investigations in selected villages of Nadia, Jessore, Burdwan, Birbhum and Hooghly confirmed the impression of large-scale depopulation in epidemic years.[36]

In contrast, population increased rather quickly in eastern Bengal. This was a consequence of the vicious cycle between 'agricultural deterioration' and epidemic malaria.

(3) Epidemic Malaria and Environmental Changes

There was also a third important factor behind outbreaks of the 'Burdwan Fever'. Obstructions to inundation considerably affected the anopheles factor as well as the human factor. Usually flooding constrained the breeding of anopheles but it is also claimed that inadequate inundation promoted proliferation of anopheles larvae.

> The inundation of the country during the monsoon is unfavourable to the multiplication of anopheles mosquitoes, in the first place, because flooding reduces the dangerous 'water-edge' which affords safe cover for mosquito larvae; in the second place, because owing to the large surface exposed to the rays of the sun the temperature of the water tends to rise so as to be exceedingly unfavourable to the life of anopheles larvae; and in the third place, because the physical and possibly the chemical character of river water is inimical to anopheles larvae.[37]

The main vector mosquito *Anopheles philippinesis* preferred 'still water for egg-laying and larval production, not heavily polluted, with rather a low water-table and a moderate (but not light-excluding) growth of aquatic plants'.[38] This was in striking contrast to the situation in Punjab. Interruptions in flooding facilitated the reproduction of anopheles, leading to the prevalence of malaria. It was concluded that hindrances to inundation affected both anopheles and human factors, resulting in 'Burdwan Fever'. It must be reiterated that this environmental change was brought about by the construction of railways and roads.

Bentley recommended the 'Bonification method' as an integrated approach to malaria control. This method, which had been tried in Italy, aimed at water regulation and agricultural improvement to ameliorate the evils of malaria, agricultural decline and depopulation.[39]

However, it seems that this improved understanding did not have much effect on malaria control practices in British India.

4. Concluding Remarks

Thus during the second decade of the 20th century some malariologists, focusing on the human factor, came to consider the problems which the Indian society faced. It was made clear that the intensified mobility of people brought about the prevalence of epidemic diseases. The socio-economic conditions of the Indian people, particularly poverty and malnutrition, were taken into account. In addition, malariologists such as C.A. Bentley recognized that environmental changes caused by 'development' were also responsible for malaria prevalence.[40] Recent studies examining epidemic malaria during the British period have basically supported these findings.

In conclusion, we will contextualize these observations into a more general story. As we mentioned in the Introduction, the period from the early 1870s to the First World War presented strong paradoxes. That is, while the rate of population growth was very low due to repeated outbreaks of famines and epidemics, there was also considerable economic growth. Recent studies have recognized that there was slight per capita economic growth,[41] and there were various developmental activities, including the building of railways and irrigation canals. We can call this period 'the Age of Development'. These developmental activities were legitimated by claims that they would mitigate the damages of famines and epidemics. Therefore we should ask why famines and epidemic so frequently occurred during this period.

Moreover, we need to reconsider the concept of 'development' in this context. In general, development means the process of bettering people's well-being, particularly in terms of per capita income growth. But we need to redefine the concept of development in order to apply it to the history of disease. C.C Hughes and J.M. Hunter defined 'development' as 'a conscious and deliberate

intervention into the empirical *status quo ante*', or as 'an intervention into the affairs of nature'. They thought that developmental activities would 'have consequences both intended and unintended' and would 'enter the scene as elements in the ecologic dialectic in which all life is enmeshed'. *The 'developo-genic diseases' – in other words the 'diseases of development' – are a corollary of developmental activities in this sense.[42]

On the other hand, O. Saito defines 'development' as 'intensification of people's activities' or the 'phenomenon that increases movement of people and goods'. He focuses upon the phenomenon of disease environment. The disease environment is an environment in which people are heavily exposed to disease-causing germs, vectors or hosts. Saito emphasizes that 'intensification of people's activities' like commercialization, migration, urbanization and intervention into nature may aggravate the disease environment. In this causational relationship 'developo-genic disease ' may occur.[43]

As a result of the commercialization of agriculture, developmental activities flourished in the western United Provinces and Punjab during the said period. In addition, the rates of industrial development, labour migration and urbanization were increasing in western and central Bengal. It has been shown that the prevalence of epidemic malaria in these regions may have resulted from these developmental activities. We can conclude that epidemic malaria was a typical case of 'developo-genic disease'.

