INDIAN JOURNAL OF MALARIOLOGY

Volume 23 **Number 1**

June 1986

MALARIA RESEARCH CENTRE
Indian Council of Medical Research
22-Sham Nath Marg
Delhi–110 054

INDIAN J. MALARIOL.

Half Yearly

Year of revival : 1981

SUBSCRIPTION RATE

Annual	India	Rs. 30.00*
	Other countries (including airmail postage)	$ 10.00

*50% discount would be admissible to the individual subscriber on annual basis.

Payments in respect of subscription may be sent by money order/bank draft/postal order/cheque crossed and marked payable to Malaria Research Centre, 22-Sham Nath Marg, Delhi — 110 054.

The 'Indian Journal of Malariology' is indexed by 'BIOSIS', 'Drugs and Pharmaceuticals Current Indian Titles', 'Index Medicus', 'Indian Science Abstracts', 'Review of Applied Entomology', 'Protozoological Abstracts', 'Quarterly Bibliography of Major Tropical Diseases' and it is selectively abstracted by 'Tropical Diseases Bulletin'. This journal is also accessible on the CAB Computer Database and MEDLINE.

INDIAN JOURNAL OF MALARIOLOGY

INDIAN JOURNAL OF MALARIOLOGY

CONTENTS

Volume 23
Number 1
June 1986

Short Notes

Indian Journal of Malariology
Vol. 23, June 1986, pp. 1–18.

Splenomegaly in Malaria

H.A.H. MASHAAL[1]

INTRODUCTION

Splenic enlargement is one of the three main signs characteristic of malaria infection (fever, anaemia, splenomegaly). It is of great diagnostic aid to every physician. To both the epidemiologist and the malariologist it is of immense value in evaluating and assessing the malaria situation within an area.

In spite of the fact that spleen examination can yield valuable information, contradictory results have been published. This is largely attributed to the widespread employment of non-medical personnel who are utilized extensively in the execution of several surveys. These non-medical personnel frequently miss the slightly enlarged spleen, particularly if it is soft in consistency. The determination of spleen consistency is difficult for the inexperienced physician; for a non-medical person, it is almost impossible to detect. In 1965–1966, the author evaluated the results of a non-medical team which had conducted spleen surveys in Southeast Asia for decades and the results were published and analysed. A school in one village in Pakistan was surveyed by the non-medical staff. When the author checked the same school, it was noticed that all the slightly enlarged spleens were missed as well as the very soft ones which yielded blood positive results. This drew the attention of the author to the fact that many publications on spleen surveys are to be critically evaluated and contradictory statements on the value of spleen examination may be largely attributed to the extensive utilization of non-medical staff as well as inexperienced physicians for spleen examination.

In India in 1847, Dempster (Russell *et al.*, 1963) had been the first to show that the correlation between an enlarged spleen and malarial fever is of practical value as a measure of malaria endemicity. Early in this century, spleen indices were commonly utilized for diagnosis, evaluation and assessment of malaria. Boyd, 1949, elaborated on the subject.

However, since the development of the Malaria Eradication Policy in 1955, some desk malariologists have unfortunately come to regard spleen examination merely as a rough index for malaria diagnosis and assessment. The author found the spleen examination of young children an outstanding tool in malaria evaluation, even in countries with schistosomiasis as the spleens here show enlargement after a lapse of long periods i.e., about a year or more.

Accepted for publication: 15 January 1986.
[1]Member WHO Expert Advisory Panel
Consultant Malariologist
P.O. Box 2968
Winnipeg, Manitoba, Canada R3C 4B5.

The spleen examination was not only utilized (by the author), in the malaria control programmes, but it was applied equally efficiently in advanced malaria eradication programmes in different countries. It was possible to get several malaria cases confirmed microscopically at a later date. In one instance (East Nepal), the author was able to detect several falciparum cases in a few days by spleen examination in selective areas projected for maintenance phase. Among the countries examined by the author which yielded excellent results for spleen examination in malaria assessment are: Gaza territory, Saudi Arabia, Syria, Pakistan, Bangladesh, Philippines, Taiwan, Sudan, Burma, Thailand, Nepal and the Solomon Islands.

Immunodiagnostic techniques are developing very fast nowadays. This is because the parasite rates do not reflect a true picture of the malaria situation among immune communities. The examination of the spleen has the advantage of giving a clearer picture of the malaria situation, if its size as well as its *consistency* are determined.

The following views are stated by renowned malariologists on the importance of spleen examination and its superiority to blood examination.

(i) According to Russell, Ross, Darling and Boyd (Russell, 1952) a spleen index above 5% is not found in non-malarious cities in the absence of schistosomiasis and kala-azar.

(ii) After the first infection is over, the enlarged spleen may decrease in size so rapidly that it will not be palpable two months later. But in chronic malaria or in patients who have had repeated infections the decrease in size is much less rapid (Russell, 1952).

(iii) In a nonimmune patient, the spleen is large and soft. With the development of immunity the spleen becomes harder. At the highest level of immunity it tends to become smaller again (Russell *et al.*, 1963).

(iv) The spleen rate is easier to obtain than the parasite rate. It includes recent/past, as well as present malaria and it is frequently more reliable in practice than the examination of blood films (Russell *et al.*, 1963).

(v) Once the individual has ceased to suffer acutely from malaria infection, enlargement of the spleen tends to subside but not to disappear while the infection persists (Russell *et al.*, 1963).

The author presents several examples of the outstanding value of spleen examination in evaluation and detection of asymptomatic cases in consolidation areas or even in areas recommended for maintenance.

STRUCTURE OF THE SPLEEN

The spleen is the largest ductless gland of the body. It is located in the posterior portion of the upper left abdominal quadrant beneath the 9th, 10th and 11th ribs. It is an oval, spongy, flattened, fist-sized organ. It is roughly 12 centimetres by 8 by 4. It has normally a dull purple colour which is due to its content of blood. It is soft and more friable than most organs. One surface lies against the kidney, one against the diaphragm, another against the stomach and another in contact with the intestine but it is separated from all of them by the peritoneum.

In healthy adults, the anterior margin usually cannot be felt under the costal border. However in small infants the anterior margin of a normal spleen is often palpable.

The spleen has an average weight, expressed as 3.9 gm per kilogram of body weight. An average man of 60 kg body weight has a spleen of 234 gm. Bruce-Chwatt reported a mean adult spleen weight of 265 gms for both sexes in his African series (Russell *et al.*, 1963).

By the naked eye, two kinds of pulp may be noticed. The white pulp is distributed as tiny firm gray islands, each less than 1 mm in diameter.

The soft red pulp fills the remaining space.

MICROSCOPIC STRUCTURE OF THE SPLEEN

The spleen is largely made up of branched connective tissue cells called reticular cells and associated reticular fibres. The parenchyma or the pulp of the spleen consists of the following:

(a) *White pulp*: It is immunologically competent; it sequesters lymphocytes, macrophages and antigen and permits them to interact (Fig 1).

The central artery enters the white pulp. As it runs through the pulp it is surrounded by a reticular sheath tightly packed with lymphocytes (the peri-arterial lymphatic sheath). The lymphocytes of the peri-arterial sheath migrate to the spleen from the thymus. These T-lymphocytes (or T-cells) are a heterogeneous population of cells which have undergone a period of maturation in the thymus and which bear the thymus surface marker.

Lymphatic nodules (Malpighian corpuscles) lie within the peri-arterial sheath. They are compact spherical collections of lymphocytes. These are rich in B-lymphocytes. In a few hours after entering the spleen, the B-lymphocytes reach and enter the lymphatic nodules and are concentrated in its periphery (corona or mantle zone). In the centre of the lymphatic nodules, germinal centres appear. They are sites of high level antibody production. Surrounding a lymphatic nodule there are vast numbers of red blood cells in its meshes. The meshes are designed to act as a filter.

As the central artery runs through the red pulp, it gives rise to several branches. Out of these (i) few end within the white pulp (ii) some supply the germinal centres and mantle zones of the lymphatic nodules (iii) the majority terminate at the periphery of the white pulp and are emptied at the marginal zone (iv) some leave the white pulp passing into the marginal zone and end in the ellipsoid and (v) some emerge from the white pulp, passing through the marginal zone and terminate in the cord of the red pulp. From the cord, cells can reach the sinusoids (see Fig. 1). The sinusoids of the red pulps drain and finally reach pulp veins which in turn drain into trabecular veins.

Some arterioles in the white pulp pass the peri-arterial lymphatic sheath and before they terminate they may be ensheathed by a tight ellipsoidal nodule whose dominant cell type is the macrophage. Plasma cells develop in both the white pulp and the red pulp. They do not enter the circulation but remain in the spleen.

(b) *Red pulp*: It is composed of splenic sinusoids, separated by splenic cords. The framework of the red pulp consists of a mesh of reticular cells and reticular fibres (Fig. 1).

The thin wall of each sinusoid is made of long, narrow reticulo-endothelial cells lying side by side and bulges into the lumen. The basement membrane of each sinusoid is perforate rather than a full sheath surrounding the outside of the endothelium.

In the cytoplasm of the reticulo-endothelial cells (both of the wall and the free macrophages) one may see the haemoglobin of phagocytosed erythrocytes which are broken down to an iron-containing pigment called haemosiderin and an iron-free pigment haematoidin, bilirubin or bile pigment. Haematoidin is readily soluble and diffuses out of cells as quickly as it is made.

The lumen of the sinusoid is a storage space for erythrocytes, granulocytes, platelets etc. It is also a place for testing cells, phagocytosis and cell maturation. After straining and scrutinization, the normal cells enter the circulation again. It is probable that most of the monocytes of the circulating blood are formed in the sinusoids and cords of the red pulp.

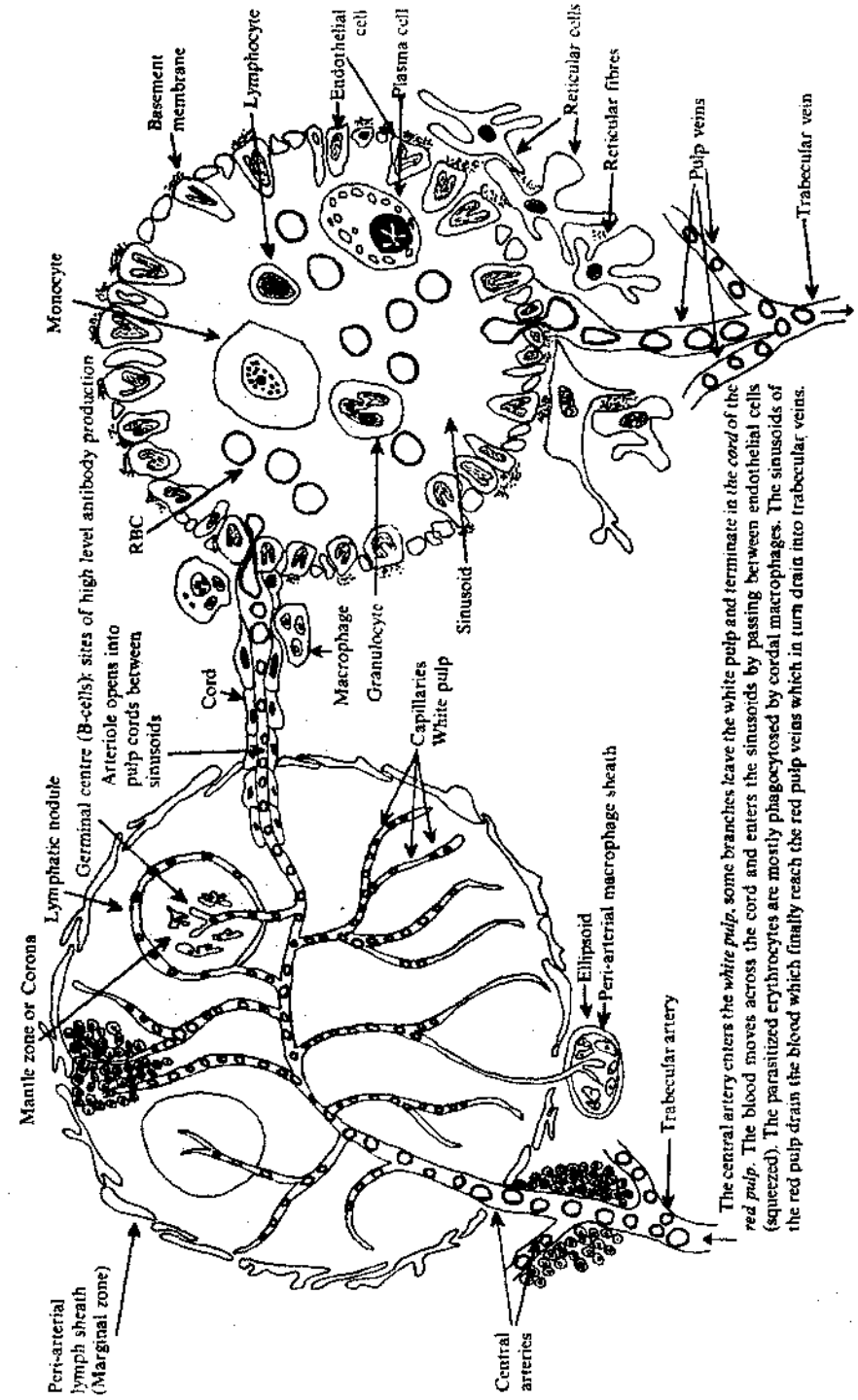


Fig. 1: Simplified diagram of white and red pulps of the spleen.

Only during embryonic life, both erythrocytes and granular leukocytes are formed in the red pulp. Haematopoiesis occurs in the spleen only in embryonic life, with the exception of some rare pathological situations.

In the red pulp, between the sinusoids, exist thin splenic cords. Each cord is a reticular meshwork with intercellular spaces. It is a cavernous system rich in macrophages. The terminal arterial vessels leaving the white pulp terminate in the cords. The blood moves across the cords and enters the sinusoids by passing between endothelial cells (Fig. 1). If the erythrocytes are not healthy or normal or contain rigid structures such as Heinz bodies or malarial parasites, they may cross to sinusoid wall with difficulty and take much longer time in the cord and are mostly phagocytosed by cordal macrophages. One important mechanism of splenomegaly is the blockage of the inter-endothelial slits to erythrocytes containing malarial parasites or other rigid particles and this leads to congestion of the cords.

SPLEEN FUNCTIONS

For practical purposes, the spleen can be considered as being composed of two organs (a) an immune one i.e., the white pulp, consisting of peri-arterial lymphatic sheaths and germinal centres (lymphatic nodules) and (b) a reticuloendothelial one i.e., the red pulp which is composed of phagocytic macrophages and granulocytes lining vascular spaces (cords and sinusoids).

(1) *T-lymphocytes and B-lymphocytes*

In the white pulp of the spleen, both T and B-lymphocytes exist. The peri-arterial lymph sheath around the central artery and its arterioles are packed with T-lymphocytes (thymus origin).

The B-lymphocytes are manufactured mainly in the bone marrow but within the white pulp of the spleen exist lymphatic nodules (Malpighian corpuscles). They are germinal centres, heavily surrounded by B-lymphocytes. The germinal centres are sites of high level antibody production.

The function of T-lymphocytes and B-lymphocytes differ. The T-cell can recognize foreign markers (antigens) which are usually collected by macrophage cells and get attached to them. Once the T-lymphocytes recognize the antigens on the macrophages, they are activated and send signals to the B-lymphocytes to produce antibodies. These antibodies coat the parasites or bacteria making them more susceptible to attacks and destruction by the leukocytes (Schwabe & Co., 1979).

Normally, in the spleen, the lymphocytes leave the blood vessels, enter the white pulp (surrounding the arterioles) and then later emerge through the veins. That traffic increases when a foreign body appears to challenge the immune system.

T-lymphocytes and B-lymphocytes can be separated from the blood in a few seconds in modern laboratories nowadays by a fluorescent activated cell sorter.

(2) *The principal functions of white pulp*

(a) Production and maturation of the T and B-lymphocytes and plasma cells as in other lymphoid organs. The spleen contains about 25% of the total body lymphoid tissue and produces specific antibodies.

(b) Formation of humoral antibodies according to the circulating antigens. Antibody response appears to be normal after subcutaneous or intra-muscular injection of an antigen. On exceptional basis autoantibodies to circulating blood elements are made, example (i) immune thrombocytopenic purpura (ITP), (ii) Commspo-

sitive, (iii) immune haemolytic anaemia (Schwabe & Co., 1979).

(c) The spleen produces "Opsonins" that coat the bacteria and enhance their rate of phagocytosis. Deficient opsonic activity has been demonstrated in the sera of patients with sickle cell anaemia.

(d) Produces a tetrapeptide called "Tuftsin" which is a leukocyte modulating hormone. Tuftsin coats neutrophils and enhances their ability to engulf bacteria. This non-specifically increases neutrophil phagocytosis and chemotaxis. In its absence in splenectomized cases or in congenital asplenic persons, this may impair their normal phagocytic function. Consequently these individuals are susceptible to infection.

(3) *The principal functions of red pulp*

(a) Removal of bacteria or unwanted blood elements. Phagocytosis of antibody-coated cells by red pulp macrophages and their destruction; phagocytosis of bacteria occurs even in the absence of specific antibodies. The red pulps act as filters for removal of debris and parasitic invaders.

(b) Selection and removal from the circulation of inclusion bodies such as Heinz bodies, Howell-Jolly bodies and nucleated erythrocytes. After splenectomy an increase of circulating nucleated erythrocytes is noticed along with an excess of Howell-Jolly bodies, reflecting the loss of this function of the spleen.

(c) A storage depot of red cells, the spleen is a reservoir for blood leukocytes and platelets. These can be released into the circulation by epinephrine. The volume of the spleen diminishes temporarily during and after excercise, when it assumes a size from one-third to one-half or less of its resting size. The shrinking of the spleen during excercise would account for 20% of the normal blood volume, in addition to the oxygen-carrying capacity which is of great importance during stress. The splenic blood has a greater concentration of erythrocytes than the system blood. The spleen enlarges a little under increased blood pressure and for a few hours after meals.

(d) Aged blood cells, distorted in shape, are destroyed by the macrophages. Their iron content is removed and made available again in the blood stream in the form of a protein called ferritin. This goes back to the bone marrow to be incorporated into new red blood cells.

(e) Under abnormal conditions the spleen can initiate haematopoietic functions. Normally, haematopoiesis takes place in the spleen during foetal intra-uterine life. Just before birth the bone marrow takes over that function. However, in exceptional situations, for example in bone marrow damage or in myelofibrosis, the haematopoietic stem cells in the spleen and liver get activated.

PATHOLOGICAL CHANGES OF THE SPLEEN IN MALARIA

During each febrile paroxysm of malaria, the spleen is swollen, tender and becomes hyperaemic. After the commencement of the acute attack the spleen is palpable within three or four days. During treatment it subsides in a few days.

According to Ross, splenomegaly occurs in 85 to 100 per cent of all malaria cases. Repeated malaria infections yield greater degree of splenomegaly. Therefore, as malaria endemicity increases, the average enlargement of the spleen is greater (Russell *et al.*, 1963).

(1) *Macroscopical appearance*

By the naked eye, the malaria spleen is dark. In acute malaria, the capsule is not thickened and can be easily torn. The pulp is diffluent. In chronic malaria it is firm or hard. In acute attacks, after death, the spleen may weigh as much as 700 gm. In chronic malaria especially vivax infection, it may weigh 5000 gm or more (Russell *et al.*, 1963).

(2) *Microscopical appearance*

There is an abundance of haemozoin in the form of granules, rods, blocks or masses. They are seen in the lymph and pulp spaces, blood vessels and capillaries. They are also commonly situated in the phagocytes and endothelial cells of blood vessels, sinusoids and cords (Boyd, 1949).

The dark yellow haemosiderin is seen in the spleen pulps but not in the Malpighian corpuscles.

All the stages of development of malaria parasites are seen in the reticular and red blood cells. *P. falciparum* schizonts are also commonly seen. There is an excess of phagocytic cells, including giant macrophages, endothelial cells. Erythrophagocytosis is extensive by both free macrophages and reticulo-endothelial cells (Boyd, 1949).

Thrombosis of the capillaries is seen in some cases. Foci of toxic necrosis in splenic pulps may occur. Haemorrhages and infarctions are not uncommon (Russell, 1952).

Splenic sinusoids and cords are congested and distended with blood. During acute malaria attacks, there is no increase in the connective tissue but in chronic malaria this is greatly increased (Russell *et al.*, 1963).

Torsion of the splenic pedicles with venous thrombosis has been reported. Spontaneous rupture of the spleen was reported in some cases (Russell *et al.*, 1963).

Within the walls of the venous sinusoids, the reticular-like cells show some degree of hyperplasia. Leukocytes and erythrocytes may adhere to the surface of these masses of reticular-like cells as the circulation tends to be slower. This results in occlusion, obstruction and subsequent infarction.

