AGENCY FOR INTERNATIONAL DEVELOPMENT
WASHINGTON, D. C. 20523

BIBLIOGRAPHIC INPUT SHEET

FOR AID USE ONLY
Batch 39

1. SUBJECT CLASSIFICATION
A. PRIMARY: TEMPORARY
B. SECONDARY:

2. TITLE AND SUBTITLE
A seroepidemiologic study of malaria in the Republic of the Philippines by the indirect hemagglutination test

3. AUTHOR(S)
Mathews,H.M.; Fried,J.A.; Kagan,I.G.

4. DOCUMENT DATE	5. NUMBER OF PAGES	6. ARC NUMBER
1970	6p.	ARC RP614.532.M429

7. REFERENCE ORGANIZATION NAME AND ADDRESS
PHS

8. SUPPLEMENTARY NOTES (Sponsoring Organization, Publishers, Availability)
(In Am.j.of epidemiology,v.92,no.6,p.376-381)

9. ABSTRACT

(Health R & D)

10. CONTROL NUMBER	11. PRICE OF DOCUMENT
PN-AAC-444	
12. DESCRIPTORS	13. PROJECT NUMBER
	14. CONTRACT NUMBER: PASA RA(HA)-5-68 Res.
	15. TYPE OF DOCUMENT

AMERICAN JOURNAL OF EPIDEMIOLOGY
Copyright © 1970 by The Johns Hopkins University
Vol. 92, No. 6
Printed in U.S.A.

A SEROEPIDEMIOLOGIC STUDY OF MALARIA IN THE REPUBLIC OF THE PHILIPPINES BY THE INDIRECT HEMAGGLUTINATION TEST[1]

HENRY M. MATHEWS, JANET A. FRIED AND IRVING G. KAGAN

(Received for publication June 17, 1970)

Mathews, H. M. (Parasitology Section, CDC, Atlanta, Ga. 30333), J. A. Fried, and I. G. Kagan. A seroepidemiologic study of malaria in the Republic of the Philippines by the indirect hemagglutination test. *Amer. J. Epid.*, 1970, 92: 376–381.—Sera from residents of the Republic of the Philippines were tested by an indirect microhemagglutination test for the presence of antibodies to malaria. The antigen employed was prepared from *Plasmodium knowlesi* maintained in rhesus monkeys, and serum titers of 1:16 or greater were considered positive. Positive reactors were found in all parts of the three provinces studied. The percentage of positive reactors in three areas in Cotabato was much higher than surrounding communities. Analysis of the data supports the distribution pattern of malaria as determined by malaria surveillance methods.

hemagglutination; malaria; Philippines

Various malariometric methods, spleen index surveys, fever surveys, and mass blood slide examinations, have been used classically to evaluate the epidemiology of malaria in areas where the disease exists. The methods are excellent but suffer from certain disadvantages: the need for well trained personnel to palpate spleens, the problems of determining accurate fever histories, and the time and expense of reading large numbers of blood films. Blood examination may often fail to detect persons with low parasitemia. In our laboratory, serodiagnostic methods are being evaluated as field tools for malaria surveillance in an effort to supplement classical methods of malaria epidemiology (1). Blood collected on filter paper in the field can be rapidly processed by a central laboratory to yield information on the distribution of malaria antibody.

The two serologic methods that are being actively studied today in malaria serology are fluorescent antibody and hemagglutination tests. The indirect fluorescent antibody test is the technique of choice for the diagnosis of malaria (2). This method, however, is technically difficult and not well adapted to mass surveys, although it has been used for this purpose on a limited scale (3, 4).

The indirect hemagglutination (IHA) test for malaria has been used for mass screening of populations. Desowitz and Saave (5) and Desowitz et al. (6) employed an IHA test using *Plasmodium cynomolgi* and *P. coatneyi* antigens adsorbed onto tanned, formalinized, sheep cells to survey populations in Australian New Guinea for malaria antibody. They found this method to be a useful adjunct to classical methods. In our laboratory an IHA test using *P. knowlesi* antigen adsorbed onto tanned human "O" red blood cells (7) has been employed in a large survey of approximately 13,000 young military recruits from

[1] From the Center for Disease Control, Atlanta, Georgia 30333.

