CROSS-SECTIONAL SEROEPIDEMIOLOGICAL SURVEY OF MALARIA IN ENDEMIC AREAS WITH DIFFERENT ACTIVITIES OF MALARIA CONTROL

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INTRODUCTION

Seroepidemiology of malaria has been extensively studied to gather information on period prevalence of this disease. The tests popularly employed are the indirect fluorescent antibody test (IFA), the indirect haemagglutination test (IHA) and the enzyme-linked immunosorbent assay (ELISA). Results of malaria seroepidemiology have been shown to have values in the establishment of malarial endemicity, assessment of changes in the degree of malaria transmission usually during or after malaria eradication or control operation, specific assessment of epidemiological factors and identification of areas or individuals requiring action especially during the later stages of malaria control programme (WHO, 1972). In view of its wide applications, serological survey has been considered for use as a supplement to the existing measures practised in the National Malaria Programme in Thailand. The objectives of this study were (a) to determine whether a single cross-sectional serological survey could be used to delineate malaria endemicity in Thailand and (b) to compare the usefulness of ELISA and IHA in the assessment of malaria endemicity.

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MATERIALS AND METHODS

Place of study: The study was carried out in April, 1982 in Klang District, Rayong

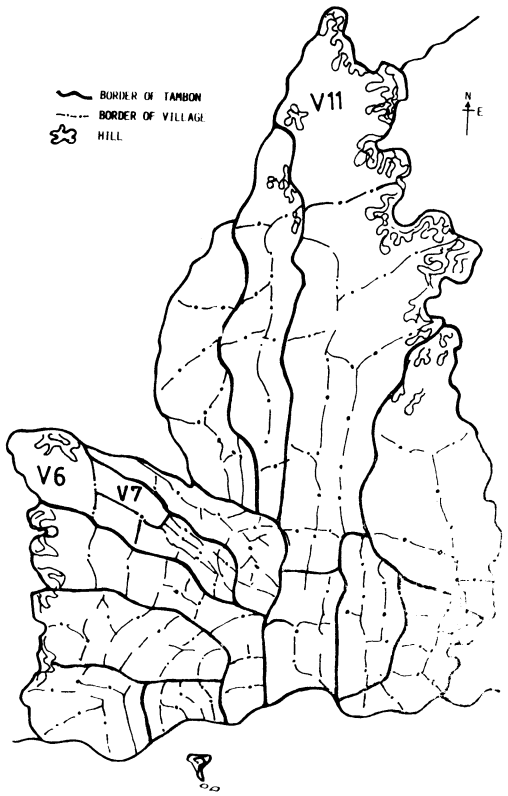

Fig. 1—A map showing locations of villages under study in Klang District, Rayong Province.
V11 = Village 11, Tambon Tung-Kwai-Kin
V6 = Village 6, Tambon Wang-Wa
V7 = Village 7, Tambon Wang-Wa

Table 1

Annual parasite positive rates in villages 11, 6 and 7, Klang District under different phases of the malaria control programme.

Village	Population in 1981	1980			1981			1982		
		No. exam.	No. positive (%)		No. exam.	No. postitive (%)		No. exam.	No. positive (%)	
			All ages	≤9 yr		All ages	≤9 yr		All ages	≤9 yr
11	11,578	3,943	931 (23.6)	193 (4.9)	7,713	1,256 (16.3)	325 (4.2)	6,357	969 (15.2)	216 (3.4)
6	755	20	4 (20.0)	0	58	1 (1.7)	0	302	22 (7.3)	2 (0.7)
7	846	115	7 (6.1)	1 (0.9)	84	3 (3.6)	0	267	6 (2.2)	0

Province, 220 km east of Bangkok. The areas selected for the study were village 11 of Tambon Tung-Kwai-Kin, villages 7 and 6 of Tambon Wang-Wa, the locations of which are shown in Fig. 1. Village 11 was a control area in the early attack phase, where DDT spraying, active and passive case detections were in operation. Village 7 was also a control area in the late attack phase where DDT spraying had been ceased since 1976, but active and passive case detections were still in practice. Village 6 was in a consolidation phase. The number of population and the slide positivity rates are shown in Table 1. The predominant parasite species was *Plasmodium falciparum*.

