PNG Med J 2002 Sep-Dec;45(3-4):200-205

Complex patterns of malaria epidemiology in the highlands region of Papua New Guinea

IVO MUELLER[1,2], JOHN TAIME[1], ERVIN IBAM[3], JULIUS KUNDI[4], MOSES LAGOG[5], MOSES BOCKARIE[5] AND JOHN C. REEDER[1]

Papua New Guinea Institute of Medical Research, Goroka and Madang, Western Highlands Department of Health, Mount Hagen, Papua New Guinea, Malaria Surveillance and Control Unit, Goroka, Papua New Guinea and University of Queensland, Brisbane, Australia

SUMMARY

A cross-sectional malaria survey of the Pabrabuk area in the Western Highlands Province found that all 4 human malaria species were present in a single village, with an overall parasite prevalence rate of 27%. *Plasmodium falciparum* was the most frequently detected infection (14%) followed by *P. vivax* (11%), *P. malariae* (5%) and *P. ovale* (3%). 10 of the 51 infections were mixed. *Anopheles punctulatus* was the most frequent vector species in the area, but both *An. farauti* no. 6 and *An. karwari* were also present in low numbers. This diversity in both parasite and vector populations indicates that complex malaria patterns are found in Papua New Guinea even at the moderate transmission levels found in low-lying inter-montane valleys.

Introduction

Papua New Guinea (PNG) is renowned for the complexity of its malaria situation (1). All 4 human *Plasmodium* species are present and transmission intensities range from highly endemic in some lowland areas to complete absence of local malaria transmission in areas above 2000 m (2). At present, *P. falciparum* is the dominant malaria parasite, but both *P. vivax* and *P. malariae* can reach high prevalence in selected areas, and mixed infections are common (3-5).

Most of the *Anopheles* mosquitoes found in Papua New Guinea are highly anthropophilic and in some villages people experience up to 500 bites/person/night (6). The main malaria vectors belong to the *Anopheles punctulatus* group which consists of at least 12 species (7,8), 7 of which make up the *An. farauti* complex. The sibling species within the *Anopheles farauti* complex are morphologically very similar and can only be reliably differentiated using allozyme and DNA-based techniques (9,10). 9 of the 12 members of the *An. punctulatus* group including 5 of the 7 known sibling species within the *An. farauti* complex are found in Papua New Guinea (8,9,11). Mosquitoes of the *Anopheles punctulatus* group are distributed from the coastal and inland regions to the highlands. The most widely distributed is *An. farauti* s.s., which is found throughout the coastal areas. *Anopheles farauti* no. 2 and no. 4 are found in coastal and inland areas while *An. farauti* no. 5 and *An. farauti* no. 6 have only been recorded in the highlands (8,9,11). Other known malaria vectors in Papua New Guinea include *An. bancroftii* and *An. karwari* (12).

1 Papua New Guinea Institute of Medical Research, PO Box 60, Goroka, EHP 441, Papua New Guinea

2 Tropical Health Program, ACITHN, School of Population Health, University of Queensland Medical School, Herston, Queensland 4006, Australia

3 Department of Health, PO Box 129, Mt Hagen, Western Highlands 281, Papua New Guinea

4 Malaria Surveillance and Control Unit, Department of Health, PO Box 778, Goroka, EHP 441, Papua New Guinea

5 Papua New Guinea Institute of Medical Research, PO Box 378, Madang, Madang Province 511, Papua New Guinea

Most recent studies on malaria epidemiology were focused in low-lying areas in the north (Sepik and Madang Provinces) and the south-west of the country (Western Province). Less is known about the complexity of malaria in the malarious areas of the highlands. Apart from *P. ovale*, for which there are no previous published accounts, all human plasmodial species have been found in the PNG highlands (13,14). There is, however, a marked decrease in malaria endemicity with increasing altitude (14,15), most profoundly in *P. malariae* and least in *P. vivax* (16). All major vector ecology studies in the PNG highlands (17) predate the revised identification of species within the *An. farauti* complex (9). However, the fact that individuals of *An. farauti* no. 5 and no. 6 have so far only been found in highlands collections indicates both a diverse and distinct highlands anopheline fauna.

Here we present data from a survey conducted in a single village in the Pabrabuk area of Western Highlands Province (WHP) that confirms the existence of all 4 *Plasmodium* species and documents the high diversity in both parasites and vectors found in parts of the PNG highlands.

Materials and Methods

The survey described here was conducted in September 2001 in Kungmul village near Pabrabuk in the Nebilyer District of the WHP. The village is situated at 1300 m above sea level on flat, mostly cultivated land between two small rivers and is within walking distance (<2 km) of the Highlands Highway.

