Malaria and *Anopheles spp* in the villages of Salubarana and Kadaila, Mamuju District, South Sulawesi Province, Indonesia

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Abstrak

Epidemiologi malaria di desa Salubarana dan Kadaila, Kabupaten Mamuju, Provinsi Sulawesi Selatan, Indonesia telah diteliti selama bulan Juli sampai December 2002 untuk mendapatkan data dasar yang berkaitan dengan faktor parasit, vektor, inang, dan lingkungan. Survei malariometrik bulanan selama kurun waktu 6 bulan menemukan suatu prevalensi malaria yang relatif tinggi di Kadaila dibandingkan dengan Salubarana. Kadaila terutama dihuni oleh transmigran dari Jawa, Bali, Lombok dan dari kabupaten lain di Sulawesi Selatan, sedangkan Salubarana dihuni oleh penduduk asli suku Mandar. Pada analisis 1.113 apusan darah yang diperoleh dari individu-individu yang dilibatkan secara acak pada survei ini ditemukan 59 positif malaria, terdiri dari Plasmodium falciparum dan P. vivax. Kedua spesies tersebut mendominasi apusan darah yang diperiksa dan kadang-kadang ditemukan sebagai infeksi campuran. Anopheles barbirostris dikonfirmasi sebagai vektor malaria pada kedua desa, sedangkan 7 spesies lainnya An. barbumbrosus, An. parangensis, An. vagus, An. crawfordi, An. pseudobarbirostris, An. tessellatus dan An. subpictus harus pula dipertimbangkan sebagai vektor. Di kedua desa, An. barbirostris mendominasi dan pada umumnya vektor ini menggunakan genangan air di sungai dan tanah sebagai tempat perindukan serta memiliki kebiasaan istirahat di dalam dan di luar rumah. Temuan-temuan ini dapat digunakan untuk penyusunan suatu program penanggulangan malaria yang berbasis bukti di daerah tersebut. **(Med J Indones 2003; 12: 252-8)**

Abstract

Malaria epidemiology in Salubarana and Kadaila villages, Mamuju District, South Sulawesi Province, Indonesia was studied from July-December 2002 to obtain baseline data related to the parasite, mosquito vector, human host, and environmental factors. Monthly malariometric surveys conducted during the six- month period revealed a relatively high prevalence of malaria in Kadaila in comparison to Salubarana. Kadaila was mainly inhabited by migrants from Java, Bali, Lombok, and from other districts of South Sulawesi. Salubarana, on the other hand, was inhabited mainly by indigenous Mandarese. Analysis of 1,113 blood smears taken from individuals randomly involved in the survey revealed 59 positive samples, consisting of Plasmodium falciparum or P. vivax. These two species predominated the samples examined and were occasionally found as mixed infection. Anopheles barbirostris was confirmed as a vector for malaria in this area whereas 7 other species An. barbumbrosus, An. parangensis, An. vagus, An. crawfordi, An. pseudobarbirostris, An. Tessellatus, and An. subpictus should be considered as potential vectors. The first mentioned species predominated, using mainly riverbeds and ground pools as breeding places with a habit of indoor or outdoor resting. These findings could be useful for establishing evidence-based malaria control program in the area. **(Med J Indones 2003; 12: 252-8)**

Keywords: *malaria epidemiology, parasite, vectors, environmental factors, Indonesia*

Malaria remains a major public health problem in Indonesia. During 1999, the annual parasite incidence (API) at the provincial level in Java and Bali ranged from 0.12 - 9.97%. Annual malaria incidence (AMI) in other islands ranged from 3% in Aceh to 16.8% in East Nusa Tenggara.[1] Currently, about 46.2% of the total 210.6 million Indonesian population reside in malaria endemic areas and among these, 56.3 million are inhabiting moderate to high risk areas. The malaria incidence increased during the last few years as evidenced by the outbreak and re-emergence of malaria in areas previously declared free.

