SEROLOGICAL AND PARASITOLOGICAL OBSERVATIONS ON MALARIA IN SOUTHERN IRAN*

Gh. H. Edrissian** A. Afshar**

ABSTRACT

In the course of serological and parasitological studies of malaria, 1,018 persons were examined. They were from four different areas (A, B, C and D) in the region of Bandar Abbas and Minab, southern Iran, where malaria is still endemic. For the serological examinations, the IFAT was used with *P. falciparum* and *P. vivax* antigens.

Several FA-positive reactions were observed in young children, most probably due to congenital antibodies.

In areas A, B, and particularly in area C, the sero-positivity rates indicate that malaria control measures have been relatively effective in recent years. The sero-positivity related to increasing age, and virtually negative in the younger age groups. Parasitological findings show that more positives were found by concentration technique than by the routine examination of thick blood films. This indicates that routine blood examination of fever cases is not able to show the real infection rate in areas where malaria is still endemic.

A comparison between the serological readings obtained with extracts from 204 blood specimens on filter paper and those produced by genuine plasma samples has yielded practically identical results.

---

* These studies were supported by the School of Public Health and Institute of Public Health Research, University of Teheran, and by the Public Health Research Project of the Ministry of Health and Plan Organization. An abstract has been published in Abstracts of Communications, Ninth International Congress of Tropical Medicine and Malaria, Athens, 1973, Vol. II, 191/192, and the complete text in — WHO/MAL/73.820.

** Department of Epidemiology and Pathobiology, School of Public Health and and Institute of Public Health Research, University of Teheran, P.O. Box 1310, Teheran, Iran

INTRODUCTION

In Iran, a malaria eradication program was implemented in 1957. The campaign was successful in the northern parts of the country, but response in the south was less satisfactory due mainly to technical problems such as nomadism, resistance to DDT and dieldrin in *Anopheles stephensi,* and the exophily and exophagy of *A. fluviatilis* and *A. dthali* (MEO, Iran 1968; Manouchehri *et al.,* 1972a, 1972b; Edwards, 1972).

The indirect fluorescent antibody (IFA) method was referred to as a potentially valuable technique for mass-surveys of malaria (Voller, H. & O'Neill, 1971) and may be useful for the assessment of malaria endemicity and the malaria infection rate (Draper *et al.,* 1972). We used the IFA test in the first sero-epidemiological studies of malaria in Bandar Abbas and Minab, where malaria is still prevalent, with the objective of developing the application of this technique for malariological studies in Iran.

MATERIAL AND METHODS

Areas Studied

The Bandar Abbas and Minab areas are situated in southern Iran, on the northern coasts of the Persian Gulf and Omar Sea, between approximately 55°-57° east and 27°-28° north. The temperature varies between about 45°-50°C in summer and rarely drops below 10°C in winter. The annual rainfall is 100-170 mm and the range of humidity is usually between 40 and 80%.

Malaria had been hyperendemic in this area up to 1950 and transmission occurred throughout all months of the year. The malaria control program, using DDT indoor spraying from 1950, has considerably reduced the malaria incidence, but the occurrence of resistance in *A. stephensi* to DDT in 1957 and to dieldrin in 1959 has again elevated the malaria incidence.

From 1957 onwards spraying continued with a combination of malathion and DDT, applying two or three rounds malathion in the plain areas and two rounds malathion plus two rounds DDT in the mountainous areas every year. These measures combined with some antilarval work such as oiling and use of larvivorous fish, mass drug distribution and surveillance have again reduced the incidence of malaria (Manoochehri, 1972b; Edwards, 1972), but they failed to interrupt malaria transmission entirely. The prevailing plasmodia species in these areas are *P. falciparum* and *P. vivax*. *P. malariae* has rarely been observed.

The following areas were selected for study:

Area A : Two villages (Chello and Ghaleh-Zangi) situated near each other in the plain area of Minab, with 555 population, where the main vector is *A.* stephensi and the annual parasite incidence (API) during four years from 1968 to 1971 was 10, 3, 26 and 4 respectively. This area was subject to rather wide

use of antimalarial drugs, especially mass drug administration though at irregular intervals. Three-hundred-and-thirty-one subjects in this area were studied.

Area B: Two villages (Gholreh and Ghishan) situated near each other in the mountainous area of Bandar Abbas, with 891 population, where the vectors are *A. stephensi, A. fluviatilis* and *A. d'thali*. The APA in these two villages from 1968 to 1971 was 17, 25, 9 and 1 respectively. Three-hundred-and-fourteen subjects were studied in area B.