DISEASES ASSOCIATED WITH SPLENIC ENLARGEMENT

1. *Infections*

—Viral: e.g., infectious hepatitis, infectious mononucleosis, chicken pox, measles, mumps.
—Rickettsial: e.g., Rocky mountain spotted fever, typhus.
—Bacterial: e.g., subacute bacterial endocarditis, typhoid, scarlet fever, brucellosis, tuberculosis, peritonitis, tularaemia.
—Spirochaetal: e.g., relapsing fever, congenital syphilis.
—Protozoal: e.g., malaria, kala-azar, toxoplasmosis.
—Metazoal: e.g., schistosomiasis.
—Mycotic: e.g., histoplasmosis, blastomycosis, coccidioidomycosis.

2. *Congestive splenomegaly*

—Congestive heart failure.
—Portal hypertension: cirrhosis of the liver, Banti's syndrome.
—Thrombosis of portal or splenic veins or partial occlusion.
—External pressure on splenic or portal veins by glands, tumours etc.

3. *Acquired haemolytic anaemias*

—Acquired haemolytic anaemia of the newly born.
—Anaemia due to bone marrow replacement, e.g., pernicious anaemia.

4. *Congenital haemolytic anaemias*

—Red cell shape abnormalities: e.g., hereditary spherocytosis and elliptocytosis.
—Acute haemolytic anaemia (Lederer anaemia).
—Haemoglobinopathies: e.g., (i) Thalassaemias, (ii) Sickle Hb variants (e.g., Hb S-C disease), (iii) Congenital Heinz body haemolytic anaemia.
—Red cell enzymopathies e.g., Pyruvic kinase deficiency.

5. *Lympho- and myelo-proliferative diseases*

—Lymphomas: including Hodgkins.
—Leukemias: specially chronic lymphocytic and chronic myelocytic.
—Polycythemia vera.
—Myelofibrosis with myeloid metaplasia.

6. *Reticulo-endotheliosis*

—Lipoid: e.g., Gaucher's, Niemann-Pick, Schuller-Christian disease.
—Non lipoid: e.g., Letterer-Siwe disease.

7. *Collagen and connective tissue diseases*

—Systemic lupus erythematosus.
—Poly-arteritis nodosa.
—Fety's Syndrome.

8. *Tumours*

—Primary tumours: (i) Haemangioma (ii) Lymphangioma (iii) Lymphangio-sarcoma.
—Metastatic tumours: extremely rare.

9. *Splenic cysts*

—Echinococcus.
—Due to resolution of previous intra-splenic haematoma.

10. *Miscellaneous*

—Sarcoidosis
—Amyloidosis
—Haemochromatosis.

In temperate climates the causes of splenomegaly commonly encountered are lympho-proliferative, myelo-proliferative and connective tissue diseases, while in the tropics, the predominant causes are infectious diseases particularly malaria and kala-azar.

Serious problems in differential diagnosis from malaria, are common only with epidemic relapsing fever (acute infection) and in chronic infections of schistosomiasis and kala-azar. But fortunately schistosomiasis and kala-azar have a relatively restricted known endemic distribution and occur in older age-groups unlike malaria infections.

DIFFERENTIAL DIAGNOSIS OF SPLENOMEGALY

Splenomegaly may occur under a great variety of diseases and circumstances. The spleen is basically composed of lymphoid and reticulo-endothelial tissue. Any pathological condition that causes hyperplasia of the lymphoid or reticulo-endothelial tissues may cause splenomegaly. This may take place in infections, malignancy, metabolic, immunologic or idiopathic diseases. Extra-medullary haematopoiesis may take place and this causes enlargement. Interference with its venous drainage as in increased portal pressure, portal vein thrombosis or splenic vein thrombosis, leads to splenic engorgement and distension of sinusoids and splenomegaly (Blaustein, 1963).

In several cases of splenomegaly, hypersplenism may occur as manifested by anaemia, neutropenia and thrombocytopenia.

Primary splenic enlargement is quite rare. Most splenomegalic cases are manifestations of a widespread process and of course the determi-

nation of the cause depends on the diagnosis of the underlying condition.

A careful history, a detailed physical examination and routine laboratory studies will serve to establish the diagnosis in many cases of splenomegaly. In a few complicated cases, certain diagnostic procedures may be required. These are blood culture, serologic procedures, sternal marrow examination, tests of liver function, X-ray studies of bone or other organs, study of serum proteins by electrophoresis, lymphoid biopsy and liver biopsy etc.

Any associated findings are of extreme importance. The coexistence of lymphadenopathy, hepatomegaly, jaundice, ascitis etc., will serve to narrow down the possibilities.

The diagnosis of several diseases associated with splenomegaly such as leukemia, pernicious anaemia, congenital haemolytic icterus and others can be made by studies of the peripheral blood.

Palpation of the splenic notch is helpful in identification of the spleen from other organs. By auscultation one can detect a bruit or friction rub. Several other masses detected in the left side of the abdomen may be difficult to differentiate from the spleen. Occasionally a large kidney may descend with respiration as the spleen, but it is situated more deeply and does not have a sharp edge. Sometimes the left renal displacement is produced by splenomegaly. These cases are mostly vertical and sometimes of medial displacement. Dowse (1962), reported a case of displacement of the left kidney across the midline due to splenomegaly. In doubtful cases pyelograms are diagnostic. Other conditions in the same area are pancreatic cyst, ovarian cyst, omental mass with tuberculous peritonitis, mesenteric cyst, retroperitoneal tumour and adrenal tumour.

The diagnosis of splenic cyst and the exclusion of other possibilities of splenomegaly is a difficult clinical problem. Selective angiography affords a direct, accurate and safe approach to the differential diagnosis of masses in the left upper quadrant (King *et al*., 1968).

Splenic abscess should be regarded as a rare complication of bacteraemia, trauma and neoplasms of the colon or stomach (McSherry and Dineen, 1962). In non-parasitic cyst of the spleen, laboratory investigation is of little value. With the aid of modern techniques in roentgenology, the diagnosis of symptomatic cysts should present no problem in the majority of cases (Qureshi *et al*., 1964).

Once the mass has been identified as the spleen, then its size is an important aid in diagnosis as outlined in the following:

(i) *Marked splenomegaly*: This is most common in chronic malaria, kala-azar, schistosomiasis, chronic granulocytic leukaemia, polycythemia vera, tumours and cysts, myeloid metaplasia. In younger age groups it may be massively enlarged in cases of congestive splenomegaly with portal hypertension. Rarely, haemorrhage beneath the capsule produces a massive tumour. This may take place in infectious mononucleosis or after trauma, and also in some cases of lipoidosis such as Gaucher's disease.

(ii) *Moderately enlarged spleen*: The problem of diagnosis is more difficult, this is because at some stage in all of the diseases mentioned above, the organ is not very large in the early stages. Moderately enlarged spleen is also common in subacute infections, Boeck's sarcoid, portal hypertension, chronic haemolytic anaemia, chronic lymphocytic leukaemia and lymphoma.

(iii) *Slight spleen enlargement*: This is common in both the above categories in the very early stages, also in acute, subacute infections, pernicious anaemia, acute haemolytic anaemia and acute leukaemia.

CLINICAL FINDINGS ASSOCIATED WITH SPLENOMEGALY

The findings associated with splenomegaly are quite important in differential diagnosis. The following are some examples.

1. *When splenomegaly is accompanied by fever*

Infection must be considered, as malaria, typhoid, brucellosis, subacute bacterial endocarditis, infectious mononucleosis and many others. This combination may also be noticed in patients with leukaemia, lymphoma or systemic lupus erythrematosus.

2. *When pallor and icterus are associated with splenomegaly*

One may think of falciparum malaria, haemolytic anaemias of different types, hepatic diseases. When pallor is present but without icterus, one may suspect malaria, hypersplenic anaemia, leukaemia or lymphomas.

3. *In case of hepato-splenomegaly*

One may consider malaria, schistosomiasis, kala-azar, liver cirrhosis, haemolytic anaemias, extra-medullary haematopoiesis, syphilis, chronic leukaemias, granulocytic leukaemias, polycythemia vera and Gaucher's disease.

4. *Splenomegaly with generalized lymphadenopathy*

The following are considered; infectious mononucleosis, lymphocytic leukaemia, lymphomas as Hodgkins, lymphosarcoma, sarcoidosis.

5. *Splenomegaly associated with bone disease*

This is in osteosclerosis, Gaucher's disease, Hand-Schüller Christ disease, lymphoma (occasional) and multiple myeloma (rare).

6. *When the patient has a plethoric appearance* (excess erythrocytes)

One may consider polycythemia vera. The splenic enlargement occurs only in the primary type and not in the secondary type.

7. *Splenomegaly with slight hepatomegaly and leukopaenia*

This may occur in typhoid, viral diseases, leukopaenia with massive splenomegaly occurs in hypersplenism.

8. *Splenomegaly with leukocytosis*

The commonest is infection, also occurs in one of the types of leukaemia, myelo-proliferative disorder or one of the haemolytic anaemias.

THE ROLE OF SPLEEN EXAMINATION IN MALARIA ACTION PROGRAMMES

Under normal health conditions, the spleen is not palpable except in infants below the age of 12 months. The spleen is only palpable after its enlargement two or three times its size. This is because the early enlargement takes place in a superior and posterior direction before it becomes palpable subcostally. Once the spleen is palpable the direction of its further enlargement is directed downwards towards the right iliac fossa.

In minor degrees of enlargement, the spleen is felt as a swelling with a smooth rounded border; later when the spleen further increases in size, it is usually located as a firm swelling beneath the left subcostal margin in the left upper quadrant of the abdomen. On inspiration the spleen moves downwards. In the lower medial border of the spleen a notch can often be felt. On percussion the spleen is dull. The enlarged spleen may reach the level of the pelvic inlet and therefore its examination should begin in the left iliac fossa to avoid missing the edge of the enlarged spleen.

Several malariologists have stated that the object of spleen examination is to determine the per-

centage of individuals with demonstrable enlargement of spleen as well as its degree of enlargement. The results are presented as spleen rates as well as average enlarged spleen in different age groups and sexes. The classification of endemicity of malaria on the basis of spleen rates has been adopted by WHO in 1955.

Thomas *et al.* (1981) conducted a seroepidemiological study on Aborigine children in Orang Asli Malaysia. The examination of 143 children revealed that the falciparum antibody prevalence rate was 84.6%, compared to 81.8% spleen and 43.3% parasite rates. Both *P. falciparum* and *P. vivax* were present in children. One can notice that the positive correlation between seroepidemiological study and spleen results are significant particularly in the age group upto 9 years old.

With a few precautions the utilization of the spleen examination as a tool of malaria evaluation is considered by the author as one of the most valuable practical, simple and economical methods existing which yield immediate and practical results in several antimalaria programmes.

1. *Spleen, blood surveys and advanced research methodology*

Several longitudinal studies in endemic malarious areas revealed that in many persons, the blood parasitaemia can only be demonstrated intermittently. Discrepancies between the spleen index and blood parasite index are commonly noted (Marsden *et al.*, 1967). Hackett, 1944, recorded a poor correlation between splenomegaly and parasitaemia (Boyd, 1949).

Malaria surveys based on the microscopic examination of blood do not always detect chronic low grade infections due to the development of immunity. Since immunity suppresses the density of parasites to low numbers, a few blood drops from a malaria case could yield a negative result by standard procedures. The number of false negatives is further augmented by loss of parasites during preparation of a slide or failure to find them by incompetent microscopists. An alternative approach for estimating the true prevalence of infection is based on repeated tests for the presence of parasites (Aron, 1982).

Many failures in different malaria eradication and control programmes were due to adoption of advanced techniques which could not be applied except by experienced technicians and thus dissipated the anti-malaria efforts. Advanced techniques, e.g., seroepidemiological methods and the determination of vectorial capacity are of high practical importance but due to their complexity in interpretation of results or in sampling techniques and to lack of staff, their use in small malaria control projects is limited. On the other hand they are of tremendous value in research projects, and in certain circumstances (e.g., serodiagnosis in areas freed from malaria).

2. *Spleen examination and classification of its enlargement*

During malaria surveys, spleen palpation is usually conducted in schools, homes or in the open in villages. Before starting spleen palpation the examiner (preferably a physician) ought to approach the community tactfully and to gain their confidence. This is the key to a successful spleen examination. The examinee should lie on his back and flex the legs to relax the abdominal muscles. The spleen examination of subjects in a standing position is not advisable because in this way the smallest enlargements are commonly not felt. If the spleen is not palpable during normal respiration, the examinee is asked to breathe more deeply or to blow his belly up. As the subject inspires, the examiner applies slight pressure inwards and upwards.

Classification of enlarged spleen

The most practical classification of enlarged spleen utilised nowadays is after Hackett (Fig. 2).

0 = The spleen is not palpable on deep inspiration (normal).

1 = The spleen is only felt on deep inspiration below the costal margin.

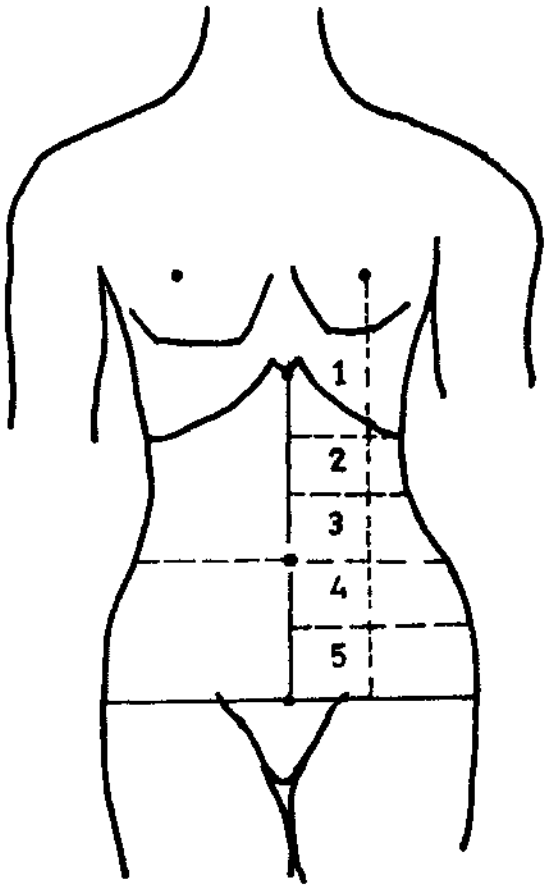


Fig. 2: Classes of spleen sizes (after Hackett)

2 = The spleen is palpable on normal breathing but not projecting below a horizontal line halfway between the costal margin and a horizontal line drawn from the umbilicus and measured along a vertical line dropped from the left nipple.

3 = The spleen is felt with its lower palpable point projecting more than halfway to the umbilicus but not beyond it.

4 = The spleen is detected below the umbilical level but not projected more than halfway towards a horizontal line through the symphysis pubis.

5 = The spleen extends beyond the lower limit of class 4.

3. *The average enlarged spleen (AES)*

This is determined by multiplying the number of individuals of each class by the spleen class number. The total is added and divided by the number of individuals having enlarged spleens.

Example: The examination of 200 children age 2–9 years yielded the following:
Spleen 0 (negative = 82, palpable spleen = 118)

Class 1 = 93
Class 2 = 11
Class 3 = 7
Class 4 = 5
Class 5 = 2

$$\text{Spleen Rate} = \frac{118}{200} \times 100 = 59\%$$

$$\text{AES} = (93 \times 1) + (11 \times 2) + (7 \times 3) + (5 \times 4) + (2 \times 5)$$
$$= 166 \text{ divided by } 118 = 1.4.$$

Significance of AES

In a malarious area, during each malaria infection, the spleen size increases. When immunity develops (adult) the spleen size subsides again. Therefore, AES reveals considerable difference in hyper- or holo-endemic malarious areas compared to areas of malaria epidemics. In

both, the spleen rates are high. But in the holo- or hyper-endemic areas the AES in age group 2–9 years is high and in the epidemic areas AES is low in the same age group. Also in holo- or hyper-endemic areas the AES among adults gradually decreases with age due to rising immunity, while in epidemic situations AES is also high among the old-age group.

After interruption of malaria transmission in highly endemic areas spleen rates may remain the same for months or diminish insignificantly. On the other hand the AES remarkably decreases, and therefore is a more sensitive tool in evaluation than the spleen rate alone.

The spleen rate taken among the 2–9 years age group and the determination of the average enlarged spleen are easier to obtain than the parasite rate. It includes recently past as well as present malaria and it is frequently more reliable in practice than the examination of blood films (MacDonald, 1957). But competence in the examination of the spleen requires at least one week's apprenticeship under the guidance of an experienced malariologist.

The list of causes of splenomegaly is an imposing one. However in malarious areas, when spleen examinations are made in schools and public places rather than in hospitals and dispensaries, confusion may arise only from measles, chicken-pox, and in some areas in higher age groups from schistosomiasis and kala-azar. Therefore in malarious areas, the spleen can be of considerable epidemiological value.

4. *Spleen Consistency Index (suggested by the author)*

The spleen surveys determine the spleen rate and AES in different age groups and sexes. In addition, the determination of "Spleen Consistency" is of great epidemiological value. During the examination of the spleen one has to determine whether the spleen is (i) soft, or (ii) medium or (iii) hard in consistency. The epidemiological value of the spleen consistency is outlined as follows:

(i) *Soft Spleen:* This indicates very recent transmission (from few days to few weeks). Very early epidemics can easily be determined by this (soft spleen in different age groups). Foci of recent transmission and detection of vectors with sporozoites are also encountered in areas with very soft spleen.

(ii) *Medium Spleen:* This reveals the existence of malaria for some time and persistence of transmission. In case a younger group has soft spleen and an older group medium spleen, there is evidence of recent transmission as well as old transmission.

(iii) *Hard Spleen:* Sometimes in a younger group (5–9 years) the spleen is hard and in older groups the spleen rate is low but the consistency is stony hard. This is interpreted as old cases of malaria (at least 3 or more years without exposure to the disease) or the acquisition of very high immunity in holo-endemic areas.

In acute primary malaria infection, the enlarged spleen is very soft in consistency and is commonly missed during examination. In chronic malaria, the consistency of the spleen is hard.

Although spleen enlargement starts as early as the first primary attack, yet majority of the examiners cannot detect it due to its soft consistency. However, after the onset of symptoms, by a week or 10 days, the examiner can detect the enlargement of the spleen when the size increases and the consistency is not very soft.

In malaria epidemics, the largest proportion of persons examined by inexperienced physicians yield blood positives but the enlargement of the spleen is detected in only a few cases. This is due to the missing of the slightly enlarged spleens, which are very soft in consistency. It is impossible for inexperienced medical or non-

medical examiners to detect these cases.

In acute untreated malaria infections, the degree of splenic enlargement is directly proportional to the duration of the clinical attack. In case the period of commencing clinical activity is not followed by recrudescences or relapses, the enlarged spleen diminishes and is not palpable within a few weeks.

In case splenomegaly continues without diminution in size, it is probably due to recrudescence or relapse. Between long intervals of inoculation, the spleen may return to normal or become too small to be detected clinically. On the other hand, when transmission is repeated with different species or even strains, it seems that each infection acts independently and produces splenic reaction which results in chronic enlargement (Boyd, 1949).

The author has used the spleen method with success in several regions of the world. Gaza strip was declared malaria free by UNRWA in 1955. Investigations in 1956 using spleen examination and consistency revealed residual foci of *P. vivax* and remedial action was taken.

In Saudi Arabia (Bisha area) in 1956 conflicting results were obtained from spleen examination and entomological surveys in one village. Complete absence of the main vector *A. gambiae* was observed. Blood examination supported the spleen results that indicated a recent epidemic and further studies revealed the presence of *A. gambiae* larvae in valleys adjacent to the village. Similarly in 1957 in Rabigh town (160 km north of Jeddah) spleen examination led to the inference of a threatening malaria epidemic (low spleen rate and AES, consistency very soft in all age groups). Though very few *A. gambiae* larvae were detected, there had been unusual rain and hence new breeding places may have been created. The authorities however, did not agree with the author's forecast which was proved correct when one of the worst epidemics took place in that area within a few weeks.

The Kattan area south of Damascus in Syria was under advanced consolidation. Case detection has yielded negative results for several years. Spleen survey of several villages was conducted and a few splenomegalic cases were traced to Hemrit village. In Hemrit village, the spleen rate was very high, AES in children reached 2 and the spleen consistency was hard, indicating long exposure to malaria. Blood examination revealed four indigenous *P. falciparum* cases. Entomological surveys carried out by one senior malariologist and one entomologist of the WHO revealed the absence of any vector. Further spleen surveys in the vicinity revealed malaria transmission and high vector density in a neighbouring village Makrousa.

Spleen examination also helped to solve a baffling case of induced malaria in a 1½ year old child in Damascus. The child contracted malaria from a professional blood donor who was an asymptomatic carrier from Kamishlie (Northeast Syria) a highly endemic malarious area.

In 1962, a confusing *P. vivax* case of a two year-old child in Taiwan was elucidated by the spleen method.

Out of dozens of interesting field investigations in Pakistan three cases are cited as examples.

An area near Lahore was selected by a leading American University for the evaluation of DDVP in comparison to DDT. Almost a total blood survey of the 4000 population revealed a parasite rate more than 80%. On the basis of spleen survey, which revealed high spleen rate, low AES and soft consistency, the author opined that the area was not suitable for long-term research due to the instability of malaria in that region. The project operated for one year and then had to close down after heavy financial losses.