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[2] K. Wakimura, 'Famines, Epidemics and Mortality in Northern India, 1870-1921', in P. Robb, K. Sugihara and H. Yanagisawa (eds.), *Local Agrarian Societies in Colonial India: Japanese Perspectives*, Surrey, 1996.

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[4] British malariologists were mainly doctors or medical scientists who did research on malaria and its control. Research on malaria was the leading part of tropical medicine at that time. They were very active during the early 20th century in the tropical colonies in the British Empire. Particularly British India had become the center of their research. R. Ross was a pioneer of malariologosts. S.R. Christophers and C.A. Bentley, who are main figures in this paper, emphasized the human factor in the etiology of malaria. See K. Wakimura, 'Anopheles Factor and Human Factor: Malaria Control under the Colonial Rule, India and Taiwan', in M. Hasan and N. Nakazato (eds.), *The Unfinished Agenda: Nation-Building in South Asia*, New Delhi, 2001.

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[6] S.R. Christophers, *Malaria in the Punjab*, Calcutta, 1911.

[7] *Ibid.*, p. 112.

[8] Wakimura, 'Famines, Epidemics and Mortality in Northern India, 1870-1921'.

[9] Maharatna, *The Demography of Famines*, p. 81.

[10] Whitcombe, 'Famine Mortality', p. 1178. She also give us a very interesting insight. When famine occurred, many cattles died. Then usually zoophilous anopheles tended to feed exclusively on humans.

[11] Zurbrigg, 'Hunger and Epidemic Malaria in Punjab, 1868-1940'.

[12] *Ibid.*, p. 15.

[13] *Ibid.*, p. 16.

[14] S. Guha, *Health and Population in South Asia: From Earliest Times to the Present*, London, 2001, p. 84.

[15] Christophers, *Malaria in the Punjab*, p. 103.

[16] S. Watts, 'British Development Policies and Malaria in India 1897-*c.*1929', *Past and Present*, No. 165, Nov. 1999, p. 167.

[17] C.A. Gill, 'The Relationship of Canal Irrigation and Malaria', *Records of the Malaria*

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[20] *Ibid.*, p. 206.
[21] K. Mankodi, 'Political and Economic Roots of Disease: Malaria in Rajasthan', *Economic and Political Weekly*, January 27, 1996.
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[28] Christophers and Bentley, 'The Human Factor', p. 82.
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[32] R.M. Prothero, *Migrants and Malaria*, Longmans, 1965.
[33] Christophers and Bentley, *Malaria in the Duars*, p. 89.
[34] Bentley, *Report on Malaria in Bengal*, p. 73.
[35] Bentley, *Malaria and Agriculture in Bengal.*
[36] Bose, *Peasant Labour and Colonial Capital*, p. 25.
[37] Bentley, *Malaria and Agriculture in Bengal*, pp. 48-49.
[38] Learmonth, *Disease Ecology*, p. 5.
[39] *Ibid.*, p. 125.
[40] We find the same kind of approach in reports of the Malaria Commission of Health Organization, League of Nations. See H. Evans, 'European Malaria Policy in the 1920s and 1930s: The Epidemiology of Minutiae', *Isis*, No. 80, 1989.
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分担研究報告書

集団治療実施に関連するヒト・熱帯熱マラリア原虫の分子疫学的研究
～ビクトリア湖島嶼マラリア撲滅に向けて～

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研究要旨
ビクトリア湖島嶼マラリア撲滅に向けた集団治療に関連して熱帯熱マラリア原虫の薬剤耐性関連遺伝子の分子疫学的解析を行う。本年度は集団治療実施前であり、2012 年 1-2 月、8 月及び 2013 年 8 月に行ったフィールド調査で収集した血液濾紙サンプルを用いて、解析システムの整備と *pfcrt* 及び *pfmdr1* の一部のシークエンス解析を行った。解析は現在進行中であり、結論を述べるには今後の解析結果を待つ必要がある。尚、現段階での解析内容は本文に記載した。