The authors thank Dr. Alfred Evans, Director, WHO Reference Serum Bank, Yale University, New Haven, Connecticut, for making available the serum used in this study. This study was supported in part by the U. S. State Department Agency for International Development, Participating Agency Service Agreement (PASA No. RA(HA) 5-68).

four countries in the Western Hemisphere. The test was found to be a rapid and sensitive method for determining the prevalence of malaria antibody (8). The present study evaluated both the antibody status of a large number of people living in four provinces in the Philippines and the feasibility of using the IHA test in epidemiologic surveys for malaria in populations comprised of all age groups.

Materials and methods

Source of serum

Serum samples were drawn in 1963 by a World Health Organization Treponematosis Advisory Team in the Republic of the Philippines. The survey was designed to assess the prevalence of yaws in 37 areas in the islands of Samar, Leyte, the southern part of Mindanao, and the northern part of Panay. A three stage stratified sample consisting of municipalities, barrios, and clusters of 200–250 inhabitants was selected. All children under 15 years of age and 20 per cent of the adults were examined (9).

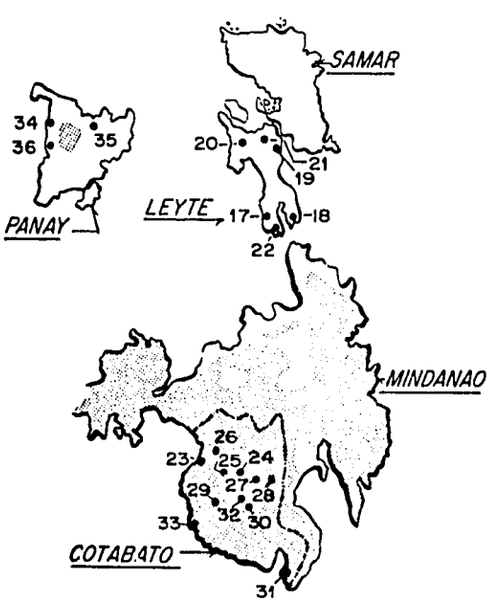

Figure 1. The location of sera sampling points in Leyte, Mindanao (Province of Cotabato) and Panay. Shaded areas indicate the distribution of malaria as of January 1961. ● Indicates sample sites, 1963.

Table 1

Age distribution of persons tested in Leyte, Cotabato, and Panay, Republic of the Philippines, 1963

Province	Municipality	Survey No.	Age (years)			
			<5	5-9	10-14	≥15
Leyte	Matalom	17	13	25	26	21
	Saint Bernard	18	9	26	12	3
	Palo	19	22	31	28	20
	Kananga	20	17	38	28	26
	Jaro	21	5	14	21	10
	Macrohon	22	19	23	20	10
Cotabato	Dinaig	23	32	48	36	73
	Pagalungan	24	21	70	61	92
	Tumbao	25	15	46	52	16
	Nuling	26	39	72	53	47
	M'Lang	27	45	71	45	70
	Makilala	28	35	47	33	46
	Insulan	29	33	62	34	112
	Tacurong	30	44	85	54	46
	Glan	31	41	56	29	35
	Lambayong	32	50	75	63	50
	Lebak	33	16	37	36	39
Panay	Culasi	34	27	37	34	26
	Mambusao	35	24	61	50	51
	Laua-an	36	4	9	13	13

A portion of the collection was made available to us by the WHO Reference Serum Bank, Yale University, New Haven, Connecticut, through the courtesy of the WHO Reference Serum Bank in Prague, Czechoslovakia. A total of 2,987 sera representing 20 of the 37 sampling points were evaluated for malaria antibody. The sera were from three areas: Leyte, Mindanao (Province of Cotabato) and Panay. The location of these sampling points is given in figure 1, and the age distribution for each point is found in table 1. The sample size from each cluster ranged from 39 to 244 sera. We had received 3,110 sera, but the first 16 sampling points were represented by only 123 sera, and these areas were eliminated from the study.

Serologic test

The IHA test for malaria using *P. knowlesi* as antigen was used to titrate malaria

antibody. Sera were diluted in twofold steps and a titer of 1:16 or greater was considered positive for malaria antibody (7).

The criteria used to compare antibody prevalence rates from different sampling points were the percentage of the population with positive titers (≥1:16), the frequency of antibody titers in children, the geometric mean reciprocal titer of the total population, and the mean titer of the positives. For calculating the mean titer of the total population, the negatives were arbitrarily treated as 1:2 dilutions; thus a mean titer of 2.0 indicates all negative sera. This system was adopted because plasma eluted from filter papers used in other surveys is approximately a 1:16–1:20 dilution. A negative titration could fall in the range below 1:16. A titer of 1:16 was chosen as a positive because previous evaluation by Rogers et al. (7) has shown this to be the most specific.

