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Serological Malaria Surveys in Nigeria*

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A parasitological and serological malaria survey of 2 large and 2 small areas of Nigeria was carried out in connexion with the activities of the WHO Treponematoses Epidemiological Team.

The results, based on data obtained from 1082 subjects, showed that all the areas were holoendemic with the usual pattern of malariometric indices, and that the differences between the parasite rates of the two large areas were due to the different timing of the survey in relation to the seasonal wave of transmission.

The fluorescent antibody test was positive (>1 : 20) in 92% of the 914 sera collected from these 2 areas.

The serological profile of the population in the 2 areas was similar, but the immunofluorescence titres were higher in all age-groups in the area south of the Benue river, indicating the antibody response to the previous endemic wave rather than the actual amount of transmission taking place at the time of the survey.

This study confirms the value of the immunofluorescent technique for large-scale malaria surveys, but indicates the need for caution in interpreting the results and stresses the importance of good knowledge of the local epidemiology of malaria before embarking on application of serological methods.

INTRODUCTION

Immunological surveys of infections with tuberculosis, diphtheria, syphilis and other diseases have provided much information without which the planning of appropriate public health measures would have been impossible.

Large-scale surveys of arthropod-borne virus infections and especially that of yellow fever in Africa (Bonnel & Deutschman, 1954; Smith, 1965) may be quoted as classical examples of serological epidemiology (Paul, 1966). Today these surveys cover a wide field of communicable diseases and human genetics; the techniques used have progressed so much that about 100 different determinations of blood groups, genetic enzymopathies and immune responses to various infections can be carried out on a few millilitres of blood from one individual (Paul, 1966).

The modern concept of epidemiological surveillance [3] of communicable diseases described by Langmuir (1965) and Raška (1966) is based on the constant development of immunological methods and covers all the activities which assess the importance, origin and spread of communicable diseases and which guide the control programmes. The Technical Discussions held during the 21st World Health Assembly [4] stressed the value of this concept on an international scale.

The use of laboratory methods on a large scale is particularly valuable in countries where vital statistical data are difficult to obtain or where the infection produces mild symptoms, or may be asymptomatic, in a proportion of the population

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[3] The term "epidemiological surveillance" used in this context is somewhat different from the surveillance used in malaria eradication programmes, where it implies a considerable amount of control activity (World Health Organization, 1963).

[4] An unpublished report on the technical discussions at the Twenty-first World Health Assembly, 1968, on *National and global surveillance of communicable diseases* (A21/Tech. Disc./5). A limited number of copies of this document is available to persons officially or professionally interested on request to Distribution and Sales, World Health Organization, 1211 Geneva, Switzerland.

(Taylor & Knowelden, 1964). The steadily growing interest in the application of serological methods in protozoan diseases generally (Garnham, 1963; WHO Expert Committee on Immunology and Parasitic Diseases, 1965) led to the reassessment of the practical value of these methods in trypanosomiasis (Lumsden, 1967) and in malaria (WHO Scientific Group on the Immunology of Malaria, 1968).

In the advanced phases of malaria eradication programmes, the techniques available for case-detection are of limited reliability, especially in Africa, while mass blood-surveys of populations are cumbersome and do not yield results commensurate with the work involved. Serological techniques could be of use in such situations.

The epidemiological characteristics of the acquired collective immunity to malaria were described by Sinton (1939), Wilson, Garnham & Swellengrebel (1950), Bruce-Chwatt (1952, 1956, 1963), McGregor (1960, 1967), McGregor & Gilles (1960), McGregor, Carrington & Cohen (1963), McGregor et al. (1965, 1968) and others. The first malaria infection in young African children may be mild, but later parasitaemia becomes dense and severe clinical illness is common from about 6 months of age until about 3 years of age. With advancing age the symptoms lessen, although parasitaemia persists. The first manifestation of acquired immunity is the modification of clinical illness without marked effect on parasitaemia. Henceforth the parasite densities slowly decline until adults seldom present a parasite rate of more than 25% and parasite density is always extremely low, although in pregnancy some disturbance of the immune response seems to occur.

Traditional malariometric data, obtained in tropical areas, indicate that functional immunity to malaria reaches its plateau relatively early, generally at adolescence if not before.

The new phase of our understanding of the immune response to malaria infection can be traced to the finding, in the 1950s, of a constant relationship between a high degree of malaria endemicity and hypergammaglobulinaemia (McGregor et al., 1956; Gilles & McGregor, 1959a, 1959b). Subsequently, the protective action of gamma-globulin obtained from immune subjects has been demonstrated (Cohen, McGregor & Carrington, 1961; Cohen & McGregor, 1963). The application to malaria of the fluorescent antibody technique (Tobie & Coatney, 1961; Voller & Bray, 1962) indicated the possibility of measuring the immune response (McGregor, Carrington & Cohen, 1963; McGregor et al., 1965) and subsequent studies revealed the range of sensitivity and specificity of this method.

The practical aim of serological surveys in malaria is to find evidence of past or present malaria experience in a population and to assess the degree of immunity in such populations. Further refinements, such as the identification of species and strains of the infecting parasite, would also be desirable. Serological diagnosis of infection and of the immunity in individuals would also be of value (Saliou, 1964). Valuable epidemiological data can be obtained from serological surveys, however, even when the tests used are not applicable to individual diagnosis.

Smith (1965) defined the essential requirements of a satisfactory arbovirus-antibody survey as being designed to answer specific questions, and to be comparable with surveys of other population groups from the point of view of age, sex, etc. These requirements were also mentioned by Paul (1966) and apply equally to serological surveys of malaria under field conditions.

The limitations and merits of the different techniques employed will now be considered separately.

