

AGENCY FOR INTERNATIONAL DEVELOPMENT WASHINGTON, D. C. 20523 BIBLIOGRAPHIC INPUT SHEET		FOR AID USE ONLY
1. SUBJECT CLASSIFICATION	A. PRIMARY Public Health	
	B. SECONDARY Malaria	
2. TITLE AND SUBTITLE Persistence of malaria antibody in Tobago, West Indies, following eradication, as measured by the indirect hemagglutination test		
3. AUTHOR(S) Mathews, H.M.; Fisher, G.U.; Kagan, I.G.		
4. DOCUMENT DATE 1970	5. NUMBER OF PAGES 6 p.	6. ARC NUMBER ARC
7. REFERENCE ORGANIZATION NAME AND ADDRESS Public Health Service, Center for Disease Control, Parasitology Branch, Atlanta, Georgia 30333		
8. SUPPLEMENTARY NOTES (Sponsoring Organization, Publishers, Availability) (In American Journal of Tropical Medicine and Hygiene, v. 19, no. 4, p. 581-585)		
9. ABSTRACT Inhabitants of the island of Tobago, West Indies, lived in an endemic area of malaria until an eradication program begun in the early 1940s was intensively pursued beginning in 1947. A slight outbreak of malaria occurred in an isolated area in 1966, involving 38 persons, but was quickly suppressed. The efficacy of the eradication program is manifested by the fact that between 1954 and 1966 no cases of malaria were detected by blood-film examination. Seroepidemiologic studies of the people living in Tobago indicate that in an area under malaria eradication the IHA antibodies fall to negligible levels within 15 years after the last clinical case is detected. Serum collected in 1955 by the Trinidad Regional Virus Laboratory indicated a high prevalence of malaria antibodies, whereas serum obtained in 1969 showed negligible levels of antibody. Persons involved in a recent focal outbreak of malaria had a higher percentage of antibodies than the general population, but the titers were not increased in this latter group. The serologic studies made in 983 inhabitants confirm the fact that malaria has been eradicated in Tobago.		
10. CONTROL NUMBER PN-AAC-631		11. PRICE OF DOCUMENT
12. DESCRIPTORS		13. PROJECT NUMBER
		14. CONTRACT NUMBER PASA RA(HA)-5-68 Res.
		15. TYPE OF DOCUMENT

TD
614.532
M429

PAST: PA(HA)-5-68 Res.
PN-AAC-631

PERSISTENCE OF MALARIA ANTIBODY IN TOBAGO,
WEST INDIES, FOLLOWING ERADICATION, AS MEASURED
BY THE INDIRECT HEMAGGLUTINATION TEST

HENRY M. MATHEWS, GEORGE U. FISHER, AND IRVING G. KAGAN

Reprinted from *THE AMERICAN JOURNAL OF TROPICAL MEDICINE AND HYGIENE*
Vol. 19, No. 4 July 1970
pp. 581-585
Printed in United States of America
Copyright © 1970 by The American Society of Tropical Medicine and Hygiene

PERSISTENCE OF MALARIA ANTIBODY IN TOBAGO, WEST INDIES, FOLLOWING ERADICATION, AS MEASURED BY THE INDIRECT HEMAGGLUTINATION TEST*

HENRY M. MATHEWS, GEORGE U. FISHER,† AND IRVING G. KAGAN
U.S. Public Health Service, Health Services and Mental Health Administration,
National Communicable Disease Center, Parasitology Section, Laboratory Division,
and Parasitic Diseases Branch, Epidemiology Program, Atlanta, Georgia 30333

ABSTRACT: After a malaria-eradication program, no known autochthonous cases of malaria had occurred on Tobago, West Indies, from 1954 to 1966. In 1969, serum collected on filter-paper rectangles from 983 residents of the island was titrated for the presence of malaria antibody by the indirect hemagglutination test, with *Plasmodium knowlesi* as antigen. Serum was collected from three groups: 40 persons previously sampled in 1955; 27 persons that were infected with *Plasmodium malariae* in 1966; and 916 residents of the island selected from the general population. For the 40 persons resampled, antibody prevalence fell from 79% in 1955 to 10% in 1969. Of the 27 persons involved in the quartan-malaria outbreak in 1966, five (18.5%) had detectable antibody. In the sample of the general population, only 1% had demonstrable antibody and, with one exception, the positive reactions were in serum from older persons. These results indicate that hemagglutination antibodies become negligible in a general population within 15 years after eradication of malaria.

(Accepted 8 January 1970)

Tobago, a small island near Trinidad and the coast of Venezuela, is 32 miles long, from 7 to 11 miles wide, with a total land area of about 116 square miles. Because of a central range of mountains, most of the estimated 30,000 inhabitants live along the coast and are concentrated on the southwest end of the island (Fig. 1).