A. 研究目的

本研究の研究代表者が計画するビクトリア湖島嶼マラリア撲滅に向けた集団治療という介入に関連して、対象地域のマラリア原虫の薬剤耐性関連遺伝子の変遷を観察、解析するという目的を有する。ケニアでは 2004 年から Artemisinin-based-combination therapies (ACTs)が熱帯熱マラリアを治療する際、第一選択薬として推奨されている。しかしながら、近年、カンボジアではACTsの感受性低下を示す症例が多数報告されており、K-13 propeller の遺伝子変異との関連が強く示唆されている（*N Engl J Med* 2009, A.M.Dondorp et al., *Nature* 2014, F. Ariey et al.)。また、ケニアの東海岸沿岸部からの報告によるとACT s による熱帯熱マラリアの治療効果が低下している症例が認められるようである（*PLoS ONE* 2011, S. Borrmann et al.)。本研究の集団治療は ACTs を主軸とするものであり、ACTs の治療効果に関連した原虫の分子疫学的情報を把握することが必要である。また、併せて既存の抗マラリア薬の耐性に関連した遺伝子の網羅的解析も行う。

B.研究方法

[研究材料]

a. 研究フィールド：ケニアのビクトリア湖島嶼及び沿岸（Ngodhe 島、Kibuogi 島、Takawiri 島、Mfangano 島及び沿岸の村落 Ungoye）

b. 期間：2012 年 1-2 月、8 月、2013 年 8 月、2014 年 3 月、以後も年 2 回で行う予定

尚、本年度は解析システムの整備と集団治療前の事前調査を行った。

[方法]

対象地域の住民にマラリア調査を行うことを周知し、参加希望者にはインフォームドコンセントを行い、同意を得て濾紙採血、G6PD Assay Kit-WST 検査を行った。

血液濾紙は後日まとめて DNA 抽出を行っているところである（QIAamp DNA Mini Kit, QIAGEN)。また、マラリア原虫の有無と種の同定を行うためマラリア原虫 MtDNA の検出を PCR 法で行う予定である。

熱帯熱マラリアの薬剤耐性に関連すると考えられる *pfcrt, pfmdr1, pfmrp, pfdhfr, pfdhps* に関しては耐性関連 SNP を検出するためにシークエンスを施行中である。また、2014 年に Ariey らが報告しアルテミシニン耐性に関連する可能性のある K13-propeller 遺伝子の解析の対象とした。

C. 研究結果

現段階において解析は進行中であり、この項目に記載するものは中間報告である。現段階での *pfcrt* 及び *pfmdr1* のシークエンスに基づく解析結果を以下の表 1、表 2 に記載する。

表 1. *pfcrt*(K76T)の発現頻度

期間		*pfcrt*(K76T)		
		解析数	変異株数 *	変異率
Islands	2012.1-2	45	19	42.2
	2012.9	30	5	16.7
Ungoye	2012.1-2	60	18	30

＊混合感染は除く

表 2. *pfmdr1* の変異発現頻度（86, 181 及び 1246 位）

		pfmdr1 86			*pfmdr1* 181			*pfmdr1* 1246		
	期間	解析数	変異株数 *	変異率	解析数	変異株数 *	変異率	解析数	変異株数 *	変異率
Islands	2012.1-2	32	4	12.5	34	10	29.4	38	8	21.1
	2012.9	27	2	7.4	27	9	33.3	28	6	21.4
Ungoye	2012.1-2	55	5	9.1	54	23	42.6	54	6	11.1

＊混合感染は除く

D.考察

マラウィでの報告によるとマラリア原虫のクロロキン耐性株はクロロキンの使用を中止し薬剤圧をなくすことにより大幅に減少し、野生株の割合が増えたとある（*J Infect Dis* 2010, M.K.Laufer et al.）。現段階での解析結果では島嶼部で得られた2時点において特にクロロキン耐性と強い関連性が指摘される *pfcrt*76T（42.2→16.7%）及び *pfmdr1* 86Y(12.5→7.4%)の割合が大幅に低下していた。このことは、対象地域でクロロキンの消費が低下していることを示唆するものかもしれないが、季節（雨季、乾季）の違いを反映している可能性もあり、今後の継続的な解析が待たれる。

