

Identification of malaria endemicity in rural areas of Nepal: A malariometric and seroepidemiological study in children

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Abstract

A cross-sectional study was performed to determine malaria morbidity and malaria endemicity in children between 2-9 years of age in three villages of Southern Nepal. Malariometric survey and seroepidemiology were compared. A total of 604 children from the three villages were tested. Tulashi and Chisapani villages were found to have equal endemicity with high parasitaemia (>12%), high level of malaria antibody (>40%) and high rate of spleen (>60%) indicating a hyperendemic area. In comparison Lumbini village had low parasitaemia (<5%), low antibody level (<25%) and low spleen rate (<25%) indicating a mesoendemic area. This study would be useful for the surveillance system in identifying endemicity and effective planning of malaria control programme in these areas. The variation in endemicity for these villages are multifactorial such as human behaviour in relation to vector behaviour and gradual deterioration of health care system for malaria, which has been discussed in this study.

Keywords: Malariometric survey; Sero-epidemiology; Children; Nepal.

Introduction

The place of seroepidemiological studies as a means for describing the malaria situation in an endemic area has been discussed by several authors (McWilson *et al.*, 1975; Tharavanij *et al.*, 1986; Greenwood *et al.*, 1987). Determination of antibody levels in a population can be used to estimate the level of endemicity to evaluate malaria control programmes and to delineate malarious areas requiring antimalarial interventions. The characteristics of immunity have been described in details in different endemic areas (McGregor, 1986) and, although the precise timing of events varies with differing degrees of transmission, the general features are constant. In areas of high transmission the incidence of malaria attacks is approximately 40 times higher in children of less than 10 years than in adults, and the differential between age groups tends to decrease in areas of low transmission; in older children and in adults, the incidence of clinical attacks is liable to be higher in areas of low transmission than of high transmission (Trape *et al.*, 1993).

The present malariometric survey including seroepidemiological data was performed in the age group 2-9 years from 3 different villages. This age group represents children from the school where the study was performed and children from the villages who assembled in the same school. Hence, this study is an assessment of endemicity of malaria in three rural communities of Southern Nepal.

Materials and Methods

Prior to the study, the head of the villages, parents and school teachers were informed to assemble the children of this age group 2-9. This age group consists of children in the village and those attending school. In order to facilitate the study the school was utilized as a convenient place to collect blood samples. A cross-sectional malariometric and a seroepidemiological survey were carried out from all children in this age group. A total of 604 children, 181 from Tulashi village (ward 1-4, total population 1771), 153 from Chisapani village (total population 1172) and 270 from Kapilvastu village (total population 1450) was included.

Clinical survey

Name, age, sex, current fever and any abnormality observed at physical examination, were recorded for each child. Spleen examinations were carried out in all individuals with subjects lying on their back and the knees flexed in order to ensure maximum relaxation. Besides the examination for determining the presence or absence of an enlarged spleen, the size or degree of enlargement was measured according to WHO classification (WHO, 1988).

Blood samples were collected by finger prick. Blood smears were made, dried and stained with Giemsa. Smears were examined for malaria parasites under a

microscope in 100 fields using a x 1000 magnification.

Parasite density was calculated by counting the number of parasites against 200 leucocytes and multiplied by 40 to obtain a density per cu mm. Parasite rates as well as positive parasite density indices (PPDI) were calculated using the scale defined by Bruce Chwatt (1988).

In order to perform the seroepidemiological tests, ELISA and IFAT, the blood samples were also collected in heparinized capillary tubes and the methods used for this test (ELISA and IFAT) followed the procedures described (Sherchand *et al.*, 1995). Packed-cell volume (PCV) was determined with a micro haematocrit centrifuge.

Results

Kapilvastu village

This village is 3 kms away from Lumbini garden, the birth place of Lord Buddha and is 12 kms from the Indo-Nepal border. There was a total of 270 children aged 2-9 years, in which 182 were male and 88 were female.