Non-specific tests

These had a great popularity some 30 years ago because of the ease with which they could be performed. The tests depended on the relative changes in the serum protein constituents and ranged from the simple flocculation of serum by the addition of distilled water to the use of melanin suspensions advocated by Henry in 1927 (Le Berre, 1968). The frequent positivity of Henry's test is due to the flocculation of melanin suspension in the presence of immunoglobulin (IgM) the level of which is raised in some populations in the tropics and increases with age (Trensz & Raab, 1965, 1966; Turner & Voller, 1966; Rowe et al., 1968). In view of the fact that cross-reactions occur in this test with trypanosomiasis, kala-azar, hepatitis and other conditions, the test is of little value.[1]

Complement-fixation tests

These are attractive since many laboratories are equipped to carry them out in other contexts, and if a reliable malaria antigen were available it would

[1] Voller, A. (1966) *Melano-flocculation test in malaria.* Unpublished document WHO/Mal/66.556. A limited number of copies of this document is available to persons officially or professionally interested on request to Distribution and Sales, World Health Organization, 1211 Geneva, Switzerland.

be possible to handle large numbers of sera. Early workers used non-malarial antigens and encountered many cross-reactions. Improvement of the antigen by extracting it in various ways from malarial parasite material has shown that the test can be quite specific and d'Antonio et al. (1966) found that there was then no cross-reaction between *Plasmodium knowlesi* antigen and *Plasmodium falciparum*. Voller & Schindler (1967) showed that an antigen from *P. c. bastianellii* which reacted well with the homologous antisera did not react with *P. falciparum* although yaws gave some false positive results.

Assuming that satisfactory antigen preparations can be obtained, it is possible that complement-fixation tests may be of some value as a species diagnostic tool in serological surveys. They are unlikely to be of use in serological estimations of endemicity since a negative reaction can be obtained even when the parasites are still demonstrable (Schindler & Voller, 1967). Positivity of the complement-fixation test seems to be related to recent parasite multiplication in the peripheral blood on a substantial scale.

Precipitation techniques

The double-diffusion method of Ouchterlony in agar gel has proved to be eminently suitable for large-scale serological surveys in the hands of McGregor and his associates (1966, 1968) who demonstrated a complex of *P. falciparum* antigens, some isolated from infected cells and others from the plasma of recently infected individuals. The antigens for gel-precipitation prepared from fully-grown schizonts of *P. falciparum* yielded a test which may find wide application in the measurement of malaria endemicity, in view of the fact that the tests are suitable for large-scale use and require little antigenic material. The fact that antibody to the soluble antigen is only rarely detected in children under 6 years of age may mean that such antibody is to some degree an indicator of effective immunity. If this were so, this technique would be of great value in estimations of population immunity.

Agglutination techniques

Recently Brown & Brown (1965) demonstrated the high specificity of agglutination tests. This is likely to be the key factor in subsequent field applications of this technique and may lead to a precise determination of the number of antigenic-variant types of a malaria parasite strain or species in any given area. Difficulties are encountered at present in the preparation of antigens from human malaria parasites (McGregor, 1967).

The technique of haemagglutination has much to recommend it as a serological tool for epidemiological studies. Large numbers of sera can be dealt with, very little antigen is required, and the tests can easily be performed under such laboratory conditions as are frequently encountered in the tropics.

An indirect haemagglutination test, involving the use of tanned formolized sheep erythrocytes sensitized by a plasmodial antigen, was developed by Desowitz & Stein (1962) and Stein & Desowitz (1964). Bray & El Nahal (1966) encountered difficulties in producing a reliable antigen and Desowitz (1966) indicated that the reaction was genus- rather than species-specific although homologous titres were usually higher. Using a slightly different technique, and a more purified *P. knowlesi* antigen, Mahoney et al. (1966) reduced the cross-reactivity to relatively low levels. With the antigen prepared from *P. cynomolgi* and *P. coatneyi*, the method of haemagglutination was applied in an epidemiological survey of malaria in the Territory of Papua and New Guinea (Desowitz & Saave, 1965). The results of this survey confirmed that high mean titres of antibody were present in the population which was exposed to the higher degree of transmission. However, the differences between the frequency distribution of high titres in various age-groups of the " protected " and " unprotected " populations were small and a fairly high proportion of positive tests was found in groups that were exposed to a lower degree of transmission. Desowitz (1966) concluded that frequent antigenic stimulation was necessary to maintain high levels of humoral antibody. It is probable that this technique will find increasing application both for individual case-detection and for the indication of malaria endemicity when a more specific antigen has been prepared. A successful use of this test with an antigen prepared from mature schizonts of *P. knowlesi* was recently reported by Rogers et al. (1968).

Immunofluorescent technique

This method will be dealt with in more detail not because it is superior to the others mentioned earlier, but because more reports of its use in laboratory and field work are available (WHO Scientific Group on the Immunology of Malaria, 1968). The technique is potentially applicable to the identification of parasites used as antigen or to the quantitative assay of malarial antibody in serum. To perform the latter assay, the test serum is pro-

gressively diluted and the end-point is considered as being that dilution at which the fluorescence is decreased to a minimal level (Voller, 1962, 1964b).

The minor variations in technique have been well reviewed by Sodeman & Jeffery (1966) and it is not proposed to deal with them here. Each of the technical variations has its supporters and critics and their arguments are frequently based on the magnitude of the titres. These comparisons are, however, of little value and do not necessarily mean that the technique giving the highest titre with a given serum is the best. Of more importance is the comparison of relative titres, for instance, between different age-groups or different populations or throughout an infection. The results of technical variations of immunofluorescent methods are broadly similar when compared on this basis. However, while so many different procedures are in use in different laboratories, no direct comparison of the reported results is possible. This is why we have continued to employ the straightforward method (Voller, 1964b), using hydrochloric acid treatment of antigen without counterstain.

At an early stage it was clear that considerable cross-reaction occurred between the various species of malaria parasites (Tobie et al., 1962; Voller, 1964a) and these observations have been put on a quantitative basis by Collins et al. (1966) and by Diggs & Sadun (1965), who showed that, in general, the heterologous titres were lower than those in the homologous system. A word of caution should be introduced here: a difference of titre between two antigens should be accepted only when each can be tested with homologous antiserum, as certain antigen preparations may sometimes be less reactive against all the antisera tested (Meuwissen, 1966).

