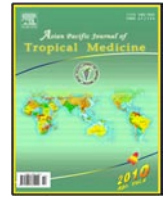




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Malaria epidemiology in the Democratic Republic of East Timor

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ABSTRACT

Objective: To investigate malaria prevalence and socio-economic conditions in East Timor. **Methods:** Blood samples were collected from 650 individuals distributed among six districts in East Timor. 434 and 216 individuals were sampled by passive and active case detection, respectively (PCD, ACD). **Results:** The results showed that the plasmodium infection prevalence was 18.9% with predominance of *Plasmodium falciparum* (*P. falciparum*) infections (60.2%). The majority of cases were detected in children between 2–14 years old in both PCD and ACD. Participants residing in Same and Lospalos were at a higher risk of malaria. The percentage of infections associated to the lack of bed net use was twice as high as users (25.2% vs 12.5%). The prevalence of malaria among participants who lived in brick/cement houses (8.3%) was two times less than those who lived in other types of houses (3.7%). There was a significantly lower prevalence of infection detected in individuals living in complete housing constructions with absence of domestic animals in or near the houses. **Conclusions:** The major risk factors for malaria in East Timor are age, lack of bed net use, incomplete housing constructions and exposure to mosquitoes.

1. Introduction

Malaria is one of East Timor's major public health problems. The disease is endemic throughout most of the country, with the highest morbidity and mortality rates reported in children^[1]. It is estimated that at least 150 people die yearly as direct consequence of malaria^[2]. Eighty-five percent of the populations exposed to malaria live in rural areas, distant from easy access to health services, particularly during the rainy season. Until 1998, malaria vector control was based upon house spraying with DDT (1982–1991) and Bendiocarb (since 1992).

Around 10 000 cases of malaria were diagnosed in 1998^[3], but among 2000–2006, malaria cases increased considerably^[4]. The total number of deaths was 132 in 2000 (www.searo.who.int/en/Section10), 100 in 2003, 65 in 2004, 88 in 2005 and 68 in 2008^[4]. *Plasmodium falciparum* (*P. falciparum*) and *Plasmodium vivax* (*P. vivax*) malaria are equally represented^[1].

In 2003, the national malaria control program was established with three main objectives 1) to guarantee that the entire population would have access to prompt and effective treatment, 2) to provide malaria prevention

measures (ITNs) to young children and pregnant women and 3) to ensure the use of appropriate vector control methods as well as dissemination of information and community education (RBM monitoring and evaluation 2005).

Although the malaria control programme was initiated, a number of factors such as socio-economic and environmental, lack of knowledge on the causes of malaria, animal breeding very near households, low educational levels and poor housing structures significantly affected its success^[5,6]. A study by Gamege–Mendis *et al*^[7], reported a strong association between malaria incidence and the type of house construction, independent of the house location.

No previous study has assessed and quantified the effect of socio-economic factors on the distribution of *P. falciparum* and *P. vivax* infection in East Timor. Therefore, in the present work we evaluated some socio-economic conditions as potential risk factors for malaria prevalence, assessed the types of houses in determining the abundance of indoor resting malaria vectors and characterized the prevalence of malaria transmission in six districts, in East Timor.

2. Materials and methods

2.1. Study area and population

The Democratic Republic of East Timor is situated on the eastern part of the island of West Timor–Indonesia, with a

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surface area of 14.610 square kilometres, divided into 13 districts, 67 sub-districts and 498 villages.

This study was carried out in 6 districts classified into three different zones which differ in ecological and climatic features which may contribute directly or indirectly to malaria transmission: North (Dili and Liquiça), South (Suai, Same and Viqueque) and East (Lospalos).

2.2. Study population

The study was conducted from 15 December 2004 to 9 March 2005. Blood sample collections were carried out from a total of 650 individuals from all ages. Overall, sex distribution in the six districts was similar ($\chi^2=3.9$, $P>0.05$). Approximately half of the participants (55.7%) were more than 15 years old, thus considered as adults. The remaining which included all participants with an age up to 14 years old, were considered as children, and distributed as follows: 7.2% below 2 years of age, 14% between 2–4 yrs, 12.3% between 5–9 yrs, 10.8% between 10–14 yrs. A total of 216 individuals were sampled by active case detection (ACD) from 71 households. According to active and passive case detection, participants were also stratified into five age groups: under two years old, two to four years old, five to nine years old, teen to fourteen, and adults. The percentages of participants in each age group were 2.3%, 7.5%, 12%, 12%, 66.2% for ACD and 9.7%, 17.3%, 12.4%, 10.1% and 50.5% for PCD. Sample collections were made from all inhabitants within each household after procedure explanation, study justification and informed consent, in local dialect. Participants were given a questionnaire on travel patterns in and out of the study area, occupation, educational level, family members, habit of bed net use, malaria history and antimalarial drug intake within the previous 6 months. Other observations were made on type of housing and house construction materials, presence of animals in the compound and/or mosquito breeding sites in/ close to the house. All these participants were asymptomatic as no fever was detected at time of blood collection.