Malaria parasitaemia, episodes of fever and spleen rate

Out of 270 children, 25 had current fever of which 14 had malaria parasitaemia. All the children with positive parasitaemia had fever $>37.5^{\circ}\text{C}$; this corresponds to a parasite rate of 5.2% and parasite density index or crude parasite rate of $57/14=4.07$.

Density class		Number		x class
1 = $<100/\mu\text{l}$		1		x 1 = 1
2 = 101 - 200		1		x 2 = 2
3 = 201 - 400		2		x 3 = 6
4 = 401 - 800		4		x 4 = 16
5 = 801 - 1600		4		x 5 = 20
6 = >1601		2		x 6 = 12
		14		57

For the determination of the degree of enlarged spleens 61 had a splenic enlargement. (i.e Spleen rate $61/270 \times 100 = 22.6\%$).

Spleen class		Number		X class
0		209		X 0 = 0
1		22		X 1 = 22
2		13		X 2 = 26
3		20		X 3 = 60
4		6		X 4 = 24
5		0		X 5 = 0
		61		132

Average enlarged spleen was $132/61 = 2.16$.

Anaemia and malaria

The packed cell volume (PCV) was measured for 270 children in which 62 children were anaemic with a PCV of less than 30%, of which 8 were in 2-4 year age group and 54 in 5-9 year age group. Of the 14 children who had malaria, 6 had a PCV of less than

30%, 6 had a PCV between 31 to 34% and 2 had a PCV 38% and 40% respectively. The association between malaria infection in children with anaemia (<30% PCV) and without anaemia showed no significant difference ($P>0.05$).

Serological findings

The serological test results using ELISA and IFAT showed that 62 children (23%) were ELISA positive and 65 (24.1%) were IFAT positive. There was no significant difference between in age specific group and among ELISA and IFAT test (Table I).

Table I: Results of ELISA and IFAT test using *in vitro* cultured *P. falciparum* antigen in age specific groups of children.

Age (yrs)	ELISA positive	ELISA negative	IFAT positive	IFAT negative
2-4	10	37	10	37
5-9	52	171	55	168
Total	62/270*	208	65/270*	205

* χ^2 , $P>0.05$.

Antimalarial antibodies absorbance (O.D.) values using ELISA test

The positive ELISA test result showed an absorbance (O.D.) value of 0.25 (cut off titre) or above. Of a total of 270 cases, 208 (77%) had an absorbance value of less than 0.25, whereas 62 (23%) children had absorbance value of 0.25 or above (Figure 1). There was no significant difference between antibodies absorbance values in age specific groups of children ($P>0.05$).

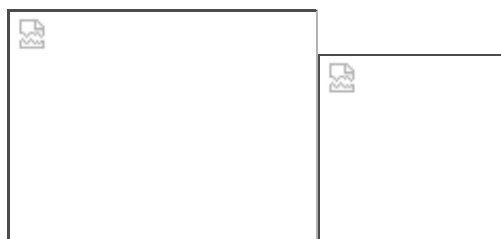


Fig. 1: Distribution of anti-*P. falciparum* ELISA absorbance values in children.



Fig. 2: Frequency distribution of IFAT antibody titres (in different age groups of children).

Antimalarial antibody titre using IFAT test

Out of 270 children, 65 (24.1%) children had a positive antibody titre (i.e. $>1/40$). The highest antibody titre (1/640) was found in 6 children who had *P. falciparum* malaria. No significant difference was found in the level of antibody titres in age specific groups of children ($P>0.05$). The frequency distribution on antibody titres by age specific groups of children is shown in Figure 2.

Tulashi village

Of 181 children from Tulashi village, 83 were male and 98 were female. There were 88 children aged between 2-4, and 93 children aged between 5-9.

Malaria parasitaemia, episodes of fever and Spleen rate

Out of 23 malaria positive cases, 13 children had an episode of fever, and 10 children who had malaria, had no episode of fever and corresponds to a parasite rate of 12.7% with parasite density index (89/23) of 3.86.