論　文

研究成果の刊行に関する一覧表

書籍

該当なし

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Kaneko A, Chaves LF, Taleo G, Morris K, Isozumi R, Wickremasinghe R, Perlmann H, Takeo S, Tsuboi T, Kimura M, Bjorkman A, Troye-Blomberg M, Tanabe K, Drakeley C.	Characteristic Age Distribution of Plasmodium vivax Infections after Malaria Elimination on Aneityum Island, Vanuatu	Infection and Immunity	82(1)	243-252	2014
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Cavaco I, Hombhanje FW, Gil JP, Kaneko A	Frequency of the functionally relevant aryl hydrocarbon receptor repressor (*AhRR*) Pro185Ala SNP in Papua New Guinea.	Drug Metab Pharmacokinet	—	*Accepted*	2013

Infection and Immunity

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

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

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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.

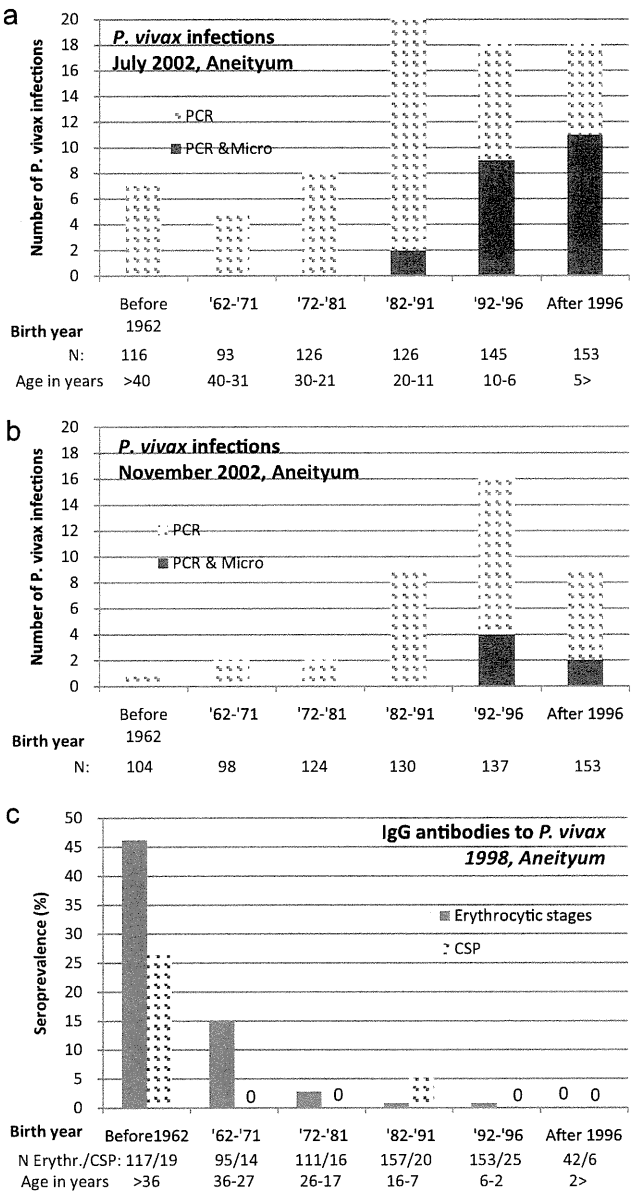

FIG 1 (a and b) Age-specific prevalence profiles for *P. vivax* infections in July (a) and November (b) 2002. Red bars represent the numbers of *P. vivax* infections detected by both microscopy and PCR, and blue bars represent the numbers detected only by PCR. All microscope-positive individuals were 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

