigns	Cues	Am	An
	Community-based campaigns	Cues		An
	ITN prevention (malaria)	Benefits	Am	An
	ITN protection (mosquitoes)	Benefits	Am	An
	ITN prevention (other diseases)	Benefits	Am	An
	ITN protection (other pests)	Benefits		An
Beliefs or factors associated with non-use	Time and cost to replace nets (keep-up)	Barriers	Am	An
	Insufficient or a lack of ITNs in the home	Barriers	Am	An
	ITNs in poor condition	Barriers	Am	An
	Inconvenience of hanging	Barriers	Am	An
	Excessive heat in the net (discomfort)	Barriers	Am	An
	Side effects of the chemical	Barriers	Am	An
Personal factors (modifying factors and self-efficacy)				
Beliefs or factors associated with access and use	Willingness or confidence to use ITNs	Self-efficacy	Am	An
	Knowledge	Modifying factors	Am	An
	Experiences (malaria history)	Modifying factors	Am	An
	Healthy habits (consistent ITN use)	Modifying factors	Am	An
	Motivation for ITN use in an effort to eliminate malaria (healthy life)	Modifying factors		An
Beliefs or factors associated with non-use	Unwillingness to buy or use ITNs	Self-efficacy	Am	An
	The absence of financial resources	Modifying factors	Am	An
	Insufficient knowledge	Modifying factors	Am	An

Am: Ambae, An: Aneityum.

Perceptions of disease severity and the potential risks of malaria infections (importation and outbreak risk [14]) were linked to sustained use of ITNs on Aneityum. However, the perceived absence of malaria was linked to non-use of ITNs on Ambae in spite of a low but still present risk of malaria transmission, suggesting a potentially detrimental effect on sustained ITN coverage and use in the context of disappearing disease. Perceived low mosquito density during the dry season, hampered consistent use of ITNs even on Aneityum. This outcome agrees with previous results on seasonality that may predict variation in ITN use [3-5,7,8,10-13]. Thermal discomfort during the hot, wet season was not mentioned as a barrier to ITN use on both islands, indicating that

the perceived higher risk of malaria outweighs the perceived discomfort of being hot during the wet (rainy) season, consistent with a previous study in Zanzibar, Tanzania [5]. Participants did, however, that ITNs were uncomfortable during the dry season, a tendency which could compromise malaria control efforts where malaria transmission still occurs. Psychological, physical or financial burden of services were directly linked to non-use of ITNs on both islands in spite of the malaria-prevention and non-malaria prevention benefits of ITN use. Routine access to new nets (keep-up strategies) had negative influences on ITN ownership on both islands despite free mass distribution (catch-up strategies) and campaigns (cues to actions). Expanding access to keep-up nets may improve coverage and use [2]. As cost significantly dampens demand and decreases access to health services among the poor at greater risk, ITNs should be provided via the public sector as a public good, like vaccines with a generous donation, because malaria is linked to poverty [34-37].

Results from focus group discussions, key informant interviews, and in-depth interviews showed that a lack of resources, discomfort, dislike, unwillingness and insufficient knowledge had negative influences on individual decision making regarding ITN use on both islands, while knowledge of preventive measures against importation and outbreak risk, healthy habits and the motivation had positive influence on decision making on Aneityum. Although a previous study in Burkina Faso has shown that the motivation for the use of new ITNs can decrease within a year due to inhabitants' conception of malaria and the inconvenience of using ITNs [38], this study showed that the sustained motivation for ITN use in an effort to promote healthy life and to eliminate malaria influenced net-use behaviours on Aneityum, indicating that sustained motivation continuously increases knowledge, encourages healthy habits and creates demand for ITNs regardless of circumstances (local culture, a hot, humid tropical climate, low risk infection, costly services in resource-poor settings and poverty) behind net-use behaviours. This study implies the primary factors that predict high ITN coverage and use on Aneityum are individual and collective motivations and engagement in elimination activities. Unlike the one-off vaccination campaigns, ITN programmes require a high degree of participation and practices to be sustained over time [39]. Community-based campaigns including information, education and communication (IEC)/behavioural change communication (BCC) minimize risk behaviours on Aneityum, where a participatory process maintains high ITN coverage and use at the community level toward malaria elimination. The findings from Aneityum provide clues to sustainable ITN use and malaria elimination in areas of reduced malaria transmission.

Limitations

The HBM was employed to explore perceptions and beliefs about malaria and ITN use. This study focused on individuals' beliefs and net-use behaviours without fully taking into account social, economic and emotional factors that may also influence preventive health behaviours. Time was limited and the number of interviews that was feasibly performed was small. In particular, the results from interviews (FGDs, KIIs and IDIs) are limited to be generalized to the wider population of each island. As the survey and interviews were performed during the dry season, the results may have differed during the wet season when mosquitoes are abundant. Some responses relating to ITN use may be subject to social desirability bias. Finally, there may be a certain degree of loss of nuances and depth as a result of the direct translation from Bislama to English in conducting interviews (FGDs, KIIs and IDIs).

Conclusions

The results on Ambae highlight the challenges of motivating communities to engage in elimination efforts when transmission continues to occur, while the results from Aneityum suggest the possibility of continued compliance to malaria elimination efforts given the threat of resurgence. Where a high degree of community engagement is possible, malaria elimination programmes may prove successful.

Additional files

Additional file 1: A questionnaire.
Additional file 2: Interview questions.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

NW designed the survey. AK, JKL, GT, SY and HL coordinated the field work. JKL, PSL, SY, HL, TT and NW conducted KAP survey. PSL conducted analysis of the KAP survey instrument. NW conducted interviews (FGDs, KIIs and IDIs) with SY and HL. NW, SY, HL conducted analysis of interviews (FGDs, KII and IDIs) with support from PSL and AK. Parallel analysis and manuscript drafting was carried out by NW with contributions from PSL and AK. All authors read and approved the final manuscript.

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Author details
[1]Department of Parasitology, Osaka City University Graduate School of Medicine, Osaka, Japan. [2]Department of Microbiology, Tumor and Cell Biology, Karolinska Institutet, Stockholm, Sweden. [3]Ministry of Health, Port Vila, Vanuatu. [4]Department of Infectious Disease Control, Healthcare Center of Kobe, Kobe, Japan. [5]Departments of Anthropology and Biological Sciences, Binghamton University, Binghamton, NY, USA. [6]Nagasaki University Institute of Tropical Medicine, Nagasaki, Japan. [7]University of Michigan School of Natural Resources and Environment, 440 Church Street, Ann Arbor, MI, USA.