In the 1940s malaria was endemic in Tobago and was regarded as the foremost public-health problem.¹ During the early 1940s control measures were directed toward swamp drainage, application of larvicides, and other methods designed to destroy the malaria vector, *Anopheles aquasalis*. In 1946 preliminary eradication work was begun with residual spraying of DDT. This work was expanded in 1947 to include the spraying of principal villages on the island, and in 1949 a full-scale malaria-eradication program was initiated. This program was so successful that no autochthonous cases of malaria were found on Tobago from 1954 to 1966. In 1966 there was a focal outbreak of 38 cases of quartan malaria. The severity of these cases and the thoroughness of the epidemiologic studies suggest that malaria

had been previously eradicated and that an excellent surveillance system was in operation.²

The persistence of hemagglutinating antibodies to malaria in former endemic areas has not been carefully studied to date. Tobago was an ideal site for such a study because we had 84 samples of serum drawn in Tobago in 1955, shortly after complete eradication, plus the names of 38 persons who had had clinically proved cases of malaria 3 years earlier, and a population to study that had lived in an area free of malaria for about 15 years.

MATERIALS AND METHODS

The 84 serum samples, drawn in 1955 by the Trinidad Regional Virus Laboratory, were supplied by Dr. W. G. Downs of the Yale Arbovirus Research Unit, Yale University School of Medicine, New Haven, Connecticut. Other serum samples were obtained in February 1969 from 1) 40 persons previously bled in 1955; 2) 27 persons infected in the quartan outbreak in 1966; and 3) 916 persons from the general island population.

In 1969 blood was collected on filter paper (Ropaco #1023-.038,* Rochester Paper Com-

* 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.

† Present address: Stanford University Hospital, Palo Alto, California.

* Use of trade names is for identification only and does not constitute endorsement by the Public Health Service nor by the U.S. Department of Health, Education, and Welfare.

pany, Rochester, Michigan) cut into 1×3 inch rectangles and imprinted with a 14-mm circle. A full circle of blood was obtained by finger-prick. The rectangles were placed in a slide box and allowed to dry overnight at ambient temperature. The following day they were interleaved with strips of glassine paper, tied into bundles of 50 to 100, and sealed in small plastic bags. When several hundred rectangles were collected, they were air-mailed to our laboratory at the National Communicable Disease Center where they were frozen at -20°C . The rectangles were removed from the freezer, and a 10-mm disc from the blood-filled circle was cut out with a paper punch. Each filter-paper disc was placed in a 13×85 mm tube, and 0.2 ml of phosphate-buffered saline solution (PBS), pH 7.2, was added. The papers were allowed to stand in the solution for 30 minutes at room temperature (27°C), then the tubes were shaken vigorously for 1 minute. The filter papers were discarded after the fluid was expressed from them with a small metal rod. The dark-brown eluate was diluted in twofold steps in a microtitration system. The initial dilution of eluate represented a 1:16 dilution of serum.

The indirect hemagglutination (IHA) test, with *Plasmodium knowlesi* as the antigen and human type O erythrocytes as carriers,⁴ was used to titrate antibody. Titers of less than 1:16 were considered negative and were assigned an arbitrary titer of 1:2. Titers of 1:16 or greater were considered positive. The prevalence of malaria was based on two criteria: the percentage of the

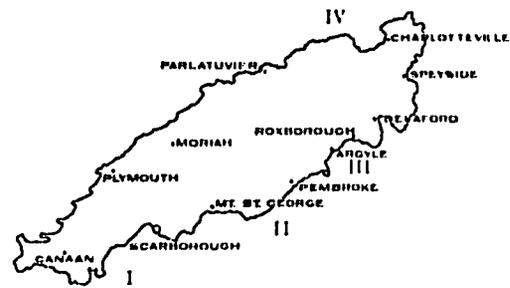


FIGURE 1. Map of Tobago showing four sampling areas.

population with positive titers, and the geometric-mean reciprocal titer (GMRT).

To show the geographic distribution of antibodies, we divided the island into four districts (Fig. 1). Area I, at the southwest end of the island, was the most populous part and is largely flat land. Areas II and IV were made up of a number of small villages. Area III includes the villages of Argyle, Roxborough, and Delaford, and was the site of the outbreak of quartan malaria in 1966.

RESULTS

The distribution of titers obtained from 84 persons sampled in 1955 and 40 of the same persons sampled again in 1969 is shown in Table 1. The 1955 collection yielded 78.6% positive serum samples, with a GMRT of 52.5. The highest titer was 1:2,048. The 40 persons sampled in 1969 show a decline in prevalence to 10%, and a GMRT of 2.7. The positive samples occurred only in the oldest age category (>44 years).