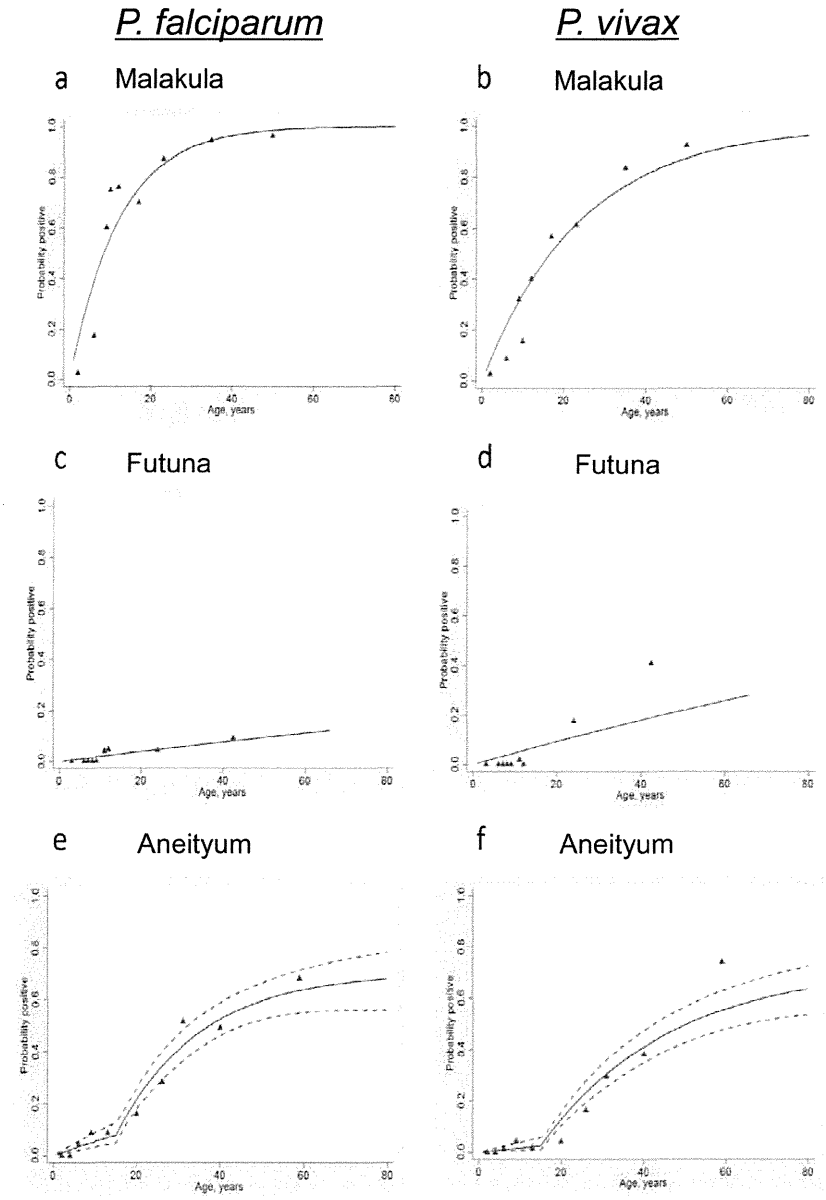

FIG 2 Seroprevalence curves of IgG antibodies to schizont extracts for Malakula Island (an area of mesoendemicity), Futuna Island (with no endemicity), and Aneityum Island (where an integrated elimination program was implemented in 1991) in Vanuatu. Results from Malakula (1998) (a and b), Futuna (1997) (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-