Area C: One village (Sar-Rick) in the plain area with 471 population, where no malaria cases were reported for five years from 1967 to 1971. One-hundred-and-sixty-eight subjects in this village were studied.

Area D: In Bandar Abbas city, 205 out-patient fever cases, suspected of having malaria were examined. These patients were either from Bandar Abbas city itself or from villages of the immediate neighbourhood.

The blood samples were collected in December 1971 and during the first week of January 1972.

Blood sampling

From each subject, routine thick and thin blood films were taken and blood samples collected in two heparinized micro-haematocrit capillary tubes.

One of the capillary tubes was sealed at one end with plasticine and each batch of 10 or 20 tubes was put in small screw bottles and transferred on ice in a thermos flask to the laboratory of the Field Research Station, Institute of Public Health Research, Bandar Abbas. They were centrifuged at 2000-3000 rpm for five minutes in a portable electric centrifuge. The tubes were nicked with an ampule file and broken directly above the cellular part. The part containing plasma was sealed at both ends and every batch of 10 or 20 tubes was packed in a small screw bottle and transferred to the freezer (—20° to —30°C) at Bazargan Agricultural Products Co. in Bandar Abbas city. The superior layers of the cellular portion of blood in the tube were used for the preparation of thick smears for detection of low parasitaemia (Edrissian & Afshar, 1973).

The blood from the second capillary tube (0.05 ml) was immediately transferred onto filter paper (Whatman No. 1) producing a spot of 1.5-2 cm diameter. These blood samples were dried, packed in plastic bags and transferred to the freezer (—20° to —30°C).

Parasitologcial examination

All routine blood slides were processed by the personnel of the local laboratory of the Malaria Eradication Organization. The slides were stained using Giemsa stain; the thick films were examined for approximately five minutes each.

In all cases with a positive reaction (1/20+) in the IFAT the concen-

trated thick films, stained with Giemsa stain, were examined for 15 to 30 minutes each at the Laboratory of Protozoology, School of Public Health and Institute of Public Health Research, Teheran. In two cases, where the parasite density in the concentrated thick films seemd to be rather high, re-examination of the routine thick films yielded positive results.

Serological examination

Malaria antigens for the IFA test were prepared as thick smear slides from washed cells according to Sulzer et al. (1969) using *P. falciparum* and *P. vivax* parasitized erythrocytes collected from malaria out-patients at the Field Research Station in Bandar Abbas. Each batch of 10 slides was wrapped in "onion skin" paper, packed in a small plastic bag and freighted together with the collected plasma samples and the blood specimens on filter paper in an ice-box containing dry ice (solid CO_2) to the central laboratory of the School of Public Health and Institute of Public Health Research, Tehran, where they were kept in an ultra-low temperature cabinet (—70°C).

The IFA test was done following the technique of Sulzer et al. (1969). The plasma was diluted in two-fold steps starting at a dilution of 1:10 to 1:5120. "Wellcome" antihuman serum conjugate was used at a dilution of 1:10 to 1:20 with 0.1% Evans-blue. The films were examined under a Leitz Ortholux fluorescent microscope equipped with a Philips transformer, a high-pressure mercury vapour lamp (Philips CS150W), a combination of exciter filters BG38, BG12 and UG1, and a Leitz 470 μ barrier filter.

Fluorescence was graded from negative to 4+ and a reading of 2+ in dilutions of 1:20+ was considered positive.

RESULTS

The results of the serological and parasitological examinations in 1018 subjects from areas A, B, C and D in the southern part of Iran are summarized, respectively, in Tables 1, 2, 3 and 4. The age grouping is the same as that used by Draper et al. (1972). The tables contain, separately for each age-group, the following data: number of subjects examined, number of subjects positive for fluorescent antibodies, percentage of seropositives and geometric mean of reciprocal titres (GMRT) of FA-positive cases and the logarithm of the GMRT (Log. GMRT), related to *P. falciparum* and *P. vivax* antigens. Also included are the results of the examinations of routine and concentrated thick smears and the species diagnosis of plasmodia observed: *P. falciparum*, *P. vivax* or unknown species. The latter pertains to cases of low parasitaemia with scanty and deformed malaria parasites which were detected in concentrated thick smears and most probably forms of *P. vivax* or *P. faciparum*.