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Infection and Immunity

Characteristic Age Distribution of Plasmodium vivax Infections after Malaria Elimination on Aneityum Island, Vanuatu

Akira Kaneko, Luis F. Chaves, George Taleo, Morris Kalkoa, Rie Isozumi, Renu Wickremasinghe, Hedvig Perlmann, Satoru Takeo, Takafumi Tsuboi, Shin-Ichiro Tachibana, Masatsugu Kimura, Anders Björkman, Marita Troye-Blomberg, Kazuyuki Tanabe and Chris Drakeley
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Characteristic Age Distribution of *Plasmodium vivax* Infections after Malaria Elimination on Aneityum Island, Vanuatu

Akira Kaneko,[a,b,c] Luis F. Chaves,[c,d] George Taleo,[e] Morris Kalkoa,[e] Rie Isozumi,[b] Renu Wickremasinghe,[f] Hedvig Perlmann,[g] Satoru Takeo,[h] Takafumi Tsuboi,[i] Shin-Ichiro Tachibana,[j] Masatsugu Kimura,[k] Anders Björkman,[l] Marita Troye-Blomberg,[g] Kazuyuki Tanabe,[j]† Chris Drakeley[m]

Island Malaria Group, Department of Microbiology, Tumor and Cell Biology, Karolinska Institutet, Stockholm, Sweden[a]; Department of Parasitology, Graduate School of Medicine, Osaka City University, Osaka, Japan[b]; Institute of Tropical Medicine, Nagasaki University, Nagasaki, Japan[c]; Programa de Investigación en Enfermedades Tropicales, Escuela de Medicina Veterinaria, Universidad Nacional, Heredia, Costa Rica[d]; Malaria and Vector-Borne Disease Control, Ministry of Health, Port Vila, Vanuatu[e]; Department of Parasitology, Faculty of Medical Sciences, University of Sri Jayewardenepura, Gangidawila, Nugegoda, Sri Lanka[f]; Molecular Biosciences, The Wenner-Gren Institute, Stockholm University, Stockholm, Sweden[g]; Division of Tropical Diseases and Parasitology, Department of Infectious Diseases, Faculty of Medicine, Kyorin University, Tokyo, Japan[h]; Cell-Free Science and Technology Research Center, Ehime University, Ehime, Japan[i]; Research Institute for Microbial Diseases, Osaka University, Osaka, Japan[j]; Radioisotope Centre, Graduate School of Medicine, Osaka City University, Osaka, Japan[k]; Department of Medicine, Karolinska Institutet, Stockholm, Sweden[l]; Department of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, London, United Kingdom[m]

Resurgence is a major concern after malaria elimination. After the initiation of the elimination program on Aneityum Island in 1991, microscopy showed that *Plasmodium falciparum* disappeared immediately, whereas *P. vivax* disappeared from 1996 onward, until *P. vivax* cases were reported in January 2002. By conducting malariometric surveys of the entire population of Aneityum, we investigated the age distribution of individuals with parasites during this epidemic in the context of antimalarial antibody levels and parasite antigen diversity. In July 2002, *P. vivax* infections were detected by microscopy in 22/759 individuals: 20/298 born after the beginning of the elimination program in 1991, 2/126 born between 1982 and 1991, and none of 335 born before 1982. PCR increased the number of infections detected to 77, distributed among all age groups. Prevalences were 12.1%, 16.7%, and 6.0%, respectively ($P < 0.001$). In November, a similar age pattern was found, but with fewer infections: 6/746 and 39/741 individuals were found to be infected by microscopy and PCR, respectively. The frequencies of antibody responses to *P. vivax* were significantly higher in individuals born before 1991 than in younger age groups and were similar to those on Malakula Island, an area of endemicity. Remarkably low antigen diversity (h, 0.15) of *P. vivax* infections was observed on Aneityum compared with the other islands (h, 0.89 to 1.0). A *P. vivax* resurgence was observed among children and teenagers on Aneityum, an age distribution similar to those before elimination and on islands where *P. vivax* is endemic, suggesting that in the absence of significant exposure, immunity may persist, limiting infection levels in adults. The limited parasite gene pool on islands may contribute to this protection.

Recently, the scaling up of malaria control efforts in countries where the disease is endemic has shown some promising results (1, 2). This has led to renewed interest in malaria elimination, with 39 countries stating their commitment to achieve elimination (3). Since these countries are all positioned along the margins of areas of endemicity, the prevention of reinfection and resurgence is an integral component of any elimination campaign. In the Asia Pacific region, the unique challenge for elimination relates to the relatively high proportion of *Plasmodium vivax* infections (4). Islands provide natural ecological experiments with great potential for intervention studies and have demonstrated some early successes in malaria elimination (5). Vanuatu consists of 68 islands in the Southwest Pacific with a high linguistic diversity. Despite different waves of human colonization, unstable malaria transmission has continued probably since the first human settlement 4,000 years ago (6). Aneityum, the southernmost island in Vanuatu, is located at the southeast edge of the range of malaria transmission in the Pacific. To examine the feasibility of malaria elimination, an integrated control program, combining mass drug administration (MDA) with vector control, was initiated on Aneityum in 1991. Eight years later, it was concluded that malaria can be eliminated from isolated islands if there is a high degree of community commitment (7). One major concern is the possible reintroduction of infection due to interisland human movement. To our knowledge, Aneityum is the only island in recent times where malaria elimination has been successfully maintained for more than a decade. Thus, observations from Aneityum can offer important insights into concerns regarding the loss of antimalarial immunity following elimination and how this might impact disease burdens in potential resurgences. An epidemic of *P. vivax* on Aneityum in 2002 provided us with an opportunity to investigate the age patterns of individuals with newly detected infections in the context of population-level antibody responses to *P. vivax* and parasite antigen diversity. Individuals

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born before elimination had considerably fewer episodes of parasitemia than those born after elimination. Our findings indicate that protective immunity against *P. vivax* infections persists for a long time, at least 10 years, after the initiation of malaria elimination efforts and thus the absence of exposure to recurrent infections.

MATERIALS AND METHODS

Interventions and surveys. Weekly MDA of chloroquine, pyrimethamine-sulfadoxine, and primaquine to the entire population (718 inhabitants) of Aneityum Island was carried out for 9 weeks in 1991, before the onset of the rainy season. Simultaneously, insecticide-treated bed nets (ITNs) were distributed to the entire population. Larvivorous fish were also introduced into several identified *Anopheles farauti* breeding sites (7). Since 1991, community-based surveillance and vector control measures, including larvivorous fish and the original universal distribution of ITNs (0.94 net per villager), with annual reimpregnation, have been maintained continuously, even after the disappearance of indigenous malaria cases (7). After the MDA, annual microscopy surveys of the whole island population showed the complete absence of *Plasmodium falciparum*, while *P. vivax* disappeared from 1996 onward. Two imported cases were documented: 1 *P. vivax* case in February 1993 and 1 *P. falciparum* case in June 1999 (7). In January 2002, a malaria epidemic was reported through passive case detection by community microscopists. In 2001, only 1 parasite-positive case among a total of 247 blood slides examined was recorded, compared with 67 positive cases in 240 slides during the first 3 months of 2002 (G. Taleo and A. Kaneko, unpublished data). Here we present the results of malariometric surveys for the entire population of Aneityum in August 2000 and in June and November 2002, conducted according to the protocol described in reference 6. Additionally, serological responses to *P. falciparum* and *P. vivax* parasites were assessed from surveys conducted in 1998 on Aneityum as well as on Malakula and Futuna Islands (overall sample size, 1,313). Molecular analysis of antigen diversity for *P. vivax* infections was also carried out by comparing the results of surveys conducted in 2002 on Aneityum with those of previous surveys on 6 other Vanuatu islands. During the surveys, finger prick blood samples collected on filter paper (31 ET Chr; Whatman, Maidstone, United Kingdom) were stored desiccated at −20°C for serological analysis, molecular analysis of the antigen diversity of *P. vivax* in infected samples, and detection of submicroscopic parasite infections by PCR.

Written informed consent was obtained from all subjects or, for children (individuals <15 years old), from their guardians. The standard doses of chloroquine and primaquine were administered to microscopy-positive individuals. Individuals with detected positive cases were asked about their history of interisland movement within the past 1 year. This study was approved by the Vanuatu Department of Health and by the Ethical Research Committee of Karolinska Institutet.