A second study group of 434 individuals was included, at the local health centre/hospital facilities with symptoms of headache and fever, and sampled for malaria in passive case detection (PCD) method. Here no notifications of household characteristics were made. The results of 650 blood samples analyzed by both optical microscopy (OM) and Polymerase Chain Reaction (PCR) were compared.

2.3. Data collection

Finger-prick collected blood was spotted on Whatman#4 for DNA preparation in parallel with Giemsa stained blood smears for microscopical examination. Parasite DNA was obtained by the phenol–chloroform method^[8] followed by ethanol precipitation. *P. falciparum* and *P. vivax* detection was performed by nested-PCR amplification of the small subunit ribosomal RNA genes^[9].

2.4. Data analysis

Statistical analysis was performed using SPSS 11.5 for Windows (SPSS, Inc.) and a significance level of 5% was considered for all tests. Differences between groups were assessed using Pearson's χ^2 Test. Whenever 25% of expected counts were less than 5, significance was determined by Fisher's Exact Test at $P<0.05$. Odds ratio, 95% confidence intervals and level of significance were calculated to estimate the risk factors of malaria infection.

2.5. Ethical approval

The study had been approved by Ministry of Health under

the process Ref.:MS/VM/PESQUISA/XII/04.

3. Results

3.1. Infection detection

Of the 650 samples, 123 were positive by PCR, though only 50 of these were diagnosed as positive by OM (40.7%). In addition, OM failed to reveal any mixed infections, which constituted 4.9% (6/123) of all positives as identified by PCR.

Overall malaria prevalence was 18.9% with predominance of *P. falciparum* infections (60.2% *P. falciparum*, 35% *P. vivax*, and 4.8% with both species). The prevalence of mixed infections [6 (4.8%)] detected by PCR in this area was very low and only detected in Dili. The percentages of infection prevalence detected in the <2 yrs, 2–4 yrs, 5–9 yrs, 10–14 yrs, and adults were 14.9%, 24.2%, 27.5%, 24.3% and 15.2%, respectively. In general the prevalence of malaria was higher in children than adults but the difference was only significant when considering the group of 5–9 yrs old (27.5% versus 15.2%, $\chi^2=9.71$, $P<0.05$).

3.2. Socio-economic factors and the malaria infection

Of the 216 participants where samples were collected by ACD in 71 houses, 107 (49%) lived in cement or brick houses, 61 (28%) in rudimentary constructions with clay and branches (stocking houses) and 48 (22%) of individuals slept under other type of houses (similar to huts made of bamboo branches or other greens). This latter sort of construction was mostly seen in Dili (72.2%), Liquiça (50%) and Same (60%), and it was less frequent in Suai, Viqueque and Lospalos (34.7%, 28.6% and 40%, respectively).

Most houses, however, showed incomplete structures allowing for openings that facilitate contact with the mosquito vectors (85.2%). In all districts 88.9% of participants lived in houses with animal presence (cock/hem, pigs, dogs, cattle, buffaloes, cows).

In relation to professional activities, most of the participants were farmers (35.4%) followed by housewives (31.7%), pre-professions (22.7%) and a number of different activities associated to health, teaching or business, etc. (10.2%). The pre-profession category included students with age more than 15 years old which were not included in any of the professional categories.

Reported mosquito net use among participants was relatively low (49.2%) but varied significantly ($\chi^2=27.9$, $P<0.05$) between the six districts with the highest coverage in Dili (54.3%), Liquiça (58.9), Same (54.5%) and 60.6% in Viqueque. Suai and Lospalos had the lowest coverage (30% and 44.4% respectively).

Concerning schooling, 21.5% participants had access to primary school, 45.9% to secondary school, 2.5% to university and 30.1% had no access to education (excluding children under 5 years old). Dili was the district with the higher proportion of university graduate participants (5.1%). The result of the odds ratio analysis (Table 1) showed that, compared to Liquiça district, where the lowest prevalence, participants residing in Same and Lospalos were at a higher risk of malaria. Comparisons made between the regions (south and east zones) showed no significant risk differences. Bed net use had an important effect on the risk of malaria infection. The percentage of infections associated to the lack of bed net use was 25.2% compared to users (12.5%).

There was no statistically significant differences between risk of malaria and education levels (secondary schooling: 20%, university: 7.7% and others/no schools: 15%) when compared to primary (20.9%). By professional categories, malaria affected mostly farmers than others workers (Table

1).