Density class		Number		X class
1 = <100/ul		2		x 1 = 2
2 = 101 - 200		2		x 2 = 4
3 = 201 - 400		4		x 3 = 12
4 = 401 - 800		6		x 4 = 24
5 = 801 - 1600		7		x 5 = 35
6 = >1601		2		x 6 = 12
		23		89

Examination for spleen enlargement

Out of 181 children aged between 2-9 years, 115 had a splenic enlargement.

(i.e. Spleen rate $115/181 \times 100 = 63.5\%$).

Spleen class		Number		X class
0		66		x 0 = 0
1		65		x 1 = 65
2		23		x 2 = 46
3		16		x 3 = 48
4		10		x 4 = 40
5		1		x 5 = 5
		115		204

Average enlarged spleen was $204/115 = 1.77$.

Anaemia and malaria

Out of 181 children, 73 were anaemic with a PCV of less than 30% of which 34 were in 2-4 year age group and 39 in 5-9 year age group. Of the 23 children who had malaria, 13 had a PCV of less than 30%, 8 had a PCV between 31 to 34% and 2 had a PCV between 35-38%. The association between malaria infection in children with anaemia (<30% PCV) and without anaemia showed no significant difference ($P > 0.05$).

Serological findings

Out of a total of 181 specimens, 66 (36.5%) children were ELISA positive and 59 (32.6%) children were IFAT positive. In age specific group of children between 2-4 years, 32 (17.7%) were ELISA and 29 (16%) were IFAT positive. In 5-9 year age group 34 (18.8%) and 30 (16.6%) were ELISA and IFAT positive respectively (Table II). Out of a total of 181 cases, 115 (63.5%) had an absorbance value of less than 0.25, whereas 66 (36.5%) children had absorbance value of 0.25 or above (Figure 3). In the case of IFAT test, 59 (32.5%) had an absorbance value above 1/40 (Figure 4).

Table II: Results of ELISA and IFAT test using *in vitro* cultured *P. falciparum* antigen in different age groups of children.

Age (yrs)	ELISA positive	ELISA negative	IFAT positive	IFAT negative
2-4	32	56	29	58

5-9	34	59	30	64
Total	66/181*	115	59/181*	122

* $\chi^2 = 0.60$; $P > 0.05$



Fig. 3: Distribution of anti-P. falciparum ELISA absorbance values in children.



Fig. 4: Frequency distribution of IFAT antibody titres (in different age groups of children).

Chisapani village

Out of a total of 153 children from Chisapani village, 65 (42.5%) were between 2 to 4 years, and 88 (57.5%) were between 5 to 9 years. Of this 74 were male and 79 were female.

Malaria parasitaemia, episodes of fever and spleen rate

Out of 153 children, 28 (18.3%) had episodes of fever and 125 (81.7%) had no episodes of fever. Those children who had an episode of fever were 14 (9.1%), and were malaria positive. 5 (3.3%) cases of malaria positive had no episode of fever. There was no significant difference in two group of children having malaria with and without fever ($\chi^2 = 2.47$; $P > 0.05$); however, a high rate of malaria positivity was found in children with fever. The parasite rate is 12.4% and the crude parasite rate or density index is (79/19) 4.15.

Density class		Number		X class
1 = <100/ul		1		x 1 = 1
2 = 100 - 200		2		x 2 = 4
3 = 201 - 400		1		x 3 = 3
4 = 401 - 800		6		x 4 = 24
5 = 801 - 1600		7		x 5 = 35
6 = >1601		2		x 6 = 12
		19		79

Examination of spleen enlargement

Out of a total of 153 children, 103 children were found to have different degrees of spleen enlargement. The spleen rate being: $103/153 \times 100 = 67.3\%$.

Spleen class		Number		X class
0		50		x 0 = 0
1		61		x 1 = 61
2		20		x 2 = 40

3		13		x 3 = 39
4		9		x 4 = 36
5		0		x 5 = 0
		103		176

Average enlarged spleen was $176/103 = 1.708$.

Anaemia and malaria

Out of 19 malaria children, 15 had PCV less than 30%; 3 had PCV between 31-34%, and 1 child had a PCV between 35-38%. The association between malaria infection in children with anaemia (<30% PCV) and without anaemia showed significant difference ($P < 0.05$).