The cross-sectional malaria survey included 190 individuals from 59 households, representing approximately 60% of all household members. From each individual, a thick and thin blood film was prepared, the spleen palpated in the lying position, axillary temperature taken and haemoglobin level measured. Symptomatic individuals were tested with Optimal test kits (Diamed, Switzerland) and those found positive were treated with chloroquine plus Fansidar. A short questionnaire on current symptoms and past malaria episodes was administered to each subject or their guardian. Giemsa-stained blood films were examined under the microscope for 100 thick film fields under oil immersion before being declared negative. The parasite species in positive films were identified and densities recorded as the number of parasites/200 white blood cells (WBC). Densities were converted to the number of parasites/μl of blood assuming 8000 WBC/μl. The slides were read independently by two experienced microscopists at the Papua New Guinea Institute of Medical Research (PNGIMR) in Madang.

Mosquitoes were collected using the CDC light-trap and the landing catch methods as described previously by Bockarie et al. (18). CDC light-traps were operated inside 2 houses between 1800 and 0600 hours and landing catches were conducted outdoors at the same time. Sampling was carried out for two consecutive nights. A rapid, nonquantitative larval survey was conducted on water bodies in the vicinity of the village. Mosquitoes were stored dry in silica gel, larvae in ethanol; all samples were transported to the PNGIMR laboratory in Madang for identification. Mosquitoes were first identified morphologically according to the keys described by Belkin (19). Specimens identified as belonging to the *An. punctulatus* group were then processed by polymerase chain reaction (PCR) following the method of Beebe and Saul (10).

Parasite densities were log-transformed. As haemoglobin levels are known to be age and gender dependent, nonlinear regression was used to obtain residual scores which are corrected for these effects. Differences in categorical variables were tested using χ^2 and Fisher's exact test and continuous variables using t-test and ANOVA. All statistical analyses were done using STATA statistical software (Version 6, Stata Corp. TX).

Results

Out of the 190 blood slides taken in Kungmul, 51 (27%) were positive by microscopy (Table 1), 27 (14%) people were infected with *P. falciparum*, 20 (11%) with *P. vivax,* 9 (5%) with *P. malariae* and 5 (3%) with *P. ovale*. Of these infections 10 (20%) were mixed (Table 1). Malarial infection was

TABLE 1

RESULTS OF PARASITOLOGICAL SURVEY (N=190)

Species	Cases	Parasite density (parasites/µl)		
		Geometric mean	95% Confidence limits	Number >2500
P. falciparum	18	523	[201, 1363]	4
P. vivax	12	588	[274, 1264]	1
P. malariae	7	204	[93, 447]	0
P. ovale	4	320	[52, 1955]	0
P. f./P. v.	7	640[a] 308	[159, 2574] [113, 844]	1
P. f./P. m.	1	21000[a] 8320		1
P. f./P. o.	1	640[a] 120		0
P. v./P. m.	1	80[a] 440		0
All infections	51	526	[337, 821]	7

[a] first number denotes density of first mentioned species

significantly associated with age (χ^2=20.7, df=3, p<0.001): it was most prevalent in the 2-4 year old children (58% positive slides), followed by the 5-9 year olds (45%) and <2 year olds (33%). The prevalences in adolescents (10-19 years) and adults (>20 years) were 16% and 18%, respectively.

Most of the infections were of very low density, with only 7 (14%) reaching more than 2500 parasites/µl (Table 1). Mixed infections had the highest densities (geometric mean of 1101/µl combined) and *P. malariae* and *P. ovale* infections the lowest. However, these differences did not reach statistical significance, due to the low number of such infections observed.

While over 70% of people with positive blood slides showed some form of malaria-related morbidity, only 20% reported acute illness (ie fever during the last 3 days). However, haemoglobin levels in infected people were reduced by 1.3 g/dl (CI 0.7-1.9, p<0.001) as compared to noninfected people. As a consequence, 50% had mild (Hb<10 g/dl) and 10% moderate (Hb<7.5 g/dl) anaemia compared with only 23% mild and 3% moderate anaemia in uninfected individuals. 50 (27%) of 185 people checked had enlarged spleens (average spleen size 2.6 following Hackett's grading); 17 (34%) of these had concurrent infections and 5 (10%) reported a recently treated malaria episode. As with the pattern of malaria infection, enlarged spleens were strongly age dependent (p<0.001), reaching more than 50% in children under 10 years. Anaemia was significantly more common and profound in mixed than in single infections (9/10 vs 21/40, p=0.015) and enlarged spleens tended to be more common in infection with *P. malariae* and *P. ovale* (8/14 vs 11/36, p=0.08).