Serological analysis. Humoral responses to malaria parasites were evaluated in samples collected on Aneityum in 1998, 7 years after the initial intervention in 1991, and, for comparison, in samples collected on Malakula, an area of mesoendemicity, in 1998 and on Futuna, where malaria is not endemic, in 1997 (7). For erythrocyte-stage antigens, all 688 residents of Aneityum were studied, while 332 Malakula and 293 Futuna subjects were selected by following a stratification by age and place from our survey records (i.e., samples were randomly selected in proportion to total samples for a given age and location). A similar stratification was employed to study the responses to circumsporozoite proteins (CSPs) in 100 residents of Aneityum and in 99 residents each of Malakula and Futuna.

Antigens. *In vitro* cultures of the *P. falciparum* parasite (laboratory strain F32) were synchronized, and sonicates of late-stage-infected erythrocytes were used as crude *P. falciparum* antigen, as described previously (8). Crude *P. vivax* antigen was prepared from acutely ill *P. vivax* patients as described previously (9). After estimation of the protein content, the crude extract was stored at −80°C until use. Recombinant *P. vivax* CSPs (VK210 and VK247 types) were expressed and affinity purified as described previously (10).

Serological methods. Sera were extracted from blood samples spotted onto filter paper and were eluted into 500 μl (final serum dilution, 1:100) of phosphate-buffered saline containing 0.05% Tween and 0.5% bovine serum albumin as described previously (11). Samples were further diluted 1:10, resulting in a 1:1,000 dilution for the determination of antimalarial IgG antibodies by enzyme-linked immunosorbent assays (ELISAs) as described previously (12).

A cutoff for antibody positivity was defined by pooling values from all sites, using a mixture model (13). The mixture model uses the antibody binding data from all samples tested and fits 2 Gaussian distributions, a narrow distribution of "seronegative" results and a broader distribution of "seropositive" results, using maximum-likelihood methods. The mean ELISA values of the Gaussian distribution corresponding to the seronegative population plus 3 standard deviations (SD) were used to define the cutoff for seropositivity.

Data analysis. To compare levels of *P. falciparum* and *P. vivax* transmission between the islands studied, the seroconversion rate (SCR; the rate at which individuals become antibody positive per year, a metric analogous to the force of infection) was estimated by fitting a simple reversible catalytic model to the measured seroprevalence, with age as a continuous variable, using maximum-likelihood methods (13). For these models, only individuals aged 1 year or older were included, in order to exclude the effect of maternally derived antibodies in infants. Additionally, for Aneityum, confirmation of temporal changes in malaria transmission was explored by fitting models in which the SCR is allowed to change at a single time point. The statistical significance of the change was identified using likelihood ratio tests against models with no change, and profile likelihoods were plotted in order to determine confidence intervals (CIs) for the estimated time of the change (14).

Molecular analysis of parasite antigen diversity. Parasite genetic diversity was examined by sequencing of the *P. vivax* merozoite surface protein 1 gene (*Pvmsp1*) and circumsporozoite protein gene (*Pvcsp*) for the *P. vivax* cases detected by microscopy in 2 malariometric surveys of the entire population of Aneityum during the 2002 outbreak (n, 28) and comparing the results with those detected by microscopy during previous surveys on 6 other Vanuatu islands (1996 to 2002) (n, 178). The parasite rates detected during the surveys on these islands are shown in Fig. 3.

Parasite genomic DNA was extracted from blood spots on filter paper by using a QIAamp DNA Blood Minikit (Qiagen, MD). A DNA fragment covering the 5′ region of *Pvmsp1* was amplified by PCR using forward and reverse primers PvF0 (5′-CCAGTGTTCGTACATCTTTAAACC-3′) and PvR5 (5′-GTTGTACTTGTCAATTTGG-3′) (15), respectively, followed by nested PCR amplification using primers PvF0-2 (5′-CGTACATCTTT AAACCCCACACACT-3′) and PvR5. The PCR conditions have been described previously (15). The nested PCR product was purified using a QIAquick PCR purification kit (Qiagen), and an ~0.4-kb region (blocks 5 and 6) beginning at nucleotide position 1996 of *P. vivax* strain Sal-I (GenBank accession AF435593) was sequenced using the BigDye Terminator cycle sequencing kit (version 3.1) (Applied Biosystems, Foster City, CA) in an ABI 3100 sequencer (Applied Biosystems). Full-length *Pvcsp* was amplified by PCR using forward and reverse primers PvCSP-F1 (5′-TGTTACATCCGTTCGAACAAGTTCTGTTCT-3′) and PvCSP-R1 (5′-TCATATCGTGTTTTCTAGAATTGCACAACT-3′), respectively, and was sequenced as described above.

Of the 206 *P. vivax* cases, 165 (27 from Aneityum and 138 from other islands) and 125 (25 and 100) cases were successfully sequenced for *Pvmsp1* and *Pvcsp*, respectively. Mixed infections, as detected from overlapping peaks in electropherograms, were excluded from further analysis, but those isolates showing clearly separable major and minor peaks (where the minor peak height was less than 40% of the major peak height) were recovered (23 isolates for *Pvcsp* and 6 for *Pvmsp1*), in which only major peaks were adopted. Genotype diversity or expected heterozygosity (h) was calculated as described previously (16).

Detection of submicroscopic parasite infections by PCR. DNA was extracted from filter paper blots by using a QIAamp DNA Minikit (Qiagen, CA, USA). A mitochondrial-DNA-based PCR was newly designed to detect the 4 human malaria species. By use of test samples from Vanuatu and Kenya, the sensitivity of the new PCR method for each of the 4 human species was slightly improved over that of an alternative method (17) (see text and Tables S1 to S4 in the supplemental material). The prevalences of infection (as determined by PCR or microscopy) for the different age groups were compared using the chi-square test.

Nucleotide sequence accession numbers. The sequences reported in this study have been deposited in the DDBJ/EMBL/GenBank database (accession no. AB539022 to AB539045 and AB539540 to AB539553).

RESULTS

Malariometric surveys on Aneityum Island. In a survey of the entire population on Aneityum in August 2000, a total of 903 individuals were examined. They consisted of 619 Aneityum islanders and 284 visitors from other islands, staying temporarily for a church meeting on Aneityum. Among the Aneityum islanders, no parasite-positive cases were detected by microscopy, but 2 *P. vivax* infections were detected by PCR (2/617 individuals [for 2 islanders, samples were not available]), for an 11-year-old girl and a 24-year-old male with no recent travel history. Among the visitors, we confirmed 1 case of *P. falciparum* infection by microscope and 28 positive cases (28/283 [for 1 visitor, no sample was available]) by PCR, which consisted of 20 *P. vivax*, 5 *P. falciparum*, 1 *Plasmodium malariae*, and 2 mixed (*P. falciparum* and *P. vivax*) infections. The mixed infections were not double-counted in the total number of positive cases (see Table S5 in the supplemental material).