Serological findings

Of the total of 153 specimens, 41 (26.8%) were IFAT positive and 48 (31.4%) were ELISA positive. In 2-4 years age group, 28 (18.3%) were ELISA positive and 19 (12.4%) were IFAT positive, whereas in 5-9 years age group, 20 (13.1%) were ELISA positive and 22 (14.4%) were IFAT positive (Table III). A positive ELISA titre above 0.25 was 31.4% (Figure 5) and IFAT titre above 1/40 was 26.8% (Figure 6).

Table III: Results of ELISA and IFAT test using *in vitro* cultured *P. falciparum* antigen in different age groups of children.

Age (yrs)	ELISA positive	ELISA negative	IFAT positive	IFAT negative
2-4	28	37	19	46
5-9	20	68	22	66
Total	48*	105	41*	112

N=153, ELISA positive 48 (31.4%), N=153, IFAT positive 41 (26.8%).

* $\chi^2 P > 0.05$

Use of *P. vivax* antigen and comparison with *P. falciparum* antigens

P. vivax antigen prepared by local strain of Tulashichauda village, was used. Of the total of 604 samples, 510 samples were included for ELISA due to paucity of samples. Similarly the IFAT could not be performed. The ELISA test for anti-*P. vivax* antibodies was tested on 180 samples from Tulashi village, 140 samples from Chisapani village and 190 samples from Kapilvastu village.

With the ELISA using *P. falciparum* and *P. vivax* antigens from 3 villages, the samples had detectable antibodies levels. However, antibodies were detected higher with *P. falciparum* antigen (29.1%) compared to *P. vivax* antigen (23.1%) (Figure 7).

ELISA positive rate by *P. falciparum* antigens was $176 \times 100 / 604 = 29.13\%$ and ELISA positive rate by *P. vivax* antigens was $118 \times 100 / 510 = 23.13\%$. However, there was no statistical significant difference between two antigens for ELISA test ($P > 0.05$), but a higher positivity rate was found in the samples tested by using *P. falciparum* antigen compared to same samples tested by *P. vivax* antigen.



Fig. 5: Distribution of anti-*P. falciparum* ELISA absorbance values in children.



Fig. 6: Frequency distribution of IFAT antibody titres (in different age groups of children).



Fig. 7: Comparison of ELISA (OD) values using *P. falciparum* and *P. vivax* antigens (Tulashi, Chisapani and Kapilvastu villages).

Overall comparison between malario-metric and serology surveys

The overall comparison of malaria parasitaemia, spleen rate, parasite density index, and antimalarial antibodies by ELISA and IFAT from three villages are shown in Table IV.

Table IV: An overall comparison.

Villages	Parasitaemia	Spleen rate	Parasite	Antibodies	
N=604		(%)	density index	ELISA +ve	IFAT +ve
Kapilvastu n=270	14 (5.2)	22.6	4.07	62 (22.9)	65 (24.1)
Tulashi n=181	23 (12.7)	63.5	3.86	66 (36.5)	59 (32.6)
Chisapani n=153	9 (12.4)	67.3	4.15	48 (41.7)	41 (26.8)

Note: Figures in the parenthesis give percentage.

Estimation of malaria endemicity

On the basis of the World Health Organisation's classification on endemicity and the results in Table IV, it is concluded that the estimation of endemicity of 3 villages indicates mesoendemic to hyperendemic areas.

Kapilvastu village:

parasite rate=5.2% represented hypoendemic area, spleen rate (22.6%) and antibody positivity rate (ELISA = 22.9%; IFAT=24.1%) indicate mesoendemic area.

Tulashi village:

parasite rate=12.7% and antibody positivity rate (ELISA=36.5%; IFAT=32.6%) indicate mesoendemic area whereas spleen rate (63.5%) indicates hyperendemic area.

Chisapani village:

parasite rate=12.4% and antibody positivity rate (ELISA=41.7%; IFAT=26.8%) indicate mesoendemic area, whereas spleen rate (67.3%) indicates hyperendemic area.