The two nights of landing catches yielded a total catch of 27 anopheline mosquitoes including 26 *An. punctulatus* and 1 *An. karwari*. Only one anopheline mosquito, *An. farauti* no. 6, but many culicines were caught in the light traps. Hundreds of *An. punctulatus* larvae were found in murky sunlit pools made by pigs along the dirt road through the village.

Discussion

The work presented here confirms that all 4 human malaria species occur in a PNG highlands area with moderate to low transmission and gives an indication of the complexity of the vector fauna in these areas.

All 4 human malaria species have been found before in large surveys in highly endemic areas around Madang and East Sepik (5) and the highlands of West Papua, Indonesia (20,21). But to our knowledge, this is the first published account of *P. ovale* in the PNG highlands. While *P. ovale* was clearly present in PNG before the second world war (22) relatively little is known about the actual distribution of this species in the country. When found, it was in the lowlands mainland areas (1) and at low frequency. In a recent survey in the East Sepik Province, *P. ovale* was not observed microscopically but detected by PCR diagnosis in 16% of the samples, all in mixed infections (5). The fact that *P. ovale* has not been noted in the PNG highlands before may be due to the common use of thick smears in many earlier surveys and routine hospital microscopy. Differentiation of *P. ovale* from *P. vivax,* however, needs careful reading of the thin smear by an experienced microscopist (23).

The overall prevalence rate of over 20%, the strong age dependence of infection and the high number of low-level infections without acute disease all indicate endemic malaria transmission in the area, which leads to at least a partial build-up of immunity. Malaria is clearly a major source of morbidity in the area, although much of it is in the form of anaemia and enlarged spleens rather than acute febrile illness. Because of the small sample size, however, little can be said about the difference in morbidity associated with infection by different *Plasmodium* species.

The diversity in parasite species is matched by the entomological situation, with 3 different malaria vectors. Although the number of mosquitoes caught in this study was small, the species composition, biting rate and relative abundance of the *Anopheles* species is of great epidemiological significance to the current malaria situation in the highlands. *Anopheles punctulatus*, the most commonly caught mosquito in Kungmul, was uncommon in the highlands region in the 1950s and 1960s when *An. farauti* s.l. was the predominant human biting mosquito (12,24). Human biting rates of *Anopheles* species were relatively low compared to the coastal region (<5 bites/person/night in WHP, 1958-1960). In the present survey a much higher biting rate of 13 bites/person/night was observed for *An. punctulatus* alone. The increase in the relative abundance and biting rate of this species may be related to forest clearing and soil disturbance for housing and cultivation of vegetable gardens or to an increase in pig husbandry, all of which result in many sun-lit temporary pools that favour the establishment of *An. punctulatus*. Such disturbances were correlated with the unusually high densities of *An. punctulatus* which subsequently became the dominant local species (25-27). *Anopheles punctulatus* is a highly efficient vector of malaria because it is highly anthropophilic and anthropophagic. It is also strongly endophilic and endophagic. Recent reports of escalation of malaria transmission intensity in the highlands region may therefore be related to the increase in the biting rates for this species.

While *Anopheles farauti* s.l. was the most common mosquito in the WHP in the 1950s and 1960s (17), it was evident that the ecology and behaviour of the *An. farauti* s.l. found in the highlands was different from the type species of *An. farauti* s.l. found in the coastal region (12). The highlands *An. farauti* s.l. were also larger than the coastal ones (17). In recent surveys the only members of the *An. farauti* complex observed so far in the highlands are *An. farauti* no. 5 and no. 6 (8,9) and more recently *An. farauti* no. 2 (11). Their respective roles in malaria transmission in the highlands are yet to be established.

Anopheles karwari, a known malaria vector elsewhere in PNG, has not been previously recorded in the PNG highlands. *Anopheles karwari* is probably not indigenous to Papua New Guinea. According to Lee et al. (28), it may have been introduced to West Papua in the 1920s from South-East Asia where it is widespread. Since that time it has been caught in human biting catches in the Wosera area, East Sepik Province (29).

The complexity of both parasites and vector species documented here might be linked to several different characteristics of the survey area. Malaria is clearly endemic in Kungmul, even though it is situated at 1300 m. However, as observed in other parts of the PNG highlands, transmission is likely to be seasonal. Both the local health staff and the community mentioned that malaria is much more frequent in the drier season. Strong seasonal differences in transmission are likely to favour parasites with either prolonged liver stages (*P. vivax* and *P. ovale*) or long-lasting, often subpatent infections (*P. malariae*) (30). The higher number of enlarged spleens in cases of *P. malariae* and *P. ovale* might be an indication of such chronic infections.