TABLE 1
Distribution of malaria IHA titers by age group for residents of Tobago in 1955 and 1969

Age group (years)	Titer									Total	GMRT
	<16	16	32	64	128	256	512	1,024	2,048		
Samples collected in 1955											
20-29	4	3	3	2	4	1	—	—	—	17	25.1
30-44	9	2	4	2	2	6	3	4	—	32	47.2
>44	5	3	7	3	2	7	5	2	2	35	82.7
Total	18	7	14	7	8	14	8	6	2	84	52.5
Samples collected in 1969											
30-44	7	—	—	—	—	—	—	—	—	7	2.0
>44	29	2	—	1	1	—	—	—	—	33	2.9
Total	36	2	—	1	1	—	—	—	—	40	2.7

TABLE 2
Distribution of malaria IHA titers in residents of Tobago by age and 1966 malaria status

Age group (years)	No known history, 1966					Infected, 1966		
	Titer					Titer		
	<16	16	32	64	128	<16	16	32
<2	2	—	—	—	—	—	—	—
2-4	27	1	—	—	—	1	—	—
5-9	38	—	—	—	—	5	1	—
10-14	164	—	—	—	—	7	1	—
15-19	310	—	—	—	—	4	—	—
20-29	90	—	—	—	—	1	1	—
30-44	128	2	—	—	—	4	1	—
>44	187	4	1	1	1	—	—	1
Total	946	7	1	1	1	22	4	1

Table 2 gives the age, titer distribution, and 1966 malaria status for the entire collection made in 1969. In the 27 persons infected in 1966 during an epidemic of malarial malaria a seropositivity rate of 18% was observed, whereas in 956 persons not known to have been infected in 1966 the seropositivity rate was only 1%. The GMRT values for the two groups are not greatly different, 3.0 and 2.1. Nine of the 10 positive specimens in the noninfected group were from persons 30 years and over, and only one positive specimen (titer 1:16) was found in a 3-year-old girl with no history of malaria.

The distribution of titers for the four geographic areas is shown in Table 3. Antibody prevalence in Area I, representing the largest population sample, was 1.1%, and the GMRT was 2.1. Area III, the area included in the 1966 outbreak, had the highest seropositivity rate of all areas, 2.4%. The GMRT, however, was not elevated. The five persons with positive antibody titers in this area had all been infected in the outbreak in 1966. Areas II and IV had posi-

TABLE 3
Distribution of malaria IHA titers by geographic area in Tobago, 1969

Area	Titer					Positive (%)	GMRT
	<16	16	32	64	128		
I	466	3	1	1	—	1.1	2.1
II	141	2	—	—	—	1.4	2.1
III	200	4	1	—	—	2.4	2.1
IV	161	2	—	—	1	1.8	2.1
Total	968	11	2	1	1	1.5	2.1

TABLE 4
Results of blood slide examinations for malaria in Tobago, 1944-1967

Year	No. slides examined	No. slides positive	Percent positive
1944	1,460	723	49.5
1945	1,266	410	32.4
1946	1,924	816	42.4
1947	6,254	860	13.8
1948	5,363	282	5.3
1949	5,995	329	5.5
1950	3,268	71	2.2
1951	2,633	47	1.8
1952	1,597	6	0.4
1953	1,392	12	0.9
1954-1965	40,087	0	0
1966	24,360	38	0.15
1967	9,147	0	0

tivity rates under 2%, and in neither instance was the GMRT elevated.

Table 4 gives the results of blood-film examinations on the island of Tobago from 1949 to 1967, the last year for which data were available. Malaria incidence was shown by blood-film examination to decline from 1946 to 1953. From 1954 to 1966 over 40,000 slides were examined, and no cases of infection were detected. Thirty-eight cases were discovered in the population in 1966, but no cases have been found since then.

DISCUSSION

For evaluation of the significance of the indirect hemagglutination test, the persistence of this antibody in serum required evaluation. The large number of positive serum samples in military recruits in three countries of the Western Hemisphere, as compared with the small number of positive blood films from these areas, suggests that the IHA test is measuring long-term experience with malaria parasitism.¹

A small survey of three villages in the Republic of Panamá under intensive surveillance also suggests that antibodies could exist in the absence of infection for at least 5 years (our unpublished data). U.S. military personnel returning from Vietnam with a history of malaria show that the persistence of malaria IHA antibody is directly related to the number of previous attacks of the disease. Thus, persons with a history of only one attack of malaria generally had no antibody in 6 to 12 months following effective chemotherapy, whereas those with histories of several attacks tended to maintain IHA titers at rather high levels

for at least 12 months following treatment (our unpublished data). Persons in Tobago infected in the 1966 outbreak of malaria also showed a rapid decline of serologic positivity after effective chemotherapy (Table 2). The sensitivity of the HIA test in the current work in detecting 18.5% of the persons 3 years after a malaria outbreak suggests that the serologic method will detect focal outbreaks of malaria in an area where eradication has been successful.