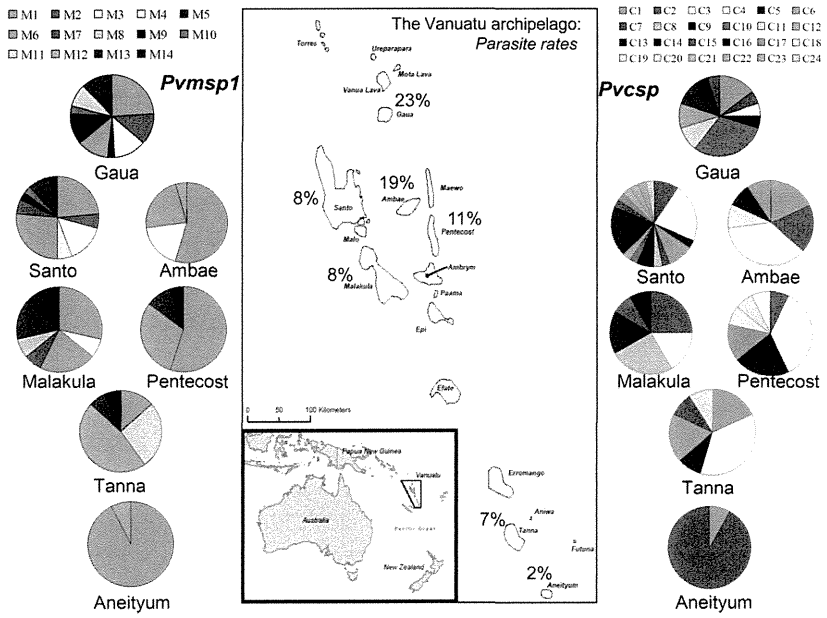

FIG 3 Distribution of *Pvmsp1* and *Pvcsp* haplotypes among *P. vivax* parasites in the Vanuatu archipelago. Shown are results for Aneityum Island during the outbreak in 2002, a decade after the beginning of the malaria elimination program (*n*, 27 and 25 for *Pvmsp1* and *Pvcsp*, respectively), and 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]: 2017	2079	2082	2088	2095	2098/9	2107	Poly(Q) in block 6[b]	Distribution in islands (from north to south)[c]: 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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Single nucleotide polymorphisms in *Plasmodium falciparum* V type H^+ pyrophosphatase gene (*pfvp2*) and their associations with *pfcrt* and *pfmdr1* polymorphisms

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ABSTRACT

Background: Chloroquine resistance in *Plasmodium falciparum* malaria has been associated with *pfcrt* 76T (chloroquine resistance transporter gene) and *pfmdr1* 86Y (multidrug resistance gene 1) alleles. *Pfcrt* 76T enables transport of protonated chloroquine out of the parasites digestive vacuole resulting in a loss of hydrogen ions (H^+). V type H^+ pyrophosphatase (PfVP2) is thought to pump H^+ into the digestive vacuole. This study aimed to describe the geographic distribution of single nucleotide polymorphisms in *pfvp2* and their possible associations with *pfcrt* and *pfmdr1* polymorphisms.

Methods: Blood samples from 384 patients collected (1981–2009) in Honduras (n = 35), Colombia (*n* = 50), Liberia (*n* = 50), Guinea Bissau (*n* = 50), Tanzania (*n* = 50), Iran (*n* = 50), Thailand (*n* = 49) and Vanuatu (*n* = 50) were analysed. The *pfcrt* 72–76 haplotype, *pfmdr1* copy numbers, *pfmdr1* N86Y and *pfvp2* V405I, K582R and P711S alleles were identified using PCR based methods.

Results: Pfvp2 was amplified in 344 samples. The *pfvp2* allele proportions were V405 (97%), 405I (3%), K582 (99%), 582R (1%), P711 (97%) and 711S (3%). The number of patients with any of *pfvp2* 405I, 582R and/or 711S were as follows: Honduras (2/30), Colombia (0/46), Liberia (7/48), Guinea-Bissau (4/50), Tanzania (3/48), Iran (3/50), Thailand (1/49) and Vanuatu (0/31). The alleles were most common in Liberia (*P* = 0.01) and Liberia + Guinea-Bissau (*P* = 0.01). The VKP haplotype was found in 189/194 (97%) and 131/145 (90%) samples harbouring *pfcrt* 76T and *pfcrt* K76 respectively (*P* = 0.007).

Conclusions: The VKP haplotype was dominant. Most *pfvp2* 405I, 582R and 711S SNPs were seen where CQ resistance was not highly prevalent at the time of blood sampling possibly due to greater genetic variation prior to the bottle neck event of spreading CQ resistance. The association between the *pfvp2* VKP haplotype and *pfcrt* 76T, which may indicate that *pfvp2* is involved in CQ resistance, should therefore be interpreted with caution.

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