The geographical reconnaissance (GR) in Sheikhupura zone of one million population was excellent and all normal malaria eradication

indices, through good sampling were carried out. Few malaria cases were detected and their classification was indefinite. The author examined some of the cases and followed thoroughly the cases with soft spleen. The investigations guided us to scattered huts that were never sprayed, and were not existing in the geographical reconnaissance maps. In spite of the outstanding GR, several *P. falciparum* cases were detected in the area that was under control.

Though an excellent plan was drafted for Malaria Eradication in West Pakistan in 1960, Karachi was excluded from the programme based on the views of late Col. Afridi. The programme was very successful in Sialkot zone, but when the area shifted into consolidation a few indigenous cases were reported. Some of the soft spleen cases detected by the author were traced to Karachi.

In Karachi, further studies showed that transmission was taking place. An engineering error which prevented the tide from entering a large section of beach had created undisturbed breeding places for *A. stephensi*. The Karachi malaria problem was discovered by spleen examination and here spleen consistency was the determining factor, not enlargement.

In Thailand (1968-73) the author was instrumental in spotting unsprayed foci of malaria in the forests of Chiangmai and Pitzanaluck.

SPLENECTOMY

Removal of the spleen is associated with high mortality. This is mainly from overwhelming pneumococcal septicaemia. The studies of Robinette and Fraumeni of the veterans of the 1939-45 war who underwent splenectomy revealed a significant late mortality from ischaemic heart diseases associated with high blood viscosity (Robertson *et al.*, 1981).

Robertson *et al.* (1981) studied the blood viscosity following splenectomy in 20 cases compared with control. It was noticed that after splenectomy, whole blood viscosity is increased. It is associated with decreased red cell deformability. The red cell deformability is determined by properties of the (i) cell membrane (ii) the viscosity of the intracellular contents and (iii) the maintenance of the biconcave shape. Splenectomy leads to abnormality of all these factors. These abnormalities increase the viscosity of the intracellular milieu and hence of whole blood. The high platelet counts are not an important factor influencing whole-blood viscosity since there is no correlation between platelet count and viscosity. Although the increase in viscosity after splenectomy is small, yet over several years this may lead to increased mortality from occlusive vascular diseases. The increased erythrocyte rigidity has a direct effect on the microcirculation causing diminished perfusion which may be a factor permitting overwhelming infection to take place after splenectomy (Robertson *et al.*, 1981).

In splenectomized cases, it seems that T-lymphocytes are not adequately produced. This suggests that an appreciable number of T-lymphocytes that migrate into the blood, come from the spleen, and they are essential in defence.

Splenectomy does not affect the life expectancy directly, but it does make the splenectomized person prone to infections which may include meningitis, septicaemia and pneumonia. Splenectomy also makes malaria worse i.e., non-lethal infections may become lethal and latent infections may relapse. *P. falciparum* infection becomes a life-threatening disease following splenectomy (Wyler *et al.*, 1977). In humans the mechanism of host defence against malaria is not fully known, but there are reported cases of recrudescence of malaria infection in patients who have undergone splenectomy many years after leaving malarious areas.

In fatal cases of blackwater fever, almost all cases were associated with inadequate prophylaxis or none at all (Ellis, 1981). Maharaj *et al.* (1982),

reported a case who had taken regular and adequate prophylaxis but after splenectomy, he developed blackwater fever. He concluded that the patient was rendered more susceptible to infection by splenectomy.

Studies by De Virgilis in 1981 showed a higher incidence of malariae induced malaria in splenectomized rather than in non-splenectomized patients with thalassemia major. This finding suggests that splenectomized subjects are more prone to develop overt *P. malariae* transfusion malaria rather than non-splenectomized patients receiving the same life-long transfusion treatment from the same pool of blood donors.

The splenectomized patients are recommended to be immunized against pneumococcal infections. Some recommend antibiotic prophylaxis e.g., benxathine penicillin (1.2 million unit/adult) given intramuscularly once monthly.

RUPTURE OF THE SPLEEN

Splenic rupture may occur spontaneously in the course of splenomegaly due to (i) disease or (ii) secondary to trauma. Traumatic rupture is usually due to non-penetrating injuries to the abdomen or lower left side of the thorax. It may be immediate or delayed.

Spontaneous rupture occurs as a complication of acute febrile diseases associated with splenomegaly. Malaria is the most common single cause of spontaneous rupture of the spleen (Blaustein, 1963). In infectious mononucleosis, it is also not uncommon. Less common causes of spontaneous rupture of the spleen are due to congestive splenomegaly, leukaemia or torsion. Rupture has also been reported in amyloidosis, typhoid, tularaemia and myelo-proliferative diseases.

The mechanism of rupture is basically a weakening of the spleen capsule and trabeculae causing infarction or haemorrhage. Subcapsular haematomas give way under increased intra-abdominal tension e.g., during straining at stool or frequent manual palpation of the spleen by the physician (De Saram and Townsend, 1943).

When rupture of the spleen takes place, it is accompanied by prompt evidence of peritoneal irritation and blood loss. During the early hours of rupture, the symptoms and signs may consist of little pain in the left upper quadrant or inability to stand up straight. However, when the blood loss continues, rigidity of the abdomen is evident and abdominal tenderness becomes more diffuse. Signs of haemorrhage occur, namely: the pulse is elevated, the blood pressure is lowered, the haemoglobin falls, the erythrocyte count drops and leukocytosis takes place. The case should be managed urgently by prompt blood replacement followed by splenectomy (De Saram and Townsend, 1943).

In some cases the rupture is delayed. This is caused by the sudden giving way of a contained subcapsular rupture. This usually takes place 48 hours or more after splenic injury. A period of apparent well-being is noticed. This is followed by sudden onset of pain, peritoneal irritation and shock. Diagnosis can be confirmed by X-ray which reveals the loss or enlargement of splenic outline and by indentation or displacement of gastric outline with downward displacement of the splenic flexure of the colon. Also the presence of elevation and fixation of the left hemidiaphragm and displacement of the left kidney downward, loss of the outline of the kidney and psoas muscle and widening of the left paravertebral shadow. Once the diagnosis is made or even suspected, splenectomy should be done before intraperitoneal rupture and shock occur.

In some accident victims, the spleen may be preserved by suturing the rupture pieces together. In modern surgery it seems the spleen can be repaired more frequently than was previously thought.

Spontaneous rupture of the spleen in falciparum malaria may occur with lack of localising signs which are usually present in subcapsular haemorrhage. Also the late and sudden onset of the signs of major abdominal haemorrhage may be lacking beside the local absence of any history of trauma (Dowse, 1962).

SPLEEN AND LIVER ENLARGEMENT IN MALARIA

In Southwest Pacific, it was shown (Aron, 1982; Black, 1954) that the liver increased in size with increasing degrees of malaria endemicity. In different age groups (excluding 0–1 year), it was found that there was a significant positive correlation between the size of the spleen and the size of the liver.

Black concluded that in the malarious areas of the Southwest Pacific the liver enlarges as well as the spleen. Also in an area of high malarial endemicity, the liver enlargement is proportionate to that of the spleen (Black, 1954; Black, 1955).

The investigations conducted by Black, revealed that in the areas studied there were no signs of high malnutrition among the population investigated. Other endemic diseases that commonly cause hepatomegaly were also absent. However, the pathogenesis of hepatomegaly is not clear.

CONGENITAL MALARIA SPLENOMEGALY

Several congenital malaria cases were reported in USA since the last 3 decades and majority of these cases had splenomegaly.

Among the rare congenitally acquired malaria infections, hepatomegaly is common and also jaundice and haemolytic anaemia. The clinical symptoms in the mother are masked or asymptomatic. The clinical manifestations in the newly born infant seldom appear before 3 weeks of age. The occurrence of hepato-splenomegaly, jaundice and haemolytic anaemia in an infant whose mother originates from endemic malarious areas or has had a history of previous residence in a malarious country, should direct the attention to the possibility of congenital malaria. The diagnosis is confirmed only by detection of the malarial parasite in the peripheral blood of the infants (Thompson *et al.*, 1976).

CONCLUSIONS

If one uses spleen rates, average enlarged spleen (AES) and spleen consistency in the field and covers different age groups and sexes, it would be easy for the investigator to have a good idea of the malaria history of the area, the present epidemiological situation and more or less a forecast on future malaria happenings. The method can be applied in malaria control programme, in advanced eradication or even in the maintenance phase. It is simple and results are immediate on the same day, which makes this method a most practical one in the evaluation of anti-malaria programmes.

Unfortunately, spleen examination in modern malariology is out of fashion. Sophisticated entomological indices, (e.g., vectorial capacity), and serological techniques are nowadays the latest trend in spite of the difficulty in their routine application in running control programmes in developing countries. They ought to be limited to research projects.

There is also a need for the training of medical officers and junior malariologists assigned in different programmes, in order to be able to detect small and soft splenomegalic cases which are seldom detected by inexperienced malariologists. The enlargement of the spleen due to schistosomiasis or kala-azar can be differentiated.

Spleen examination in running malaria control programmes can explain on the spot the epide-

miological situation, the degree of immunity, the laboratory efficiency, previous history and future prospects of the disease.

REFERENCES

1. Aron, J.L. (1982). Malaria epidemiology and detectability. *Trans. R. Soc. Trop. Med. Hyg.*, **76**(5): 595–601.
2. Black, R.H. (1954). Enlargement of the liver due to malaria in natives of the Southwest Pacific area. *Trans. R. Soc. Trop. Med. Hyg.*, **48**(3): 226–233.
3. Black, R.H. (1955). Comparison of the liver and spleen in a community in Netherlands, New Guinea suffering from malaria of a high degree of endemicity. *Trans. R. Soc. Trop. Med. Hyg.*, **49**(1): 62–63.
4. Blaustein, A. (1963). *The spleen.* (The Blakiston Division, McGraw-Hill Book Company Inc., New York, Toronto, London).
5. Boyd, M.F. (1949). *Malariology*, v. 1 & 2 (W.B. Saunders Company, Philadelphia and London).
6. De Saram, G.S.W. and R.F. Townsend (1943). Spontaneous rupture of the spleen in malignant tertian malaria. *Lancet*, **5**: 584–585.
7. De Virgilis, S., R. Galanello and A. Cao (1981). *Plasmodium malariae* transfusion malaria in splenectomized patients with thalassemia major. *J. Pediatr.*, **4**: 584–585.
8. Dowse, J.L.A. (1962). Left renal displacement due to splenomegaly. *British J. Surg.*, **5**: 114–116.
9. Ellis, C.J. (1981). Diagnosis not to be missed: Malaria. *British J. Hosp. Med.*, **26**(11): 400–403.
10. King, M.C., B.W. Glick and A. Freed (1968). The diagnosis of splenic cyst. *Surg. Gynaecol. Obstet.*, **127**: 509–512.
11. Macdonald, G. (1957). *The epidemiology and control of malaria.* (Oxford University Press, London).
12. Maharaj, D., G.A. McDonald and J.W. Dobbie (1982). Splenectomy and blackwater fever. *British J. Haematol.*, **51**(4): 663–664.
13. Marsden, P.D., D.H. Connor, A. Voller, A. Kelly, E.D. Schofield and M.S.R. Hutt (1967). Splenomegaly in New Guinea. *Bull. WHO*, **36**: 901–911.
14. McSherry, C.K. and P. Dineen (1962). The significance of splenic abscess. *American J. Surg.*, **103**(5): 618–623.
15. Qureshi, M.A., C.D. Hafner and J.R. Dorchak (1964). Nonparasitic cysts of the spleen. *Arch. Surg.*, **89**: 570–674.
16. Robertson, D.A.F., F.G. Simpson and M.S. Losowsky (1981). Blood viscosity after splenectomy. *British Med. J.*, **283**(8): 573–575.
17. Russell, P.F. (1952). *Malaria, basic principles briefly stated. American Lec. Ser. 126* (Blackwell Scientific Publications, Oxford).
18. Russell, P.E., L.S. West, R.D. Manwell and G. McDonald (1963). *Practical Malariology*, 2nd ed. (London University Press, New York, Toronto).
19. Schwabe & Co. AG, Basel (1979). Role of the spleen in the immunology of parasitic diseases. *Trop. Dis. Res. Ser. 1* (UNDP/World Bank/WHO Special Programme for research and training in tropical diseases).
20. Schwabe & Co. AG, Basel (1979). The membrane pathobiology of tropical diseases. *Trop. Dis. Res. Ser. 2* (UNDP/World Bank/WHO Special Programme for research and training in tropical diseases).
21. Thomas, V., S.K. Hock and Y.P. Leng (1981). Serocpidemiology of malaria. *Trop. Doctor*, **11**(4): 149–154.
22. Thompson, D., C. Pegelow, A. Underman and D. Powars (1976). Congenital malaria: A rare cause of splenomegaly and anaemia in an American infant. *Pediatrics*, **60**(2): 209–212.
23. Wyler, D.J., L. Miler and L. Schmidt (1977). Spleen function in quartan malaria (due to *Plasmodium inui*): evidence for both protective and suppressive roles in host defence. *J. Infect. Dis.*, **135**(1): 86–93.
24. Wyler, D.J. and J.I. Callin (1977). Spleen-derived mononuclear cell chemotactic factor in malaria infections: A possible mechanism for splenic macrophages accumulation. *J. Immunol.*, **118**(2): 478–484.

Indian Journal of Malariology
Vol. 23, June 1986, pp. 19-25.

Studies on the Detection of Malaria at Primary Health Centres. Part II. Age and Sex Composition of Patients subjected to Blood Examination in Passive Case Detection

A.E. BELJAEV[1], G.K. SHARMA[1], J.A. BROHULT[1] and M.A. HAQUE[2]

This study analyses age and sex composition of patients from the Passive Case Detection (PCD) carried out in 39 PHCs in Orissa. Age-sex representation was compared to the census data and correlations were computed between groups by Spearman's Rank Correlation method.

Women (20–49 years) and young children (0–9 years) were seen to be severely under-represented in the PCD. This was related to the over-representation of adult males, whose proportion showed negative correlation with representation of almost all female age groups.

Factors which operate against representation of young children, are not sex-selective in Orissa, because baby girls were only slightly less represented than baby boys (this may not be true in the demographic situation of Northern India). There was strong correlation between the proportion of young children and of women of 25–39 years.

The influence of women's under-representation on measured malaria incidence is discussed. Equal representation of the sexes (without change in the age structure of PCD) would have increased the blood examination rate by 23%.

INTRODUCTION

Every year in India, about 20 million slides are collected through the Passive Case Detection (PCD). In 1984, this source of collection contributed to 32% of total blood slides examined and generated 52% of positive cases.

The epidemiological information which is being generated by the PCD has important biases, i.e., inequal representation of different groups of population due to geographical and behaviouristic factors. However, a realistic picture may still be obtained from data having inherent biases, if the directions and magnitude of those biases are known. This study focuses on some aspects of this problem, notably the influence of age and sex on the probability of a person being screened by the PCD system. The information utilized was

Accepted for publication: 31 March 1986.
[1]National Malaria Eradication Programme
22-Sham Nath Marg
Delhi-110054, India.
[2]Plasmodium falciparum Containment Programme
(Zone 2)
333-Shaheed Nagar
Bhubaneshwar, India.

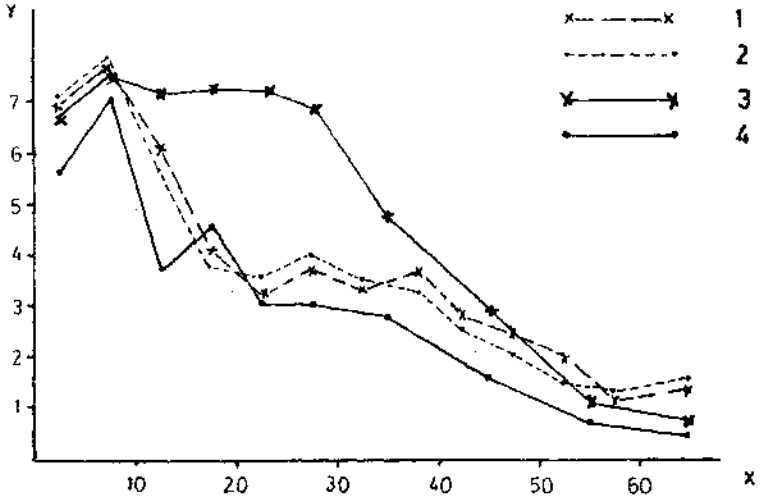


Fig. 1: Age distribution of males and females in the population of Sambalpur, Mayurbhanj and Phulbani districts (1971 census) and in the sample examined.
Legend
1. Males, census; 2. Females, census; 3. Males. PCD sample; 4. Females, PCD sample.
The total number of individuals of both sexes in the total population and in the PCD sample, respectively, is taken for 100%.

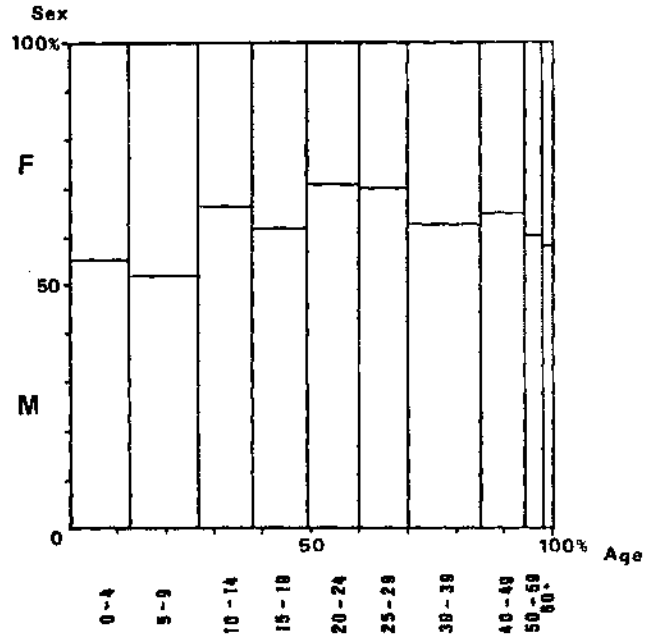


Fig. 2: Age distribution in the PCD sample and representation of males in each group.
X-axis—proportion of the age groups in the total sample.
Y-axis—proportion of males in each age group.

collected during a study on the accuracy of malaria diagnosis by decentralized laboratories (Beljaev *et al.*, 1985).

MATERIAL AND METHODS

The study was conducted in 39 PHCs in Sambalpur, Mayurbhanj and Phulbani districts of Orissa. In short, the design was to collect 100 to 200 slides in duplicate from outpatients of each PHC and to process them independently in the PHC and at the Project laboratory (Beljaev *et al.*, 1985).

A total of 4115 slides were collected in the course of this study. For 4029 specimens the information on age and sex was available. This study covers them all, including those for whom the results of the parasitological diagnosis by the Project laboratory was not available (they comprise about 15% of the sample).

Population was subdivided into ten age groups: six groups at 5 year intervals from 0 to 29, three groups at 10 year intervals from 30 to 59 and a group of 60 and above. Calculations were done initially for each of these groups. On the basis of results of the initial analysis, the groups were amalgamated for future analysis.

To analyse the composition of the groups of patients, the age/sex distribution of PCD cases was compared with the census data of 1971 (detailed data of 1981 census was not yet available). For processing the results, mostly non-parametric statistical methods were used, as described by Sprent (1981). The data was processed using a pocket computer Sharp PC 1251.

RESULTS

The age and sex distribution of the patients was compared with the age/sex distribution of the rural population of Sambalpur, Mayurbhanj and Phulbani districts as per 1971 census (Fig. 1). In this graph, the total population of both sexes was taken for 100%. The census data was plotted at the centres of 5-year intervals, except for the group of 60 + years for which the average age (about 65 years) was taken. In PHC data there was a strong tendency to record the age rounded to decade, therefore, double i.e., 10 year intervals were taken. To make the data of those double groups comparable to the 5-year groups, the corresponding strengths of the double groups were divided by two.

The age distribution of males and females in the general population is similar, but strikingly different in the PCD sample. Compared with the proportion of the particular age group in the total populations, the males were slightly under-represented at the age of 0–9 and over 50 years, but grossly over-represented at the age of 10–39 years. As to females, they were under-represented in all age groups, except 15–19 years.

The age/sex composition of the total sample is shown in Fig. 2. On the x-axis, the percentage of each age group in the total population is shown, and the proportion of males in each of the groups is given on the y-axis.

To measure correlations in the representation of various age/sex groups, the total number of patients of both sexes in each PHC sample was taken for 100%, and the percentage of each of the 20 age/sex groups was calculated. Some of the groups were amalgamated so as to obtain 12 groups of the ages 0–4, 5–9, 10–14, 15–19, 20–24 and 25 and above years, of each sex. All 39 PHCs were ranked according to the relative strength of each group, and 12 rankings were compared with each other by calculating the Spearman's rank correlation coefficient (RCC). As a threshold value of RCC, 0.312 was taken which, for this number of observations, corresponds to more than 95% significance level in two-tailed test. Out of a total of $12 \times 11/2 = 66$ comparisons, only 10 showed strong or moderate correlation, as given in Table 1.