All fluorescent antibody tests show a fair range of cross-reactions which do not imply any degree of protective immunity of the host against the parasite used as antigen. Vaccination experiments with simian malaria parasites have also revealed a discrepancy between antibody levels and protective immunity (Targett & Voller, 1965).

Much of our present understanding of the immunological response to malaria indicated by fluorescent antibody techniques comes from the careful studies of induced malaria in individuals (Tobie et al., 1962, 1966; Collins, Jeffery & Skinner, 1964a, 1964b; Garin, Ambroise-Thomas & Saliou, 1965; Lunn et al., 1966). In general fluorescent antibodies became detectable within a few days of the onset of patent parasitaemia, reached a peak after 3–4 weeks and then declined to a lower level which was maintained for a long time.

Dynamics of the antibody response

Studies on the persistence of specific antibody (Collins et al., 1964a, 1964b; Coudert et al., 1965; Jeffery, 1966; Kuvin & Voller, 1963) showed that the low antibody levels that persist during periods of low parasitaemia are insufficient to prevent superinfection with a homologous strain and that antibody rises to a high level after antigenic stimulation. The results of studies on induced malaria suggest the presence of species-specific and strain-specific antibodies, the first type of antibody being stimulated by a heterologous antigen early in the course of the infection and the rise of the strain-specific antibody occurring later.

The dynamics of the antibody response to multiple infections, with a large number of strains of the different species of malaria parasites encountered in highly endemic areas of the world, are much more complicated. Desowitz (1966) discussed some infrequent, but nevertheless important, shortcomings of the various methods of immunological diagnosis and called for a realistic assessment of their promise and for an investigation of the apparent inconsistencies which occur when different methods are used.

MATERIALS AND METHODS

The immunological survey of malaria formed part of a multipurpose field study carried out by the WHO Treponematoses Epidemiological Team which operated during 1965–67 in the Northern, Western and Mid-Western Regions of the Federal Republic of Nigeria, and the organization of this survey has already been described (Schindler & Voller, 1967). Although the primary aim of the team was to evaluate the yaws situation in Nigeria, aliquot samples of sera collected in the field were used for serological studies on arthropod-borne viruses, and for studies on genetic factors of blood-groups, haemoglobin variants, enzyme systems and serum proteins. Serum samples were transported from Africa by air and stored in liquid nitrogen; this appears to be the method of choice for such studies (Guthe, 1965, 1966). After being divided into aliquots for several serological tests, the samples were stored at −70°C. Aliquots designated for fluorescent antibody assay were tested according to the method described by Voller (1964a). The antigen was the simian parasite *P. cynomolgi bastianellii* maintained

in *Macaca mulatta* and *Erythrocebus patas*; the thin films on slides were kept at −70°C until the day of use. The sera were tested at dilutions of 1: 20 – 1: 5120 and those not reacting at 1 : 20 were designated as negative. A standard serum of known titre, obtained from an immune adult in Nigeria, was included in each day's tests. If there was any marked deviation in the expected titre of the control serum, the relevant series of tests was repeated.

An extended survey was carried out during the period August 1966–January 1967 in 2 large and 2 small areas in Nigeria. Malariometric data were collected at the time of the survey by the staff of the Malaria Unit of the Northern Region of Nigeria. The present report gives an account of the final results of this study.

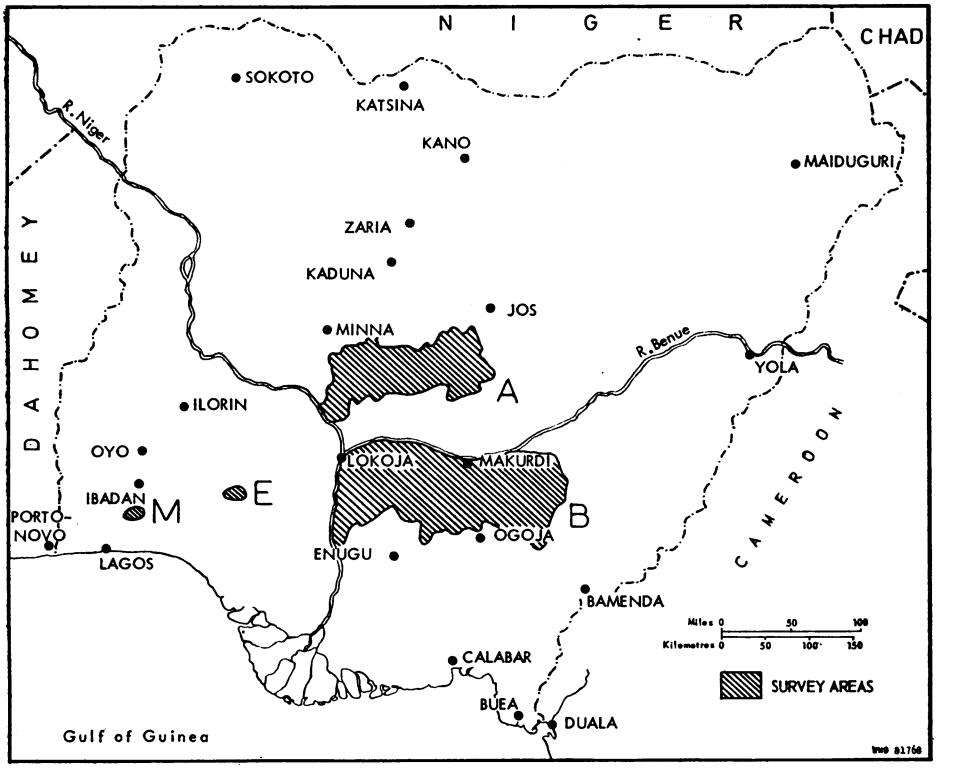
An initial study designed to indicate the suitability of *P. cynomolgi bastianellii* as an antigen for complement-fixation and immunofluorescence tests showed the greater reliability of the latter (Voller & Schindler, 1967) and only the results of this latter test will be reported here. The presence, and levels, of the *Toxoplasma* antibody and the hookworm antibody were assayed by other workers and the results will be reported elsewhere.