During the survey conducted in July 2002 on Aneityum (Fig. 1a), which covered a total of 759 islanders, 22 *P. vivax* infections were confirmed by microscopy: 20 infections among 298 children born after 1991 (aged 0 to 10 years) and 2 infections among 126 teenagers born between 1982 and 1991 (11 to 20 years). Parasite counts for these infections ranged from 80 to 3,840 parasites/μl of blood (median, 400). No microscopy-positive infections were seen among the 339 adults born before 1982 (older than 20 years). A total of 77 *P. vivax* infections were detected by PCR and were more evenly distributed among all age groups than those detected by microscopy only (Fig. 1a). All microscopy-positive cases were PCR positive. Thus, the total parasite positivity rates were 12.1%, 16.7%, and 5.97% for children, teenagers, and adults, respectively (P, <0.001 [χ^2, 11.46] for comparison of children and teenagers with adults). In a subsequent survey conducted in November 2002 (Fig. 1b), 6 *P. vivax* infections were confirmed by microscopy only among 290 children born after 1991. Parasite counts ranged from 80 to 7,840 parasites/μl of blood (median, 560). A total of 39 *P. vivax* infections were detected by PCR; these were distributed among all age groups (Fig. 1b). Again, all microscopy-positive cases were PCR positive, and the *P. vivax* positivity rates were 8.71%, 6.92%, and 1.54% for children, teenagers, and adults, respectively, with relations similar to those for the July survey (P, <0.001 [χ^2, 15.95] for comparison of children and teenagers with adults). Seven individuals (3 children and 4 teenagers) were positive by PCR in both the July and November surveys.

For these *P. vivax*-positive individuals on Aneityum, no recent travel history was recorded. One *P. falciparum* infection of a 26-year-old male with a history of recent travel to Tanna Island was identified by PCR in the November survey. The *P. vivax* infections detected by microscopy and PCR in 2002 were distributed over the whole area of Aneityum Island. Among the total of 28 microscopy-positive cases identified in 2002, only one 2-year-old girl was symptomatic. Treatment was not given to those who were found positive only by PCR, since they were not symptomatic, and the PCR tests were done later using stored samples.

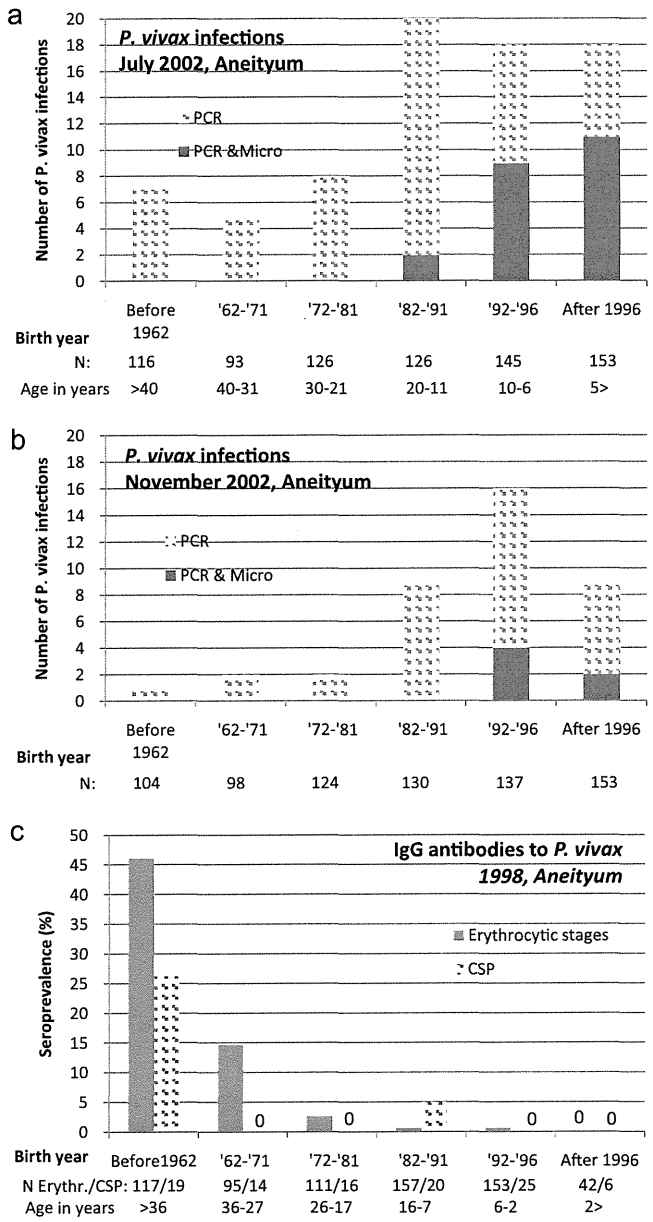

FIG 1 (a and b) Age-specific prevalence profiles for *P. vivax* infections in July (a) and November (b) 2002. Red bars represent the numbers of *P. vivax* infections detected by both microscopy and PCR, and blue bars represent the numbers detected only by PCR. All microscope-positive individuals were also PCR positive. (c) IgG antibodies to *P. vivax* antigens in 1998 on residents of Aneityum Island, where *P. falciparum* malaria transmission had been interrupted since 1991 and *P. vivax* malaria transmission had been interrupted since 1996. Blue bars represent seropositivity rates for antibodies to *P. vivax* erythrocyte-stage antigens, and orange bars represent those for antibodies to recombinant *P. vivax* CSPs, either VK210 or VK247.

Seroepidemiology. In 1998 on Aneityum (Fig. 1c), IgG antibodies for *P. vivax* erythrocyte-stage antigens were detected in 73