The morbidity rate of malaria in South Sulawesi Province within the last five years showed a relatively

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stable figure at 0.48%.[2] Twenty districts were reported endemic for malaria. Although its AMI is relatively low in comparison to other provinces in Indonesia, the level of malaria endemicity is quite high in certain rural areas, most notably in Mamuju District, where the AMI in the year 2002 was reported at level 1.17%. Entomological studies conducted over several decades had identified more than 80 species of Anopheline mosquitoes in Indonesia. Of these, 21 species have been confirmed as malaria vectors in various endemic areas. On the island of Sulawesi, 7 species consisting of *Anopheles subpictus, An. barbirostris An. minimus, An. nigerrimus, A. sundaicus, An. flavirostris* and *An. lulowae* were confirmed as vectors.[3-6]

Mamuju is one of the 26 districts of South Sulawesi that has long been known endemic for malaria and filariasis.[7-8] Three species of malaria parasites, *Plasmodium falciparum, P. vivax,* and *P. malariae* have been reported from this area. The main vector for malaria is *An. barbirostris* whereas for filariasis it is *Mansonia uniformis*. Although there had been several reports indicating that *An. barbirosstris* could transmit both malaria and Brugian filariasis[9-14], it remains unclear as to whether in this area *An. barbirostris* serves as vector for both diseases.

The establishment of an effective and suitable malaria control program is highly dependent on the availability of valid data on the malaria parasite, mosquito vector, human host and environment. However, very few if any, data are available related to the aforementioned factors. Until now, available data on malaria incidence in the area were based on clinical symptoms of malaria without microscopic confirmation. Therefore, health professionals in this area have difficulties in monitoring the efficacy of different malaria treatment.

The study was intended to obtain baseline data regarding the parasites, mosquito vector, human and environmental factors in the Kadaila village, Mamuju District, South Sulawesi Province, Indonesia. Data obtained will be important for the establishment of an evidence-based malaria control program in this area.

METHODS

Study site, population and environments. Mamuju District is the northernmost district of South Sulawesi Province that shares a border with the Central Sulawesi Province and is located along the 400-km length of the west coastal line of Sulawesi. Mamuju district occupies a vast area of 11,057 km^2 and is divided into 15 subdistricts (Figure 1). The marshland along its vast coastal line had been either cultivated or converted into fishponds whereas the inland part is a tropical rain forest. Most of the area is used for cacao, coconut and palm oil plantations, however rice is also cultivated. In line with the government policy to re-distribute people from the over-populated islands of Java, Bali and Lombok, several forest areas in Mamuju were opened for human settlements in 1992. This included re-settlement of the local Mandarese ethnic group of Salubarana Village to the area along the newly established trans-Sulawesi road connected the district to the rest of the provinces of Sulawesi and the migration of the Buginese, Javanese, Balinese and Lombokese to the newly established Kadaila Village in 1995. The majority of the 292,987 residents of Mamuju are farmers.

The study was conducted in Salubarana and Kadaila villages, Karossa sub-district from July to December 2002. Salubarana has a population of 1,461 people consisting predominantly of Mandarese ethnic group. Kadaila, located about 6 km from Salubarana, is inhabited by about 1,826 migrants, consisting of 4 main ethic groups: Torajan from South Sulawesi and Javanese, Balinese and Lombokese ethnic groups from other islands of Indonesia. Both villages have been reported as endemic foci for malaria and filariasis in Mamuju with mesoendemic status for infection by *P. vivax* and *P. falciparum*. The two villages were selected for this study based on their different population characteristics, in which Salubarana was inhabited by native indigenous Mandarese whereas Kadaila was inhabited mainly by migrants. The climate in both villages is typically tropical, warm and humid throughout the year with little variation in temperature. There are two main seasons, dry season between April and September and monsoon occurring between October and March. Both villages are located in the lowland but the Kadaila Village in particular is characterized by peat soil. The average temperature during the survey in both Salubarana and Kadaila villages was relatively similar. The temperature was between 25-30^o C whereas the humidity between 80-95%.