The serologic survey of a small number of adults bled in 1955 suggests that this island was endemic for malaria since the percentage of those with elevated levels of malaria antibody was high. The 1969 collection of blood from a much larger group of the general population suggests that after 15 years of no exposure to infected mosquitoes, antibody titers decline to negligible levels. The data from the serum collected in 1955 and the results obtained from a sample of these same persons in 1969 also show a dramatic drop in prevalence of circulating antibody (from 79 to 10%, respectively) as well as a marked drop in the GMRT (52.5 to 2.7, Table 1).

The outbreak of quartan malaria in 1966 allowed us to evaluate the sensitivity of the test in detecting antibody of recent origin in an area where malaria has been eradicated. The persons infected in the outbreak of 1966 were of all ages. Titers of 1:16 or less were obtained except for one person over 44 years of age who had a titer of 1:32. The survey of the general population showed only one questionable positive reaction, in a person under 29 years of age, and titers in the oldest age group (>44) ranged to 1:128.

The prevalence of antibodies to malaria among persons infected in the 1966 quartan outbreak was higher than in the general population (18.5% to 1%), but the GMRT was not significantly different (3.0 to 2.2). The persons infected in the 1966 outbreak had been detected rapidly and treated immediately. In addition, the villages affected were placed under immediate surveillance, intensive spraying, and mass therapy. This protocol was extended to cover the entire island and thus helped restrict the outbreak to a few cases in one area. The success of these activities is evidenced by the absence of antibodies to malaria in the outbreak area in persons not diagnosed. The clinical nature of the disease in the infected persons was severe enough to cause them to seek medical attention,² suggesting a lack of

immunity due to the eradication program. The rapid and effective treatment of these persons probably did not allow for an extended period of antibody production; hence, high levels of antibody were not produced.

The serologic survey of the general population made in 1969 confirms the epidemiologic studies made in the area,² that malaria has been eradicated in Tobago. Some persons will maintain low positive titers for long periods of time. These are the older persons in the population who have had the longest experience with malaria before eradication. This is apparent when one looks at the age and titer distribution of the positive reactors in the general population sampled. Positive titers are found only in persons over the age of 30 (with a single exception). Among the five persons infected in 1966 who had positive titers after 3 years, the only titer greater than 1:16 was in a woman over the age of 44 who may have had malaria experience before 1966.

SUMMARY

The inhabitants of the island of Tobago, West Indies (Fig. 1), lived in an endemic area of malaria until an eradication program begun in the early 1940s was intensively pursued beginning in 1947. A slight outbreak of malaria occurred in an isolated area in 1966 involving 38 persons but was quickly suppressed. The efficacy of the program is manifested by the fact that between the years of 1954 to 1966 no cases of malaria were detected by blood-film examination. Sero-epidemiologic studies of the people living in Tobago indicate that in an area under malaria eradication the HIA antibodies fall to negligible levels within 15 years after the last clinical case is detected. Serum collected in 1955 by the Trinidad Regional Virus Laboratory indicated a high prevalence of malaria antibodies, whereas serum obtained in 1969 showed negligible levels of antibody. Persons involved in a recent focal outbreak of malaria had a higher percentage of antibodies than the general population, but the titers were not increased in this latter group. The serologic studies made in 983 inhabitants confirm the fact that malaria has been eradicated in Tobago.

ACKNOWLEDGMENTS

The authors wish to thank Dr. Wilbur G. Downs for providing the 1955 serum collection; Dr. O. H. Siung and Dr. A. Santiago, Ministry

of Health and Housing, Trinidad and Tobago, for making this study possible, and Janet A. Fried for technical assistance.

PUBLICATIONS CITED

1. Downs, W. G., Gillette, H. P. S., and Shannon, R. C., 1943. A malaria survey of Trinidad and Tobago, British West Indies. *J. Nat. Mal. Soc.* (Suppl.), 2: 1-44.
2. Siung, O. H., 1968. Report on an outbreak of quartan malaria on the island of Tobago, West Indies - 1966. World Health Organ. document WHO Mal/68.667.
3. Rogers, W. A., Jr., Fried, J. A., and Kagan, I. G., 1968. A modified, indirect microhemagglutination test for malaria. *Am. J. Trop. Med. & Hyg.*, 17: 804-809.
4. Kagan, I. G., Mathews, H. M., Rogers, W. A., Jr., and Fried, J. A., 1970. Seroepidemiologic studies by indirect hemagglutination tests for malaria. 1. Military recruit collections from the United States, Brazil, Colombia, and Argentina. *Bull. World Health Organ.*, in press.
5. Waterman, J. A., 1967. Malaria and its eradication in Trinidad and Tobago. *Carib. Med. J.*, 19: 19-35.