Table 1. Representation of various age/sex groups

Sl. No.	Groups Compared	RCC	Correlation
a)	*Within the same sex*		
1.	Males 0–4 vs. males 25 + years	−0.41	strong negative
2.	Females 0–4 vs. females 5–9 years	0.34	moderate positive
b)	*Between two sexes*		
3.	Males 0–4 vs. females 0–4 years	0.58	strong positive
4.	Males 0–4 vs. females 15–19 years	−0.45	strong negative
5.	Males 5–9 vs. females 15–19 years	−0.42	strong negative
6.	Males 5–9 vs. females 25 + years	−0.33	moderate negative
7.	Males 25 + vs. females 0–4 years	−0.49	strong negative
8.	Males 25 + vs. females 5–9 years	−0.38	moderate negative
9.	Males 25 + vs. females 20–24 years	−0.38	moderate negative
10.	Males 25 + vs. females 25 + years	−0.39	moderate negative

Table 2. Influence of female representation on that of young children

Sl. No.	Groups Compared	RCC	Correlation
a)	*Between children 0–4 years and females*		
1.	Children vs. females 25–39 years	0.37	moderate positive
2.	Children vs. females 50 + years	0.33	moderate positive
b)	*Between the groups of females*		
3.	25–39 vs. 40–49 years	0.31	moderate positive
4.	25–39 vs. 50 + years	0.34	moderate positive

Table 3. Three groups of situations in respect of the representation of young children and women of 25–39 years

Group	Number of PHCs	No. of Women 25–39 years/ number of both sexes of 25–39 years (percentage)		No. of Children 0–4 years/ total population (percentage)	
Q	12	55/277	(19.9)	102/1069	(9.5)
R	16	154/451	(34.1)	220/1796	(12.2)
S	11	138/275	(50.2)	174/1164	(14.9)
Total		347/1003	(34.6)	496/4029	(12.3)

To test the hypothesis that the representation of the youngest children may be influenced by the representation of females, essentially the same method was used. For each of the PHCs, the following six indices were calculated, (i) proportion of children of both sexes aged 0–4 years in the total samples; (ii) proportion of females in the following age groups: 15–19, 20–24, 25–39, 40–49 and 50 and above. All the PHCs were ranked in respect of each of these six indices. Each of the six rankings obtained in this way was compared with each other (15 comparisons). Significant correlations (RCC >0.312) are shown in Table 2.

It was observed that the situations with good representation of children of 0–4 age group or women of 25–39 age group are essentially the same. The reverse is also true. Essential characteristics of these groups are given in Table 3.

DISCUSSION

The term PCD is used here in its initial meaning, i.e., comprising only of slides collected in medical institutions. This remark is needed because in some states, blood slides collected through volunteers are also included into PCD category, which makes the picture less clear (Ray and Beljaev, 1984).

This study has its limitations. First, the geographical aspect was not covered. It is well-known that in developing countries, distance is a critical determinant of medical care. The average number of outpatient attendances per person decreases very fast as distance increases. Examples from a few developing countries including India given by Jolly and King (1966) indicate that it halves itself about every 1/2 to 2 miles (0.8–3.2 km). As a result, PCD covers mostly the population of the immediate neighbourhood of the PHC.

Another factor which was not studied here is the time factor. At each point, the collection of slides usually continued for few weeks only and, therefore, it is not known whether the observed differences between the PHCs were of a permanent or of a temporary nature. Therefore, we refer to classification of situations rather than to classification of PHCs.

The observed distribution of patients is a result of selection on two levels. First, there is self-selection by patients (or their custodians, in case of children), second—selection by PHC workers.

Various factors are involved in self-selection. The degree of disability caused by the disease is probably the most important: a mild disease may not induce a person to undertake a long trip to an institution, at the cost of a working day. On the other hand, if the symptoms are very severe, the limited mobility of the patient may make his travel to the institution difficult.

Awareness that the curative facilities are existing and readiness to use them are two different things. At the present time, even illiterate people are aware of the medical facilities in their areas, but they do not always believe in the effectiveness of modern treatment and often prefer traditional systems of medicine.

These factors are particularly relevant where women are concerned. As Murthy (1982) put it: "Women in India, because of their household responsibilities, and out of ignorance, tend to neglect their illness until they become too sick to move around.... Often, too, they are dependent on others in the family to get them needed medical attention.... Thus, women do not seek medical help when they ought to; and by the time they are sick enough to know they should consult a doctor, it is much more difficult for them to do so". Not only adult women are in disadvantage. Less attention which is traditionally paid in some communities to the welfare of girls compared with boys may contribute to inequal representation by sex from a very young age (Lopez, 1984).

As to malaria itself, we could not find any evidence that its prevalence affects the age/sex distribution in the PCD. No sex difference in malaria incidence could be demonstrated (Beljaev *et al.*, 198.). The proportion of the youngest children is not elevated in samples with a high SPR, as one could expect, rather an opposite trend is observed, though not statistically significant.

The second group of factors is that which influences the selection of patients for blood examination by the PHC workers. Some medical

officers do not send patients for blood examination because they rely heavily upon their own clinical judgement and reject diagnosis of malaria straightaway, if they think that they have found some other cause of the fever. In case of shortage of antimalarials, which occurs sometimes at the periphery because of the faulty distribution, the blood examination may decline because of inability to provide presumptive treatment to every patient examined for malaria. Sometimes the blood examination in outpatients drops when the laboratory is overloaded or the technician is absent or the doctor feels that the results produced by the laboratory are unsatisfactory and do not tally with his clinical observations.

In the Indian subcontinent males outnumber females. In India, this phenomenon is less pronounced in rural areas compared with cities, but even in rural populations the ratio of females to males is 951 to 1000 (1981 census). This is often explained by an environmental disadvantage of women (Bhatia, 1983; Lopez, 1984).

In the Orissa population the proportion of females is relatively high. In this respect Orissa is nearer to the Southern than Northern states. Among 15 states of India with a population of more than 10 million each (which together constitute 96.2% of the population of India) Orissa ranks second after Kerala, with 999 and 1034 females per 1000 males, respectively (1981 census), being followed by Maharashtra (987), Tamilnadu (987), Andhra Pradesh (984) and Karnataka (978).

Female/male ratio for the population of Sambalpur, Mayurbhanj and Phulbani districts is 978 per 1000, i.e., well within the limits observed normally in the South of India. As follows from Fig. 1, in these districts there is no gross deficit of females which is observed in Northern India. In the age below 10, the numbers of females and males are almost the same. Later, males start to outnumber females which is probably explained by environmental disadvantage of females. Slight edge that females have in the groups of 20–35 years is probably due to emigration of young adult men: the population of Orissa being a main supplier of unskilled and semi-skilled labour for many of the other and often remote states.

This study revealed that women, particularly at the age group of 20–49 years are severely underrepresented in the PCD. So also are young children (0–9 years). This under-representation is connected with over-representation of adult males, the proportion of whom shows a negative correlation with the representation of almost all age groups of females.

There is only a slight under-representation of baby girls compared with baby boys in the sample studied. It seems that the factors which operate against the representation of young children in general are not sex-selective, the correlation between the representation of boys and girls in 0–4 age group being strong (if proportion of young boys in the sample is high, so is the proportion of young girls, and vice versa). In Northern India, parents are usually less concerned with the health of daughters compared with sons, which explains higher mortality in girls (Bhatia, 1983). Probably, this discrimination is less pronounced in Orissa. This is in agreement with the high proportion of females in the population studied, compared to Northern India.

The proportion of young children shows a strong correlation with the representation of women of 25–39 years. The fact that young children are brought by their mothers is not significant, it is more relevant that those who bring their children probably use this opportunity to get themselves examined. It looks as if their own disease, by itself, is not a sufficiently strong motivation to go to the hospital. The proportion of young children also correlates, to a lesser extent, with the proportion of females in the 50+ age group (possibly, grandmothers are involved in the same

way as mothers), but with none of the remaining groups of the women. Women of 40–49 are not involved possibly because their own children are already grown up, but only few have grandchildren.

It follows from this observation that the samples with good representation of females are also better balanced in respect of the age structure. This may help in analysing the surveillance data, when only a breakdown by sex, and not by age is available.

The females are under-represented among the outpatients. This is not because they are less affected by malaria. Studies (Beljaev *et al.*, 198.), indicate that the prevalence of malaria among the outpatients of both sexes is about the same. A question arises about the number of cases missed due to this factor.

Suppose that the number of females in the PCD becomes equal to the number of males. In this case, the percentage of increase in total collection may be measured by a formula

$P = (100 - F)/(100 + F) \times 100$, where:

F is the observed ratio of females per 100 males.

In this particular study, $F = 62$ per 100, therefore, an equalisation of the representation of the sexes (without any change in the age structure of PCD) would increase the blood examination rate by 23%.

REFERENCES

1. Beljaev, A.E., J.A. Brohult, G.K. Sharma, M.A. Haque and K.C. Samantaray (1985). Studies on the detection of malaria at Primary Health Centres. Part I. Reliability of parasitological diagnosis by decentralized laboratories. *Indian J. Malariol.*, **22**(2): 85–103.
2. Beljaev, A.E., J.A. Brohult, G.K. Sharma and K.C. Samantaray (198.). Studies on the detection of malaria at Primary Health Centres. Part III. Parasitological profile of population surveyed for malaria through Passive Case Detection (in preparation).
3. Bhatia, Sh. (1983). Traditional practices affecting female health and survival: evidence from countries of South Asia. In *Sex differentials in mortality*, edited by A.D. Lopez and L.T. Ruzicka (Misc. Ser. No. 4, Deptt. of Demography, Australian National University, Canberra).
4. Jolly, R. and M. King (1966). The organization of health services. In *Medical care in developing countries*, edited by M. King (Oxford University Press 1982 ELBS reprint).
5. Lopez, A.D. (1984). Sex differentials in mortality. *WHO Chronicle*, **38**(5): 217–224.
6. Murthy, N. (1982). Reluctant patients – the women in India. *World Health Forum*, **3**(3): 315–316.
7. Ray, A.P. and A.E. Beljaev (1984). Epidemiological surveillance: a tool for assessment of malaria and its control. *J. Com. Dis.*, **16**(3): 197–207.
8. Sprent, P. (1981). *Quick statistics–An introduction to non-parametric methods*. Penguin Books): 1–364.

Indian Journal of Malariology
Vol. 23, June 1986, 27-38.

Impact of DDT and HCH Spraying on Malaria Transmission in Villages with DDT and HCH Resistant *Anopheles culicifacies*

V.P. SHARMA[1], R.K. CHANDRAHAS[1], M.A. ANSARI[1], P.K. SRIVASTAVA[1], R.K. RAZDAN[1] C.P. BATRA[1], K. RAGHUVENDRA[1], B.N. NAGPAL[1], S.C. BHALLA[2] and G.K. SHARMA[3]

The impact of spraying DDT and HCH on *A. culicifacies* resistant to DDT and HCH was assessed in Loni PHC, Ghaziabad, U.P. Monitoring of entomological and parasitological indices revealed that correct insecticidal dosage application and increasing the insecticidal coverage brought about significant reduction in vector densities and the incidence of malaria. The study also showed that there was no advantage of enhancing HCH dosage from 200 to 500 mg/m² and that DDT performed better in this area.

INTRODUCTION

Anopheles culicifacies is a primary vector of rural malaria in India. The first report of high tolerance to DDT in *A. culicifacies* came from Panch Mahals district Gujarat in 1959 (Rahaman *et al.*, 1959) and to dieldrin resistance from Thane district in Maharashtra in 1953 and 1957 (Patel *et al.*, 1958). Since then DDT resistance in *A. culicifacies* has become fairly widespread (Raghavan *et al.*, 1967). In areas of high DDT resistance, a changeover to HCH spraying was initially effective but over the years *A. culicifacies* also developed resistance to HCH. In areas of resistance to both classes of organochlorine insecticides, malathion spraying was initially effective in malaria control, but its continued use for several years has resulted in resistance to it also, mainly in Gujarat and Maharashtra (Sharma, 1984). In such areas changeover to another insecticide such as fenitrothion or propoxur was considered, but the replacement insecticides are very expensive and more toxic to humans. It seems likely that mosquitoes would develop resistance to these compounds also in due course of time. In view of these problems the continued use of cheaper insecticides such as DDT and/or HCH was desirable as long as the spraying of these insecticides was effective in reducing malaria transmission.

It is however well-known that the spraying of DDT or HCH or malathion is producing only a marginal impact on malaria transmission in many areas. Reasons for this poor response are not only the development of resistance in the vector species but also the faulty spraying, and at

Accepted for publication: 21 April 1986.
[1]Malaria Research Centre (ICMR)
22-Sham Nath Marg, Delhi-110054.
[2]Directorate of Health, U.P. Govt.
Jawahar Bhawan, Lucknow, U.P.
[3]National Malaria Eradication Programme
22-Sham Nath Marg, Delhi-110054.

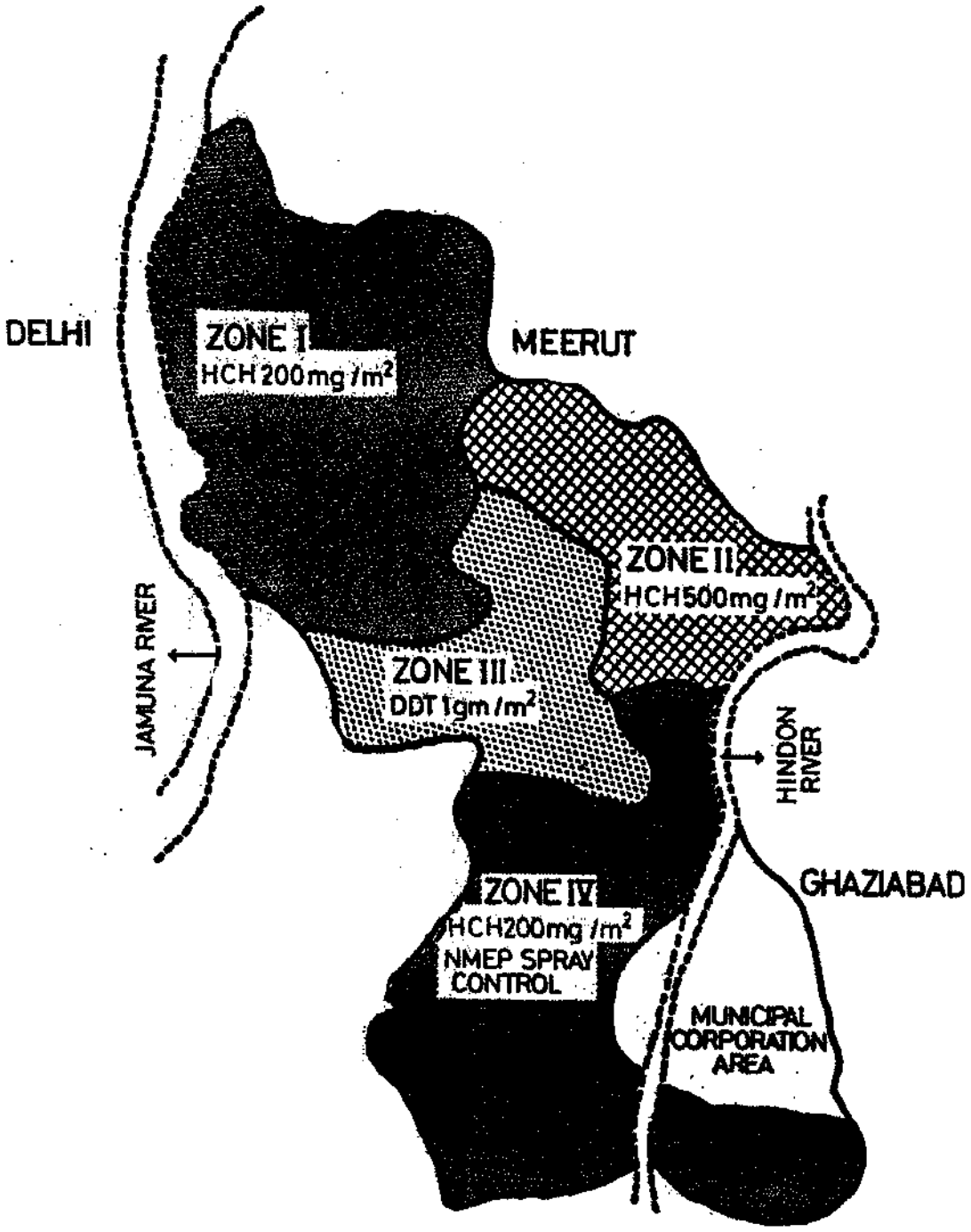


Fig. 1: Map of Loni Primary Health Centre showing the four zones.

times the misuse (e.g., diverting supplies to agriculture) of insecticides. This was shown by the fact that considerable impact on malaria transmission was obtained by improving the DDT spraying operation on a DDT resistant *A. culicifacies* population in Faridabad villages (Sharma *et al.*, 1982). With this background field experiments were carried out to see whether spraying of DDT or HCH at normal and enhanced dosages would control malaria in areas where spraying of HCH at 200 mg/m^2 was proving ineffective, and DDT spraying at 1 gm/m^2 had been discontinued in favour of HCH due to the detection of DDT resistance in *A. culicifacies*. It may be noted that Rawlings *et al.* (1981) had shown that spraying of an enhanced dosage of HCH i.e., 530–880 mg/m^2 in experimental huts killed the heterozygotes and most of the resistant homozygotes for upto 13 weeks after spraying. The lowest dosage (270 mg/m^2) killed all the susceptibles but not all the heterozygotes and few of the resistant homozygotes. Thus there was direct evidence of the effectiveness of an enhanced dosage of HCH in the control of HCH resistant *A. culicifacies*. Field experiments were therefore carried out by spraying villages with HCH at 200 or 500 mg/m^2 and this was compared with DDT coverage at 1 gm/m^2. Results of this study are reported in this paper.

MATERIAL AND METHODS

Loni Primary Health Centre (PHC) villages of Ghaziabad district (U.P.) were selected for the field experiments. Studies carried out by the Malaria Research Centre (MRC) in Ghaziabad (U.P.) villages during the last few years showed that the incidence of malaria was extremely high (MRC Annual Report, 1983–84, pp. 99–103) and the routine spraying of HCH had little or no impact on malaria transmission. *A culicifacies* was incriminated as the vector of malaria (Choudhury. 1984) and it showed varying levels of resistance to DDT and HCH. Loni, Razapur and Muradnagar PHC villages were the worst affected by malaria within Ghaziabad district. Loni PHC is situated in the trans-Jamuna area on the Delhi-U.P. border. Excluding the municipal limits of Ghaziabad town, there are 70 villages with a population of 1,40,000 covered by the PHC. The entire PHC area was divided into four zones (Fig. 1), each containing a population of 30,000 to 35,000. Villages of zone I were sprayed with HCH at 200 mg/m^2, those of zone II with HCH at 500 mg/m^2 and those of zone III with DDT at 1 gm/m^2, and zone IV was initially held as a comparison area. Unfortunately, it was not possible to keep any area as an entirely unsprayed control because of the high level of malaria transmission, and the comparison area was sprayed by the routine procedure of the NMEP using HCH at 200 mg/m^2. This was the same target dose as in zone I but zone I as well as zones II and III were sprayed under the supervision of the scientific staff of the Malaria Research Centre (MRC). It should be pointed out that the District Malaria Officer of Ghaziabad had agreed that spraying in the comparison area would be done according to the standards prevailing in other parts of the district and in the Loni PHC areas in previous years. However, later spraying was intensified in an attempt to achieve the maximum coverage. Therefore, the comparison area was neither an unsprayed control nor even an area with the usual standards of spraying under the NMEP. Insecticides, stirrup pumps and spraying squads were provided by the NMEP.

Parasitological and entomological indices were collected by the MRC staff from all four zones. Parasitological surveillance was carried out on weekly basis and data pooled for the month. In each zone, one village in the centre and four villages i.e., one each to the north, south, east and west were selected for the collection of these indices. In the first three months (June-Aug), the same five villages in each zone were held for monitoring but in later months (from Sept-Dec), the four outlying villages in each zone were changed randomly at each collection while the central village remained fixed. Efforts were made

Table 1. Insecticidal spraying coverage achieved in the experimental villages

Spray Round	Zone I					Zone II					Zone III				
	Spray dates	HCH 200 mg/m²				Spray dates	HCH 500 mg/m²				Spray dates	DDT 1.0 gm/m²			
	1984	HD	R	CS	TS	1984	HD	R	CS	TS	1984	HD	R	CS	TS
First	May 1–	5766	21591	211	872	May 12–	3631	15391	197	172	May 17–	4716	17964	169	183
	June 26	(75)	(61)	(91)	(83)	June 17	(89)	(70)	(84)	(82)	July 4	(78)	(62)	(96)	(91)
Second	June 27–	5382	19832	182	502	July 7–	3680	15037	178	202	Aug 1–	5144	20381	147	186
	Aug 20	(76)	(60)	(93)	(89)	Aug 26	(70)	(53)	(94)	(88)	Sept 25	(74)	(60)	(92)	(97)
Third	Aug 21–	4432	15993	179	265	Aug 27–	3680	15037	171	87	—	—	—	—	—
	Oct 10	(69)	(52)	(93)	(91)	Sept 30	(55)	(46)	(96)	(100)	—	—	—	—	—

HD—Human Dwellings
R—Rooms
CS—Cattlesheds
TS—Temporary Structures

Notes: 1. Spraying time synchronized with the NMEP spraying.
2. Percentage coverage shown in parentheses.
3. In calculating % coverage in HD, even if some area in a house was sprayed it was scored as sprayed whereas in calculating % room coverage, only the sprayed rooms were scored.

to collect as many blood smears as possible. For this active surveillance from febrile cases was carried out in each village. Adult mosquito collections were made using torches and suction tubes. In each village indoor collections were made from sprayed rooms for two hours (15 mins. each in four room and 15 mins. each in four cattlesheds) at 15-day intervals and the number of mosquitoes collected per man hour were calculated for each zone. Insecticide susceptibility tests were carried out using the field collected *A. culicifacies* gravid females. Mosquitoes were exposed on 4% DDT impregnated papers for 1 hour or 4% dieldrin impregnated papers for 2 hours, and mortality of adults recorded after a 24 hour exposure period. It may be pointed out that the 4% dieldrin exposure is survived only by homozygotes for the gene conferring resistance to both dieldrin and HCH.