Influx of malarial infections from other areas may also contribute to diversity. The Pabrabuk area is adjacent to lower-lying, malarious areas in East Kambia (WHP) and Gulf Province. Moreover, the increase of travel on the Highlands Highway is likely to have increased the number of infections introduced from coastal areas (31). The potential role of the different vector species in maintaining parasite diversity is difficult to assess since very little is known about the possible interactions between individual vector and parasite species. However, it is well established that anopheline mosquitoes can simultaneously be infected with more than one *Plasmodium* species (32, 33) and can thus potentially transmit multiple infections with a single inoculum.

The malaria situation found in Kungmul is comparable to the one observed earlier in the highlands of West Papua, Indonesia (26). There, a heightened malaria transmission was observed following the construction of new houses and drainage ditches which resulted in increased breeding of *An. punctulatus*. As in our survey, malaria was endemic and all 4 malaria species were present.

Changes in both human ecology and behaviour may therefore have created favourable conditions for complex patterns of malaria epidemiology in the New Guinea highlands regions by increasing both the introduction of infections from the lowlands and the breeding potential of *An. punctulatus*. These changes may have greatly influenced the epidemiology of malaria in the PNG highlands and may lead to an aggravation of the malaria problem in these areas.

ACKNOWLEDGEMENTS

This work was approved by the Papua New Guinea Medical Research Advisory Committee. Financial support was provided by the World Health Organization through the Regional Office for the Western Pacific and the Global Roll Back Malaria Initiative. I. Mueller is supported by the Swiss National Science Foundation, the Rudolf Geigy Foundation and P. Mueller.

REFERENCES

1 **Cattani JA.** The epidemiology of malaria in Papua New Guinea. In: Attenborough RD, Alpers MP, eds. Human Biology in Papua New Guinea: The Small Cosmos. Oxford: Clarendon Press, 1992:302-312.

2 **van Dijk WJOM, Parkinson AD.** Epidemiology of malaria in New Guinea. *PNG Med J* 1974;17:17-21.

3 **Genton B, Al-Yaman F, Beck HP, Hii J, Mellor S, Narara A, Gibson N, Smith T, Alpers MP.** The epidemiology of malaria in the Wosera area, East Sepik Province, Papua New Guinea, in preparation for vaccine trials. I. Malariometric indices and immunity. *Ann Trop Med Parasitol* 1995;89:359-376.

4 **Bruce MC, Donnelly CA, Alpers MP, Galinksi MR, Barnwell JW, Walliker D, Day KP.** Cross-species interactions between malaria parasites in humans. *Science* 2000;287:845-848.

5 **Mehlotra RK, Lorry K, Kastens W, Miller SM, Alpers MP, Bockarie M, Kazura JW, Zimmerman PA.** Random distribution of mixed species malaria infections in Papua New Guinea. *Am J Trop Med Hyg* 2000;62:225-231.

6 **Burkot TR, Graves PM, Paru R, Wirtz RA, Heywood PF.** Human malaria transmission studies in the *Anopheles punctulatus* complex in Papua New Guinea: sporozoite rates, inoculation rates and sporozoite densities. *Am J Trop Med Hyg* 1988;39:135-144.

7 **Beebe NW, Cooper RD, Morrison DA, Ellis JT.** Subset partitioning of the ribosomal DNA small subunit and its effects on the phylogeny of the *Anopheles punctulatus* group. *Insect Mol Biol* 2000;9:515-520.

8 **Foley DH, Bryan JH, Yeates D, Saul A.** Evolution and systematics of *Anopheles*: insights from a molecular phylogeny of Australasian mosquitoes. *Mol Phylogenet Evol* 1998;9:262-275.

9 **Foley DH, Paru R, Dagoro H, Bryan JH.** Allozyme analysis reveals six species within the *Anopheles punctulatus* complex of mosquitoes in Papua New Guinea. *Med Vet Entomol* 1993;7:37-48.

10 **Beebe NW, Saul A.** Discrimination of all members of the *Anopheles punctulatus* complex by polymerase chain reaction-restriction fragment length

polymorphism analysis. *Am J Trop Med Hyg* 1995;53:478-481.

11 **Cooper RD, Waterson DGE, Frances SP, Beebe NW, Sweeney AW.** Speciation and distribution of members of the *Anopheles punctulatus* (Diptera: Culicidae) group in Papua New Guinea. *J Med Entomol* 2002;39:16-27.