In Table 5 the global data of area A, B, C and D are compared.

The variation of sero-positivity rates according to age is illustrated for

area A, B, C and D, respectively, in Figure 1-4, with *P. falciparum* and *P. vivax* malaria antigens.

In 204 out-patient fever cases in Bandar Abbas city (area D), where the parasite rate and the sero-positivity rate were 15% and 26% respectively, the serological results attained with the extracts from blood specimens on filter paper were very similar to those produced by genuine plasma samples from capillary tubes.

DISCUSSION

Although in view of the type of antigen used in the IFAT an interpretation of the results must be made with caution, our preliminary serological studies of malaria in the southern part of Iran do permit the following conclusions: several FA-positive reactions with *P. falciparum* and *P. vivax* antigens among young children of up to two years of age in areas A, B and C were apparently due to congenital antibodies (WHO, 1968).

The difference between the standardized sero-positivity rates in the IFAT with *P. falciparum* antigen observed in the 2-16 years old of area A (17%) and area B (3%) is statistically significant ($X^2 = 24.533$; $P < 0.001$), while the difference was less pronounced in the IFAT with *P. vivax* antigen, area A 17%; area B 10% ($X^2 = 6.279$, $P < 0.05$). Considering the much wider use of antimalarial drugs in area A this points to a different exposure to malaria infections, in particular to *P. falciparum*, which could very well be an indicator of better operational success in area B as the original epidemiological situation was the same in both areas. The comparatively low parasitological positivity in area A (2.7% as against 3.2% in area B) and the virtual absence of *P. falciparum* infections in area A could be explained by the effect of antimalarial drugs which lowered parasitaemia but did not eliminate the parasites and prevent antibody production.

In area C where no malaria cases were reported since 1967, only two of 68 subjects with the age of 2-16 years were sero-positive, one of them having a low parasitaemia. Both persons have a history of travelling to other malarious areas.

In areas A and B the sero-positivity rates are positively related to increasing age (Figs 1-4). This is generally also the case with the GMRT. In area C, it is only the age-group of 31+ years which has a significant sero-positivity rate. This phenomenon may reflect the result of an accumulative effect of repeated malarial infections and/or age dependant immunilogical capacity (Voller, 1971), but it is nevertheless evident that in area C the malaria control measures of recent years were relatively effective. This also follows from the results in the younger age-groups in area A and B, where the specific immunological experience of adults may have resulted in producing immunological tolerance to malaria.

These studies also show that in areas A, B and C the number of malaria parasitaemia cases detected by parasitological routine and concentration techniques (Edrissian. H. + Afshar, 1973) was with 19 cases, considerably higher than the number found by surveillance carried out by the local malaria eradication unit during the year 1971 (five cases). Most of these infections, 13 out of 19, had a low parasitaemia detected in concentrated thick blood smears only. Some of this discrepancy may be due to inadequacies of surveillance, to factors inherent in the routine examination of normal thick blood smears or due to interference caused by a wide use of antimalarial drugs and a still rather high prevalence of malaria (parasite rates between 1.3 and 3.2%) in areas A, B and C, and thus a considerable antibody residue apt to interfere with clinical symptomatology which usually is the criterium for case detection activities.

Under the circumstances prevailing in area A, B and C, it must be concluded that case detection by routine blood film examination of fever cases was not able to show the real prevalence of malaria. While parasite concentration methods permit to estimate the reservoir level much closer to its true value, it is serological techniques, in particular the IFAT, that provide retrospective information on man's contact with the parasite.

In 204 out-patient fever cases in Bandar Abbas city suspected of having malaria, the parasite rate was 15%. There was considerable correlation between sero-positivity and parasitaemia in the different age-groups. The sero-positivity rate (26% P.f. and 23% P.v.) was less than that observed in area A (42% P.f. and 29% P.v.), but parasitaemia was much more frequent. This and the fact that routine blood examination detected the parasites in 25 out of 30 cases indicate that these were in majority acutely ill patients and that passive case detection is a valuable and essential component of surveillance.

ACKNOWLEDGEMENT

The authors would like to thank Dr. M.A. Faghih, Dean of the School of Public Health and Director of the Institute of Public Health Research, University of Teheran, whose support and guidance encouraged us in these studies.