RESULTS AND DISCUSSION

Table 1 gives details of the percentage coverage achieved in zones I-III of the Loni PHC area. In the first round, coverage in all the zones varied from 60–90%. It was higher in cattlesheds and temporary structures (80–96%) as compared to human dwellings (75–89%) and rooms (61–70%). In the second round, coverage remained more or less the same but in the third round, it was reduced in houses and rooms. It may be noted that every effort was made to maximize spraying and the coverage reported in Table 1 was the maximum possible which could be achieved in this area. To achieve this we followed the procedure of convincing village people at all levels, of the advantage of spraying. This was done by the staff of the centre and the spray supervisors. Statistically, no significant difference was found in coverage achieved in the three experimental zones. The coverage in the control zone was poor and rough estimates showed that it was around 50–60% in the first round and gradually declined to 30–40% in the later rounds. Table 2 provides information on the susceptibility status of *A. culicifacies* to DDT and HCH. Exposure of adults to 4% DDT papers for 1 hour produced 10–40% mortality, but exposure of adults on 4% dieldrin paper for 2 hours produced 0–10% mortality. The vector *A. culicifacies* showed resistance in both tests but the frequency of the gene for dieldrin/HCH was higher than that for DDT. One likely reason for the relatively higher susceptibility to DDT was the fact that Loni PHC was under HCH spray for the past 6 to 7 years. Withdrawal of DDT since 1977 may have allowed selection for the DDT susceptibility allele to have produced partial reversion to DDT susceptibility in the population.

The entomological and parasitological assessment data has been presented in the form of (i) pooled data of five villages and (ii) central village. This became necessary because till August the four selected villages besides the

Table 2. Susceptibility of *A. culicifacies* to DDT and Dieldrin

Villages	DDT 4% 1 hr exposure		Dieldrin 4% 2 hr exposure	
	Number exposed	Corrected mortality (%)	Number exposed	Corrected mortality (%)
Pre-spray status (April, 1984)				
1. Sirora	30	10.0	30	0.0
2. Bhoop Kheri	15	33.3	30	10.0
3. Pachaira	15	40.0	15	6.7
4. G. Karaya	15	13.3	30	0.0

Table 3. *A. culicifacies* densities in each zone*

1984	Fortnightly collection	Per man hour density in each zone			
		HCH 200 mg/m²	HCH 500 mg/m²	DDT 1 gm/m²	Control**
JUNE	I	1.65	6.40	0.75	9.95
	II	4.45	13.10	5.56	24.45
JULY	I	4.60	11.35	9.60	36.75
	II	15.70	4.75	9.90	20.75
AUG	I	8.10	6.45	5.35	36.05
	II	8.45	10.25	2.80	25.40
SEPT	I	8.05	2.20	4.90	10.75
	II	10.15	8.75	1.60	18.85
OCT	I	3.05	1.75	0.70	14.55
	II	0.70	7.50	0.85	15.50
NOV	I	3.70	2.95	0.70	14.00
	II	0.65	0.25	0.25	19.15
DEC	I	0.50	10.95	0.35	3.35
	II	1.10	0.15	0.40	5.95

* Average of 5 villages.
**Villages sprayed with HCH 200 mg/m² by the NMEP.

central village were fixed villages, one each in north, east, west and south, and in later months they were changed and selected randomly. Table 3 shows man hour density for five pooled villages and Table 4 for the central villages.

The application of t-test for inter-comparison of per man hour density of all the three spraying zones paired with control zones showed significant differences. However, the difference in zones with HCH 200 mg/m² and HCH 500 mg/m² was not significant even at 20% level (Fig. 2) which clearly means that the enhanced dose of 500 mg/m² HCH was in no way better than 200 mg/m². The difference in zones of HCH 200 mg/m² and HCH 500 mg/m² with DDT was found statistically significant at 20% and 5% level respectively.

Though central villages did not exhibit significant difference in control of vector density in areas with HCH 200 mg/m² and HCH 500 mg/m² paired with control area, DDT area showed better control of *A. culicifacies* (Fig. 3). The trend of t-values for inter-comparison of central villages of each zone was however found to be the same as in inter-zone comparison.

Tables 5 and 6 give slide positivity rate (SPR) and slide falciparum rate (SfR) of the five pooled villages and central villages in each zone respectively.

In the inter-zonal and inter-village (central) comparisons of SPR and SfR, all values were found significant at 1% level (Figs. 4 and 5). The trend of Z-values for different pair for com-

Table 4. *A. culicifacies* densities in central villages

1984	Fortnightly collection	Per man hour density in each zone			
		HCH 200 mg/m²	HCH 500 mg/m²	DDT 1 gm/m²	Control*
JUNE	I	0.70	4.75	1.00	1.75
	II	0.75	13.75	0.50	9.75
JULY	I	4.00	6.25	3.25	8.50
	II	5.75	3.50	5.25	3.25
AUG	I	5.25	9.25	1.75	5.00
	II	9.50	6.75	0.75	1.25
SEPT	I	6.25	2.00	1.50	6.75
	II	6.75	3.75	1.75	16.00
OCT	I	1.50	2.25	0.75	8.25
	II	1.75	3.00	0.25	4.50
NOV	I	1.75	2.75	1.50	2.75
	II	2.25	0.75	1.25	1.25
DEC	I	1.50	1.50	1.50	1.00
	II	0.00	0.75	0.00	0.50

*Villages sprayed with HCH 200 mg/m² by the NMEP.

parison again show that HCH 500 mg/m² ($Z = 0.60$ paired with control) was in no way better than HCH 200 mg/m² ($Z = 4.55$ paired with control). The SfR Z-values for zone I, II and III paired with zone IV were found higher than SPR Z-values which lead to the conclusion that per cent falciparum cases were reduced more than the per cent total cases as a result of spraying.

Thus the comparatively large t and Z-values of DDT with control for different malariological parameters showed a relatively high degree of resistance to HCH in the field population of *A. culicifacies* and proved that DDT was definitely a better choice.

It is noteworthy to mention that control zone in the Loni PHC was sprayed with HCH 200 mg/m². This spraying reduced the vector densities and interrupted malaria transmission at least to some extent. If the control zone was held unsprayed (which was not possible due to ethical reasons), the impact of DDT or HCH spraying would have been most remarkable. This could be seen by the malaria situation prevailing in the adjacent areas. Active surveillance during 1983–84 in Razapur PHC adjacent to the experimental zones which is a contiguous similar geographic terrain revealed very high SPR (Fig. 6) which is the normal situation of malaria in Razapur or Loni PHC. It is therefore, obvious that spraying of DDT and HCH in areas with double resistance (DDT + HCH) in *A. culicifacies* was effective in reducing vector densities and the incidence of malaria. The study further brought out the importance of correct dosage spraying and maximizing coverage to achieve better impact of residual spraying. DDT spraying compared to HCH 200 or 500 mg/m² produced

Table 5. Results of parasitological surveys in Loni PHC*

1984	Zone I HCH 200 mg/m²			Zone II HCH 500 mg/m²			Zone III DDT 1gm/m²			Zone IV HCH 200 mg/m²		
	B.S.	SPR	SfR	B.S.	SPR	SfR	B.S.	SPR	SfR	B.S.	SPR	SfR
JUNE	541	43.99	02.58	691	30.39	01.44	660	44.54	00.75	385	27.53	01.29
JULY	654	39.44	03.52	484	39.87	03.51	567	37.91	03.52	586	20.98	03.92
AUG	636	39.62	09.27	398	35.92	08.54	484	44.42	09.29	553	34.35	18.26
SEPT	418	33.01	15.55	436	26.83	11.01	378	36.24	15.87	416	33.17	20.19
OCT	266	24.43	19.92	198	30.80	17.67	170	30.00	14.70	194	43.29	35.05
NOV	140	20.71	15.71	117	09.40	05.12	63	26.98	15.87	135	47.00	44.44
DEC	86	17.44	10.46	101	13.86	11.88	53	24.52	20.75	101	27.72	27.72

*Data of 5 villages from each zone pooled.

Table 6. Results of parasitological surveys in the central villages

1984	Zone I HCH 200 mg/m²				Zone II HCH 500 mg/m²				Zone III DDT 1 gm/m²				Zone IV HCH 200 mg/m²			
	Total B.S.		SPR	SfR	Total B.S.		SPR	SfR	Total B.S.		SPR	SfR	Total B.S.		SPR	SfR
JUNE	198	3	31.81	01.51	152	0	33.55	0.00	227	2	53.74	00.88	82	0	24.39	00.00
JULY	196	11	31.63	05.61	125	6	33.60	4.80	114	2	45.61	01.75	47	1	10.63	02.12
AUG	217	13	25.81	05.99	80	7	23.75	7.50	113	6	46.90	06.19	93	16	29.03	17.20
SEPT	170	20	26.47	11.76	124	10	24.19	8.06	107	17	34.57	15.88	102	25	39.21	24.51
OCT	172	29	19.76	16.86	91	16	27.47	17.58	77	12	35.06	15.58	101	36	43.56	35.64
NOV	99	14	20.20	14.14	59	2	05.08	03.38	25	3	20.00	12.00	66	29	43.93	43.93
DEC	56	5	16.07	08.92	47	8	19.14	17.02	22	5	22.72	22.72	44	14	31.81	31.81

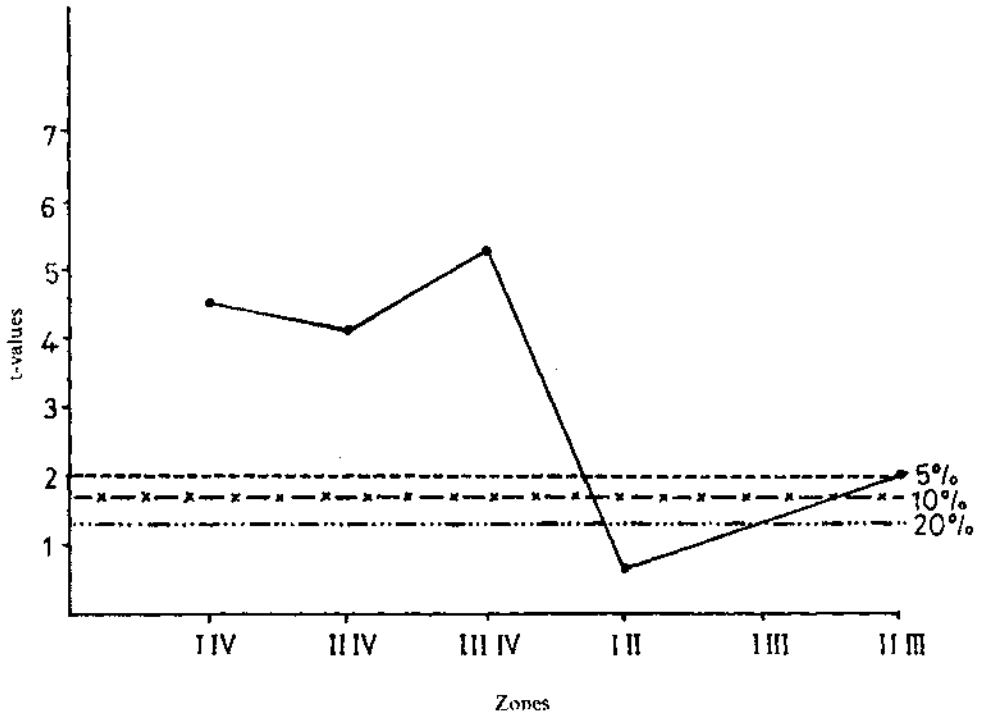


Fig. 2: t-test values for inter-comparison of *A. culicifacies* per man hour density of the 4 zones (pooled data of 5 villages).

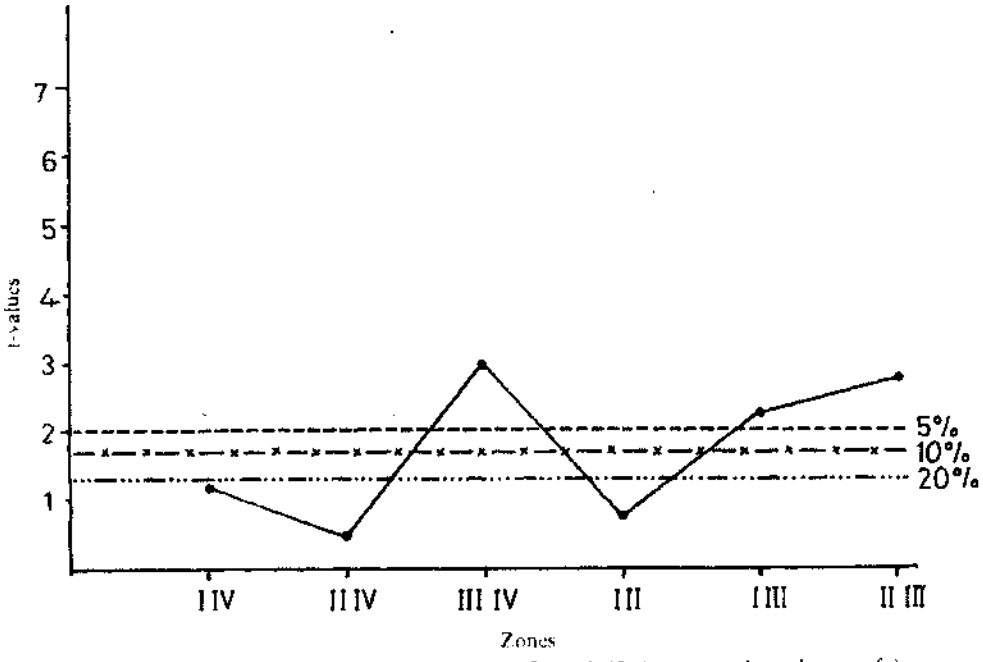


Fig. 3: t-test values for inter-comparison of *A. culicifacies* per man hour density of the 4 zones (data of central villages).

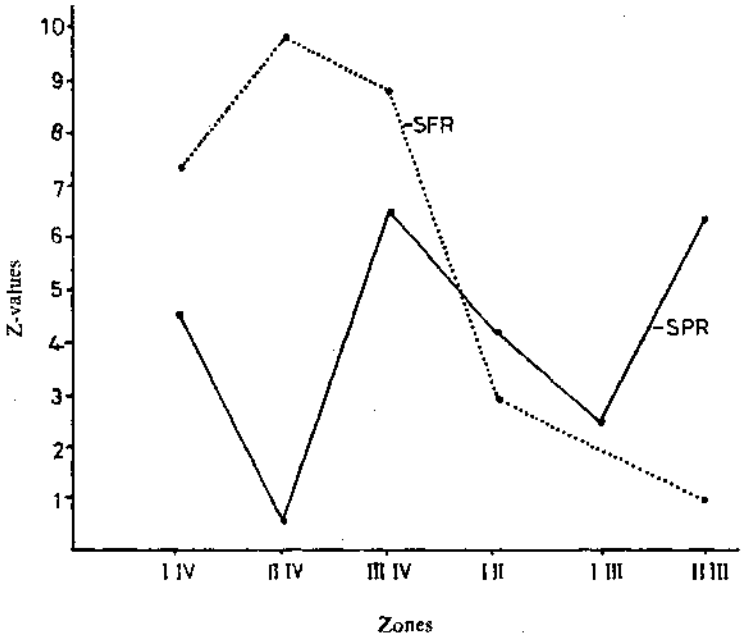


Fig. 4: Z-test values for inter-comparison of the SPR and SfR of the 4 zones (pooled data of 5 villages).

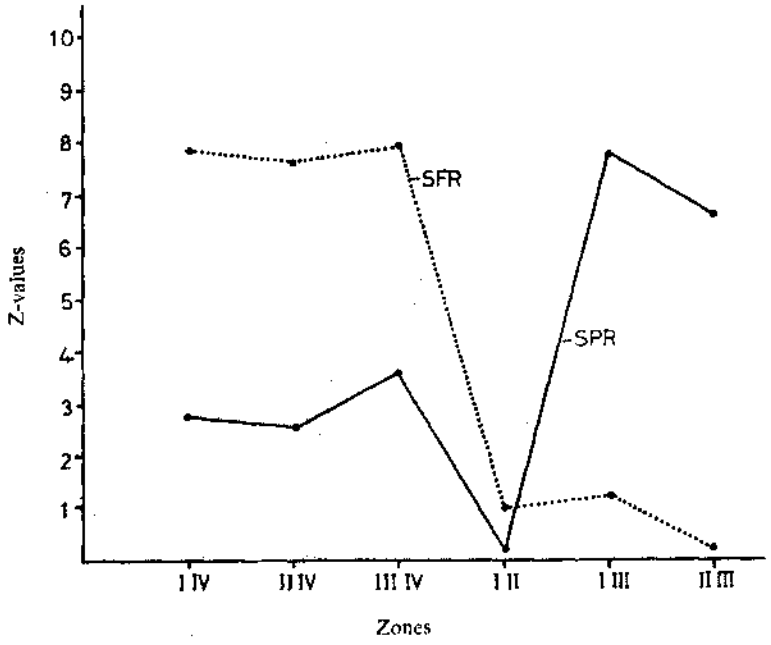


Fig. 5: Z-test values for inter-comparison of SPR and SfR of the 4 zones (data of central villages).

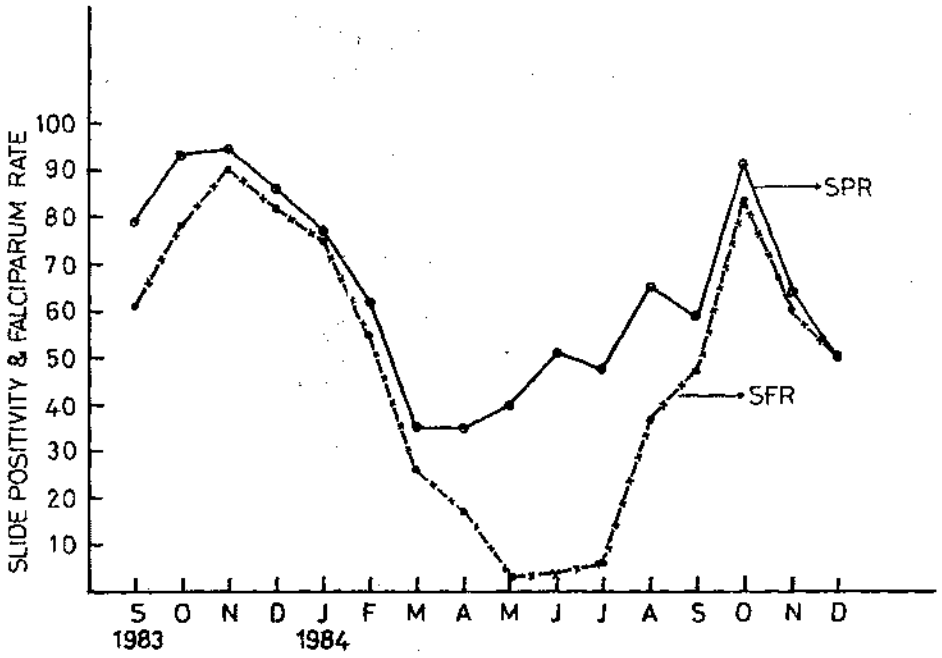


Fig. 6: Slide positivity rate in Razapur PHC.

statistically significant impact, both on the vector densities and malaria transmission. The similarity of results with HCH 200 mg or 500 mg disagrees with the expectation from the results of Rawlings *et al.* (1981). This might be because the Loni PHC population contains a "strong" dieldrin/HCH resistance gene of the kind found in some populations which is recognized by the fact that heterozygotes can survive 4% dieldrin exposure for 2 hours.