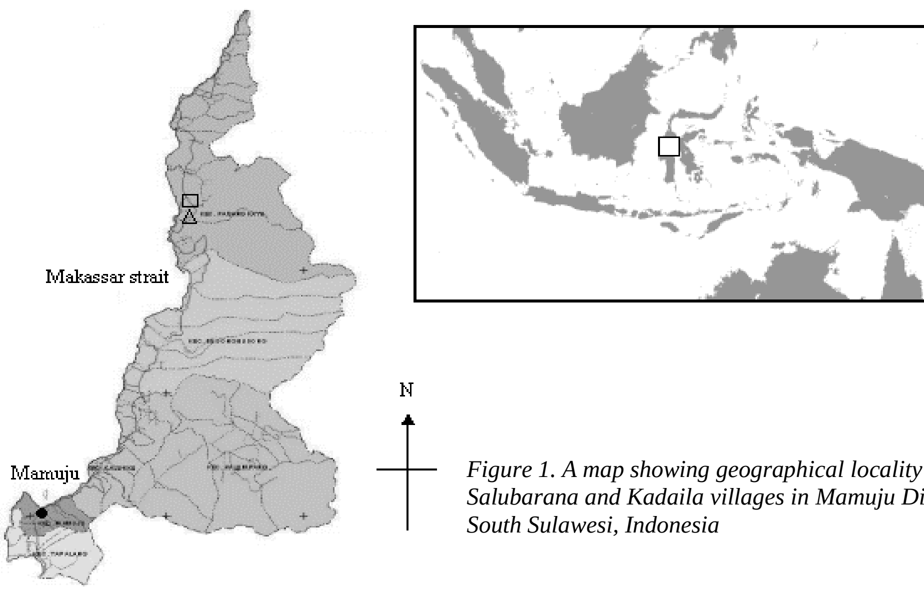

Figure 1. A map showing geographical locality of Salubarana and Kadaila villages in Mamuju District, South Sulawesi, Indonesia

Health care services. Like in other parts of Indonesia, Karossa sub-district has a primary health center located in Salubarana to serve all villages within the sub district including Kadaila. A physician is responsible for the clinical care and outpatient clinic whereas at the village level, there is a sub-health center, which is run by a nurse or midwife. The system provides health services for all residents of the Karossa sub-district. At the time of the survey, no microscope was available at the health center. Therefore, malaria diagnosis in this area was based on clinical signs and symptoms followed by treatment, usually given without monitoring.

Malariometric surveys. Monthly malariometric surveys were conducted between July and December 2002 in both villages, consisting of the examination of Giemsa-stained thick and thin blood smears. In addition, molecular analyses of parasites were performed using blood blots. Spleen palpation was not done considering that most of the sample population were adults. The samples were determined by using stratified random sampling at which each household was represented by at least one sample. An average of 200 subjects, age ranging from 9 months to 65 years, from both villages who presented a written informed consent, were involved in each survey. A total of 1,113 blood samples consisted mostly of the same subjects, 446 of Salubarana and 667 of Kadaila were collected during the six-month period. Slide positive individuals were treated with the standard regimen of either chloroquine or sulfadoxine-pyrimethamine as recommended by the Ministry of Health at each survey. Treatment outcomes and molecular analyses of the parasites will be discussed elsewhere.

Parasite detection. Thick and thin blood smears, taken during the malariometric survey and stained with Giemsa, were subsequently examined under light microscope. Parasite density was determined by counting the number of asexual parasites per leukocytes in 100x magnification in a Giemsa-stained thick film, assuming an average of 20 leukocytes per microscopic field and 8000 leukocytes/μl of blood. Slides were declared negative if parasite could not be detected in 100 microscopic fields. Ethical clearance regarding the involvement of human subjects was obtained from the Ethical Committee on Biomedical Research, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia.