Results

Table 2 lists the prevalence of positive reactors by age in the 20 cluster areas studied, with the total geometric mean reciprocal titer (GMRT) and the GMRT of the positive samples in each category. Seropositivity rates range from 0 per cent to 80 per cent and the total mean titers range from 2.0 to 101. The mean positive titers range from 16 to 750. Although these ranges are wide, there is a strong tendency for both per cent positive and GMRT to increase with age. Samples from Leyte and Panay have generally lower values than samples from Cotabato, indicating a higher prevalence of malaria in this latter province.

The relationship of the total GMRT and

Table 2

Distribution of mean titers and per cent positive reactors in three provinces of the Philippines by age group

Province	Survey No.	<5 Years			5-9 Years			10-14 Years			≥15 Years		
		%+	GMRT Total	GMRT Positives	%+	GMRT Total	GMRT Positives	%+	GMRT Total	GMRT Positives	%+	GMRT Total	GMRT Positives
Leyte	17	0	2.0	—	8.0	2.6	45.3	7.7	2.6	64.0	9.5	2.6	32.0
	18	11.1	2.5	16.0	15.4	3.1	38.1	33.3	5.7	45.3	33.3	4.0	16.0
	19	0	2.0	—	12.9	2.6	16.0	21.4	3.2	18.0	10.0	2.9	90.5
	20	41.2	4.6	16.0	15.8	2.8	16.0	10.7	2.6	25.5	15.4	2.9	22.6
	21	0	2.0	—	7.1	2.3	16.0	14.3	2.9	25.5	30.0	4.9	40.2
	22	0	2.0	—	30.4	4.0	19.6	20.0	3.2	22.6	0	2.0	—
Cotabato	23	3.1	2.1	16.0	10.4	2.7	32.0	13.9	2.9	32.0	24.6	4.0	33.4
	24	4.8	2.3	32.0	5.7	2.3	19.0	1.6	2.1	16.0	19.6	4.0	72.0
	25	0	2.0	—	41.3	4.9	17.3	19.2	3.4	32.0	43.8	9.1	64.0
	26	5.1	2.4	90.5	5.6	2.3	16.0	18.9	3.4	34.3	40.4	8.3	69.1
	27	11.1	2.7	32.0	22.5	4.4	66.7	42.2	12.7	160	55.7	31.3	274
	28	8.6	2.5	32.0	8.5	2.5	22.6	15.2	3.2	48.5	28.3	5.0	51.6
	29	33.3	8.3	145	38.7	11.2	171	35.3	11.8	304	80.4	101	263
	30	4.5	2.4	128	7.0	2.8	228	7.4	3.1	750	28.3	5.6	75.1
	31	12.2	3.3	111	28.6	6.3	112	41.4	11.7	144	40.0	14.5	282
	32	2.0	2.1	16.0	0	2.0	—	4.8	2.3	16.0	30.0	5.1	44.3
	33	0	2.0	—	0	2.0	—	8.3	2.4	16.0	12.8	2.7	21.0
Panay	34	3.7	2.2	32.0	13.5	2.8	24.3	11.8	2.8	38.1	3.8	2.2	16.0
	35	4.2	2.2	16.0	0	2.0	—	2.0	2.1	16.0	9.8	2.5	18.4
	36	0	2.0	—	0	2.0	—	0	2.0	—	30.8	4.0	19.0

GMRT = Geometric mean reciprocal titer.