Our earlier studies in Faridabad villages (1982) and the present study in Loni PHC have provided sufficient proof of the usefulness of DDT in areas where *A culicifacies* is resistant to DDT and/or HCH. What is needed is good coverage and the application of correct dosages of insecticides. It may also be noted that the cost of insecticidal spraying per million population in 1985 was Rs. 3.4 million with DDT, Rs. 3.7 million with HCH and Rs. 19.9 million with malathion. Because of the cost considerations alone, change of insecticide should be resorted to as a last measure when good spraying fails to produce the desired level of epidemiological impact on malaria transmission.

ACKNOWLEDGEMENTS

The authors are grateful to Dr. (Mrs.) Aruna Srivastava, Malaria Research Centre for statistical analysis of the data. Thanks are also due to the Officers of the malaria department of U.P. Govt. for their help in the completion of this study.

REFERENCES

1. Choudhury, D.S. (1984). Studies on resurgence of malaria in parts of northern India. In *Proceedings of the Indo-UK Workshop on Malaria*. Nov. 14-19, 1983. edited by V.P.

Sharma. (Malaria Research Centre, ICMR, Delhi): 61–71.

2. Patel, T.B., T.R. Rao, A.V. Halgeri and R.B. Deobhankar (1958). A preliminary note on a probable cause of dieldrin resistance in *Anopheles culicifacies* in Thane district, Bombay state. *Indian J. Malariol.*, **12**: 367–370.
3. Raghavan, N.G.S., B.L. Wattal, V.N. Bhatnagar, D.S. Choudhury, G.C. Joshi and K.S. Krishnan (1967). Present status of susceptibility of arthropods of public health importance to insecticides in India. *Bull. Indian Soc. Mal. Com. Dis.*, **4**: 209–245.
4. Rahaman, J., M.L. Roy and Kuldip Singh (1959). Development of increased tolerance to DDT in *A. culicifacies* Giles in the Panch Mahals district of Bombay state (India). *Indian J. Malariol.*, **13**: 125–130.
5. Rawlings, P., G. Davidson, R.K. Sakai, H.R. Rathor, M. Aslamkhan and C.F. Curtis (1981). Field measurements of the effective dominance of an insecticide resistance in anopheline mosquitoes. *Bull.WHO*. **59**(4): 631–640.
6. Sharma, G.K. (1984). Review of malaria and its control in India. In *Proceedings of the Indo-UK Workshop on Malaria*, Nov. 14–19, 1983, edited by V.P. Sharma. (Malaria Research Centre, ICMR, Delhi): 13–40.
7. Sharma, V.P., H.C. Uprety, Nutan Nanda, V.K. Raina, S.K. Parida and V.K. Gupta (1982). Impact of DDT spraying on malaria transmission in villages with resistant *Anopheles culicifacies*. *Indian J. Malariol.*, **19**(1): 5–12.

Indian Journal of Malariology
Vol. 23, June 1986, pp. 39-42.

Mosquitoes of Goa

S.M. KULKARNI[1], J. DHANPAL[1] and V.M. NAIK[1]

During a mosquito survey conducted in Goa in August 1983, 2096 adult mosquitoes belonging to 29 species were collected. *Culex tritaeniorhynchus* was the most abundant species forming 36 per cent of the total collection. *Aedes aegypti* and *Ae. gubernatoris* were collected as immature stages; the former being recorded for the first time in Goa.

The overall parity rate of the four potential Japanese encephalitis (JE) vector species viz., *Culex tritaeniorhynchus*, *C. pseudovishnui*, *C. bitaeniorhynchus* and *C. gelidus* was 39 per cent. Thirteen per cent of the parous mosquitoes showed 2-3 dilatations in their follicular relics.

The role of the suspected vectors of malaria and JE is highlighted. With the present study, the number of mosquito species recorded so far in Goa has risen to 51 comprising *Anopheles*-25, *Armigeres*-1, *Aedes*-6, *Culex*-14, *Heizmannia*-1 and *Mansonia*-4 species.

INTRODUCTION

The information on the mosquito fauna of the union territory of Goa is scanty. Until 1963, 24 species of anophelines were recorded in connection with malaria and its control (Borcar *et al.*, 1967). An outbreak of Japanese encephalitis (JE) occurred in 1982 for the first time in Goa (Choudhary *et al.*, 1983; Mohan Rao *et al.*, 1983). During the epidemic investigation, the former authors encountered five species of anophelines which includes an additional record of *An. leucosphyrus* 'group' and 10 species of culicines. Since no data is available on the vector mosquitoes of JE in Goa, a preliminary survey was undertaken to assess the species composition and relative abundance of mosquitoes between 14 and 27 August, 1983. This communication presents the data collected during this survey and an updated list of mosquito fauna of Goa.

MATERIAL AND METHODS

Topographical and climatological features of Goa district have been described earlier (Choudhary *et al.*, 1983). The mosquitoes were collected from 29 localities within a radius of 50 sq km from Panaji township using standard methods (Kaul *et al.*, 1982). The mosquitoes were identified following Christophers (1933), Barraud (1934), Knight and Stone (1977) and other recent publications. Samples of incriminated and potential vector species of JE were dissected and examined for their parity rates and age composition employing the methods described by Detinova (1962).

Accepted for publication: 7 May 1986.
[1]National Institute of Virology
Pune-411001, India.

Table 1. Species composition of adult mosquitoes collected from Goa with additional earlier records

Sl. No.	Species	No. collected	Percentage of total
1.	*Anopheles (Cellia) annularis** Van der Wulp	2	N
2.	*An. (Cel.) fluviatilis** James	1	N
3.	*An. (Cel.) jamesii** Theobald	247	11.8
4.	*An. (Cel.) jeyporiensis** James	2	N
5.	*An. (Cel.) karwari** (James)	144	6.9
6.	*An. (Cel.) subpictus** Grassi	279	13.3
7.	*An. (Cel.) vagus** Doenitz	255	12.1
8.	*An. (Anopheles) peditaeniatus** (Leicester)	1	N
9.	*Armigeres (Armigeres) subalbatus* (Coquillett)	44	2.1
10.	*Aedes (Stegomyia) aegypti*** (Linnaeus)	—	—
11.	*Ae. (Ste.) albopictus* (Skuse)	10	0.5
12.	*Ae. (Ste.) vittatus* (Bigot)	1	N
13.	*Ae. (Finlaya) gubernatoris*** (Giles)	—	—
14.	*Ae. (Aedes) pseudomediofasciatus* (Theobald)	1	N
15.	*Ae. (Aedimorphus) vexans* (Meigen)	3	0.1
16.	*Culex (Culex) ambiguus* Theobald	8	0.4
17.	*C. (C.) bitaeniorhynchus** Giles	19	0.9
18.	*C. (C.) cornutus* Edwards	9	0.4
19.	*C. (C.) fuscocephala* Theobald	14	0.6
20.	*C. (C.) gelidus** Theobald	49	2.3
21.	*C. (C.) infula* Theobald	12	0.6
22.	*C. (C.) pseudovishnui** Colless	165	7.9
23.	*C. (C.) quinquefasciatus** Say	45	2.1
24.	*C. (C.) tritaeniorhynchus** Giles	752	35.8
25.	*C. (C.) vishnui* 'group'	7	0.3
26.	*C. (C.) whitmorei** (Giles)	13	0.6
27.	*C. (Culiciomyia) pallidothorax* Theobald	7	0.3
28.	*C. (Eumelanomyia) pluvialis* Barraud	1	N
29.	*Heizmannia* sp.	1	N
30.	*Mansonia (Coquillettidia) crassipes* (Van der Wulp)	1	N
31.	*Man. (Mansonioides) uniformis** (Theobald)	3	N
	Total	2,096	—

N = <0.1

* = Recorded earlier also

** = Recognised from larval collection and rearing.

Additional Earlier Records of Mosquito Species

32. *Anopheles (Cellia) aconitus* Doenitz; 33. *An. (Cel.) culicifacies* Giles; 34. *An. (Cel.) kochi* Doenitz; 35. *An. (Cel.) leucosphyrus* 'group'; 36. *An (Cel.) maculatus* Theobald; 37. *An. (Cel.) majidi* Young and Majid; 38. *An. (Cel.) minimus* Theobald; 39. *An. (Cel.) pallidus* Theobald; 40. *An. (Cel.) philippinensis* Ludlow; 41. *An. (Cel.) pulcherrimus* Theobald; 42. *An. (Cel.) stephensi* Liston; 43. *An. (Cel.) tessellatus* Theobald; 44. *An. (Cel.) theobaldi* Barraud; 45. *An. (Cel.) varuna* Iyengar; 46. *Anopheles (Anopheles) aitkenii* James; 47. *An. (An.) barbirostris* Van der Wulp; 48. *An. (An.) culiciformis* Cogill; 49. *Culex (Culex) vishnui* Theobald; 50. *Mansonia (Mansoniides) annulifera* (Theobald) and 51. *Man. (Man.) indiana* Edwards.

RESULTS AND DISCUSSION

The data on mosquitoes collected during the present survey and those recorded by earlier workers are presented in Table 1. During the present study, 2,096 adult mosquitoes belonging to 29 species were collected. In addition, a few samples of larvae were collected from coal tar drums and reared to adults, which yielded *Aedes aegypti* and *Ae. qubernatoris*. Thirteen species viz., *Anopheles jamesii*, *An. karwari*, *An. subpictus*, *An. vagus*, *Armigeres subalbatus*, *Culex bitaeniorhynchus*, *C. fuscocephala*, *C. gelidus*, *C. infula*, *C. pseudovishnui*, *C. quinquefasciatus*, *C. tritaeniorhynchus* and *C. whitmorei* together formed 96 per cent of the total collection. Remaining 16 species (4%) were found in very small numbers.

Of the 25 species of *Anopheles* recorded earlier, eight species were recorded during the present survey including *An. fluviatilis* which is considered to be a vector of malaria in Goa (Borcar *et al.*, 1967; Choudhary *et al.*, 1983). However, Rao (1984) has reported a distribution record of only 18 species according to different altitudes. *An. subpictus* has recently been incriminated as a vector in coastal villages of Southeast India (Panicker *et al.*, 1981) and *An. annularis* and *An. jeyporiensis* are known vectors of local importance elsewhere in India (Rao, 1984).

Out of 26 culicine species, 23 were recorded during the present survey. *C. tritaeniorhynchus* was the most predominant species and formed 35.8 per cent of the total collection (Table 1). Earlier, this species was also reported to be the most predominant species in the area (Choudhary *et al.*, 1983). It was mainly collected at dusk while biting cattle and resting on vegetation around cattlesheds. Out of 752 adults of this species, 671 adults were collected at dusk with a density of 11.4 per man hour. *Aedes aegypti*, the vector of dengue virus was recorded for the first time in Goa.

The data on the parity rate and age composition of four incriminated and potential vector species of JE are presented in Table 2. It was found that the parity rates of *C. tritaeniorhynchus* and *C. pseudovishnui* were nearly 39 per cent. About 13 per cent of these mosquitoes had 2–3 dilatations in their follicular relics. In earlier studies in Mandya district of Karnataka, the parity rates for *C. vishnui* and *C. tritaeniorhynchus* were found to vary from 50 to 85 per cent during April to June 1983 and that the epidemic had coincided with the time when these vectors had high parity rates (Mishra *et al.*, 1984).

ACKNOWLEDGEMENTS

The authors are grateful to Dr. K.M. Pavri,

Table 2. Parity rates of four potential vector species of JE in Goa

Species	No. Nulliparous	Parous with no. of dilatations			Total dissected	% Parous
		1	2	3		
Culex tritaeniorhynchus	67	25	16	1	109	38.5
C. bitaeniorhynchus	6	3	0	0	9*	—
C. pseudovishnui	31	14	4	1	50	38.0
C. gelidus	0	3	0	0	3*	—
Total	104	45	20	2	171	—
%	60.8	26.3	11.7	1.2	—	39.2

*Number dissected was too small to be of significance.

Director, NIV, Pune for facilities and encouragement. Thanks are due to the Director and other officials of the Directorate of Health Services, Government of Goa, Daman and Diu, for their help and cooperation during the mosquito survey conducted in Goa.

REFERENCES

1. Barraud, P.J. (1934). *The fauna of British India including Ceylon and Burma*. v. 5. (Taylor and Francis, London): 1–463.
2. Borcar, P.A., H.M.L. Srivastava, R.G. Roy and N.L. Mukherji (1967). Malaria eradication programme in Goa. *Bull. Indian Soc. Mal. Com. Dis.*, **4**: 45–54.
3. Choudhary, N., N.B.L. Saxena, S.R. Dwivedi and J.S. Khamre (1983). Study of the outbreak of Japanese encephalitis in Goa. *J. Com. Dis.*, **15**: 111–120.
4. Christophers, S.R. (1933). *The fauna of British India including Ceylon and Burma*. v. 4. (Taylor and Francis, London).
5. Detinova, T.S. (1962). Age grouping methods in Diptera of medical importance with special reference to vectors of malaria. *WHO Monograph Series*, **47**: 1–216.
6. Kaul, H.N., J. Dhanpal, P.K. Deshmukh and K. Banerjee (1982). A report on the mosquitoes collected during an epidemic of encephalitis in Dhanbad district, Bihar. *Indian J. Med. Res.*, **76**: 53–58.
7. Knight, K.L. and A. Stone (1977). *A catalog of the mosquitoes of the world (Diptera: Culicidae)*. (Entomological Society of America, Box A.J. 4603, Calvert Road, College Park, Maryland 20740).
8. Mishra, A.C., Jacob George, S. Ramanujam, H.R. Bhat and K.M. Pavri (1984). Mosquito vectors of Japanese encephalitis epidemic (1983) in Mandya district (India). *Indian J. Med. Res.*, **80**: 377–389.
9. Mohan Rao, C.V.R., S.R. Prasad, J.J. Rodrigues, N.G.K. Sharma, B.H. Shaikh and K.M. Pavri (1983). The first laboratory proven outbreak of Japanese encephalitis in Goa. *Indian J. Med. Res.*, **78**: 745–750.
10. Panicker, K.N., M. Geetha Bai, U.S. Bheema Rao, K. Viswam and U. Suryanarayan Murthy (1981). *Anopheles subpictus*, vector of malaria in coastal villages of South-East India. *Curr. Sci.*, **50**: 694–695.
11. Rao, T.Ramachandra (1984). *The Anophelines of India*. Rev. ed. (Malaria Research Centre, ICMR, Delhi).

Indian Journal of Malariology
Vol. 23, June 1986, pp. 43–48.

Progressive Changes in the Liver Function of Monkeys following *Plasmodium knowlesi* Infection

RAKA KHANNA[1], HARIS M. KHAN[1] and SOHAIL AHMAD[1]

This investigation was aimed at studying the extent of liver damage by experimentally induced infection of acute malaria. Rhesus monkeys were intravenously inoculated with simian malaria parasite, *Plasmodium knowlesi*. The liver damage caused by the invading parasites was demonstrated by performing several liver function enzyme tests like SGOT, SGPT, and total serum bilirubin. The enzyme levels were estimated at various parasitaemic levels in the infected animals. These values were compared with those obtained from post-chloroquine treated, infected/uninfected animals. The sera from normal saline inoculated healthy monkeys served as controls. Attempts were also made to study the pathophysiological changes in the infected liver by correlating the functional impairment with any detectable anatomical abnormality. Data obtained from a few follow-up cases has also been included.

INTRODUCTION

In spite of the enormous amount of literature on malaria, comparatively little information is available on parasite induced changes in the host liver. It is well documented that malarial infection impairs liver function (Riley and Maegraith, 1961). A few substances capable of impairing the liver function *in vitro*, have already been identified in the sera of *P. knowlesi* infected animals (Riley and Maegraith, 1961; Maegraith *et al.*, 1963). The ultrastructural and biochemical changes in the infected liver cells are, in fact, initiated during the erythrocytic phase through mediators released in the serum (Maegraith, 1966). The liver damage caused by the invading parasites during acute malaria can be more conveniently demonstrated by means of several routine liver function tests. The enzymes generally used as indicators of liver function are: serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT) and alkaline phosphatase. In a few early studies, estimation of total serum bilirubin concentration was also used as yet another parameter for assessing the liver function (Kingsbury, 1926; Ross, 1927). In this study estimation of SGOT and SGPT enzyme levels were carried out for assessing the extent of liver damage as a result of experimental infection of monkeys with *P. knowlesi*. The sera obtained from infected animals were also used for estimating bilirubin concentration. In the follow-up studies assessment of the liver damage was also investigated in the sera of chloroquine treated/untreated monkeys over a 10–40 days period. The sera of

Accepted for publication: 20 May 1986.
[1]Parasitology Laboratory
Department of Microbiology
Jawaharlal Nehru Medical College
Aligarh Muslim University
Aligarh-202001, India.

Table 1. Serum enzyme levels in experimental monkeys

Animal groups	Days after inoculation	Per cent parasitaemia ± SD	SGOT units/ml ± SD	SGPT units/ml ± SD	Total serum bilirubin mg/dl ± SD
Saline control	0	0	28 ± 2.6	24 ± 2.1	2.55 ± 0.63
	4	0	24 ± 2.4	24 ± 2.0	2.65 ± 0.63
	8	0	28 ± 2.6	26 ± 2.1	2.65 ± 0.61
	15	0	28 ± 2.6	24 ± 2.1	2.65 ± 0.65
Parasite infected	9	5.7 ± 1.36	30 ± 1.8	52 ± 4.3	5.55 ± 0.89
			P < 1.8	P < 0.01	P < 0.05
	11	10.8 ± 1.41	36 ± 1.8	52 ± 4.3	6.08 ± 0.89
			P < 0.05	P < 0.01	P < 0.05
	12	27.6 ± 3.9	36 ± 2.3	52.2 ± 3.8	15.6 ± 1.7
			P < 0.05	P < 0.01	P < 0.01
	13	80.0 ± 6.7	56 ± 3.5	72 ± 4.7	23.0 ± 2.31
			P < 0.05	P < 0.01	P < 0.01

P < 0.05 is significant.

Table 2. Serum enzyme levels in chloroquine treated monkeys

Animal groups	Days after inoculation	Days after treatment	SGOT units/ml ± SD	SGPT units/ml ± SD	Total serum bilirubin mg/dl ± SD
Saline control	0	—	28 ± 2.6	24 ± 2.1	2.55 ± 0.63
	4	—	24 ± 2.4	24 ± 2.0	2.65 ± 0.68
	8	—	28 ± 2.6	26 ± 2.1	2.65 ± 0.63
	15	—	28 ± 2.6	24 ± 2.1	2.65 ± 0.63
Uninfected, chloroquine treated	—	0	28 ± 1.6	28 ± 1.9	7.76 ± 0.69
			P < 0.1	P > 0.1	P < 0.05
	—	4	36 ± 2.8	52 ± 2.9	9.02 ± 0.83
			P < 0.05	P < 0.01	P < 0.01
	—	8	24 ± 2.5	28 ± 2.7	6.18 ± 1.21
			P > 0.1	P > 0.1	P < 0.05
	—	15	28 ± 2.5	24 ± 2.7	6.02 ± 1.21
			P > 0.1	P > 0.1	P < 0.05
Parasite infected, chloroquine treated	15	3	36 ± 2.0	52 ± 2.3	13.6 ± 1.78
			P < 0.05	P < 0.05	P < 0.01
	20	8	28 ± 2.0	28 ± 1.9	6.08 ± 0.98
			P > 0.1	P > 0.1	P < 0.05
	30	18	24 ± 1.9	24 ± 2.2	6.32 ± 0.86
			P > 0.1	P > 0.1	P < 0.05
	40	28	24 ± 2.5	28 ± 2.7	6.08 ± 0.71
			P > 0.1	P > 0.1	P < 0.05

Chloroquine was administered on 12th day of infection.
Per cent parasitaemia was zero throughout the studies.

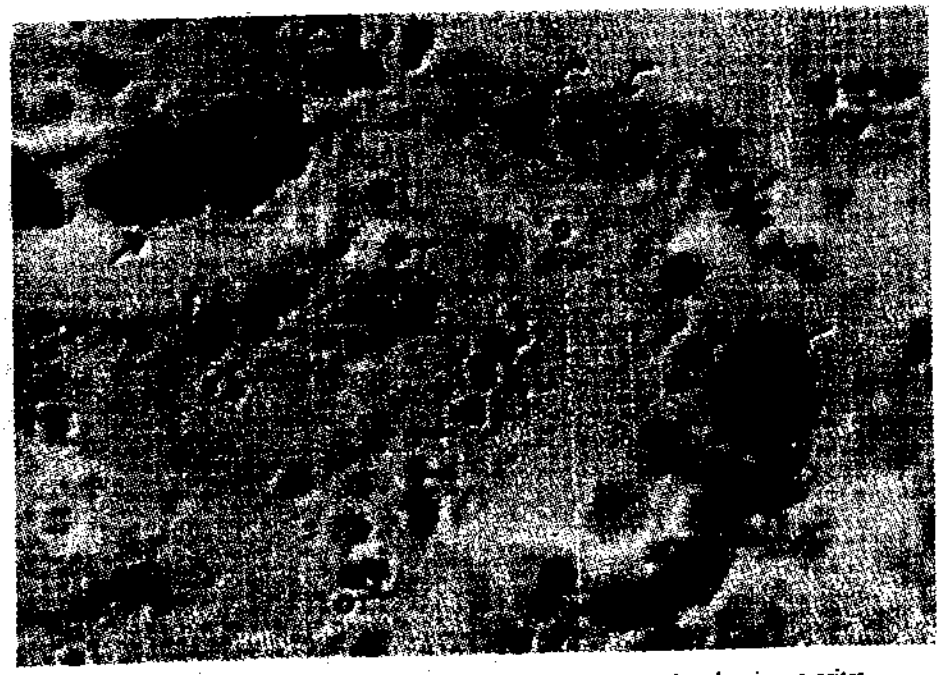


Fig. 1: Photomicrograph of a liver section from an infected monkey showing parasites (↱) and accumulation of malaria pigment (➔) (× 1000).