AREA OF STUDY

The present survey covered 2 large areas of Northern Nigeria, designated A and B; in addition, small numbers of sera were collected from Meko and Eror in the Western and Mid-Western Regions of Nigeria (M and E) as shown in Fig. 1.

The area A, north of the Benue river, covered the administrative divisions of Abuja, Jemmaa and Nasarawa of the Niger and Benue Provinces; the area B, south of the river Benue, comprised the divisions of Igala, Idoma, Tiv and Wukari of the Kabba and Benue Provinces. The survey was carried out in August–September 1966 in the area A and in December 1966–January 1967 in the area B.

FIG. 1
LOCATION OF THE SURVEY AREAS IN NIGERIA



Differences between the ecology of the two areas, while not striking, are nevertheless evident. The area A lies at an altitude of 600–1500 ft (200–500 m) and comprises wide plains with little relief except for isolated kopjes or inselbergs (Buchanan & Pugh, 1954).

The area B lies within the topographical unit defined by geographers as the " Benue trough ". It is a vast shallow land which includes the large floodplain; flat-topped hills of sedimentary origin occur as well as small volcanic cones on the eastern border. The vegetation of the two areas shows a gradual transition from the remaining patches of secondary forest of " derived Guinea savanna " in area B to the fringing forest on stream banks in " Guinea savanna " of area A.

During the wet season from May to October the rainfall averages 50 in–60 in (1270 mm–1520 mm) in the high plain of Hausaland and 60 in–70 in (1530 mm–1770 mm) in the Benue trough.

During the dry season (November–April) there is hardly ever more than 5 in (127 mm) of rain in either area. The mean maximum temperature is 90° F (32.2°C) with seasonal variations and the mean monthly minimum temperature is about 73° F (22.7°C). The mean daily range of 25° F (11°C) may increase to as much as 50° F (28°C) during the dry season when the mean absolute maximum is 103° F (39.4°C) and the mean absolute minimum 58° F (14.4°C) while the humidity may be as low as 20%–30% (Federal Government of Nigeria, 1956).

Both areas are essentially agricultural although the subsistence crops are slightly different. The population is predominantly Hausa and Nupe, with Yoruba and Fulani admixture in the northern area of the survey, while in the Benue trough there is a high proportion of the Tiv in addition to other smaller tribes. Livestock consists of cattle, goats, donkeys, horses, sheep and poultry.

The basic health services, while still inadequate, have shown a steady improvement over the past decade. There are hospitals in the large towns and health centres or dispensaries in the rural areas. No large-scale malaria-control activities had been carried out in either area and there had been no distribution of antimalarial drugs except for single-dose treatment of people with clinical symptoms of malaria.

Transmission of malaria in both areas is at the holoendemic level but with marked swings in relation to the seasons. Although the duration of the high-transmission season is never less than 6–8 months, it depends not only on the total annual rainfall, but also on its distribution in time, i.e., the number of rainy days. Local factors may be more important than meteorological conditions; thus the presence of permanent water-sources may create perennial transmission (Bruce-Chwatt, 1951).

Anopheles gambiae and *A. funestus* are the main vectors of malaria in Northern Nigeria. Their seasonal densities differ; the former increases explosively after the beginning of the rainy season and recedes to very low densities during the dry months especially in arid areas; *A. funestus* shows less pronounced swings and, while present throughout the year in much lower numbers than *A. gambiae*, it is also in evidence during the dry season (Bruce-Chwatt, 1951). Most of the transmission appears to be carried out by *A. gambiae*; its man-biting habit is 0.5 and the mean probability of survival almost 0.85.

The importance of seasonal changes in the intensity of transmission was shown by Archibald (1956) and Bruce-Chwatt & Archibald (1959). The mean daily infective density averaged 7.4 in August–September and 0.002 in April. The comparative epidemiology of malaria in southern as opposed to northern Nigeria was outlined by Archibald (1956).

The large areas dealt with in the present study are subject to the well-defined transmission waves of the savanna zone of West Africa. During the dry season, when the amount of transmission decreases, there is a general fall of spleen and parasite rates particularly marked in the young. No detailed long-term malariometric data for the two main areas of the present study are available. However, a survey carried out in 1955–56 in the Mokwa area (Bruce-Chwatt, unpublished) provides some baseline figures for area A.

The mean spleen rate in children 2–10 years of age was 59% with considerable variation between 40% and 84% from village to village. The mean parasite rate of the same group was 74%, composed predominantly of *P. falciparum*; *P. malariae* (20%) and *P. ovale* (5%) were also present, the latter mainly in very young children.

On the basis of infant parasite rates the inoculation rate (Macdonald, 1957) was estimated at 0.012. The basic reproduction rate calculated from entomological data was 1270, but this figure decreased to about 60 during the dry season. The net reproduction rate (a composite expression representing the maximum infectivity of a case within the given

TABLE 1

MALARIOMETRIC DATA OBTAINED IN 1963–64 IN AREAS CONTIGUOUS TO THE MAIN AREAS OF THE SURVEY

Age-group (years)	Benue Province			Kabba Province		
	No. examined	Spleen rate	Parasite rate	No. examined	Spleen rate	Parasite rate
<1	140	25.0	39.2	167	11.9	45.5
1–2	171	40.8	69.0	243	40.5	65.2
3–4	175	62.4	76.6	287	37.2	78.4
5–7	308	51.3	79.1	435	54.5	82.0
8–10	193	26.4	77.2	153	58.7	83.2
11–15	177	8.4	73.9	128	12.0	75.2
Adults	166	NA[a]	28.8	133	NA[a]	41.2

[a] Not available.

environment) has been estimated at 12.5 for the rainy season and 2.7 for the dry season.