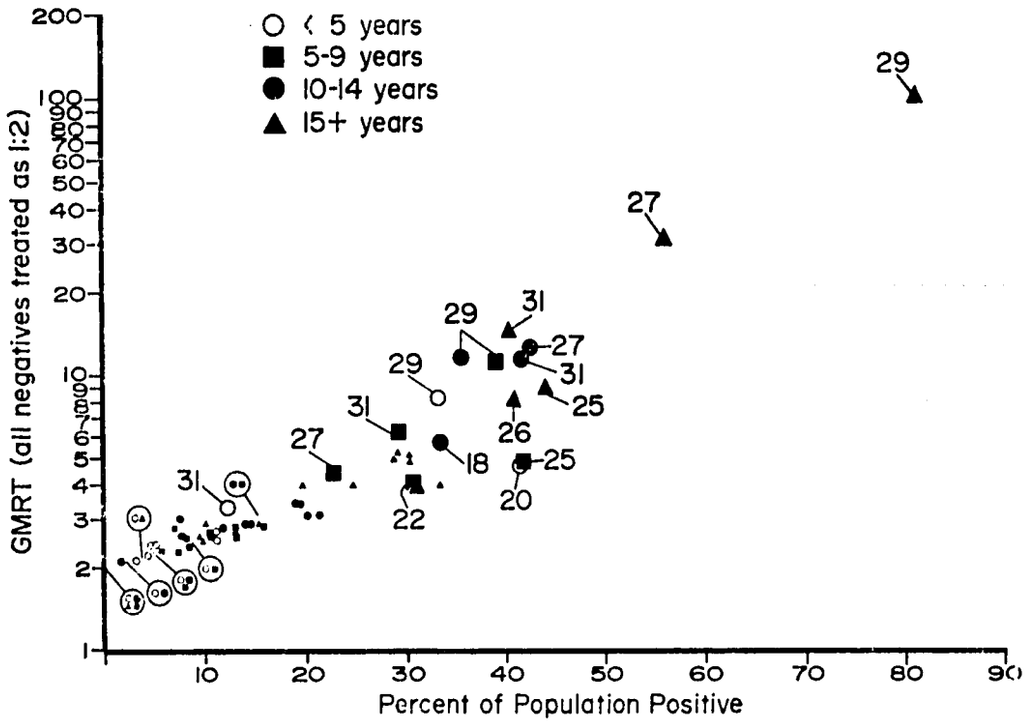

FIGURE 2. Relationship between geometric mean reciprocal titer (GMRT) and percent positive reactors by age group in selected sample sites in the Philippines. Numbers in the graph are sampling points in the survey.

per cent positive for each age group is presented graphically in figure 2. In the under five-year population the points are clustered near the origin, except for three points, 20, 29, and 31, which are elevated. In the five- to nine-year age group there is a smaller cluster near the origin and a second cluster of surveys, 22, 25, 27, 29, and 31, which have higher values. In the population of 10-14-year olds, the same type of dual clustering is evident, one group at the origin with four surveys—18, 27, 29, and 31—forming the elevated cluster. In the adult group, ≥15 years of age, there is no obvious clustering, and the points are distributed linearly on the graph. Three surveys—#'s 27, 29, 31—in this age group are noticeably higher than the remaining ones.

Figure 1 illustrates sampling points in relation to the distribution of malaria in January 1961, as determined by the Malaria Eradication Program in the Philippines (10). Malaria is classified as nonendemic on Leyte and most of Panay which has a relatively small central area of endemic malaria. Cotabato is largely endemic, but it has a central area that is considered malaria free.

Data on blood slide examinations are available only for North and South Cotabato for fiscal years 1959 and 1960. North Cotabato had slide positivity rates of 7.6 per cent and 12.6 per cent for 1959 and 1960 respectively, whereas the rates for South Cotabato were 1.46 per cent and 2.18 per cent for these years (10).

DISCUSSION

Historically, malaria distribution in the Philippines has been irregular and unstable in nature. This is largely due to the distribution of the primary vector *Anopheles minimus flavirostris*, and to the mass population movements that have occurred since

World War II. Surveillance mechanisms have not always been operating in the frontier areas where population movements have taken place. These factors result in focal outbreaks of epidemic malaria (11).

The results obtained in this serologic survey of 2,987 individuals from 20 locations in three provinces indicate that malaria antibody was present to some degree in all areas. This does not imply active transmission in all these areas, however, because it is well known that antibody to malaria may persist for long periods of time in the absence of exposure (12).