Fig. 2: Photomicrograph of a liver section from uninfected, chloroquine treated monkey showing reticular cells in sinusoids (× 1000).

also showed a slight increase in these values on day 3 following chloroquine administration. The SGOT enzyme levels increased from 28 units/ml to 36 units/ml, while SGPT levels were raised from 28 units/ml to 52 units/ml (Table 2). Similarly, the total bilirubin concentration in the serum of treated, but uninfected, animals was raised to 9.02 mg/dl on day 4. The above values returned to normal levels on day 8 in the uninfected, chloroquine treated animals (Table 2). Animals inoculated with normal saline served as experimental controls.

Histological examination revealed diffuse infiltration of the liver sinusoids by macrophages. The macrophages with engulfed haemazoin pigment appeared rounded and globular. There was necrosis of hepatocytes around the central vein. The portal triad areas showed infiltration by the neutrophils with a few lymphocytes (Fig. 1). The liver section of the uninfected and chloroquine treated monkeys (Desowitz *et al.*, 1967) showed normal appearance with little connective tissue seen in the portal tracts. The sinusoids were lined by inconspicuous reticular cells (Fig. 2).

DISCUSSION

Significant changes in the level of serum transaminases were observed in *P. knowlesi* infected monkeys. It is reported that due to the exo-erythrocytic cycle there is a continuous destruction of liver cells resulting in increased enzyme levels in the blood of an infected subject. These changes are probably more related to the erythrocytic destruction, anoxia and liver damage occurring in acute malaria (Sudan *et al.*, 1965).

An increase in bilirubin values observed in our experiments is indicative of a liver function impairment in the infected animals. Kingsbury (1926) and Ross (1927) have found elevated levels of serum bilirubin in *P. vivax*, *P. malariae* and *P falciparum* infections. Due to an infiltration of reticulocytes in the liver, the hepatocyte micro-villi are likely to get blocked. Such a situation could then be a possible cause of hyperbilirubinaemic state in malaria. Some workers are of the opinion that hyperbilirubinaemia in malaria occurs due to an abnormally high blood destruction (Fairley and Bromfield, 1934). Bhamarapravati *et al.* (1973) in subsequent investigations attributed jaundice in malaria infected animals due to an impairment of bilirubin transport. Liver function enzyme values were also slightly altered following chloroquine treatment in untreated animals. The observed alterations in the enzyme levels may quite well be due to a cumulative effect of chloroquine on the liver tissue. Whether such alterations are due to chloroquine treatment, or malaria, or both cannot be definitely ascertained only on the basis of this one study. Further investigations along these lines using chloroquine and other commonly used antimalarials will undoubtedly be helpful in determining the role of these drugs in altering such serum enzyme levels.

In prolonged infections, it is reported that the liver becomes black following accumulation of malarial pigment in the reticuloendothelial cells (Desowitz *et al.*, 1967). In an active infection, the perilobular kupffer cells have been shown to phagocytose malarial pigment and the infected erythrocytes (Aikawa *et al.*, 1968). In an earlier study, *P. knowlesi* infected liver sections showed an excessively large number of parasitized RBCs attached to the endothelial cells (Jervis *et al.*, 1972; Gutierrez *et al.*, 1976). Skirrow *et al.* (1964) found that monkeys with terminal *P. knowlesi* malaria show marked constriction of the portal vein and its branches. Liver sections from our infected animals showed cellular infiltration of lymphocytes and granulocytes in the portal triad. The data obtained on serum enzyme values, shows that even chloroquine treated animals have elevated levels of SGOT and SGPT and bilirubin. This effect was only found further enhanced in infected and subsequently treated animals.

Such findings may have some value in the early diagnosis of infection, as also for assessment of antimalarials vis-a-vis liver function impairment (Sudan *et al.*, 1965).

ACKNOWLEDGEMENT

This work was partially supported by the Council of Scientific and Industrial Research, New Delhi.

REFERENCES

1. Aikawa, M., C.G. Huff and H. Sprinz (1968). Exo-erythrocytic stages of *Plasmodium gallinaeceum* in chick embryo liver as observed electron microscopically. *American J. Trop. Med. Hyg.*, **17**: 156–169.
2. Bhamarapravati, N., S. Boonpucknavig, V. Boonpucknavig and C. Yaemboonruang (1973). Glomerular changes in acute *Plasmodium falciparum* infection. *Arch. Pathol.*, **96**: 289–298.
3. Desowitz, R.S., L.H. Miller, R.D. Buchanan, V. Yuthasastrkosol and B. Permpanich (1967). Comparative studies on the pathology and host physiology of malarias. I. *Plasmodium coatneyi*. *Ann. Trop. Med. Parasitol.*, **61**: 365–374.
4. Fairley, N.H. and R.J. Bromfield (1934). Laboratory studies in malaria and blackwater fever. III. A new blood pigment in blackwater fever and other biochemical observations. *Trans. R. Soc. Trop. Med. Hyg.*, **28**: 307.
5. Gutierrez, Y., M. Aikawa, H.N. Fremount and C.R. Sterling (1976). Experimental infection of *Aotus* monkeys with *Plasmodium falciparum*: Light and electron microscopic changes. *Ann. Trop. Med. Parasitol.*, **70**: 25–44.
6. Jervis, H.R., H. Sprinz, A.J. Johnson and B.T. Wellde (1972). Experimental infection with *Plasmodium falciparum* in *Aotus* monkeys. II. Observations on host pathology. *American J. Trop. Med. Hyg.*, **21**: 272–281.
7. Kingsbury, A.N. (1926). Some investigations of malarial fevers. *Trans. R. Soc. Trop. Med. Hyg.*, **19**: 459.
8. Maegraith, B.G. (1966). Pathogenic processes in malaria. In *The pathology of Parasitic Diseases*, edited by A.E.R. Taylor. (Blackwell, Oxford): 15–32.
9. Maegraith, B.G., K.A. Fletcher, M.G.N. Angus and D.I. Thurnham (1963). Further observations on the inhibition of tissue metabolism in malaria. *Trans. R. Soc. Trop. Med. Hyg.*, **57**: 2.
10. Riley, M.V. and B.G. Maegraith (1961). A factor in the serum of malaria-infected animals capable of inhibiting the *in vitro* oxidative metabolism of normal liver mitochondria. *Ann. Trop. Med. Parasitol.*, **55**: 489–497.
11. Ross, G.R. (1927). Bilirubinaemia in malignant tertian malaria and blackwater fever. *British J. Exp. Pathol.*, **8**: 442.
12. Skirrow, M.B., T. Chongsulphajaisiddhi and B.G. Maegraith (1964). The circulation in malaria. II. Portal angiography in monkeys (*Macaca mulatta*) with *Plasmodium knowlesi* and in shock following manipulation of the gut. *Ann. Trop. Med. Parasitol.*, **58**: 501–510.
13. Sudan, E.H., J.S. Williams, F.C. Meroney and G. Hutt (1965). Pathophysiology in *Plasmodium berghei* infection in mice. *Exp. Parasitol.*, **17**: 277–286.
14. Voller, A. and R.N. Rossan (1969). Immunological studies on simian malaria. II. Immunity to challenge and antigenic variation in *P. knowlesi*. *Trans. R. Soc. Trop. Med. Hyg.*, **23**: 507–523.

Indian Journal of Malariology
Vol. 23, June 1986, pp. 49-53.

Biochemical Method for the Detection of Chloroquine Resistance in *P. falciparum*

L.N. GRINBERG[1], D.A. NOOSHTAEV[1], F.F. SOPRUNOV[1], D.S. CHOUDHURY[2], USHA DEVI[2] and V.P. SHARMA[2]

WHO *in vivo* and *in vitro* tests are available for detection of chloroquine resistance in *P. falciparum*. Nooshtaev *et al.* (1982) showed that chloroquine resistance in *P. berghei* could be assessed by the measurement of the change in pH of the media due to the production of lactic acid by the intraerythrocytic parasites as a result of glycolysis.

In the present study it was possible to demonstrate the applicability of this biochemical test for the detection of chloroquine resistance in *P. falciparum*. Hence this test can be used in parallel with WHO *in vitro* test for determination of chloroquine resistance.

INTRODUCTION

Chloroquine resistance in *P. falciparum* is one among the many technical problems which are responsible for the widespread resurgence of malaria in many of the Southeast Asian countries.

In India chloroquine resistance in *P. falciparum* was first detected in 1973 in Assam (Sehgal *et al.*, 1973). Since then it has spread to other parts of India and presently it has been detected in ten states of the country (Sharma, 1983). In order to effectively control this problem it is essential to monitor the status of chloroquine resistance in different parts of India so that proper corrective measures can be taken up on time and effectively.

WHO *in vivo* as well as *in vitro* micro and macro tests are available at present for detecting chloroquine resistance in *P. falciparum* (Rieckmann *et al.*, 1978). However, *in vitro* tests need proper equipment, sterile conditions and at least 24 hours period of observation. It is difficult to carry out these tests under field conditions. Failure of maturation of the parasites in the control is another drawback in these tests.

Nooshtaev *et al.* (1982) showed that chloroquine resistance in *P. berghei* could be assessed by the measurement of the change in pH of the media. This change is due to the production of lactic acid by the intraerythrocytic parasites as a result of glycolysis. In the presence of chloroquine, the normal glycolysis of the parasite is inhibited in

Accepted for publication: 11 June 1986.
[1]Martsinovsky Institute of Tropical Medicine and Parasitology Moscow, USSR.
[2]Malaria Research Centre (ICMR) 22-Sham Nath Marg, Delhi-110054, India.

the susceptible strain to a higher degree as compared with the resistant strain. Therefore, by monitoring the change in pH of the media during the incubation of the parasite *in vitro*, it is possible to differentiate the chloroquine resistant strain from the susceptible one.

In order to determine whether this method is applicable for the detection of chloroquine resistance in *P. falciparum*, studies were carried out at Malaria Research Centre, Delhi with the *in vitro* culture parasites of *P. falciparum*. The data obtained in these experimental studies is presented in this paper.

MATERIAL AND METHODS

In vitro cultures of *P. falciparum*, both susceptible and resistant to chloroquine which are being maintained in continuous culture in the Centre were utilized for the test. The batches of normal erythrocytes of group A received from the local blood bank were used during the test. For determining the pH a portable sensitive pH meter and a microelectrode were used (Radelkis, Hungary).

Before the test, *in vitro* cultures of *P. falciparum* were selected keeping in mind the level of the parasitaemia and the stages of the parasites. The erythrocytes, both normal and infected were washed in the weak-buffered salt solution: (in m mol/l) NaCl-139, KCl-2.7, $CaCl_2$-1.8, $MgCl_2$-1.1, Na_2HPO_4-1.3, glucose-5.0, Tris-HCl-2.5, pH-7.30. The same solution was used for the incubation of the cells in plastic tubes with stoppered caps. The final haematocrit of the suspension was 10% and the final volume was 0.3 ml in each tube. Chloroquine diphosphate was added to the tubes before the incubation giving the final concentration of chloroquine varying from 12.5 to 100 micromol/l. The tubes were incubated at 37°C for 3 hours with stirring every half an hour. The difference in the pH before and after incubation (ΔpH) was used for the calculation of the degree of glycolysis inhibition by chloroquine according to the formula:

$$I = \frac{(\Delta pH_o - \Delta pH_x)\ inf}{(\Delta pH_o)\ inf - (\Delta pH_o)\ norm} \times 100\% \qquad (1)$$

Where I = coefficient of inhibition (%).

ΔpH_o = decrease in pH during the incubation in samples without chloroquine.

ΔpH_x = the same in the presence of chloroquine.

inf = suspension containing infected RBCs.

norm = suspension containing only normal RBCs.

RESULTS AND DISCUSSION

The experimental data is presented in Table 1. From this it can be seen that there was insignificant variation in the rate of glycolysis in normal erythrocytes (the ΔpH mean value for 22 samples was 0.135 ± 0.008). It did not depend on the duration of keeping the cells in the culture (1–3 days). Thus under the above conditions, the contribution of the uninfected erythrocytes in glycolysis of the mixed population of cells was rather stable.

It is also seen from the data that the glycolytic rate in the suspensions containing parasitized erythrocytes was two to three times higher than in suspensions containing normal erythrocytes. Extrapolating these to 100% parasitized cells, one can estimate the glycolytic rate in the infected erythrocytes (mainly trophozoites) to be 30 to 50 times more than in the normal erythrocytes. These results are in good agreement with the data obtained in experiments on synchronized culture of *P. falciparum* (Pfaller *et al.*, 1982; Yayon *et al.*, 1983; Zolg *et al.*, 1984)

Table 1. Change of pH after incubation of infected and normal (non-infected) erythrocytes in presence of chloroquine

S. No.	Type of erythrocytes	ΔpH					Parasitaemia				
		Conc. of chloroquine ($\times 10^{-5}$M)					Number of parasites per 1000 RBCs				
		0	1.25	2.5	5.0	10.0	Rings	Early trophs.	Late trophs.	Schizonts	Total
1.	Normal	0.155	—	—	—	0.150	—	—	—	—	—
	Infected (S)	0.220	—	—	0.160	0.155	15	21	—	6	42
2.	Normal	0.135	—	—	—	0.130	—	—	—	—	—
	Infected (R)	0.280	—	0.285	0.260	0.250	5	18	—	8	31
3.	Normal	0.125	—	—	—	0.125	—	—	—	—	—
	Infected (S)	0.360	—	0.230	0.155	0.130	21	33	7	0	61
4.	Normal	0.125	—	—	—	—	—	—	—	—	—
	Infected (S)	0.260	—	0.170	—	—	6	36	15	3	60
	Infected (R)	0.320	—	0.330	0.320	0.260	4	31	13	4	52
5.	Normal	0.100	—	—	—	—	—	—	—	—	—
	Infected (S)	0.580	0.505	0.510	0.415	0.325	2	13	68	1	84
6.	Normal	0.120	—	—	—	—	—	—	—	—	—
	Infected (S)	0.250	—	0.235	0.220	0.225	0	5	12	2	18
	Infected (R)	0.190	—	0.185	0.190	0.180	4	13	3	0	20
7.	Normal	0.130	—	—	—	—	—	—	—	—	—
	Infected (S)	0.285	—	0.260	0.230	0.215	0	20	14	3	37

Notes: 1. ΔpH per 3 hours incubation are given in the table (average of two samples).
2. In expt. No. 1 and 2 the total number of trophozoites (early and late) are shown together.
3. 'S' stands for susceptible and 'R' stands for resistant strains of *P. falciparum*.

and asynchronized population of *P. berghei* (Grinberg, 1982).

The above data supports the idea that the measurement of pH can be used for the determination of glycolytic rate in intraerythrocytic malaria parasites. It has been shown that lactic acid is the principal source of H^+ excreted into the medium by the infected erythrocytes under given *in vitro* conditions (Grinberg, 1982). It was also shown that the change in pH in the range 7.4 to 6.9 is proportional to the quantity of lactic acid produced by those cells. Therefore, the ΔpH per unit time can be used as the criterion for measuring the glycolytic rate in the parasite. The additional justification of this approach comes from the fact that the activities of phosphofructokinases of *P. berghei* and *P. knowlesi* do not depend upon the pH in this range (Sander *et al.*, 1982a, b). Hence the glycolysis in the parasites is maintained at a constant level under the conditions of low pH in a certain range.

The data in Table 1 shows that lactic acid production is inhibited in the presence of chloroquine in culture containing parasites while this is not so with the normal erythrocytes.

In equation 1, the denominator shows the contribution of parasitized erythrocytes into the total amount of lactic acid produced by the culture cells, i.e., the rate of glycolysis in the parasites.

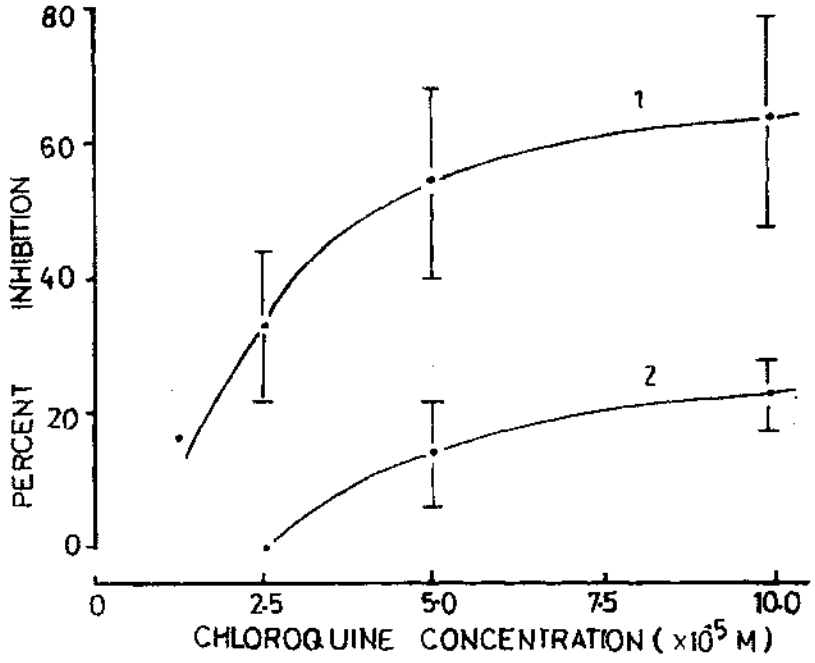


Fig. 1: Inhibition of *P. falciparum* glycolysis in the presence of chloroquine:
1 — chloroquine susceptible strain;
2 — chloroquine resistant strain.

The numerator in equation 1 shows the decrease in the rate of parasite glycolysis due to chloroquine. The inhibition coefficients (I) were calculated according to equation 1 for each concentration of chloroquine.

Fig. 1 shows the dependence of the inhibitory effect on the concentration of chloroquine. The points represent the mean values of the replicates shown in Table 1. Under given concentration of the drug, the degree of the inhibition of the glycolysis was significantly lower in the case of chloroquine resistant strain of *P. falciparum*. The graphs shown in Fig. 1 are similar to that in case of *P. berghei* (Nooshtaev *et al.*, 1982).

As can be seen from Table 1, there were variations in the ratio of the different stages of the parasites in the replicates. This might be responsible for the variations in first values in replicates because sensitivity of the malaria parasites to chloroquine depends on the age of the parasites (Polet and Barr, 1968; Yayon *et al.*, 1983). To elucidate whether that was so in present experiments, regression analysis of the experimental data was carried out with the help of a computer.

Regression analysis using the data shown in Table 1 (right) and Fig. 1 (curve 1) showed that the value of inhibition coefficient I and percentage of rings were linearly correlated. The relationship is significant (correlation coef. 0.99; regression coef. 1.76). No correlation could be found between I and percentages of the other age forms of the parasites.

These results indicate that the younger stages of *P. falciparum* are more sensitive to chloroquine than other stages under given conditions. This agrees with the data obtained in the Martsinovsky Institute with *P. berghei*. However, Yayon

et al. (1983) found that glycolysis of *P. falciparum* was inhibited in the presence of chloroquine in cultures containing mostly the mature trophozoites. For arriving at the final conclusion it is necessary to obtain reproducible results with strictly synchronous population of the parasites.

It has been seen from the results of the present experiments that it is possible to carry out this glycolysis inhibition test even with blood containing 2 to 3% parasites. But one must keep in mind that patients suffering from *P. falciparum* malaria mostly show only the rings and early trophozoites in the peripheral circulation. Work is now in progress to find out whether this method can detect the glycolysis of the rings which is but slightly higher than that of uninfected RBCs. Based on the presented data it is quite reasonable to carry out this test in parallel with WHO *in vitro* test for determination of chloroquine resistance in *P. falciparum*. This will show the relative diagnostic value of this test in the determination of chloroquine resistance in patients.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the help rendered by Dr. A.A. Luriye of the Martsinovsky Institute of Tropical Medicine and Parasitology, Moscow, U.S.S.R. in the statistical analysis of the data.