Collection of specimens: Finger-tipped blood was collected in heparinised Natleson and haematocrit tubes from people of all ages who came voluntarily to the mobile malaria clinic at the operation sites to receive not only examination and treatment for malaria but also examination and treatment for other medical illnesses. Only people who had been living in the area for one year or more were included in the study involving 189 persons from village 11; 192 persons from village 6, and 132 persons from village 7. Blood specimens were kept in an ice chest: those collected in haematocrit tubes were centrifuged in a haematocrit centrifuge for 5 min at room temperature within 10 hours of storage, the tubes cut at the interface, and the plasma kept in capillary tubes at 4°C for no more than three days and finally at −20°C until used. Blood specimens collected in Natleson tubes were centrifuged no longer than three days after ice chest storage at 500 × g for 10 min at room temperature, the tubes cut at the interface, sealed and the plasma kept at −20°C until used.

Serological tests: ELISA and IHA were used to detect antibody against *P. falciparum* according to the technique previously described (Tharavanij *et al.*, 1984). The initial dilutions were 1 : 80 and 1 : 40 for ELISA and IHA respectively. For the sake of convenience in data analysis, the negative plasma with ELISA and IHA were arbitraily given titers of 1 : 40 and 1 : 20 respectively.

Statistical analysis: Chi square with Yate's correction and Student's t test were used.

RESULTS

ELISA and IHA seropositive rates in the stratified age groups of people in these three villages are shown in Fig. 2, 3 respectively. The seropositive rate of population of village 11 (84.6%) was significantly higher than those residing in village 6 (48.9%) and 7 (28.8%) ($p < 0.05$). After age stratification, it was shown that higher seropositive rates in population of village 11 were evident in all age groups when compared with population of village 7 ($p < 0.05$) and in all ($p < 0.05$) except the ≥ 45 year age groups ($p = 0.28$) when compared with population of village 6. Similarly, the IHA seropositive rate of all age groups of population of village 11 (71.4%) was significantly higher than those of village 6 (50.5%) and village 7 (31.3%) ($p < 0.05$). In comparison with those of village 7, it was shown that the higher seropositive rates in population of village 11 were evident only in the age groups of 6-14, 15-29 and 30-44 years ($p<0.05$) but not in the age groups of ≤ 5 and ≥ 45 years. In contrast, IHA seropositive rate of population in village 11 was significantly higher than those of village 6 only in the ≤ 5 year age group ($p = 0.007$). No significant difference was found between seropositive rates in populations of villages 6 and 7 in all age groups tested. The ELISA and IHA geometric mean reciprocal titers (GMRT ± 95% confidence limits) of populations in the study villages are shown in Figs. 4, 5. The ELISA GMRT of population in village 11 (897) was significantly higher than those of village 6 and 7 (291.2 and 90.74 respectively). After age stratification, the difference was seen in every age group tested.

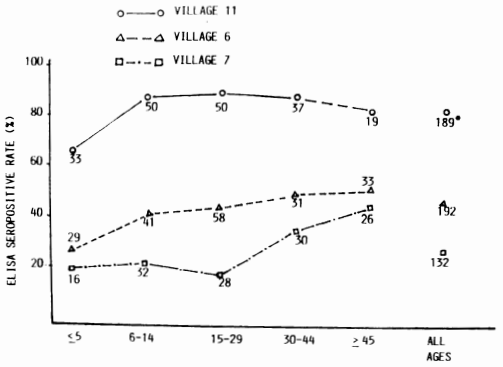

Fig. 2—ELISA seropositive rates in various age groups of population of villages 11, 6 and 7.
* Number of individuals tested.