The graphic presentation of data in figure 2 points out the wide range of malaria antibody levels in areas of the Philippines that were studied. The percentage of persons in a given age group with positive titers ($\geqslant$1:16) reflects the number of persons who have had experience with malaria at some time in the past. On the other hand, the mean titer gives an indication of more recent experience, with an increase in the titer of circulating antibody. Other studies have shown that in endemic areas malaria antibody titers increase with age achieving a plateau in adult life (13). This is seen in most of the sample areas in this study (table 2). The absence of demonstrable antibody in younger children suggests a lack of exposure to malaria. The total absence of detectable antibody in children under five years of age in seven of the 20 survey areas (table 2) suggests a lack of transmission within the past five years. No antibody was detected in children five to nine years old in four areas; in two of these, antibody was found in younger children, but at very low levels. This emphasizes again the absence of transmission in some locations in the preceding five to nine years. There were three areas with high prevalence rates and elevated mean titers in all age groups, suggestive of current or very recent malaria transmission. Survey 29 had elevated serologic titers in all age groups. The percentage positive in all age groups was also elevated in areas 27 and 31 but since the GMRT values were low, transmission was probably not as recent as in area 29 nor as intense. The pattern of response in survey number 20 is different from the patterns of other areas. The young children show relatively high levels of antibody, but persons over the age of five show relatively low levels. This pattern suggests a recent epidemic in a restricted age group, which seems unlikely, or perhaps it is a result of the sampling methods.

The samples examined from Leyte (surveys 17–22) indicate no transmission in four areas (17, 19, 21, 22), possible transmission in area 18 and probable transmission in survey 20 (see <5 age category, table 2). Cotabato, considered to have the most endemic malaria (figure 1), was found to have generally higher levels of antibody than Leyte or Panay. The level of antibody in different age groups suggests current or recent transmissions in three (surveys 27, 29, and 31) of the 11 areas studied. Note that Survey #27 is within the zone considered non-endemic for malaria (figure 1). Elevated GMRT values and numbers of seropositive individuals indicate that other areas have experienced malaria in the past. The focal nature of malaria is evidenced by the close proximity of areas of apparent recent transmission, (e.g. #27) and areas with no evidence of transmission (e.g. #24, figure 1). Panay province shows little evidence of malaria antibody, but the number of sera from this island was limited.

References

1. Kagan, I. G., Mathews, H. M. and Sulzer, A. J. The serology of malaria: Recent applications. Bull. NY Acad. Med., 1969, *45:* 1027–1042.
2. Sulzer, A. J., Wilson, M. and Hall, E. C. Indirect fluorescent antibody tests for parasitic diseases. V. An evaluation of a thick smear antigen in the IFA test for malaria antibodies. Amer. J. Trop. Med. Hyg., 1969, *18:* 199–205.
3. Collins, W. E., Warren, Mc., Skinner, J. C. and Fredericks, H. J. Studies on the relationship between fluorescent antibody response and ecology of malaria in Malaysia. Bull. WHO, 1968, *39:* 451–463.

4. Voller, A. and Bruce-Chwatt, L. J. Serological malaria surveys in Nigeria. Bull. WHO, 1968, *39:* 883–897.
5. Desowitz, R. S. and Saave, J. J. The application of the hemagglutination test to a study of the immunity to malaria in protected and unprotected population groups of Australian New Guinea. Bull. WHO, 1965, *32:* 149–159.
6. Desowitz, R. S., Saave, J. J. and Stein, B. The application of the indirect hemagglutination test in recent studies on the immuno-epidemiology of human malaria and the immune response in experimental malaria. Milit. Med., 1966, *131(Supp.):* 1157–1166.
7. Rogers, W. A., Jr., Fried, J. A. and Kagan, I. G. A modified indirect hemagglutination test for malaria. Amer. J. Trop. Med. Hyg., 1968, *17:* 804–809.
8. Kagan, I. G., Mathews, H. M., Rogers, W. A., Jr. and Fried, J. A. Seroepidemiologic studies by indirect hemagglutination tests for malaria. I. Military recruit collections from the United States, Brazil, Colombia, and Argentina. Bull. WHO, in press.
9. World Health Organization. Unpublished document INT/VDT/169/65.
10. Dy, F. J., Postiglione, M., Ch'em, C. T., Cruz, A. H., Florio, L. J. and Howard, L. M. The current status of the malaria eradication program in the Philippines. A special report to the Secretary of Health, 1961, Republic of the Philippines.
11. World Health Organization. Unpublished document WPR/427/65.
12. Mathews, H. M., Fisher, G. U. and Kagan, I. G. The persistence of malaria antibody in Tobago following eradication as measured by the indirect hemagglutination test. Amer. J. Trop. Med. Hyg., 1970, *19:* 581–585.
13. McGregor, I. A., Williams, K., Voller, A., and Billewicz, W. Z. 1965. Immunofluorescence and the measurement of the immune response to hyperendemic malaria. Trans. Roy. Soc. Trop. Med., *59:* 395–414.