The prevalence of malaria infection among participants who lived in brick/cement houses (8.3%) was two times less than those who lived in other types of houses (3.7%). Additionally, there was a significantly lower prevalence of infection detected in individuals living in complete housing constructions with absence of domestic animals in or near the houses (Table 1).

3.3. Prevalence of malaria by passive and active case detection

Table 2 presented the prevalence of infection by age in the PCD and ACD groups. There was a significant difference between PCD (25.8%) and ACD (5.1%).

Divided the populations at approximately its median into 2 groups (0–14 and older than 15 years) and compared the two age groups using Pearson's χ^2 Test and odds ratio analysis. It showed $\chi^2 = 0.9, P > 0.05$ and $\chi^2 = 7.9, P < 0.01$ for PCD and ACD, respectively. A significant association was found between the age and the infection prevalence in the individuals in ACD.

Table 1

Effect of socio economics factors on malaria infection among participants.

Socio economics factors	Number	Infected (%)	Odds ratio	95% CI	χ^2
Bednet usage					
No	330	25.2*	2.35	1.55 – 3.56	16.95
Yes	320	12.5	1.00		
Zones of residence					
South	285	21.1	1.50	0.94 – 2.31	2.90
East	117	21.4	1.50	0.86 – 2.60	2.03
North	248	15.3	1.00		
Districts					
Dili	175	17.7	2.03	0.86 – 4.85	2.62
Suai	120	17.8	2.00	0.81 – 4.97	2.29
Same	99	28.3*	3.72	1.52 – 9.09	9.06
Viqueque	66	16.7	3.72	1.52 – 9.09	9.06
Lospalos	117	21.4*	2.56	1.05 – 6.28	4.45
Liquiça	73	9.6	1.00		
Professional categories (≥ 15 years)					
Farmers	128	25.0	3.80	1.10 – 13.10	4.90
Housewives	115	3.5	0.41	0.10 – 1.9 0	1.40
Pre professions	82	19.5	2.70	0.70 – 10.10	2.50
Others (healths, teachers, business etc.)	37	8.1	1.00		
Education level (≥ 6 years)					
Secondary	235	20.0	0.94	0.53 – 1.64	0.06
University	13	7.7 Δ	0.32	0.38 – 2.52	1.32
Others (no school)	138	17.0	0.75	0.39 – 1.42	0.79
Primary	109	21.1	1.00		
Type of Houses (N=216)					
Clay & branches (Stocking houses)	61	4.9	1.33	0.29 – 6.16	–
Others	48	8.3	2.15	0.52 – 8.95	–
Brick/Cemen	107	3.7	1.00		
Housing construction					
Complete	32	0.0			
Incomplete	184	6.0	NA	NA	–
Animal presence					
No	24	0.0			
Yes	182	6.0	NA	NA	–

*: $P < 0.05$ based on Pearson's Chi-Square-Test; Δ : P -value based on Fisher's Exact Test; NA: not applicable; OR: odds ratio; CI: confidence interval.

Table 2

Prevalence of malaria infection by passive and active case detection according to age groups.

Age (Years)	Passive case detection (PCD)		Active case detection (ACD)	
	N	infected (%)	N	infected (%)
<14	215	60 (28.0)	73	8 (11)
≥ 15	219	52 (23.7)	143	3 (2.1)
Total	434	112 (25.8)	216	11 (5.1)

4. Discussion

This study presents information on the prevalence of malaria in East Timor, associated to some socioeconomic factors among six districts distributed in three different zones (North, South and East). Our results showed that in general, prevalence and risk of malaria seemed to be higher in the south and east of the island than in the north. These data confirm previous suggestions from Bragonier *et al.*^[10] about the higher levels of transmission in the southern coastal belt and in the far east of the country.

Though this is a small country, there is an effect directly related to the prolonged rainy season in the south and the east, with a visible increase of malaria transmission. In these districts where the climate is mostly wet, though rainfall occurs during a specific period of the year, the presence of permanent breeding sites (open water bodies) is likely to be the main cause for the presence of malaria vectors and consequent transmission.

The natural vegetation is lowland hill forest, but in most areas in these districts human activity is aimed towards agriculture which has mostly damaged these forests and has had, consequently, a dramatic effect on the intensity of malaria transmission. The probability of contact with mosquito vectors is also increased in areas of rice plantation, especially at the usual early hours of field work. In urban areas such as Dili the capital city there are also the usual problems of garbage such as cans or water containers which can work as breeding sites for mosquitoes.