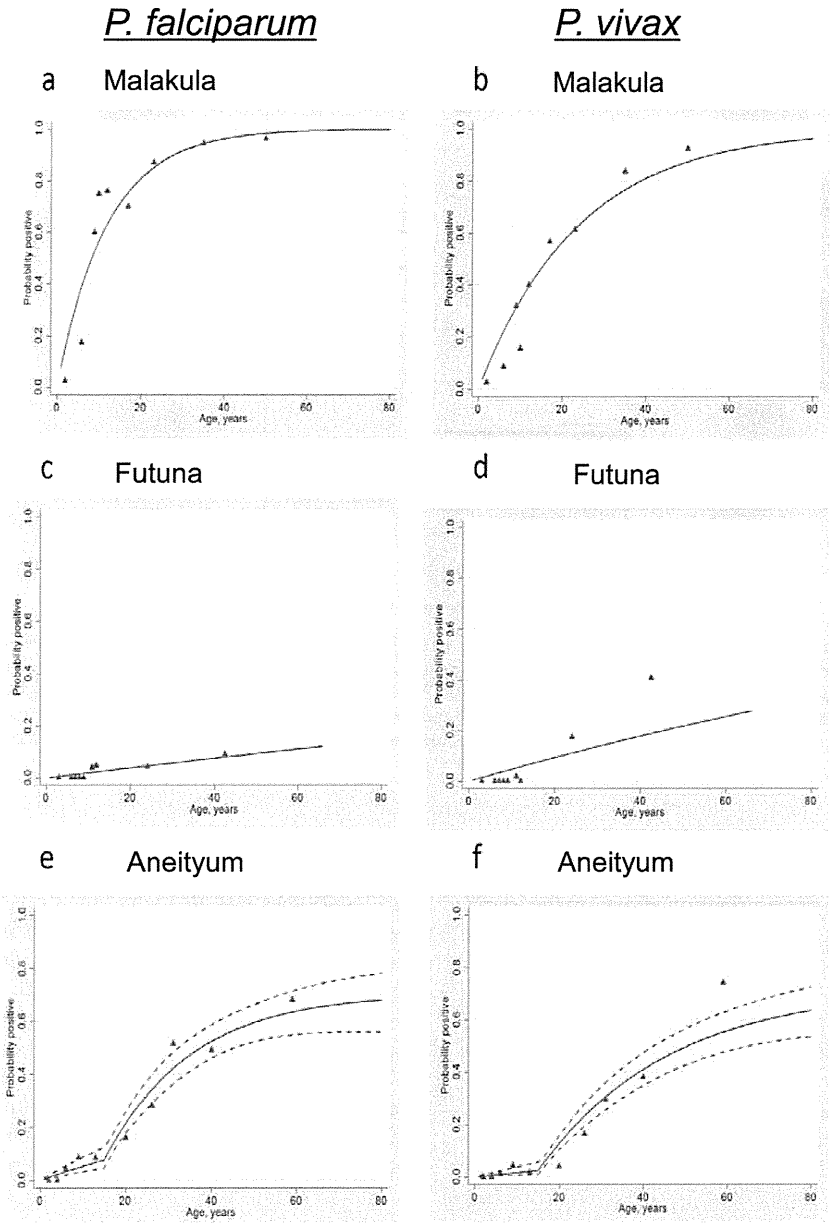

FIG 2 Seroprevalence curves of IgG antibodies to schizont extracts for Malakula Island (an area of mesoendemicity), Futuna Island (with no endemicity), and Aneityum Island (where an integrated elimination program was implemented in 1991) in Vanuatu. Results from Malakula (1998) (a and b), Futuna (1997) (c and d), and Aneityum (1998) (e and f) for *P. falciparum* (a, c, and e) and *P. vivax* (b, d, and f) are shown. In each plot, the red triangles represent observed data points (divided into deciles), and the blue lines represent the predicted value from the maximum-likelihood model. For Aneityum, a model with 2 forces of infection was plotted, with a change set at 15 years before the survey. Seroconversion rates for both *P. falciparum* (SCR, 0.08 [95% CI, 0.07 to 0.10]) and *P. vivax* (SCR, 0.040 [CI, 0.035 to 0.050]) on Malakula are significantly higher than those on Futuna (*P. falciparum* SCR, 0.002 [CI, 0.000 to 0.004]; *P. vivax* SCR, 0.005 [CI, 0.000 to 0.008]). On Aneityum, current SCRs for *P. falciparum* (0.006 [CI, 0.003 to 0.010]) and *P. vivax* (0.002 [CI, 0.000 to 0.040]) are 10- to 20-fold lower than preelimination levels (*P. falciparum* SCR, 0.04 [CI, 0.03 to 0.06]; *P. vivax* SCR, 0.030 [CI, 0.020 to 0.035]).

of 675 islanders. The seropositivity rate increased with age, from 0% (0/42) for individuals born after 1996 (newborn to 1 year old) to 46.1% (54/117) for those born before 1962 (>36 years old) (P, <0.001; χ^2 trend, 139.1). Only 1 of 195 children born after 1991 (<7 years old) was seropositive. The seropositivity rate for individuals born between 1982 and 1991 (7 to 16 years old) was as low as that of individuals born after 1991, with 1 of 157 individuals seropositive.

IgG antibodies for any recombinant *P. vivax* CSP were detected in 6 individuals out of 100 islanders. Among these seropositive individuals, 4 had antibodies for both VK210 and VK247, and 1 of these 4 was born between 1982 and 1991. The seropositivity rate for CSPs among adults born before 1962 was 26.3% (5/19) (Fig. 1c).

Seroconversion rates (SCRs) for erythrocytic antigens on Malakula (Fig. 2a and b) were higher for both *P. falciparum* and *P. vivax* than on Futuna (Fig. 2c and d). Analysis of SCRs for both parasite species on Aneityum showed that a model with 2 serocon-

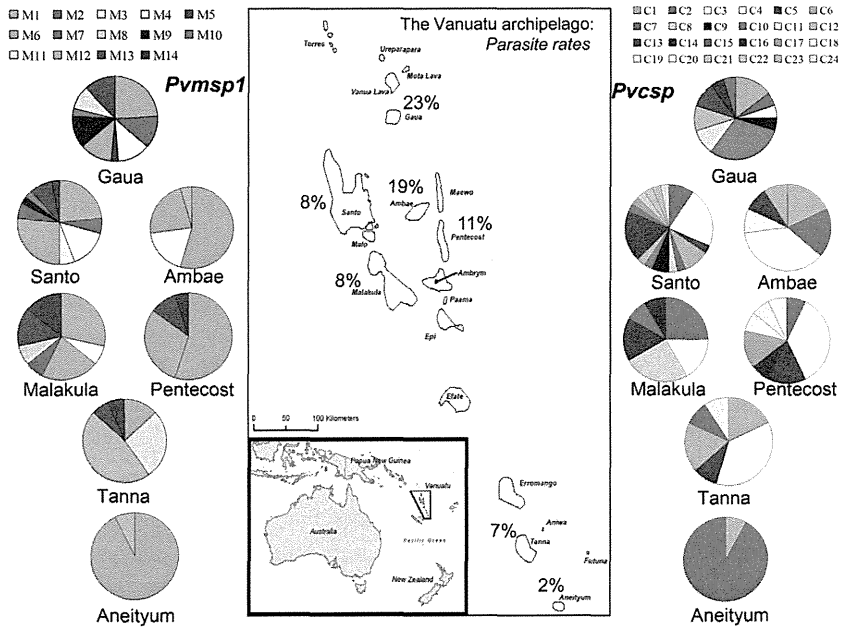

FIG 3 Distribution of *Pvmsp1* and *Pvcsp* haplotypes among *P. vivax* parasites in the Vanuatu archipelago. Shown are results for Aneityum Island during the outbreak in 2002, a decade after the beginning of the malaria elimination program (*n*, 27 and 25 for *Pvmsp1* and *Pvcsp*, respectively), and for other islands with malaria transmission: Gaua (*n*, 33 and 20) (1997), Santo (*n*, 34 and 32) (1996, 1997, and 2001), Ambae (*n*, 22 and 11) (2002), Malakula (*n*, 14 and 12) (1998 and 2001), Pentecost (*n*, 20 and 14) (1998 and 2000), and Tanna (*n*, 15 and 11) (1999 and 2002). See Tables 1 and 2 for the detailed haplotype classifications for *Pvmsp1* and *Pvcsp*, respectively. Parasite rates detected during the case selection surveys on these islands are presented on the map. The inset map shows the location of Vanuatu in Oceania.

version rates fitted better than a model with a single SCR, with the change point in the SCR set at approximately the same time as the change in transmission due to the elimination efforts on the island (Fig. 2e and f). Current SCRs for both *P. falciparum* and *P. vivax* are close to zero and are 10- to 20-fold lower than preelimination levels.