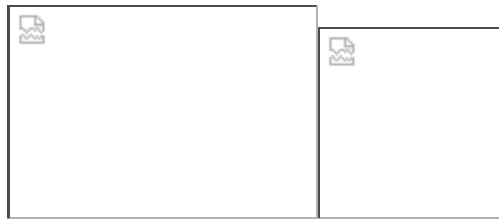


Fig. 8: Comparison of spleen rate versus antibody positive rate.

Discussion

The present study on malariometric and sero-epidemiological surveys were carried out in children aged between 2-9 years in three villages of Southern Nepal. Parasite and spleen rates have been used traditionally as the indices for malaria endemicity. The parasite rate is a measure of the proportion of the population, who at a given point in time, demonstrates a detectable parasitaemia.

In areas of stable malaria, children are usually born free of infection and "clinical" malaria is rare during the first three months of life (Bruce-Chwatt, 1952; Garnham, 1949; McGregor *et al.*, 1956). After this period, prevalence of infection rises so rapidly that by one year of age practically all the children will have been infected. This rise is accompanied by a rapid increase in the incidence and severity of clinical malaria with the main burden of severe life threatening malaria being borne by the pre-school age group (Hendrickse, 1987). By the time of school entry, the incidence and severity of "clinical" malaria mostly have been waned considerably, even though the vast majority of primary school children carry malaria parasites in their blood (Lucas *et al.*, 1969).

In the present study, the estimation of malaria endemicity by the assessment of spleen rate in the three villages, were mesoendemic to hyperendemic. Considering the limitations of blood film examination, the parasite rate is likely to be an under-estimate, since it will not take into account those individuals who are semi-immune and who exhibit low parasitaemias. The major limiting factor for the reliability of the spleen rate is that it is a subjective index and in a malaria endemic area, there maybe many other potential causes of splenomegaly eg., typhoid, leishmaniasis. Despite these problems spleen rate combined with antibody detection can give reliable estimates of endemicity.

Figure 8 elucidates a comparison of the spleen rate and antibody titre determination by ELISA in three different villages of Nepal: Kapilvastu, Tulashi and Chisapani. This data reveals a good correlation between spleen rate and antibody titre (22.6% Vs 22.9%) in Kapilvastu. However, there is a disparity between the two indices, spleen rate (63.5% and ELISA (36.5%) in Tulashi. In Chisapani also, disparity was noted between spleen rate (67.3%) and ELISA (41.7%). These disparities maybe because these two villages are hyperendemic areas as compared to Kapilvastu, which is classified as a mesoendemic area. This could possibly be a reason for higher detection of malaria in these two villages.

In highly endemic areas, thick blood smears are usually positive regardless of the clinical picture. Even if blood examination is carried out, the mere presence of parasites in the peripheral blood of semi-immune individuals does not necessarily mean that the illness manifested by the patient is due to malaria (Hendrickse *et al.*, 1971). It is difficult to distinguish childhood malaria from other common febrile disorders by parasite count alone because of the wide variation in tolerance to parasitaemia among individuals (Rougemont *et al.*, 1991). Although the determination of parasite count has been used as a means of assessing the probable role of malaria in causing a particular illness, there is no agreement about the level of parasitaemia required to produce symptoms in presumed semi-immunes (Hendrickse *et al.*, 1971).

The association between the episodes of fever and infection of malaria were recorded in all children and it was found that the majority of malaria positive cases were associated with episodes of fever. In Kapilvastu, all thick-smear positive children had some degree of fever (i.e. $>37.5^{\circ}\text{C}$), whereas in Tulashi and Chisapani, some of the cases with positive thick smears had no fever. The decrease in body temperature or no fever among parasitaemic individuals might be due to individual immunological response or low density of malaria, which is insufficient to present with clinical symptoms including fever.