In addition, the poor drainage system in the watershed areas of the Comoro River (in the west) and the Santana River (in the east), waste water from residential areas, poor sanitation, stagnant water in the street during the rain fall, corn cultivation around the houses, the practice of growing kang kung (a green, leafy vegetable crop) in city swamps areas, can additionally contribute to proliferation of mosquitoes.

A problem related to difficulties in malaria diagnosis is strongly associated to the number of health facilities (laboratory services), doctors and microscopists, which are concentrated in the Dili district (capital city), to where patients are directed and where population is more concentrated. Here, therefore, numbers are probably more accurate than in Liquiça where facilities are scarcer.

The relatively low malaria transmission in Liquiça district may be attributed to the hot and dry weather unsuitable for vectors, thereby leading to high vector mortality and reduction in infection. This is because the paucity of creeks on the northern shore and fewer valleys with spring-fed swamps. In addition, most people live above 1500 m of altitude and move to the lowlands plantations and saline planes only for work during the daytime, although a separate study would be required to confirm it.

The overall prevalence of *P. falciparum* single-infections was higher than *P. vivax* and mixed *P. falciparum*+*P. vivax* infections. These findings are consistent with previous reports by Ezard *et al.*^[11] and Kolaczinski & Webster^[12].

Our results re-enforce the notion that in malaria endemic areas where transmission of both *P. falciparum* and *P. vivax* occurs, nested PCR detection of malaria parasites can be a very useful complement to microscopical examination^[13]. Additionally, PCR was able to detect mixed infections that were missed by microscopy, as commonly reported before^{[14–}

18]. In 2000, the Medical Emergency Relief International (MERLIN) was given the task of improving diagnosis and treatment through training of microscopists^[19] but the current results strongly suggest that a continuous effort is required, in training of these technicians and other health workers. Good diagnosis requires well trained personal and prompt treatment of all malaria cases and may be one of the key factors for the prevention of future epidemics in the region.

Malaria infections were quite common in children less than 14 years of age and declined substantially by the age of 15 years. These observations are in agreement with others previously reported^[20–22], indicating increasing protection through acquired immunity. Of particular importance in this study was the prevalence of infection among asymptomatic individuals (5.1%) observed by ACD. Asymptomatic malaria infection could be a significant health problem under two circumstances in this epidemiology setting. Firstly, there may be consequences for the individual of harbouring such an infection since asymptomatic infections may be in the process of being controlled and eliminated but they may also be capable of conversion to clinical disease. Secondly, it may be a public health problem as these asymptomatic carriers could be reservoir of infection capable of infecting others both during and after the nine month dry season^[17]. In fact there were asymptomatic cases with circulating gametocytes, providing the potential to infect mosquitoes and cause additional human cases^[23]. In this context, the need for active case detection in this region must be stressed.

Bed net users in the present study had lower malaria prevalence, which could be expected due to higher levels of protection against mosquito bites (decreases man–vector contact) and hence the risk of malaria infection. These results are similar to those reported by Genton *et al.*^[24] in Papua New Guinea; Lozano *et al.*^[25] in Mexico and Pinto *et al.*^[26] in São Tomé and Príncipe.

The percentage of houses considered to be of high quality is very low. Previous studies have shown that the risk of malaria may be associated with type of house and/or housing construction^[5–7,27–29], with occupational exposure^[30] and/or with education level^[31]. In the present study, with exception of educational level, we have found evidence that other socio-economic factors are important determinants of malaria infection. The risk to getting malaria infection was apparently greater for inhabitants of the poorest type of houses, characterized by houses made from stocking wall (incomplete wall) and others (e.g. bamboos, branch of the palm materials used etc.) compared with individuals living in brick/concrete houses, but this was not statistically significant. This may perhaps be explained by the fact that most brick/concrete houses were found to have open windows which are more likely to allow passage of mosquitoes. A study by Lyindsay *et al.*^[32] found that houses with open eaves or lacking ceilings are associated with increased number of mosquitoes indoors (house entry by mosquitoes). Also, we found additional risk of infection in presence of domestic animals in or near the houses. We also found that the agriculture workers appear to be at higher risk of malaria when compared with other types of occupation (e.g. teachers, health's, business etc). This situation should be alleviated by displacement of domestic animals and clearance of vegetation around human dwellings and the agriculture workers who sleep in the fields/households

should also use bed nets.

Education on malaria and its causes would help in better planning and implementation of malaria control programmes. Particularly, the nature of socioeconomic networks involving individuals, families and communities is likely to influence the success of prevention and malaria control strategies. Detailed entomological and drug resistance studies will be needed to complement these findings and better understand malaria transmission in the region. Knowledge of these factors is important to gain a better understanding of local malaria epidemiology and implementation of appropriate tools for malaria control.

Conflict of interest statement

We declare that we have no conflict of interest.

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