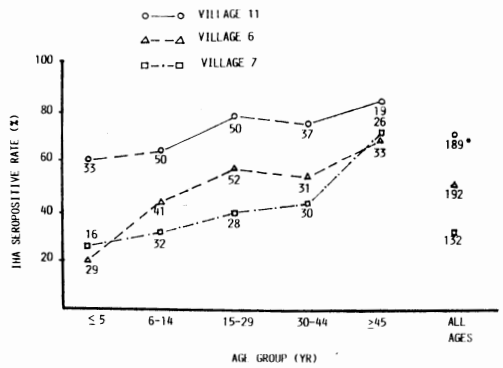

Fig. 3—Indirect haemagglutination (IHA) seropositive rates in various age groups of population of villages 11, 7 and 6.
* Number of individuals tested.

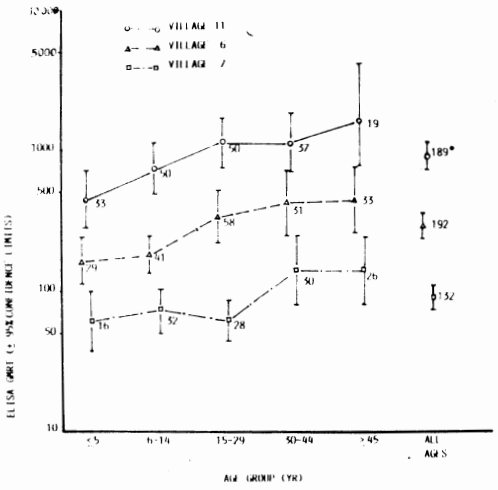

Fig. 4—ELISA geometric mean reciprocal antibody titers (GMRT + 95% confidence limits) in various age groups of population of villages 11, 6 and 7.
* Number of individuals tested.

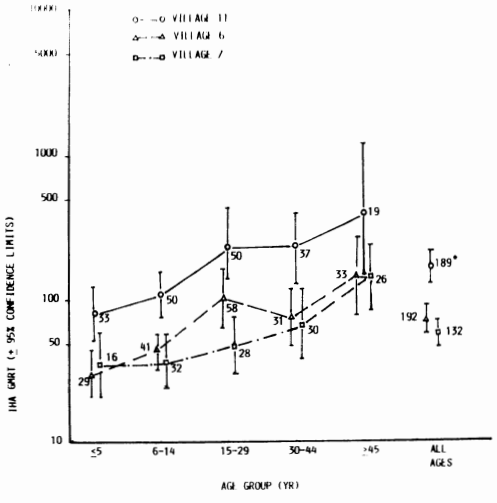

Fig. 5—IHA geometric mean reciprocal antibody titer (GMRT+95% confidence limits) in various age groups of population of villages 11, 6 and 7.

* Number of individuals tested.

Likewise population of village 6 had higher ELISA GMRT than those of village 7 in all age groups tested ($p < 0.004$). With IHA, slightly different result was obtained: though population of village 11 had significantly higher GMRT than those of village 6 and 7 in all but the ≥ 45 year age groups, the GMRTs of populations of villages 6 and 7 in all age groups are not statistically significant ($p > 0.05$).

To assess the level of malaria endemicity in these villages, the relation between the GMRT and the percent seropositive populations initially described by Kagan (1972a) was determined. It was shown (Fig. 6) by either ELISA or IHA that village 11 had higher level of malaria endemicity than those of village 6 and 7. In contrast, village 6 showed higher level of malaria endemicity than those of village 7 only with ELISA and not with IHA.

DISCUSSION

Successful applications of ELISA and IHA in malaria seroepidemiological studies have been amply documented (Kagan, 1972a, b; Meuwissen, 1974; Spencer *et al.*, 1979; Voller *et al.*, 1980; Mathews *et al.*, 1982a, 1982b; Cattani *et al.*, 1986). In general, it is shown that ELISA and IHA seropositive rates of people living in malaria endemic areas increased with increasing ages; people living in areas with high transmission have significantly higher level of malaria specific antibody than those living in areas with low transmission; and those living in areas protected from malaria by insecticide residual spraying have lower level of malaria antibody than those living in 'unprotected' areas.