The pattern of malaria infections in age specific groups in Kapilvastu was different from Tulashi and Chisapani. The parasite rate was higher in 5-9 years age group in Kapilvastu, whereas the parasitic infections rate was the highest in the age group between 2-4 in Tulashi and Chisapani. The highest spleen rate above 60% of spleen enlargement was found in Tulashi and Chisapani indicating a hyperendemic area of malaria, whereas $<50\%$ spleen rate was found in Kapilvastu indicating a mesoendemic area. In addition, a positive relationship was found between increasing on prevalence of malaria and enlargement of spleen rate. An earlier survey, done in the Rapti valley (Central Inner Terai of Southern Nepal) during 1956 to 1957 found that the infant parasite rate was 63%, child parasite rate 57 to 77%, and child spleen rate 92%. This earlier survey also showed that transmission was perennial, with a peak in the second quarter of the year (Nepal Malaria Eradication Organisation, 1966).

Supplementary information on this area is provided by a much earlier survey done in Makwanpur and Chitwan by Major Phillips of the Indian Military Services (Phillips, 1925). Phillips identified that the people in the area were made up of different tribal

groups: Tharus, an indigenous race, Rai Dhanuwar, of hill origin but who had settled in the area for some time, and Kumalaya, who were recent migrants. Philips found differing levels of morbidity and mortality amongst these groups, the Tharus being the least susceptible to malaria and the Kumalaya the most. A survey of 436 Tharu children showed that 85% had enlarged spleen. Phillips commented that this disappeared with adolescence. Amongst the Rai Dhanuwar, former migrants, 65% of 105 children had enlarged spleens, but spleens were not palpable in the majority of children after the age of 12 years. The recent migrants from the Hills seemed to be most susceptible to malaria, suffering both child and adult deaths. For example, Phillips estimated a general mortality rate in children of around 43% in migrants and 17% in Tharus. One group of settlers he described as "Doomed to extinction".

This information suggests that in children in particular part of the Inner Terai, malaria may have had the characteristics of holoendemicity, high spleen rates in children but low in adults for the indigenous population. Unfortunately, this is the only evidence that could be located on the morbidity and mortality caused by malaria before control began in 1960's. It is not known for the Terai as a whole, nor is there similar information available for hypoendemic areas. In the hypoendemic areas, it is likely that immunity in adults gave some considerable degree of protection to indigenous farmers, though at the expense of infant and child mortality. New settlers, both adults and children, seem to have been at high risk of illness and death. In hypoendemic areas, the relatively low infant and child parasite rates and predominant species (*P. vivax*) suggest that malaria prevalence in adults was relatively low and symptoms milder. Repeated relapses may have caused severe debility in a small proportion of the population. Occasional epidemics, causing morbidity and mortality, are likely to have occurred.

In a malaria hyperendemic area, most people had malaria antibody irrespective of their symptoms (Molineaux *et al.*, 1980).

In Tulashi and Chisapani (parasite rate >12%), a significant difference ($P < 0.05$) was observed on IFAT and ELISA values between positive group and negative group. In the hypoendemic area of Kapilvastu, low numbers of children (5.2%) were infected with malaria but they had high levels of antibody positivity and antibody titres, whereas negative thick smear children had low titres of antibodies. In Tulashi and Chisapani, antimalarial antibody levels were high (>60%) in children, which indicate a hyperendemic area with current or previous infection of malaria, and had high titres of antibodies. This might give

different level of ELISA and IFAT values between hyperendemic and hypoendemic.

In the Garki project, the researcher (Molineaux & Gramiccia, 1980) found that malaria serology was useful. In younger individuals there was a positive correlation between the serological results and parasitaemia, which indicated levels of exposure and infection. The converse situation was observed in adults where a negative association was taken to denote the level of immunity. The data presented in

this study was that the antibody titres against *P. falciparum* in children of Tulashi and Chisapani, are relatively high indicating an exposure to malaria earlier in life. With ELISA using *P. falciparum* and *P. vivax* antigens specimens from 3 villages had detectable antibody levels. The antibody levels were higher with *P. falciparum* antigen compared to *P. vivax* antigen. However, no significant difference was found. Serological tests used in this study found no significant difference between IFAT and the ELISA results in three different

areas in school children with *P. falciparum* antigen ($P > 0.05$). There is also no significant between two test ($P > 0.05$). The correlation coefficient ($r = 0.39$) indicates the positive correlation between the two tests.

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