Recent studies carried out in the savanna zone (Foll, 1968) showed that the first presence of malaria parasites in the blood of infants was at about 3.5–7.1 months of age, but 25% of infants less than 3 months old were already positive. Infants not infected during the dry season were rapidly infected at the beginning of the main transmission season in June.

Reliable malariometric data from areas contiguous to those of the present study were collected in 1963–64 by Dr J. S. Dodge (personal communication). These data supplement the incomplete information from the two areas of the survey and are shown in Table 1. These figures do not indicate much difference between the two areas but this conclusion may be modified when one realizes that they represent the situation during the low level of transmission in both areas and especially in the Kabba Province, which corresponds to area A. In fact, the peak transmission period in the latter area would produce much higher parasite rates and this is corroborated by the data presented later in Table 3.

RESULTS

The immunological tests were carried out on sera from a total of 1082 subjects in the present survey, and the results on those sera obtained from areas A and B were compared with the malariometric data collected by the Malaria Unit of the Northern Region of Nigeria.

Although an attempt was made to carry out a spleen survey in all areas, this proved to be difficult, because of the shortage of experienced staff. The data collected covered only some localities and some age-groups and for this reason they are not tabulated. Nevertheless, the data show that the spleen rates in the 6–10-year-old age-group were 7% higher in area B than in area A. Crude and specific parasite rates and parasite counts were obtained concurrently with the collection of sera. The number of sera tested from each age-group in the 2 large (A and B) and 2 small areas (Eror and Meko) covered by the present survey, is shown in Table 2.

Parasite rates in the 2 main areas are shown in Table 3 for the 4 standard age-groups together with the geometric mean of the reciprocal titre of the fluorescent antibody test.

The results of blood examinations indicate a crude parasite rate rather higher than that found during the previous surveys of the contiguous areas (see Table 1), especially in the young age-groups. *P. falciparum* was, as usual, the most prevalent species in all age-groups; *P. malariae* averaged 22% of all blood examinations in area A and 14% in area B with a high proportion (28%–42%) in the younger age-groups. This parasite species was generally found as a mixed infection with *P. falciparum*. *P. ovale* was found in about 5% of all blood slides, more commonly in the group less than 5 years of

TABLE 2

NUMBER OF SERA TESTED FROM EACH AGE-GROUP IN TWO LARGE (A AND B) AND TWO SMALL AREAS IN NIGERIA

Area	1–5 years	6–10 years	11–15 years	>15 years	Total
A	105	118	43	342	608
B	74	75	32	125	306
Eror (Mid-Western Region)	16	25	16	26	83
Meko (Western Region)	47	23	19	29	85
Total	209	241	110	422	1 082

TABLE 3

MALARIOMETRIC DATA AND RESULTS OF SEROLOGICAL TESTS

Area and period of survey	Age-group (years) [a]	Parasite rate			Crude parasite rate [c]	Parasite density index	Proportion of FA tests positive	Fluorescent antibody GMRT [d]
		P. falciparum [b]	*P. malariae*	*P. ovale*				
Area A Aug.–Sept. 1966	1–5 (74)	90.5 (43.2)	41.9	6.8	91.0	5.05	78.4	36.4
	6–10 (75)	88.0 (37.3)	24.0	2.7	89.0	3.91	82.5	74.2
	11–15 (32)	84.4 (34.4)	15.6	3.1	91.0	3.04	68.8	35.7
	>15 (125)	47.2 (13.6)	8.0	0	49.0	2.44	92.8	144.2
Area B Dec. 1966–Jan. 1967	1–5 (105)	77.1 (21.9)	27.6	3.8	78.0	4.70	94.0	134.0
	3–10 (118)	74.6 (17.8)	11.0	2.5	78.0	3.22	98.2	309.5
	11–15 (43)	60.5 (14.0)	14.0	2.3	65.0	2.78	97.5	439.4
	>15 (342)	31.3 (5.6)	4.4	0.3	35.6	2.14	99.8	591.0

[a] The figures in parentheses represent the number of persons examined.
[b] With gametocyte rates in parentheses.
[c] The proportion of the total number of persons examined who had malaria parasites in their blood.
[d] Geometric mean reciprocal titre.

age and as a mixed infection with *P. falciparum* or *P. malariae*. The gametocyte rate of *P. falciparum* in area B was 20% in the 0–5-year age-group, decreasing gradually to 6% in the over-15-year age-group. The respective gametocyte rate in area A was higher (34%) in the younger age-group but followed the same pattern of decrease in the older age-groups.

Table 4 shows the results of fluorescent antibody tests given as frequency distribution and as geometric mean reciprocal titres for each age-group of the two main areas surveyed.

In both areas the proportion of positive tests and the geometric mean reciprocal titre (GMRT) shows an increase according to the rising age-group but both indices are considerably higher in area B. This difference is of such magnitude that the adult levels of the titre in area A are close to those shown by the 1–5-year age-group of area B. Fig. 2 illustrates the frequency distribution of titres within age-groups in both areas.

The differences in the two main areas between the parasite rate, parasite density, the proportion of positive fluorescent antibody tests and their GMRT are emphasized by the combined data shown in Table 4.

