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AID PASA Control No. RA (HM) 5-68, Amend. #6
"Serologic Diagnosis of Malaria"

QUARTERLY REPORT
July 1 - September 30, 1971

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SEROLOGIC DIAGNOSIS OF MALARIA

PASA Control No. RA (HM) 5-68

Amendment #6

Purpose and Scope of Project

The program is designed to develop simple, rapid, sensitive, and specific serologic methods for the diagnosis of malaria which are applicable for epidemiologic and clinical purposes.

In an effort to develop methods that may be useful in the worldwide malaria eradication program for surveillance and other purposes, this laboratory has concentrated its efforts on the evaluation and standardization of the indirect hemagglutination (IHA) technique for the detection of malaria antibody. Studies are in progress to define the extent of cross reaction and sensitivity of speciation obtained with the indirect fluorescent antibody (IFA) test.

I. Studies on the Indirect Hemagglutination (IHA) Test

A. Antigen Studies

Efforts to develop a more stable antigen have continued. Antigens extracted from the plasma of Plasmodium knowlesi infected rhesus monkeys were evaluated. These antigens, prepared by Dr. M. Ristic, were similar to those extracted by Rivanol precipitation in that they showed reduced activity when tested against a battery of control sera. One of Dr. Ristic's fractions showed a relatively high rate of false positive reactions.

An antigen has been obtained from rhesus monkey with a dual P. knowlesi-Babesia infection. This antigen will be utilized in studies on relationships between these two intra-erythrocytic parasites.

B. Immunoglobulin Studies

Preliminary studies of the longevity of IgM on filter paper have been initiated. IgM levels for blood stored on filter paper are being determined by the radial immunodiffusion method. Effects of storage of filter paper samples for various periods of time and under different conditions will be investigated. Future studies will include the determination of IgG levels in papers stored under similar conditions.

C. Stabilization of the Red Cell Carrier

1. Frozen red cells

In cooperation with the Serum Bank Unit/CDC, we have initiated evaluations of human type "O" erythrocytes preserved by freezing. The freezing process, similar to the one used by the Red Cross for preserving blood for transfusion, offers promise for storage of red cells for use in malaria serology. Initial experiments with a single lot of cells frozen in this manner indicate that cells frozen for up to 8 weeks can

be thawed and successfully sensitized with malaria antigen. Furthermore, thawed cells remain usable for up to 6 weeks when stored at 4° C. This exceeds the storage period for fresh cells by 2 to 3 weeks. Further experiments are in progress to determine whether this method is reproducible with cells from different donors. Additional experiments will be conducted to determine whether sensitized red cells can be frozen and thawed without loss of activity.

2. Aldehyde fixed cells

Stable sensitized cells were prepared in small quantities and tested with negative and positive control sera. A large batch of stable sensitized cells will be prepared for evaluation with another battery of sera. A report on the results of six replicate runs of 24 sera which were tested within a 3-month period with the stable sensitized cells is being written for publication.

D. Seroepidemiologic Studies

1. Brazil. The fourth survey in Mato Grosso, Brazil, was conducted in September 1971 as a collaborative study with Central America Malaria Research Station (CAMRS), El Salvador.

2. Philippines. Over 3,300 samples from a penal colony on Palawan were tested this quarter. The five subcolonies which make up the entire colony have a range of slide positivity rate from 0.46 to 10.8 percent. Preliminary tabulations of seropositivity rates show higher levels but, in general, reflect the slide positivity rate. Serologic data are being prepared for final tabulation by the computer system.

3. Tunisia. Over 2,100 samples collected in Tunisia by Dr. Ambroise-Thomas (University of Grenoble, France) were tested by the IHA test. Dr. Ambroise-Thomas has provided the IFA test

results, and the IHA and IFA results are being tabulated by the computer. This study provides an opportunity to compare the IFA test as performed by Dr. Ambroise-Thomas with the IHA test as performed in our laboratory.

4. Nigeria. The initial serologic survey in the WHO-sponsored longitudinal malaria study in Nigeria is being conducted during September and October 1971. Dr. Hans O. Lobel spent 3 weeks in the area at the invitation of WHO.

5. CAMRS. Collaborative efforts with CAMRS in the evaluation of the IHA test in Central America have continued. Baseline data have been obtained for 10 localities in the Guayabo area near a large hydroelectric dam project. Additional studies are being planned for a coastal area (San Diego) in El Salvador and for a low malaria-incidence area (Puntarenas) in Costa Rica.

6. Epidemic Aid. In an effort to identify the source of an indigenous case of malaria in Texas, 44 serum specimens drawn from contacts were tested. It was not possible to serologically identify a source.

7. Miscellaneous. A total of over 1,325 serum specimens were tested from various sources. These include sera from experimental animals and filter paper and serum samples from foreign and domestic collaborators.

II. Evaluation and Development of the Indirect Fluorescent (IFA) Test for Malaria

A. Studies in Progress

1. Plasmodium ovale studies

A chimpanzee with negative malarial IFA test reactions has been acquired. The animal is presently being conditioned and will be splenectomized in about a month. Recovery from the splenectomy will take 1 to 2 months, when he will be infected with P. ovale.

2. Occult malaria studies

The second filter paper blood samples from Nigerian students now residing in the United States have been obtained. These sera will be tested at a later date as part of the continuing project to determine the significance of high malarial antibody titers in people from endemic areas.

3. Serology

The evaluation of the schizont antigens of human plasmodial species is continuing. Seventy-eight of the 190 sera included in the battery have been tested. This evaluation will be completed by the end of next quarter.

4. Multispecies antigen

Two lots of antigen containing P. vivax, P. falciparum, and P. brasilianum have been made. Titers obtained with the multi-species antigen are equal to or within one fourfold dilution of the greatest titer obtained with any of the single species antigens. All sera will be screened with the multi-species antigen; such screening will reduce by two-thirds the number of slides presently used. All positive sera

will be titrated with the single species antigens to try to differentiate species. A research note on this antigen has been submitted to the Journal of Parasitology.

5. Splenomegaly and malaria in South Vietnamese

The study with Dr. L. C. Butler, NAMRU-2, on Vietnamese patients with and without splenomegaly to determine the incidence and relationship of malaria and splenomegaly has been completed. In addition to the initial 50 sera tested during last quarter, 40 more sera have been tested. Dr. Butler reports that all the splenomegaly patients except one had high antibody titers, but there was also a fairly high incidence of significant titers in patients without splenomegaly.

6. Tanzania study

Selected sera from a population in Pare, Tanzania, have been tested with IFA by both Dr. C. C. Draper of the London School of Tropical Medicine and this laboratory. Comparison of results with P. falciparum antigen showed excellent agreement between the two laboratories. Results at CDC with P. vivax, P. ovale, and P. brasilianum antigens, which Dr. Draper did not use, indicate that after P. falciparum, P. malariae is the most prevalent species in this area; P. vivax is third; and P. ovale is last. Though numbers of sera tested were small, the serologic results agree with the prevalence determined by blood slide examination in the area.

General Comments

On June 28, 1971, an AID Research Review visited the project. In preparation for this review, a summary of Project Goals and Accomplishments was compiled.

A Project Proposal for FY 1973-1975 was submitted September 9, 1971, for a 3-year extension of the project. On September 24, the Annual Report for FY 1971 was submitted.

A presentation "Evaluation of the Indirect Hemagglutination Test as an Epidemiologic Technique for Malaria" was written for the Inter-American Malaria Research Symposium in El Salvador, November 1-4, 1971.