Parasite antigen diversity. *P. vivax* cases from Aneityum in 2002 showed very limited diversity in both *Pvmsp1* and *Pvcsp* compared with cases from 6 other Vanuatu islands. First, mixed-genotype infections were rarely seen on Aneityum (0/27 *P. vivax* cases had mixed *Pvmsp1* genotypes, and 1/26 cases had mixed *Pvcsp* genotypes), whereas 26% of cases (46/178) had mixed *Pvmsp1* genotypes and 56% (97/174 cases) had mixed *Pvcsp* genotypes on the other islands. Second, the number of *Pvmsp1* and *Pvcsp* haplotypes was also very small on Aneityum (Fig. 3). We detected 8 single nucleotide polymorphisms (SNPs) in the sequenced region of *Pvmsp1* block 5 in a total of 165 cases from Aneityum and 6 other islands of Vanuatu (Table 1). All of them, except for G/A at 2017, were detected as a single haplotype (Van-M-2). In contrast, the number of tandem repeats of Q in block 6 was highly variable, and thus, in total, 14 distinct *Pvmsp1* haplotypes were identified (Table 1). The number of haplotypes on Aneityum was 2, whereas it ranged from 4 to 10 on other islands (Table 1 and Fig. 3). In *Pvcsp*, there were 3 SNPs, 2 insertions/

TABLE 1 Distribution of *P. vivax msp1* haplotypes on 7 islands of Vanuatu

Haplotype	SNP in block 5 at the following nucleotide position[a]:							Poly(Q) in block 6[b]	Distribution in islands (from north to south)[c]						
	2017	2079	2082	2088	2095	2098/9	2107		Gaua	Santo	Ambae	Pentecost	Malakula	Tanna	Aneityum
Van-M-1	GGC (G)	GAC (D)	TTC (F)	CCC (P)	GAG (E)	AGC (S)	GCC (A)	Q2	8	8	12	11	4	2	25
Van-M-2	GGC (G)	GAT (D)	TTT (F)	CCA (P)	CAG (Q)	GCC (A)	ACC (T)	Q8Q2	4	2					
Van-M-3	GGC (G)	GAC (D)	TTC (F)	CCC (P)	GAG (E)	AGC (S)	GCC (A)	Q7(QQQ)2Q2	4	5	4		1		
Van-M-4	GGC (G)	GAC (D)	TTC (F)	CCC (P)	GAG (E)	AGC (S)	GCC (A)	Q6P(QQQ)3Q2		2					
Van-M-5	GGC (G)	GAC (D)	TTC (F)	CCC (P)	GAG (E)	AGC (S)	GCC (A)	Q4(QQQ)4Q2	1						
Van-M-6	AGC (S)	GAC (D)	TTC (F)	CCC (P)	GAG (E)	AGC (S)	GCC (A)	Q2	4	9	5	6	3		
Van-M-7	AGC (S)	GAC (D)	TTC (F)	CCC (P)	GAG (E)	AGC (S)	GCC (A)	Q7(QQQ)4Q2		2			1		
Van-M-8	AGC (S)	GAC (D)	TTC (F)	CCC (P)	GAG (E)	AGC (S)	GCC (A)	Q7(QQQ)2Q2					1		
Van-M-9	AGC (S)	GAC (D)	TTC (F)	CCC (P)	GAG (E)	AGC (S)	GCC (A)	Q5(QQQ)5Q2	4	1					
Van-M-10	AGC (S)	GAC (D)	TTC (F)	CCC (P)	GAG (E)	AGC (S)	GCC (A)	Q5((Q/E)QQ)5Q2	1	1					
Van-M-11	AGC (S)	GAC (D)	TTC (F)	CCC (P)	GAG (E)	AGC (S)	GCC (A)	Q5(QQQ)4Q2	3					4	
Van-M-12	AGC (S)	GAC (D)	TTC (F)	CCC (P)	GAG (E)	AGC (S)	GCC (A)	Q4(QQQ)5Q2			1			7	2
Van-M-13	AGC (S)	GAC (D)	TTC (F)	CCC (P)	GAG (E)	AGC (S)	GCC (A)	Q4(QQQ)4Q2	4	3		2	2	1	
Van-M-14	AGC (S)	GAC (D)	TTC (F)	CCC (P)	GAG (E)	AGC (S)	GCC (A)	Q4(QQQ)3Q2		1		1	2	1	
Total									33	34	22	20	14	15	27

[a] Data are codons, with changed nucleotides underlined and resulting amino acid changes in parentheses.
[b] Q, CAA; (QQQ), CAGCAACAA; (Q/E), CAG/GAG. The number of tandem repeats is listed after each motif.
[c] See Fig. 3. Values are numbers of incidences of each haplotype on each island.

TABLE 2 Distribution of *P. vivax csp* haplotypes on 7 islands of Vanuatu

Haplotype	SNP[a] at position: 112 or 113	258	Insertion between nt 285 and 286	Nonapeptide repeat type (no.)[b]	No. of GGNA repeats	Deletion at nt 838–840	Distribution in islands (from north to south)[d] Gaua	Santo	Ambae	Pentecost	Malakula	Tanna	Aneityum
Van-C-1	AAC (N)	AAT (N)	GCA (A)	VK210 (17a)	2	GGA (G)	3		2			2	
Van-C-2	AAC (N)	AAT (N)	GCA (A)	VK210 (17b)	2	GGA (G)	1	3	2	1	3		
Van-C-3	GGC (G)	AAA (K)		VK247 (19)	1		1	7	4	5	2	4	
Van-C-4	AAC (N)	AAT (N)		VK210 (15)	2	GGA (G)			1				
Van-C-5	AAC (N)	AAT (N)	GCA (A)	VK210 (17c)	2	GGA (G)		1	1			1	
Van-C-6	AAC (N)	AAT (N)		VK210 (17d)	4	GGA (G)		3	1			2	2
Van-C-7	AAC (N)	AAT (N)	GCA (A)	VK210 (17e)	4	GGA (G)		1				1	23
Van-C-8	AAC (N)	AAT (N)	GCA (A)	VK210 (17f)	2	GGA (G)		1			3		
Van-C-9	GGC (G)	AAA (K)	GGA (G)	VK247 (20)	1		1	2					
Van-C-10	AAC (N)	AAT (N)	GCA (A)	VK210 (17e)	2	GGA (G)	6	1					
Van-C-11	AAC (N)	AAT (N)	GCA (A)	VK210 (17g)	2	GGA (G)	2						
Van-C-12	AAC (N)	AAT (N)	GCA (A)	VK210 (18a)	2	GGA (G)	2	1					
Van-C-13	AAC (N)	AAT (N)		VK210 (17d)	3	GGA (G)	2						
Van-C-14	AAC (N)	AAT (N)		VK210 (18b)	2	GGA (G)	1	6		2	2		
Van-C-15	GGC (G)	AAT (N)		VK210 (18c)	2	GGA (G)	1	1			1		
Van-C-16	AAC (N)	AAT (N)		VK210 (17d)	2	GGA (G)				1	1		
Van-C-17	AAC (N)	AAT (N)		VK210 (18d)	2	GGA (G)		1		2			
Van-C-18	AAC (N)	AAA (K)		VK247 (19)	1					1			
Van-C-19	AAC (N)	AAT (N)		VK210 (17d)	5	GGA (G)				1		1	
Van-C-20	AAC (N)	AAT (N)		VK210 (17h)	5	GGA (G)				1			
Van-C-21	AAC (N)	AAT (N)	GCA (A)	VK210 (17e)	1	GGA (G)		1					
Van-C-22	AAC (N)	AAT (N)		VK210 (17i)	2[c]	GGA (G)		1					
Van-C-23	GGC (G)	AAT (N)	GCA (A)	VK210 (17j)	6[c]	GGA (G)		1					
Van-C-24	AAC (N)	AAT (N)		VK210 (18e)	2[c]	GGA (G)		1					
Total							20	32	11	14	12	11	25

[a] Data are codons, with changed nucleotides underlined and resulting amino acid changes in parentheses. Nucleotide positions are given according to the *P. vivax* Sal-I *csp* sequence (PlasmoDB ID number PVX_119355).