The surveys of the Eror and Meko zones of the Mid-Western and Western Regions covered small samples of the populations and no parasitological

TABLE 4
FREQUENCY DISTRIBUTION OF FLUORESCENT ANTIBODY TITRES OF SERA FROM TWO AREAS OF NORTHERN NIGERIA

FA test titre	Age-group (years) and survey area							
	1–5		6–10		11–15		>15	
	A	B	A	B	A	B	A	B
<20	16	9	10	2	10	1	9	4
20	13	4	13	4	3	0	11	2
40	9	10	6	1	2	0	12	6
80	17	19	12	15	6	2	23	24
160	4	20	10	20	1	7	4	33
320	6	21	10	30	5	11	27	86
640	4	8	5	19	1	8	13	48
1 280	3	8	5	22	2	12	18	68
2 560	0	1	2	3	0	1	0	25
5 120	2	5	2	2	2	1	8	46
Total	74	105	75	118	32	43	125	342
GMRT	36.4	134.0	74.2	309.5	35.7	439.4	144.2	591.0
% positives	78.4	94.0	82.5	98.2	68.8	97.5	92.8	99.8

data for these zones have been collected. The proportion of positive fluorescent-antibody tests ($\geq 1:20$) is higher (85% against 68%) in children in the former area (Eror) and there is a much higher GMRT in all age-groups but especially in the young (Table 5).

DISCUSSION

Field studies carried out in endemic malarious areas of Africa during the past 5 years have shown a striking parallelism between the age-dependent increase of immunity to malaria and the level of antibody measured by immunofluorescent techniques (Voller & Bray, 1962; McGregor et al., 1965, 1968; Voller & Schindler, 1967; Collins, Skinner & Coifman, 1967).

With advancing age and increased exposure to high degrees of transmission, the inhabitants of hyperendemic and holoendemic areas show a progressive rise of the fluorescent antibody (FA) titre. The high level of antibody in the adults goes together with their rare and discrete parasitaemia, indicating a well-established balance between the parasite and the host.

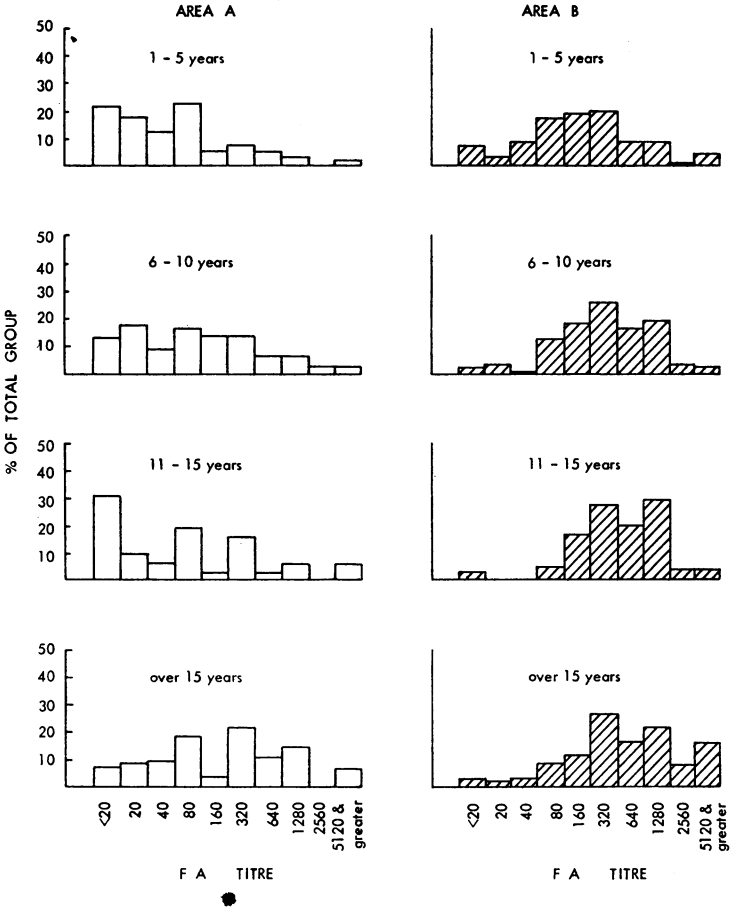
Studies on the persistence of antibody (Collins, Jeffery & Skinner, 1964a, 1964b; Jeffery, 1966) have shown the relevance of these findings to the situation in highly endemic areas, but several aspects of the collective immunological response to malaria are still little known.

The results of the present survey, based on a relatively large sample of 1082 sera collected from 4 age-groups of the African population living in highly endemic malarious areas of Northern Nigeria, correspond to the immunological profile found by those other observers. There was a 4-fold increase of the mean FA titre in adults as compared with the titre in children below 5 years of age.

It is odd that the plateau of high FA titres, in these areas, is seen in the adult population and not before. McGregor et al. (1965) indicated that the rate of increase of antibody is rapid in young children but slows down in adolescence and adult life. The mean value for any age-group represents a wide range of titres for individuals. Interpretation of individual values as indicators of the immune level of that individual would be misleading.

FIG. 2

FREQUENCY DISTRIBUTION OF MALARIA IMMUNOFLUORESCENT TITRES IN VARIOUS AGE-GROUPS IN TWO AREAS OF NORTHERN NIGERIA



A high titre probably bears some relation to the combination of density and duration of parasitaemia and to the time since the last bout of parasitaemia. However, a high FA level does not necessarily represent the concentration of the specific protective antibody (Cohen, McGregor & Carrington, 1961) and this has been repeatedly stressed by McGregor (1967). The pattern of the progressive rise of immunoglobulins G and M, reported by McGregor (1967), and studied in detail in 2 population groups by Rowe et al. (1968), may be reflected by the immunofluorescent test and in certain circumstances may give a measure of functional immunity in populations exposed to a significant amount of transmission. One should perhaps remember that the FA test, as generally carried out in the field, does not indicate the part played by different immunoglobulins. Rey, McGregor & Mattern (1967) have shown that the usual anti-human globulin serum used in the FA test gives much lower titres than an anti-human, gamma-globulin, monovalent serum.

The lack of close correlation between protective antibody and serologically demonstrable antibody may be due to the fact that considerable amounts of group-specific antibodies are produced in plasmodial infections although only those that are species- and strain-specific have a protective effect.