We would also like to express our gratitude to Professor A. Sanati, Dr. M. Ghorbani, Assistant Professor, and Mr. Asaad, Technician, of the Protozoology Unit, Department of Epidemiology and Pathobiology, School of Public Health, and to the staff of the Bandar Abbas Research Station, the Bandar Abbas and Minab Malaria Eradication Units and Bandar Abbas Bazargan, Agricultural Products Co., for their valuable help and cooperation in this investigation.

REFERENCES

1. Draper, C.C., Voller, A. and Carpenter, R.G. (1972). The epidemiological interpretation of serologic data in malaria. Amer. J. Trop. Med. Hyg. *21*:696-703.

2. Edrissian, Gh. H. and Afshar, A. (1973). Detection of low malaria parasitaemia using indirect fluorescent antibody (IFA) and parasite concentration techniques. Pahlavi Med. J. *4*:533-544.
3. Edwards, S. (1972). Report on a visit to Iran. World Health Organization, EM/Mal/114.
4. MEA, Ministry of Health, Iran (1968). Report on malaria. 8th Inter-Country ME Co-ordination Border Meeting, Baghdad, 8-11 January, 1968.
5. Manouchehri, A.V., Ghiasseddin, M. and Shahgudian, E.R. (1972a). *Anopheles dthali* Patton, 1905, a new secondary vector in southern Iran. Ann. Trop. Med. Parasit. *66*:537.
6. Manouchehri, A.V., Shahgudian, E.R., Kargar, S. and Ghiassedin, M. (1972b). A large-scale Malathion trial in the Bandar Abbas area, Iran. Iran. J. Pub. Hlth. *1*:60-68.
7. Sulzer, A.J., Wilson, M. and Hall, H.C. (1969) 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. *18*:199-205.
8. Voller, A. and Bruce-Chwatt, L.J. (1968). Serological malaria surveys in Nigeria. Bull. Wld. Hlth. Org. *39*:883-897.
9. Voller, A. (1971). The detection and measurement of malarial antibodies. Trans. Roy. Soc. Trop. Med. Hyg. *65*:111-124.
10. Voller, A. and O'Neill, P. (1971). Immunofluorescence method suitable for large-scale application to malaria. Bull. Wld. Hlth. Org. *45*:524-529.
11. World Health Organization. (1968). Immunology of malaria. Wld. Hlth. Org. Techn. Rep. Ser., No. 396.

TABLE 1. AREA A, MALARIA ANTIBODIES AND PARASITES IN TWO VILLAGES IN THE LITTORAL REGION OF SOUTHERN IRAN (1972)

Age group	Number examined	Serological results								Parasitological results (No. positive)						
		P. falciparum antigen				*P. vivax* antigen				Usual thick smears			Concentrated thick smears			Total
		No. pos. (1/20+)	% pos.	GMRT	Log. GMRT	No. pos. (1/20+)	% pos.	GMRT	Log. GMRT	P.f.	P.v.	Unknown	P.f.	P.v.	Unknown	
0-11 mos	13	0	0	0	0	2	15	20	1.30	-	-	-	-	-	-	-
12-23 mos	15	0	0	0	0	0	0	0	0	-	-	-	-	-	-	-
2-4 yrs	47	3	6	69	1.83	3	6	49	1.69	-	-	-	-	1	-	1
5-9 yrs	74	13	17	30	1.47	15	20	26	1.41	-	-	-	-	2	-	2
10-16 yrs	64	16	25	24	1.38	14	22	22	1.34	-	-	-	-	-	-	-
17-30 yrs	54	30	55	62	1.79	18	33	40	1.60	1	-	-	-	2	-	3
Over 31 yrs	64	51	79	105	2.02	30	47	52	1.71	-	-	-	-	1	1	2
Total	331	113	41.8[a]	72[a]	1.86[a]	82	28.5[a]	40[a]	1.60[a]	1	-	-	-	6	1	8

[a] Standardized for age, according to census 1966.