Mosquito surveys. Entomological surveys were conducted every month, from July to December 2002 in both Salubarana and Kadaila villages using larval and adult collection methods[15]. Adult mosquitoes were collected using light traps, capture on landing sites and human bites inside and outside the house. Larva and/or pupae were collected in various different potential habitats. For each habitat sampled, a collection record describing water temperature, conductivity, salinity and pH were measured. Aquatic stages were transported to the field laboratory and individually link-reared to the adult stage. The obtained larva and adult mosquitoes were then identified morphologically to determine the species variation using the illustrated keys of Indonesian anophelines.[4-5]

RESULTS

A total of 1,113 inhabitants of Salubarana and Kadaila were involved in this study during the period of July to December 2002. Microscopic examinations of blood smears indicated that the malaria prevalence was consistently higher in Kadaila. In both villages, the malaria prevalence was found to be relatively high in July and December whereas the lowest incidence was detected in August. The infection rates in Salubarana was 0.80% whereas in Kadaila it was 7.94% (Table 1). No malaria infection was observed during August, September and November in Salubarana.

Two malaria species were detected in both villages, *P. falciparum* and *P. vivax*, either as single or to a much lesser extent as mixed infections. In addition, infection with *P. falciparum* was more frequent than the *P. vivax*, counting for 67.0% of all infections. The parasite density, either asexual or sexual stages, was also higher in Kadaila. Gametocytes were found three of the 4 malaria positive samples in Salubarana whereas in Kadaila, the gametocyte prevalence was 24.5% (Table 1). The highest gametocyte prevalence in Kadaila was detected in November (67.0%).

Mosquitoe vectors at Salubarana and Kadaila villages

Larval collection

Larval collection using a dipper in various potential breeding sites in Salubarana and Kadaila revealed 5 species of anopheline mosquitoes: *An. barbirostris*, *An. barbumbrosus*, *An. parangensis*, *An. vagus*, *An. pseudobarbirostris* (Table. 2). In Salubarana, the breeding places were located mostly around the houses whereas in Kadaila no larvae of *Anopheles* could be found in streams, swamps, ditches or fish ponds around the houses.

Table 1. Slide positivity rates, species distribution and gametocyte frequency in Salubarana and Kadaila villages

Village	*Plasmodium falciparum*	*P. vivax*	Mixed infection	Gametocyte (%)	Total slides	Slide positivity rate
Salubarana	3		1	75%	446	0.8
Kadaila	35	17	1	24.5%	667	7.94
Total	38	17	2	28%	1113	5.1

Table 2. Larvae of *Anopheles* species collected in Salubarana and Kadaila villages and their breeding sites

Anopheline species	Breeding places				
	Riverbed pool	Ground pool	Fishpond	Swamp	Stream
An. barbirostris	+	+	-	-	-
An. barbumbrosus	+	-	-	+	+
An. parangensis	-	-	+	+	-
An. vagus	+	+	-	+	-

Adult collection

Light trap, capture on resting/landing sites and human bite collection conducted inside and outside 5 houses at both villages revealed that *An. barbirostris* was the predominant species. In Kadaila, *An. barbirostris* was the only *anopheline* species found by human baits/landing methods. In Salubarana, three other *Anopheles* species *i.e*. *An. subpictus*, *An. tessellatus* and *An vagus* accounted for 5%, 5% and 10% respectively of the total adult mosquitoes were cached., Several anopheline species were also collected by light traps in Kadaila and Salubarana (Table 3). There was a difference in the mean hour density (MHD) observation in both villages. In Salubarana, the MHD peak was observed at dawn, from 4-5 AM and at dusk 6-7 PM whereas in Kadaila the MHD peak was between 5 to 6 AM. Approximately 5,000 adult mosquitoes were collected during the 6 months observation, consisting of 4 major species, *An. barbirostris*, *An. subpictus*, *An. tessellatus* and *An. vagus* (Table 3). However, dissection of salivary glands revealed the presence of sporozoites only in *An. barbirostris*. More anopheline species were detected at Salubarana. Beside anopheline mosquitoes in both villages, *Mansonia spp, Aedes albopictus* and several *Culex* species were found. However, *Ae. aegypti* was not found so far.