12 **Spencer TET, Spencer M, Venters D.** Malaria vectors in Papua New Guinea. *PNG Med J* 1974;17:22-30.

13 **Peters W, Christian SH.** Studies on the epidemiology of malaria in New Guinea. IV. Unstable highland malaria – the clinical picture. *Trans R Soc Trop Med Hyg* 1960;54:529-536.

14 **Sharp PT.** Highlands malaria: malaria in Enga Province of Papua New Guinea. *PNG Med J* 1982;25:253-260.

15 **McMahon JE.** Malaria endemicity among the semi-nomadic people of the Karimui area of Papua New Guinea. *PNG Med J* 1974;17:99-107.

16 **Hii J, Dyke T, Dagoro H, Sanders RC.** Health impact assessments of malaria and Ross River virus infection in the Southern Highlands Province of Papua New Guinea. *PNG Med J* 1997;40:14-25.

17 **Peters W, Christian SH.** Studies on the epidemiology of malaria in New Guinea. V. Unstable highland malaria – the entomological picture. *Trans R Soc Trop Med Hyg* 1960;54:537-542.

18 **Bockarie M, Kazura J, Alexander N, Dagoro H, Bockarie F, Perry R, Alpers MP.** Transmission dynamics of *Wuchereria bancrofti* in East Sepik Province, Papua New Guinea. *Am J Trop Med Hyg* 1996;54:577-581.

19 **Belkin JN.** The Mosquitoes of the South Pacific (Diptera, Culicidae), Vol 1. Berkeley: University of California Press,1962.

20 **Bangs MJ, Purnomo, Anthony RL.** *Plasmodium ovale* in the highlands of Irian Jaya, Indonesia. *Ann Trop Med Parasitol* 1992;86:307-308.

21 **Pribadi W, Sutanto I, Atmosoedjono S, Rasidi R, Surya LK, Susanto L.** Malaria situation in several villages around Timika, south central Irian Jaya, Indonesia. *Southeast Asian J Trop Med Public Health* 1998;29:228-235.

22 **McMillan B.** Further observations on ovale malaria in New Guinea. *Trop Geogr Med* 1968;20:342-346.

23 **Hombhanje FW.** *Plasmodium ovale* species in Papua New Guinea – lest we forget. *PNG Med J* 1998;41:116-118.

24 **Peters W.** Investigations of malaria and mosquitoes in New Guinea. MD Thesis, University of London, 1965.

25 **Spencer TET.** The malaria pattern in the Territory of Papua and New Guinea. *Med J Aust* 1962;2:873-877.

26 **Anthony RL, Bangs MJ, Hamzah N, Basri H, Purnomo, Subianto B.** Heightened transmission of stable malaria in an isolated population in the highlands of Irian Jaya, Indonesia. *Am J Trop Med Hyg* 1992;46:346-356.

27 **Ebsworth P, Bryan JH, Foley DH.** Ecological distribution of mosquito larvae of the *Anopheles punctulatus* group on Niolam (Lihir) island, Papua New Guinea. *J Am Mosq Control Assoc* 2001;17:181-185.

28 **Lee DJ, Hicks MM, Griffiths M, Debenham ML, Bryan JH, Russell RC, Geary M, Marks EN.** The Culicidae of the Australasian Region, Vol 5. Canberra: Australian Government Publishing Service, 1987.

29 **Hii JLK, Smith T, Mai A, Mellor S, Lewis D, Alexander N, Alpers MP.** Spatial and temporal variation in abundance of *Anopheles* (Diptera: Culicidae) in a malaria endemic area in Papua New Guinea. *J Med Entomol* 1997;34:193-205.

30 **Gilles HM, Warrell DA.** Bruce-Chwatt's Essential Malariology, 3rd edition. London: Arnold Headline Group, 1993:45.

31 **Radford AJ, Van Leeuwen H, Christian SH.** Social aspects in the changing epidemiology of malaria in the highlands of New Guinea. *Ann Trop Med Parasitol* 1976;70:11-23.

32 **Arez AP, Palsson K, Pinto J, Franco AS, Dinis J, Jaenson TG, Snounou G, do-Rosario VE.** Transmission of mixed malaria species and strains by mosquitoes, as detected by PCR, in a study area in Guinea-Bissau. *Parassitologia* 1997;39:65-70.

33 **McKenzie FE, Bossert WH.** Mixed-species *Plasmodium* infections of *Anopheles* (Diptera: Culicidae). *J Med Entomol* 1997;34:417-425.