Our results based on a single blood survey indicate a higher parasite rate and parasite density in area A than in B, thus suggesting that at the time of the survey there was a greater degree of transmission in area A compared with B. It appears

TABLE 5
FREQUENCY DISTRIBUTION OF FLUORESCENT ANTIBODY TITRES OF SERA FROM 2 SMALL AREAS IN WESTERN (MEKO) AND MID-WESTERN (EROR) NIGERIA

FA test titre	Age-group (years) and survey area							
	1–5		6–10		11–15		>15	
	Meko	Eror	Meko	Eror	Meko	Eror	Meko	Eror
<20	9	3	3	3	2	1	3	1
20	0	2	3	3	0	0	1	2
40	0	3	4	1	3	1	1	1
80	4	5	4	4	7	1	3	2
160	0	1	2	1	2	0	2	3
320	1	1	6	4	1	7	10	5
640	0	0	1	2	1	3	1	0
1 280	0	0	0	7	1	2	8	3
2 560	0	1	0	0	1	0	0	0
5 120	0	0	0	0	1	1	0	9
Total	14	16	23	25	19	16	29	26
GMRT	5.3	36.4	55.8	128.3	100.9	289.4	184.8	486.1
% positives	35.8	81.3	87.0	80.0	89.4	93.6	89.6	96.0

from the detailed description of the two areas given earlier that in area A the transmission is more sharply seasonal and the blood samples were taken at the peak of transmission, namely, in August and September. In area B, where the transmission is more evenly spread over the year, the blood samples were taken at the time of least transmission—in December and January.

Our parasitological data do not seem to agree with the serological results and from them one would infer that the higher antibody levels in area B reflect a higher degree of malaria challenge. This conclusion is warranted if one considers the fact that the FA test represents the cumulative immunological response over a long period. Such an explanation finds some support from the evidence that the spleen rates in the 6–10-year age-groups were higher in area B than in area A.

Alternatively the difference between the two areas may be due to a high wave of malaria transmission in area B during the previous rainy season. If there were a time-lag of 2–3 months before peak antibody levels were reached then the antibody levels in area A would not have reached their maximum at the time the survey was carried out. In the 2 small areas of the Western and Mid-Western Regions the FA tests showed a much higher GMRT in all age-groups but particularly in the young. The exact cause of this difference may also be due to the fact that in the Mid-Western Region the blood collection was carried out soon after the end of the transmission season, though there were also differences in the local conditions between the two areas.

The problem of the relationship between the parasite rate and the FA titre is of particular interest in the light of other similar surveys carried out in Africa and elsewhere.

In the Gambia, McGregor et al. (1965) found that there was generally a direct correlation between the patent parasitaemia and an increased FA titre in the young age-groups (up to 5 years) even though in about 15% of these children the tests were negative (less than 1 : 25 titre) and in a few the tests were negative while the parasites were present in the blood. A significant difference between the FA response of the inhabitants of villages within the same area has been reported in this survey indicating the relevance of strictly local factors to

the transmission of malaria and serological assessment of its degree.

A survey carried out in the Gambia (Harverson, Wilson & Hall, 1968) showed good concordance between the parasite rate and FA tests in the rural areas when the transmission was at a high level. In the urban area the concordance was close in the younger age-groups but not in adults who showed a low parasite rate with a relatively high proportion of positive FA tests.

However, the difference in the over-all distribution of positive tests and titres between the rural and urban areas was considerable and was ascribed to the effect of malaria control activities.

A similar observation was made in Senegal by Rey et al. (1967) in their study of hospitalized cases of malaria. Although high titres were generally found in subjects with positive blood slides, a considerable proportion of clinical cases of malaria with high parasitaemia had FA titres often below the level of accepted positivity.

Coudert et al. (1966) carried out a study of malaria immunity in Dakar and stressed that on the average there is some correlation between the FA titres of children and their parasitaemia; however, there was no such correlation in adult Africans who had an acute attack of malaria at the time of examination and of those who were in good health.

A serological study of two villages in New Guinea where a high incidence of splenomegaly and hepatic sinusoidal infiltration was associated with endemic malaria (Marsden et al., 1967) showed that there was no direct relationship between parasitaemia and the FA titre. The group with the highest incidence of parasitaemia had FA titres within the range of 1 : 320 to 1 : 640 while in the group with FA titres of 1 : 5120 only 44% had parasites in the blood. However, high titres of fluorescent antibody were found in subjects with splenomegaly, consequent upon a sustained malaria challenge.

In a previous study in New Guinea, Curtain et al. (1964) found that the age-related rise of immunoglobulins in Melanesians living in highly endemic areas was not reflected in the level of specific malaria antibody, which remained relatively constant. This contrasts with the observations made in Africa.

A survey of malaria in the Ethiopian highlands showed an over-all low proportion of positive FA tests in a large area, but a high proportion in 2 villages where parasitological evidence later confirmed a previous epidemic (Collins et al. personal communication). In Malaysia, Collins et al. (1968) found that the presence of an infection was related to higher titres in one area, but in another area very low parasite rates were accompanied by high frequencies of positive FA tests and high FA titres.

The influence of malaria control activities on the results of immunological surveys is considerable. Thus Voller & Wilson (1964) showed in the Gambia that after administration of antimalarial drugs to mothers and to their infants, from the time of their birth for a period of about 1 year, the FA levels of the mothers were markedly reduced and the children were serologically negative. In another study in Senegal, Mattern et al.[1] confirmed and extended this observation. Its full significance from the point of view of functional immunity is not clear, but the importance of this effect in interpreting the results of serological surveys is obvious. However, there is no evidence that treatment played any part in the difference between the FA titres in the areas A and B of the present survey.

The present difficulties of direct comparison between the results of malaria serological surveys are due not only to the variations in techniques pointed out by Sodeman & Jeffery (1966) but also to other factors. This can be illustrated by the differences between our present results and those of Collins, Skinner & Coifman (1967). They examined a population from south-western Nigeria for fluorescent antibodies using *P. falciparum* as an antigen and also other human and simian antigens. Even with the homologous antigen, the FA titres they found were lower than those of the present study. Such differences may be due either to the method of collection of blood samples on filter-paper instead of separation of sera, or to the type of antigen used, or to other points of the technique of the test. It should be emphasized, however, that there is no particular virtue in obtaining very high titres; it is the degree of reproducibility which is more important.