[b] The number of 9-mer repeats of the VK210 type (GDRADGQPA) or the VK247 type (ANGAGDQPG) is given in parentheses. Distinct sequences with the same number of repeats are subtyped as a to j.

[c] For haplotypes 22 to 24, GGNA is followed by ANKKAGDAGA.

[d] See Fig. 3. Values are numbers of incidences of each haplotype on each island.

deletions, and various numbers of 9-mer oligopeptide repeats (types VK210 and VK247) and 4-mer (GGNA) repeats in a total of 125 cases from 7 Vanuatu islands (Table 2). In the nonapeptide repeats, the VK210 type was frequently seen, but the VK247 type was rare (Table 2). No VK247 types were detected on Aneityum, while both types were found on 6 other islands. In total, 24 distinct *Pvcsp* haplotypes were identified (Table 2). The number of haplotypes on Aneityum was 2, whereas it ranged from 6 to 10 on the other islands (Table 2 and Fig. 3).

When the *Pvmsp1* and *Pvcsp* haplotypes were combined (*n*, 113), the difference in the number of genotypes between Aneityum (*n*, 2) and comparison islands (*n*, 6 to 24) was more pronounced than with single-locus comparisons, with remarkably low genotype diversity (i.e., expected heterozygosity) on Aneityum (*h*, 0.15) and high genotype diversities on 6 comparison islands (*h*, 0.89 to 1.0) (Table 3). Only 2 genotypes, M1–C7 and M12–C6, were found on Aneityum; the former was not found on other islands, and the latter was found on Tanna. However, both the M1 (*Pvmsp1*) haplotype and the C7 (*Pvcsp*) haplotype were detected on both Tanna and Santo (Tables 1 and 2).

Closer looks into the distributions of SNPs of *Pvmsp1* and *Pvcsp* revealed that all SNPs were shared among islands (Tables 1 and 2). In contrast, repeat variations were extensive, and some repeat types were occasionally unique to individual islands, as were *Pvmsp1* M4 on Santo, M5 on Gaua, and M8 on Malakula and *Pvcsp* C4 on Ambae, C20 on Pentecost, and C21 to C24 on Santo (Tables 1 and 2).

DISCUSSION

During the 2002 malaria outbreak on Aneityum, 11 years after the beginning of the 1991 elimination program, *P. vivax* infections were identified by microscopy (>80 parasites/μl of blood) primarily in people born after 1991 (i.e., without any previous malaria exposure). In contrast, low-density parasite infections detected by PCR only (<80 parasites/μl of blood) were seen in individuals born before 1991. One explanation could be that older individuals have some protective immunity, which prevents and/or limits infection (18). Such long-term protection has been observed previously in studies with neurosyphilitics (19, 20). While the present study did not directly examine protection from clinical infection, these results suggest that individuals born before malaria elimination had sufficient exposure to generate persistent immunity that suppresses the level of *P. vivax* infection but not the infection itself. Moreover, the PCR positivity rate for *P. vivax* reinfection was significantly lower among individuals exposed more than 10 years previously, suggesting that the immunity that suppresses the establishment of *P. vivax* infection could also persist in this population, a phenomenon that may be related in part to seropositivity for CSPs, which was observed only in adults.

Our data are consistent with a previous report from the central highlands of Madagascar, where a falciparum malaria epidemic started in the mid-1980s in an area in which this disease had been absent for almost 3 decades (21). During this epidemic, individuals older than 40 years were more protected against clinical falciparum malaria than younger individuals. Nevertheless, older individuals were not protected from reinfection but had lower levels of parasitemia overall (22). Furthermore, in highland areas of low and unstable *P. falciparum* transmission in Kenya, parasite density was lower in the area of higher transmission only in persons ≥15 years of age, supporting the idea that control of parasitemia may

TABLE 3 Distribution of *P. vivax* genotypes on 7 islands of Vanuatu

MspI–Csp genotype[a]	No. of incidences on[b]: Gaua (n = 19)	Santo (n = 28)	Ambae (n = 10)	Pentecost (n = 13)	Malakula (n = 10)	Tanna (n = 8)	Aneityum (n = 25)
M1–C1			2				
M1–C2		1	1	1	1		
M1–C3		1	3	2			
M1–C6		1	1				
M1–C7							23
M1–C10	2						
M1–C14	1	3		2			
M6–C3		1		2	1		
M6–C6		2					
M6–C14		1			1		
M7–C3		2					
M12–C3						3	
M12–C6						1	2
M13–C1	2						
M13–C2		1			1		
M13–C3				1	1		
M14–C2		1			1		
Others	14	14	3	5	4	4	
No. of genotypes (genotype diversity)	17 (0.99 ± 0.02)	24 (0.99 ± 0.01)	7 (0.91 ± 0.08)	10 (0.96 ± 0.04)	10 (1.00 ± 0.04)	6 (0.89 ± 0.11)	2 (0.15 ± 0.09)

[a] For details on *Pvmsp1* and *Pvcsp* haplotypes, see Tables 1 and 2, respectively.
[b] Islands are ordered from north to south (refer to Fig. 3). Historically, transmission decreases as one goes south in the archipelago (6).

require immunity that comes with increased age and exposure (23).

The PCR-positive malaria infections detected in the visitors to Aneityum in August 2000 indicated a high potential for malaria reintroduction due to human movement. These cases originated from various islands (see Table S5 in the supplemental material) and roughly reflected the geographical patterns of malaria prevalence observed in these islands (6). For example, the 2 PCR-positive infections detected in Aneityum islanders during August 2000 support the idea that malaria parasites brought to the island by visitors could have triggered the 2002 epidemic. The community microscopists on Aneityum first reported an unusual increase in the number of cases in early 2002. Radical treatment with primaquine usually is not administered to patients with infections detected in peripheral health facilities, and the "Chesson" strain of *P. vivax* (24) in this region may have a short relapse pattern. Therefore, the *P. vivax* infections detected in our surveys might comprise new infections, relapses, and reinfections due to a time gap of several months between the start of the epidemic and our populationwide surveys. Although we have no detailed information on these initial cases, this time gap may explain why most of the children found positive by our surveys on Aneityum in 2002 were also asymptomatic.

Before elimination, the age patterns of parasite prevalence were initially similar on Aneityum and Malakula Islands, as reflected by the seroconversion curves. The rates of *P. vivax* parasite infection on Aneityum in 1991, before elimination, generally decreased with age; they were 23%, 10%, 1%, and 1% in the age groups 0 to 5, 6 to 15, 16 to 30, and >30 years, respectively (7). These age patterns were seen under conditions of ongoing transmission, but our results showed that they were maintained in a population with no exposure in the past 7 to 10 years. This might reflect two possible, non-mutually exclusive components: acquired immune protection in adults and/or intrinsic susceptibility to infections in children (25).