TABLE 2. AREA B, MALARIA ANTIBODIES AND PARASITES IN TWO VILLAGES IN THE MOUNTAINOUS REGION OF SOUTHERN IRAN (1972)

Age group	Number examined	Serological results								Parasitological results (No. positive)						
		P. falciparum antigen				*P. vivax* antigen				Usual thick smears			Concentrated thick smears			Total
		No. pos. (1/20+)	% pos.	GMRT	Log. GMRT	No. pos. (1/20+)	% pos.	GMRT	Log. GMRT	P.f.	P.v.	Un-known	P.f.	P.v.	Un-known	
0-11 mos	3	0	0	0	0	0	0	0	0	-	-	-	-	-	-	-
12-23 mos	8	1	12	40	1.60	0	0	0	0	-	-	-	-	-	-	-
2-4 yrs	29	1	3	40	1.60	3	10	40	1.60	-	-	-	-	-	1	1
5-9 yrs	98	2	2	28	1.44	8	8	40	1.60	1	2	-	-	-	1	4
10-16 yrs	103	4	4	56	1.74	12	12	85	1.92	-	1	-	-	-	-	1
17-30 yrs	28	13	46	52	1.71	9	32	54	1.73	-	-	-	-	1	-	1
Over 31 yrs	45	38	84	80	1.90	30	67	66	1.81	-	1	-	-	1	-	2
Total	314	59	35.6[a]	69[a]	1.84[a]	62	30.4[a]	60[a]	1.78[a]	1	4	-	-	2	2	9

[a] Standardized for age according to census 1966.

TABLE 3. AREA C, MALARIA ANTIBODIES AND PARASITES IN A CONTROL VILLAGE IN THE LITTORAL REGION OF SOUTHERN IRAN (1972)

Age group	Number examined	Serological results								Parasitological results (No. positive)						
		P. falciparum antigen				P. vivax antigen				Usual thick smears			Concentrated thick smears			Total
		No. pos. (1/20+)	% pos.	GMRT	Log. GMRT	No. pos. (1/20+)	% pos.	GMRT	Log. GMRT	P.f.	P.v.	Unknown	P.f.	P.v.	Unknown	
0-11 mos	2	0	0	0	0	0	0	0	0	-	-	-	-	-	-	-
12-23 mos	7	0	0	0	0	1	14	20	1.30	-	-	-	-	-	-	-
2-4 yrs	24	0	0	0	0	1	4	20	1.30	-	-	-	-	-	-	-
5-9 yrs	37	0	0	0	0	1	2	40	1.60	-	-	-	-	1	-	1
10-16 yrs	42	0	0	0	0	0	0	0	0	-	-	-	-	-	-	-
17-30 yrs	24	1	4	160	2.20	0	0	0	0	-	-	-	-	-	-	-
over 31 yrs	32	13	40	34	1.53	8	25	30	1.47	-	-	-	-	-	1	1
Total	168	14	12.4[a]	37[a]	1.57[a]	11	8.5[a]	29[a]	1.46[a]	-	-	-	-	1	1	2

[a] Standardized for age, according to census 1966.

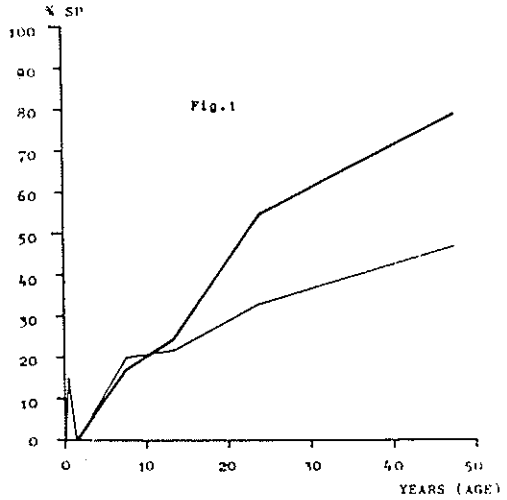
TABLE 4. AREA D. MALARIA ANTIBODIES AND PARASITES IN OUT-PATIENT FEVER CASES OF BANDAR-ABBAS IN SOUTHERN IRAN (1972)