The extent to which our present knowledge of immunology of malaria is still fragmentary may be indicated by the recent discovery of soluble antigens in the blood of Africans with severe falciparum malaria (McGregor et al., 1968). The rarity of antibodies to these soluble antigens in young children contrasts with the fact that malaria infection in this age-group is frequent and often severe and

[1] Mattern et al. (1967) *Chimioprophylaxie antipalustre et anticorps fluorescents.* Unpublished document WHO/Mal/67. 609. A limited number of copies of this document is available to persons officially or professionally interested on request to Distribution and Sales, World Health Organization, 1211 Geneva, Switzerland.

that immunoglobulins (IgG) are generally present in these children.

In the light of the results of the present survey the value of the immunofluorescent tests for epidemiological studies in malaria should be reassessed. Ever since Dempster in India introduced the " spleen rate " into malariometry some 120 years ago, this index has been widely used as a convenient, though rough, yardstick for estimating the amount of malaria in a locality or an area. Wherever malaria is " stable ", according to MacDonald's classification (1957), the spleen rate and the average enlarged spleen provide a reasonably good measure of endemicity if all the requirements for the proper use of this method are fulfilled.

Even in endemic conditions with seasonal waves, the 2 indices indicate the extent of the transmission. It has been shown in the Gambia (McGregor et al., 1965) that the spleen rates of young age-groups and their average enlarged spleen index are reflected in the frequency distribution and titres of the fluorescent antibody. Although we were unable to correlate individual spleen sizes with the FA titre we could confirm the fact that the area with higher mean FA titre (area B) had a higher spleen rate in the 6–10-year age-group.

The spleen rate is an age-dependent index and has serious limitations when applied indiscriminately to populations in some endemic zones such as West Irian (Metselaar & van Thiel, 1959). There is little doubt that the use of this index for classification of malaria endemicity is not satisfactory and the time has come for revising the present criteria (World Health Organization, 1963).

The results of blood examination for malaria parasites provide a reasonably adequate measure of the point prevalence of the infection. However, the limitation of this method in groups of populations that have achieved a degree of immunity and in which parasitaemia is very scanty, or periodical, are well known (Field, Sandosham & Fong, 1963; Raghavan, 1966; Dowling & Shute, 1966). A more complete picture can be obtained only in the course of a longitudinal survey when repeated blood examinations can be carried out (Bruce-Chwatt, 1963; Foll, 1968).

Immunological methods generally, and at present fluorescent-antibody tests in particular, provide additional evidence of the degree of malaria endemicity and reflect the period prevalence of the infection. The great advantage of this method is that it shows the degree of response of the adult age-groups to the infection and provides a serological indication of time-related trends in the immunological profile of the collective group.

The actual and potential value of the FA technique for detection of malaria infection in individuals or for mass surveys in the field has been fully recognized. A number of field projects now in progress indicate the interest aroused by this method and will better assess its importance for epidemiological studies related to malaria eradication.

The results of the present investigation confirm the potential value of the immunofluorescent technique for large-scale field surveys, but indicate the need for caution in interpreting their results and stress the importance of good knowledge of local epidemiology of malaria.

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RÉSUMÉ

En 1966–1967, une équipe épidémiologique de l'OMS pour les tréponématoses a rassemblé un nombre important d'échantillons de sérums provenant des régions nord, ouest et centre-ouest du Nigéria, en vue d'une étude préliminaire de diverses infections. Dans le cadre de cette enquête, des investigations sérologiques et parasitologiques sur le paludisme ont été menées dans quatre régions du Nigéria du Nord: deux zones étendues et deux

zones de faible superficie. Dans les deux zones étendues, les sérums ont été recueillis à deux périodes différentes: dans l'une (nord de la rivière Benue) au plus fort de la période de transmission et dans l'autre (sud de la rivière Benue) pendant la saison sèche, période de faible transmission. Des données sur les indices plasmodiques et les densités parasitaires ont été réunies et comparées aux données sérologiques.

Des quantités aliquotes de 1082 sérums prélevés sur des habitants appartenant à des groupes d'âge déterminés ont été expédiées dans l'azote liquide à Londres, où l'on a pratiqué des épreuves d'immunofluorescence en utilisant *Plasmodium cynomolgi bastianellii* comme antigène. Les sérums ont été éprouvés à des dilutions allant de 1: 20 à 1: 5120, et ceux qui ne réagissaient pas au titre le plus faible ont été considérés comme négatifs. Un sérum normalisé de titre connu, conservé à −70°C, a servi de témoin.

Les résultats de l'enquête parasitologique ont confirmé que le paludisme était holoendémique dans les deux principales zones et que *P. falciparum* était le parasite prédominant; l'épreuve des anticorps fluorescents a été positive (titre $\geqslant$ 1: 20) avec 92% des 914 sérums examinés. Le profil immunologique établi d'après la distribution de fréquence des titres a montré que le niveau de la réponse immunitaire était en relation directe avec le groupe d'âge et reflétait la structure de l'immunité collective.

La moyenne géométrique de l'inverse des titres et la proportion de titres élevés obtenues dans l'épreuve d'immunofluorescence étaient beaucoup plus fortes dans une zone (sud de la rivière Benue) après la précédente vague de transmission, bien que l'indice parasitaire ait été plus élevé dans l'autre zone (nord de la rivière Benue) où la transmission était en progression ascendante au moment de l'enquête.

La présente étude a confirmé la valeur de la réaction d'immunofluorescence pour les enquêtes paludologiques de grande envergure, mais elle a montré qu'il fallait être prudent dans l'interprétation des résultats et a fait ressortir qu'il était important de bien connaître l'épidémiologie locale du paludisme, avant de commencer à appliquer des méthodes sérologiques.

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