Antibodies typically reflect cumulative exposure and thus can potentially be used to reconstruct the history of exposure. To determine whether observed differences in parasite rates were related to antibody levels, an age-specific seroprevalence study was conducted. On Aneityum, the age-adjusted profiles of antibodies to whole-parasite extracts of both *P. falciparum* and *P. vivax* clearly showed higher levels in individuals born before the 1991 malaria elimination program, which are comparable to the antibody levels seen on Malakula, an area of mesoendemicity, indicating greater malaria exposure for individuals born before 1991. Statistical analysis of the age seroprevalence curves indicate a significant change in the SCR approximately 15 years prior to the 1998 survey, i.e., several years before the initiation of the elimination program in 1991. This discrepancy may be due to both technical and biological factors. The relatively small sample size means that the precision around the estimates of the time of change in the SCR is limited, with an SD of ±4 years. Also, it is probable that antibody responses in young children at the initiation of the elimination program would not be sufficiently established, so that these children would serorevert relatively quickly. This would lead to a change point earlier than expected, as has been shown with other serological analyses of malaria control projects (26).

SCR profiles indicate that the exposure levels for individuals born after malaria elimination on Aneityum were similar to those seen on Futuna, where malaria is not endemic. How these population-level antibody responses relate to protection from infection is not clear, although the data suggest that a greater breadth and magnitude of response to parasite antigens is advantageous (27). The seroepidemiological results and the distribution of infections

during the 2002 Aneityum outbreak among children born after 1991 would appear to confirm the low immunity of this age group, presumably reflecting a lack of exposure. This contrasts with the pattern for older individuals, who had very few infections and higher antibody responses. The prevalence of antibodies to *P. vivax* CSP antigens was lower than that to erythrocyte-stage antigens, although they showed similar age profiles. This is not surprising given that whole-parasite extracts are multiantigenic, and CSP is known to be less immunogenic than blood-stage antigens, inducing antibodies with shorter half-lives (28).

An important ancillary observation is the slight but measurable malaria antibody seroprevalence in older age groups on Futuna, where *Anopheles* mosquitoes and malaria transmission are absent (Fig. 2c and d). During the surveys on Futuna, in 1992 and 1997, no parasite-positive cases were detected (7). We believe that the mobile nature of this population can explain the seropositivity detected. Because this island is a Polynesian outlier with limited resources, many Futuna families stay off the island for periods ranging from a few months to 10 years. The village of Port Patrick on Aneityum is a Futuna community, where a parasite rate of 17% was recorded before the intervention in 1991 (7). In contrast, interisland human movement is unlikely to explain the high seroprevalence observed in adults on Aneityum, since this island has abundant resources and population movement is infrequent. However, this limited movement could explain the low seroprevalence in the children born after elimination on Aneityum.

It was not our intention to link the serological and parasitological surveys directly, given the difference in the timing of the surveys and the fact that the former assesses population-level exposure to infection rather than directly examining immunity in more detail. The precise determinants of immunity to malaria are not known, but it is widely agreed that IgG plays a major role (29). Protective levels of IgG are thought to be rapidly lost without rechallenge (30). Some field observations appear to support this idea: after nonsustained elimination attempts, *P. falciparum* resurgence has been recorded in various African islands (21, 31, 32) and resurgence of both *P. falciparum* and *P. vivax* in several Asian countries (33). In contrast, and in line with our results, malaria-specific antibodies have been found to persist in the absence of infection for at least 10 years after isolated outbreaks (22, 34), in African adults several years after emigration to countries where malaria is not endemic (35), and in Brazilian individuals after a *P. vivax* outbreak (36). Our data are also consistent with reports of the persistence of antibodies to *P. vivax* MSP-1_{19} more than 30 years after elimination (37). One model has suggested that antibody responses to *P. falciparum* MSP-1_{19} have a half-life as long as 40 years in areas of endemicity (38). Our data show that the seroprevalences of antibodies against both *P. falciparum* and *P. vivax* schizont extracts in individuals born before 1982 were still moderate (i.e., approximately 50% were seropositive) and that the seroprevalence in individuals born between 1982 and 1991 was lower on Aneityum than on Malakula, suggesting that the antibody half-life also depends on the length of previous exposures to parasites.

Although the current explanations of long-term antibody production and memory include low-grade chronic infection, antigen-antibody complexes, or cross-reactivity, all of which involve continuous antigenic stimulation, an alternative model is based on protection by long-lived plasma cells without restimulation (39). In line with the latter model, it was shown recently that individuals from an area of northern Thailand with an extremely low level of malaria transmission had antibody and B-cell memory responses to malaria antigens that were stable and were independently maintained over time in the absence of reinfection (40). Long-lasting cellular immunity has also been detected in Caucasians last exposed to *P. vivax* sporozoites as long as 49 years ago, with the persistence of T-cell memory for *P. vivax* epitopes (41).

High rates of infections with mixed parasite clones were observed in *P. vivax* cases from islands with continuous malaria transmission (6), in sharp contrast with the near-complete absence of mixed infections in 140 *P. falciparum* cases previously reported for *Pfmsp1* antigen alleles on these islands (42). Furthermore, *P. vivax* antigen haplotypes were quite diverse on islands with continuous malaria transmission. These results indicate a heterozygous nature of *P. vivax* parasites even in low-transmission settings, in agreement with previous results (43, 44).

However, infections with mixed parasite clones were almost absent among *P. vivax* cases during the 2002 Aneityum outbreak, a decade after the beginning of the elimination program (7). When haplotypes of *Pvmsp1* and *Pvcsp* were combined, infections were genetically limited, with only 1 major and 1 minor genotype. The minor genotype was also found on Tanna. Importantly, all haplotypes (two *msp1* and two *csp* haplotypes) on Aneityum were found on Tanna, and at least one of them was also found on the other five islands. Considering a situation of rapid genotype change, because of potentially frequent meiotic recombination events inferred from high rates of mixed-haplotype infections, our results suggest that recent importation of parasites via interisland human movements within Vanuatu may be the source of the 2002 malaria outbreak on Aneityum. However, we cannot distinguish whether the minor parasite line on Aneityum was generated from two independent imports or was due to heterogeneous relapses from a single import (44). A focal outbreak of *P. falciparum* malaria caused by a clonal parasite line was documented on Santiago Island, Cape Verde (32), and among Amazonian Yanomami Amerindians (45). A previous study conducted during a malaria epidemic in the eastern highlands of Papua New Guinea showed that all *P. falciparum* infections shared a single genotype, suggesting external introduction as the epidemic source, while the *P. vivax* infections were highly diverse, suggesting endemic transmission (46). To our knowledge, the 2002 Aneityum outbreak is the first documented outbreak of *P. vivax* malaria caused by a semiclonal parasite line.

Our sequence results indicate stable SNPs, but rapid evolution of repeat length polymorphisms, in the *P. vivax* antigen loci in Vanuatu with a limited gene pool. This is compatible with the previous observations of *P. falciparum* populations from Vanuatu islands (47). Probably the human population born before the beginning of the malaria elimination program in 1991 on Aneityum had previously encountered the parasite antigen haplotypes, represented by the stable SNPs, introduced during the resurgence in 2002. It is also likely that continuous parasite exposure in this age group before malaria elimination resulted in immunity that is effective across strains (48).

Taken together, our data suggest that *P. vivax*-specific antibodies persist a decade after the initiation of elimination efforts and that these antibodies may remain effective. This effectiveness may be more pronounced if the complexity and diversity of the infecting parasites are increasingly limited (19, 49), as appears to be the case on Aneityum. These study results have implications for ma-

laria elimination campaigns in areas of *P. vivax* prevalence and support the importance of protective measures against clinical diseases targeted at young populations born after malaria elimination. However, interventions that include populations of all ages may remain critical to sustaining malaria elimination, since submicroscopic infections may contribute to maintaining transmission (50, 51).

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