With a single cross-sectional seroepidemiologal survey in the present study, it was possible to determine the level of malaria endemicity in some villages in Thailand. The results (Figs. 2-5) showed that ELISA and IHA seropositive rates and GMRT in populations residing in areas with high transmission (village 11) were significantly higher than those residing in areas with low transmission (village 6,7), and the levels of antibody were age-related. When two areas with low transmission (villages 6 and 7) were compared, it was shown that population of village 6 had higher seropositive rates than those of village 7 only in the 15-29 year age group but only with ELISA and not with IHA. In addition, ELISA GMRTs of population in village 6 were significantly higher than those of population of village 7 in all corresponding age groups. With IHA, in contrast, no significant difference of GMRT was demonstrated even in the 15-29 year age group. This result indicated then that ELISA was superior to IHA in malaria seroepidemiology because it was possible with ELISA to show differences of serological responses even in areas with low transmission whilst IHA could not

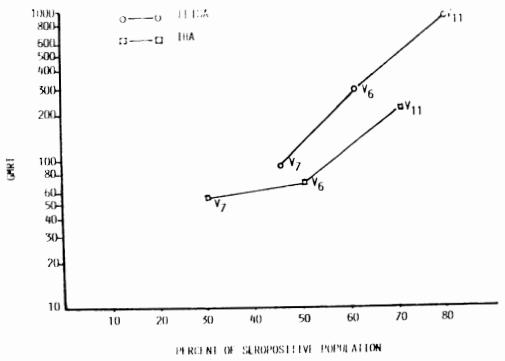

Fig. 6—Relationship between ELISA and IHA GMRT in all age groups and percent of seropositive population of villages 11, 6 and 7.
V11 = Village 11
V6 = Village 6
V7 = Village 7

(Fig. 4,5). The difference in the level of malaria endemicity in areas with different degree of transmission was more clearly discernable with ELISA than with IHA (Fig. 6). Furthermore, it is easier techniquely to establish ELISA than to establish IHA, since the latter requires a good batch of human group O cells, and absorption of sera or plasma with human red blood cells prior to testing to remove antibody against Forssmann antigen which could give rise to non-specific reaction. Besides it is possible with ELISA but with IHA to determine the immunoglobulin class of antibody so that assessment of recent exposure of malaria could be made. ELISA is therefore recommended for use in malaria seroepidemiology more than IHA.

SUMMARY

A single cross-sectional seroepidemiological survey of malaria antibody was conducted in 1982 in Klang District, Rayong Province in three villages under different phases of malaria control activity to determine whether a single survey could be used to delineate malaria endemicity in Thailand and to compare the usefulness of ELISA and the indirect haemagglutination test (IHA) in the assessment of malaria endemicity. Village 11 was a control area with high infection rate with an annual slide positive rate of 16.3% in 1981. Village 6 was also a control area but was in the late attack phase in which residual insecticide spraying has been ceased since 1976. Village 7 was a consolidation area. Finger-tipped blood was collected from 189, 191 and 132 individuals from villages 11, 6 and 7 respectively, and the plasma tested for anti-*P. falciparum* antibody with ELISA and IHA. With ELISA, it was shown that the seropositive rate in population of village 11 (84.6%) was significantly higher than those of other two villages (48.9% in village 6 and 28.8% in village 7). After age stratification, it was shown that the differences were observed in every age group except in the ≥ 45 year age group of village 6. With IHA, a significantly higher seropositive rates in population of village 11 was evident when they were compared with the corresponding age groups of 6-14, 15-29 and 30-44 years in village 7, and the age group of ≤ 5 year in village 6. The ELISA geometric mean reciprocal titer (GMRT) of population of village 11 were significantly higher than those of village 6 which were in turn higher than those of village 7 in all age groups tested. The IHA GMRT of people of village 11 were significantly higher than those of other two villages in all except in the ≥ 45 year age group. In contrast to ELISA, the IHA GMRT in populations of villages 6 and 7 were not significantly different in all age groups tested. The level of endemicity assessed by the relation between the GMRT and the percentage of seropositive population showed higher malaria endemicity in village 11 than other two villages. Comparison between ELISA and IHA indicated that ELISA was superior to IHA for two reasons. First, it gave a higher antibody titer especially in the lower age group. Secondly, it could delineate subtle differences in the level of

antibody in populations of village 6 and 7 when IHA test failed.

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