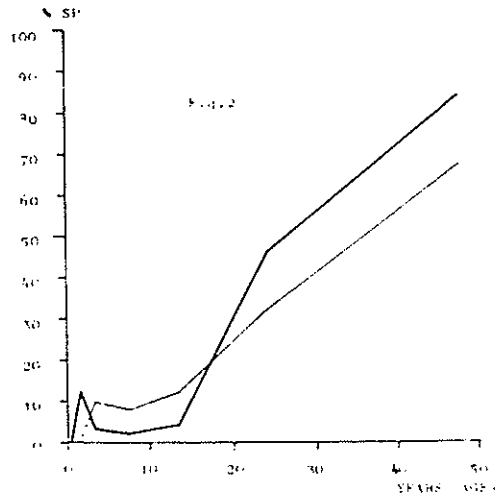
Age group	Number examined	Serological results								Parasitological results (No. positive)						
		P. falciparum antigen				P. vivax antigen				Usual thick smears			Concentrated thick smears			Total
		No. pos. (1/20+)	% pos.	GMRT	Log. GMRT	No. pos. (1/20+)	% pos.	GMRT	Log. GMRT	P.f.	P.v.	Un-known	P.f.	P.v.	Un-known	
0-11 mos	3	0	0	0	0	0	0	0	0	-	1	-	-	-	-	1
12-23 mos	5	0	0	0	0	0	0	0	0	-	-	-	-	-	-	-
2-4 yrs	18	0	0	0	0	3	16	62	1.72	-	1	-	-	-	-	1
5-9 yrs	24	4	16	190	2.27	4	16	115	2.06	4	2	-	-	-	-	6
0-16 yrs	35	7	20	140	2.14	6	17	56	1.74	4	-	-	-	1	1	6
7-30 yrs	68	26	39	56	1.74	23	33	52	1.71	5	3	-	1	1	-	10
ver 31 yrs	52	16	30	130	2.11	11	21	48	1.68	2	3	-	-	1	-	6
Total	205	53	26	100	2.00	47	23	56	1.74	15	10	-	1	3	1	30

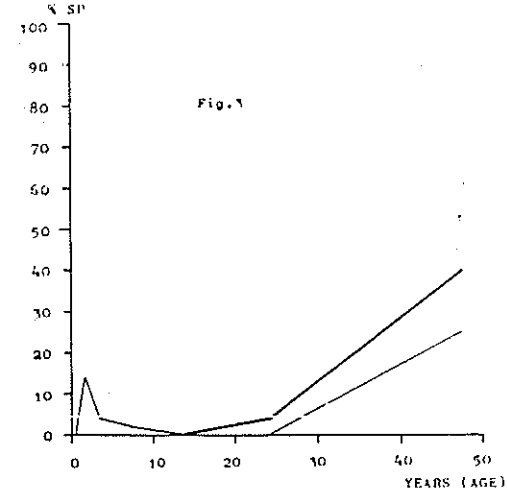
TABLE 5. MALARIA ANTIBODIES AND PARASITES IN FOUR AREAS OF SOUTHERN IRAN

Area	Type of survey	Number examined	Serological results				Parasitological results							
			P. falciparum		P. vivax		Usual thick smears			Concentrated thick smears			Total	
			% pos. (1/20+)	GMRT	% pos. (1/20+)	GMRT	P.f.	P.v.	Un-known	P.f.	P.v.	Un-known	No.	% + Ve
A	Cross-section	331	41.8[a]	72[a]	28.5[a]	40[a]	1	-	-	-	6	1	8	2.7[a]
B	Cross-section	314	35.6[a]	69[a]	30.4[a]	60[a]	1	4	-	-	2	2	9	3.2[a]
C	Cross-seotion	168	12.4[a]	37[a]	8.5[a]	29[a]	-	-	-	-	1	1	2	1.3[a]
D	Fever cases	205	25.9	100	22.9	56	15	10	-	1	3	1	30	14.6

[a] Standardized for age, according to census 1966.







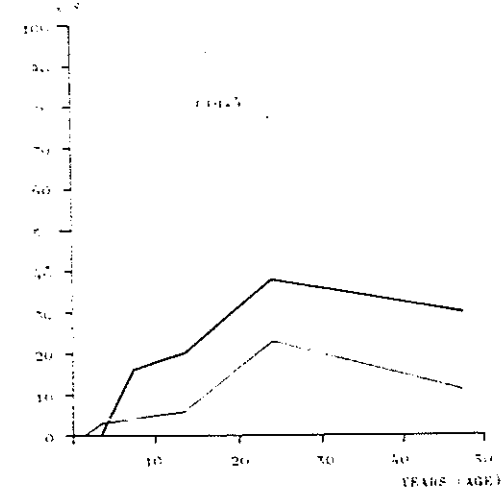


Fig.1 AGE-SPECIFIC SERO-POSITIVITY RATES (1/20+) IN THE IFAT USING P.falciparum AND P.vivax ANTIGENS, AREA A

Fig.2 " " " " " " " " " " " " " " " , AREA B

Fig.3 " " " " " " " " " " " " " " " , AREA C

Fig.4 " " " " " " " " " " " " " " " , AREA D

% SP = % SERO-POSITIVE ——— P.falciparum ANTIGEN ——— P.vivax ANTIGEN