DISCUSSION

During the 6 months observation, the slide positivity rates indicated that the malaria prevalence in Salubarana village was hypoendemic, whereas in Kadaila malaria seemed to be at the borderline level of hypo-to mesoendemic status. This means that in Salubarana the impact of malaria was relatively little on the public health but was relatively important in Kadaila.

There are several explanations that may address this disparity. First, Salubarana was inhabited by ethnic Mandarese who has been living in the area for several generations and were therefore exposed to malaria for most of their lives whereas in Kadaila, most of the inhabitants were from relatively non-malarious areas. This may attribute to the existence of naturally acquired immunity to malaria among the native Mandarese. Previous studies conducted in West Papua Province discovered similar findings. The naturally acquired immunity for malaria developed in an age-dependent pattern after two years exposure.[16-17] Second, there may be differences in the intensity of exposure to mosquito vectors *i.e*. in Kadaila, exposure to the vector may be relatively more than that of Salubarana. This fact however, is in contrast with the finding that in Salubarana more species of *Anopheles* were found around the houses and the density of the vectors collected in both villages during the indoor and outdoors landing capture were relatively similar. Considering that the inhabitants in Kadaila has already settled in the village for more than 5 years, it seems that the significant differences of malaria in Salubarana and Kadaila may be influenced by another factor such as genetic constitution of the host.

The biting habits of the mosquitoes in Kadaila seemed to peak at dawn whereas in Salubarana mosquitoes were detected both at dusk and dawn. This finding could be possibly associated to the fact that in Salubarana more species of *Anopheles* were found whereas in Kadaila only *An. barbirostris* was found around the house during the man biting experiment. Also, this finding indicates that in Kadaila *An. barbirostris* plays an important role in malaria transmission whereas in Salubarana *An. vagus*, *An subpictus* and *An tessellatus* may also be potential vectors. Previous studies in the adjacent sub-district of Babana, Mamuju, found that *Anopheles barbirostris* and *An. vagus* seemed to play a major role in malaria transmission in the area (unpublished observation).

Table. 3. Adults mosquitoes collected in Salubarana and Kadaila villages

Anopheles species	Methods of collection	Villages
An. barbirostris	Light trap, in and outdoors	Salubarana, Kadaila
An. subpictus	In and outdoors	Salubarana
An. tessellatus	In and outdoors	Salubarana
An. vagus	Light trap, in and outdoors	Salubarana and Kadaila
An. pseudobarbirostris	Light trap	Kadaila
An. crawfordi	Light trap	Kadaila
An. barbumbrosus	Light trap	Kadaila
An. parangensis	Light trap	Kadaila

During the survey in Kadaila, we could not identify the presence of any breeding sites for *Anopheles* around the houses. This finding may be associated with the environmental condition of Kadaila where most of the land is peat soil. This condition caused relative acidity of the water around the houses in Kadaila which does not favour larval breeding. It is highly possible that the *Anopheles* vector in the village flew from the nearby areas where the environment was favourable for breeding sites. This may explain why in Kadaila *An. barbirostris* biting activity peaked at a later time.

The higher incidence of malaria in Kadaila also alerted us to the need of proper planning for people resettlement programs in Indonesia. The finding that after seven years settlement, the malaria prevalence is still relatively high among the migrant people in comparison to the native people in the nearby village indicates that they have not been well protected. The fact that all malaria treatments in both villages were given without proper monitoring and confirmation by microscopy also contribute to the maintenance of transmission as revealed by the relatively high prevalence of gametocytes among the positive samples.

In conclusion, malaria is currently on the increase in many parts of Indonesia. This phenomenon was associated with several factors such as large scale of human migration due to civil conflicts, inadequate health services due to monetary crisis, resistance of the malaria parasite to the available antimalarial drugs and resistance of the mosquito to the insecticide. To circumvent the situation, a new approach in malaria control, based on the careful assessment of the local contributing factors such as the malaria parasite, the human host and the environment should be established.

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