REFERENCES

1. Grinberg, L.N. (1982). Investigations of erythrocyte glycolysis with automatic titration of lactic acid. In *Application of biochemical micromethods for the investigation of tropical disease pathogens*. (WHO, Geneva): 195–196.
2. Nooshtaev, D., F. Soprunov and L. Grinberg (1982). Biochemical method for the detection of chloroquine resistance in *Plasmodium berghei in vitro*. *Medicinskaya Parasitologia and Tropitcheskie Bolesni*. 1982. (6, C): 26–29 (Russian).
3. Pfaller, M., D. Krogstad, A. Parfuet and Dinh P. Nguyen (1982). *Plasmodium falciparum*: stage-specific lactate production in synchronized cultures. *Exp. Parasitol.*, **54**(3): 391–396.
4. Polet, M. and C. Barr (1968). Chloroquine and dihydroquinine: *in vitro* studies of their antimalarial effects upon *Plasmodium knowlesi*. *J. Pharmacol. Exp. Ther.*, **164**: 380–386.
5. Rieckmann, K., G. Campbell, L. Sax and J. Mrema (1978). Drug sensitivity of *Plasmodium falciparum*. *Lancet*, **1**(8054): 22–23.
6. Sander, B., M. Lowery and W. Kruckeberg (1982a). *Plasmodium berghei*: acid-insensitive phosphofructokinase in infected mouse erythrocytes. *Exp. Parasitol.*, **53**(1): 11–16.
7. Sander, B., K. Osborn, M. Lowery and W. Kruckeberg (1982b). *Plasmodium knowlesi*: glycolytic intermediate levels and enzyme activities of infected rhesus monkey erythrocytes. *Exp. Parasitol.*, **53**(3): 355–361.
8. Sehgal, P.N., M.I.D. Sharma, S.L. Sharma and S. Gogai (1973). Resistance to chloroquine in falciparum malaria in Assam state, India. *J. Com. Dis.*, **5**(4): 175–180.
9. Sharma, V.P. (1983). Drug resistant falciparum malaria in India. In *Proceedings of the Indo-UK Workshop on Malaria*, Nov. 14–19, 1983, edited by V.P. Sharma. (Malaria Research Centre, ICMR, Delhi): 169–184.
10. Yayon, A., J. Vande Waa, M. Yayon, T. Geary and J. Jensen (1983). Stage-dependent effects of chloroquine on *Plasmodium falciparum in vitro*. *J. Protozool.*, **30**(4): 642–647.
11. Zolg, N., A. Macleod, J. Scaife and R. Beaudsin (1984). The accumulation of lactic acid and its influence on the growth of *Plasmodium falciparum* in synchronized cultures. *In vitro*. **20**(3): 205–215.

SHORT NOTES

Indian Journal of Malariology
Vol. 23, June 1986, pp. 57–59.

Incrimination of *Anopheles culicifacies* as Vector of Malaria in Orissa

B.N. NAGPAL[1] and V.P. SHARMA[1]

Orissa state is located on the east coast of India, bounded in the north by Bihar, in the west by Madhya Pradesh, in the northeast by West Bengal and in the south by Andhra Pradesh. Topographically Orissa is divided into three main regions viz., (1) hill districts (Koraput, Kalahandi, Phulbani and Balangir); (2) plains districts (Sambalpur, Sundergarh, Keonjhar, Dhenkanal and Mayurbhanj); and (3) coastal districts (Ganjam, Puri, Cuttack and Balasore). The state is endemic for malaria with high incidence of *P. falciparum*. During 1984 there were 283927 malaria cases of which 226279 (79.69%) were *P. falciparum*. Although *P. malariae* is also found, but its distribution is localized in certain tribal areas and the proportion of *P. malariae* is low (about 2 to 5%). It is noteworthy to mention that about 35% of all new falciparum malaria cases come from Orissa alone i.e., in 1984 there were 655453 *P. falciparum* cases in India and of these Orissa contributed 226279 cases (G.K. Sharma, NMEP, Personal communication). Our preliminary studies showed that the mosquito fauna of Orissa is rich and the role of different anopheline vectors of malaria since resurgence is not known. Therefore, during our surveys from 1982–1985 to study mosquitoes of Orissa, we also dissected mosquitoes for the gut and gland infection. Results of this study are reported in this paper.

Early account by Perry (1914) on vector incrimination showed that in Koraput district (Jeypore hills) *A. listoni* (*A. fluviatilis*) was an important vector and *A. maculatus* played a secondary role in malaria transmission. Watts (1924) recorded one gland positive specimen of *A. annularis* from Singhbhum hills. Senior White (1937a, 1938) conducted studies in Jeypore hills and concluded that the members of fluviatilis group (*A. fluviatilis*, *A. minimus*, *A. varuna* and *A. aconitus*) were the only vectors of malaria. However, they also found five specimens of *A. culicifacies* and four of *A. jeyporiensis* with oocyst in their guts. Senior White and Das (1938) again incriminated fluviatilis group as a vector of malaria in Singhbhum hills and stated that *A. culicifacies* and *A. jeyporiensis* played no part in malaria transmission. There is no earlier record of vector incrimination from the five districts in plains, but recently Dash *et al.* (1982) incriminated *A. annularis* from Keonjhar and considered this to be the main vector of malaria from this area.

In coastal districts, Sarathy (1932) was the first to incriminate *A. annularis* as the vector of malaria. Senior White (1937b) discovered *A. sundaicus* in Puri district and later Senior White and Adhikari

Accepted for publication: 2 May 1986.
[1]Malaria Research Centre (ICMR)
22-Sham Nath Marg, Delhi-110054, India.

(1939) incriminated *A. sundaicus* and considered it to be the primary vector of malaria. This finding was further supported by Covell and Singh (1942) and Panigrahi (1942). In 1943 Senior White and co-workers concluded that *A. sundaicus* was the primary vector and *A. annularis* was playing secondary role in malaria transmission in coastal region of Orissa. They also found gut positive specimens of *A. culicifacies*, *A. pallidus* and *A. ramsayi*. It is noteworthy to mention that in the recent surveys reported by Nagpal and Sharma (1983) no specimen of *A. sundaicus* was found from coastal Orissa suggesting that it has been uprooted as vector of malaria from this region. Summarizing the earlier findings of the malaria vectors of Orissa, the Manual of the Malaria Eradication Operation (1960) states that "although 12 species of *Anopheles* are commonly found in this area, only *A. fluviatilis*, *A. varuna* and *A. minimus* play a part in transmission in the order mentioned. *A. culicifacies* which transmits malaria in the larger part of India does not seem to have any role in transmission in this area though the species is not absent."

We had undertaken an extensive mosquito fauna survey in the entire state of Orissa from December 1984 to March 1985, which was in addition to the coastal district villages surveyed in June-August, 1982. During this survey a total of 3148 anopheline mosquitoes belonging to four species viz., *A. annularis*, *A. culicifacies*, *A. fluviatilis* and *A. jeyporiensis* were collected from hill, plain and coastal villages and dissected for natural gut and gland infection. In hill districts a total of 956 anophelines were dissected i.e., 432 *A. culicifacies*, 26 *A. annularis*, 371 *A. jeyporiensis* and 77 *A. fluviatilis*. In the plains districts a total of 1156 anophelines belonging to three species viz., 785 *A. culicifacies*, 368 *A. annularis* and 3 *A. fluviatilis* were dissected. From the coastal districts 619 specimens of *A. culicifacies* and 417 *A. annularis* were dissected.

Results of the number of *A. culicifacies* dissected in each habitat and the numbers found positive for gut or gland infection are given in Table 1. Dissection results revealed that only *A. culicifacies*, and no other mosquito was found positive for the gut or gland infection. The incrimination of *A. culicifacies* in villages in the hills, plains and coastal areas, and in an urban area establishes the role of *A. culicifacies* in the transmission of malaria in Orissa.

The problem of *A. culicifacies* control is being complicated *inter alia* due to widespread DDT resistance. The appearance of chloroquine resistant *P. falciparum* strains (Guha *et al.*, 1979) is

Table 1. Results of vector incrimination studies in Orissa

Districts	Habitat	Date of dissection	*A. culicifacies* dissected	Gut +ve	Gland +ve	Total
Ganjam	Gopalpur urban	07-7-82	35	1	0	1
	Golenthra village	03-7-82	65	1	0	1
Cuttack	Phulnoknara village	25-7-82	73	1	0	1
Phulbani	Godipada village	08-1-85	69	0	1	1
Balangir	Sagarpalli village	15-1-85	72	0	1	1
Sambalpur	Maneswar village	29-1-85	127	0	1	1
Cuttack	Ayodhyapur village	01-3-85	64	0	1	1
	Biribati village	02-3-85	99	0	1	1
	Pingapada village	03-3-85	73	1	0	1
Total			677	4	5	9

adding a new dimension to the already complicated situation of malaria in this state. It would be interesting to study the bionomics of the *A. culicifacies* sibling species complex in representative areas of the state and integrate this knowledge with improved and intensified malaria control measures.

Earlier studies have shown that *A. fluviatilis*, *A. minimus*, *A. varuna* and *A. aconitus* were transmitting malaria in the hill districts, *A. annularis* in the plains and *A. sundaicus* was the sole vector on the coast, although *A. annularis* was also playing a secondary role in the area around Puri. But recent studies have shown that *A. culicifacies*, which was not considered a vector of malaria in Orissa before the launching of malaria eradication was found to transmit malaria in all the three terrains and also in urban areas. The role of other vectors of malaria such as *A. annularis* and *A. fluviatilis* could not be established as only a small number of mosquitoes were dissected. It is however likely that these vectors may be adding to the transmission along with *A. culicifacies*. Further studies are needed to elucidate the role of other vectors along with *A. culicifacies* in the transmission of malaria in different geographical regions of Orissa.

ACKNOWLEDGEMENTS

Authors are thankful to Mr. G.P. Shukla, Mr. S.M. Das, Mr. Om Prakash, Mr. Ram Pal and Mr. Kamal Suryan for their technical assistance during the study tour.

REFERENCES

1. Covell, G. and P. Singh (1942). Malaria in the coastal belt of Orissa. *J. Mal. Inst. Ind.*, **4**: 457–488.
2. Dash, A.P., M.S. Bendle, A.K. Das, M. Das and S.R. Dwivedi (1982). Role of *Anopheles annularis* as a vector of malaria in inland of Orissa. *J. Com. Dis.*, **14**: 242.
3. Guha, A.K., J.R. Roy, Silpi Das, R.G. Roy and S. Pattanayak (1979). Results of choloroquine sensitivity tests in *Plasmodium falciparum* in Orissa state. *Indian J. Med. Res.*, **70** (suppl.): 40–47.
4. Manual of the Malaria Eradication Operation (1960). (Ministry of Health, Govt. of India): 390.
5. Nagpal, B.N. and V.P. Sharma (1983). Mosquitoes of coastal Orissa. *Indian J. Malariol.*, **20**: 141–145.
6. Panigrahi, R.G. (1942). Malaria in Puri. *J. Mal. Inst. Ind.*, **4**: 423–428.
7. Perry, E.L. (1914). Endemic malaria of the Jeypore hill tracts of the Madras Presidency. *Indian J. Med. Res.*, **2**: 457–491
8. Sarathy, M.K.P. (1932). Observation on malaria in Puri district, Orissa. *Indian Med. Gaz.*, **67**: 254–260.
9. Senior White, R. (1937a). On malaria transmission in the Jeypore Hills, Part I. A year's dissection results. *Rec. Mal. Surv. Ind.*, **7**: 47–75.
10. Senior White, R. (1937b). The presence of *Anopheles sundaicus* (Ludlow) on the Chilka lake. *Indian Med. Gaz.*, **72**: 361–363.
11. Senior White, R. (1938). On malaria transmission in the Jeypore hills. Part II. A second year's results. *J. Mal. Inst. Ind.*, **1**: 129–145.
12. Senior White, R. and A.K. Adhikari (1939). On malaria transmission around the Chilka lake. *J. Mal. Inst. Ind.*, **2**: 395–423.
13. Senior White, R., A.K. Adhikari and V. Ramakrishna (1943). On malaria transmission on the Orissa coastal plain. *J. Mal. Inst. Ind.*, **5**: 159–189.
14. Senior White, R. and B.K. Das (1938). On malaria transmission in the Singhbhum hills. *J. Mal. Inst. Ind.*, **1**: 169–184.
15. Watts, R.C. (1924). *Report No. II. Malaria survey of the mining settlement of Singhbhum, Orissa.* (Superintendent, Govt. Printing, Bihar and Orissa, Patna).

Indian Journal of Malariology
Vol. 23, June 1986, pp. 61–63.

Comparative Effectiveness of Different Anabolic and Sex Steroids on the Development of *Anopheles stephensi*

V. SITA[1], S.S. THAKUR[1], B. KISHEN RAO[1] and D. RAGHUNATHA RAO[1]

Demands created by environmental awareness and complete insecticidal resistance in some mosquito species, encouraged the use of insect growth regulators (IGRs), as important means of pest control. Even though varying groups of compounds have been tested as IGRs with different degree of success (Lewallen, 1964; Spielman and Williams, 1966; Diekman, 1972; Marx, 1973; Anonymous, 1974; Patterson, 1974; Busvine *et al.*, 1976), one of the group of compounds which has been least exploited is that of vertebrate steroids. The present study deals with the screening of certain steroids both anabolic and vertebrate sex steroids, for the growth regulating activity on *Anopheles stephensi*.

A. stephensi was reared under controlled conditions of temperature 27 ± 1°C and relative humidity 70 ± 5% and photoperiod 12:12 hr. The larvae were raised in large white enamel troughs containing water. Fish food was provided every alternate day. The developing pupae were transferred into beakers placed in mosquito-net cloth cages (50 × 50 × 100 cm) in which adult emergence occurred. The adults were fed on 10% glucose for the first two days after emergence, thereafter guinea pig was provided for the blood meal required by the adult females. The life cycle completed in 12–15 days. The steroids used were Nandralone phenyl propionate (Durabolin-organon); hydroxy progesterone; pregnenolone and pregnenolone acetate (CSIR Centre for Biochemicals). The biological activity was assayed on the freshly moulted last instar larvae. Varying doses of the compound dissolved in acetone were mixed in 50 ml of water to make different concentrations. Various concentrations tested were 500, 250, 125, 62.5, 31.25 and 16 ppm. Twenty larvae were released in each beaker of 250 ml. Same quantity of the solvent was mixed in water for parallel controls.

Observations were recorded concerning the mortality, ecdysis and different morphogenetic changes. The mating behaviour and oviposition were also observed in the resulting forms. Scoring for the IGR activity was related to the morphogenetic deformities at the next moult, and is as follows:

(a) Normal pupae	0
(b) Pharate pupae with different degrees of adult development	1
(c) Pupae with larval exuvium	2
(d) Larvae with different degrees of melanisation and total arrest of development	3

Accepted for publication: 20 May 1986.
[1]Department of Zoology
Osmania University
Hyderabad-500007, India.

The percentage IGR activity for each compound was calculated according to Bransby-Williams (1971).

$$\frac{P(a.1) + (b.2) + (c.3)}{NX} \times 100$$

Where P = percentage of activity
a,b,c = number of insects in each category
1,2,3 = scoring allotted
N = total number of insects treated
X = maximum score given

The IGR activity data thus obtained was then subjected to probit analysis to calculate the ID50 values.

Activity of the steroids: The descending order of activity for various steroids depending on their ID50 values with their fiducial limits calculated statistically is given in Table 1.

Nandralone phenyl propionate is highly active in almost all concentrations tested. Higher concentrations induced 98% activity while lower concentrations induced 62% activity. Pregnenolone acetate is the least effective steroid when compared to other steroids tested. Higher concentrations induced 25% activity while lower concentrations induced 12% activity.

Amongst the treated larvae most of them did not undergo any moulting but showed prolongation of larval life for a period of 10-14 days at higher concentrations. Total arrest of development was observed in all such forms. In a few others, varying degrees of larval melanisation was noticed depending on the nature and concentration of the steroids used. However, blackening of the larval body was noticed with small amounts of low concentrations within a few minutes after the treatment, which resulted in total mortality. A smaller percentage of the larvae turned into larval pupal intermediates with heads bearing larval mouthparts and the abdomen with paddles at the abdominal extremity as in pupae. In certain cases, the larval exuvium could not be shed off completely and the pupae were seen with attached exuviae. Total mortality was observed during eclosion in all such forms. The pupae which appeared to be normal but had no adult emergence were the pharate pupae. However, some adults showed partial emergence. The exuviae remained attached to such adults and most of them died in the process. The successfully emerging adults which were very few, were either normal looking or with bloated abdomens. The latter were inactive, could not take to flight and were found dead on the surface of the water.

In our present investigations, we have observed the formation of intermediate forms, arrest of development and inhibition of eclosion which are considered to be typical IGR effects. The above results are in conformity with the results obtained in different species of mosquitoes by using different juvenile hormone mimics (Spielman and Skaff, 1964; Patterson, 1974; Busvine *et al.*, 1976). The fact that an ecdysone analog

Table 1. Steroid activity

Sl. No.	Compound	ID50	Fiducial limits
1.	Nandralone phenyl propionate	0.00196 µg	0.00191-0.00199 µg
2.	Hydroxy progesterone	0.0104 µg	0.00701-0.0179 µg
3.	Pregnenolone	0.0106 µg	0.0102 -0.01113 µg
4.	Pregnenolone acetate	0.10636 µg	0.10635-0.10662 µg

would inhibit larval development is not surprising in view of what is known about the hormonal control of metamorphosis. Mulder and Gijswijt (1973) showed that ecdysterone like compounds interfere with cuticle formation in insect larvae during the process of ecdysis. Nevertheless the extreme range in the sensitivity displayed by *A. stephensi* to various steroids used is quite striking.

ACKNOWLEDGEMENT

The financial assistance to V. Sita by Council of Scientific and Industrial Research, New Delhi, is gratefully acknowledged.

REFERENCES

1. Anonymous (1974). A biological survey of the insects of Canada. *Bull. Entomol. Soc. Can.*, **6**(2): 1–16.
2. Bransby-Williams (1971). Juvenile hormone activity of ethylfarnesoate dihydrochloride with the cotton stainer *Dysdercus cardinalis*. *Bull. Entomol. Res.*, **61**: 41–47.
3. Busvine, J.R., Y. Rongsriyam and D. Bruno (1976). Effects of some insect development inhibitors on mosquito larvae. *Pestic. Sci.*, **7**: 153–160.
4. Dickman, J.D. (1972). Use of insect hormones in pest control. *Proceedings of the Natural Extension of Insect Pest Management Workshop*. (Purdue University).
5. Lewallen, L.L. (1964). Effects of farnesal and zeram on mosquito larvae. *Mosq. News*, **24**: 43–45.
6. Marx, J.L. (1973). Insect control II Hormones and Viruses. *Science*, **181**: 833–835.
7. Mulder, R. and M.J. Gijswijt (1973). The laboratory evaluation of two promising new insecticides which interfere with cuticle deposition. *Pestic. Sci.*, **4**: 737–745.
8. Patterson, J.W. (1974). A comparison of the morphogenetic and sterilizing activities of juvenile hormone mimics on *Aedes aegypti*. *J. Insect. Physiol.*, **20**: 2095–2106.
9. Spielman, A. and C.M. Williams (1966). Lethal effects of synthetic juvenile hormone on larvae of the yellow fever mosquito *Aedes aegypti*. *Science*, **15**: 1043–1044.
10. Spielman, A. and V. Skaff (1964). Inhibition of metamorphosis and ecdysis in mosquitoes. *J. Insect. Physiol.*, **13**: 1087–1095.

Indian Journal of Malariology
Vol. 23, June 1986, pp. 65–67.

Impact of Malathion Thermal Fogging on Mosquito Populations in Delhi and its place in Malaria Control

V.P. SHARMA[1], G.K. SHARMA[2], M.A. ANSARI[1], P.K. MITTAL[1], R.K. RAZDAN[1] and C.P. BATRA[1]

In India malathion thermal fogging was introduced in late 1970s as an emergency measure to control Japanese encephalitis (JE) epidemics that broke out in several parts of the country. Thermal fogging machines of various makes were imported by the National Malaria Eradication Programme (NMEP) and supplied to the state governments to control JE epidemics. Since JE epidemics are unpredictable and infrequent in occurrence, the fogging machines were idle most of the time. These machines gradually found application in the urban malaria scheme (UMS) of the NMEP (Pattanayak *et al.*, 1981), although UMS strategy never envisaged the use of malathion fogging as a routine measure. Instead, the recommended procedure of malaria control in urban areas is mainly by anti-larval methods and chemotherapy. Pyrethrum space spraying inside a positive house, and in about 50 houses in the vicinity of a positive case is recommended to prevent further spread of the foci of infection.

Since the introduction of malathion fogging in some urban areas as a routine operation, it is thought to be an important method of mosquito control and also a means to eliminate malaria, by some sections of the society. At present 200 fogging machines (41 TIFA, 23 TIGA, 22 LECO, 100 VAN FOG and 14 UNI FOG) are in operation in different states of the country. The demand for more fogging machines from local bodies (municipalities/corporations and even the industrial townships etc.) is increasing as the visual impact of fogging is quite impressive. The UMS under the NMEP is functioning at present in 122 towns of the country. In addition to this there are a large number of urban areas with moderate to high mosquito nuisance requiring some measure of control, which prefer to adopt fogging because of the visual impact. In view of this situation, it was considered important to evaluate the usefulness of malathion fogging as a means to control mosquito populations and reduce malaria transmission. Results of this study are reported in this paper.

Mosquito larvae were collected from their breeding sites in Delhi colonies and also from villages of Ghaziabad district (UP). The immatures were brought to the laboratory and held at room temperature until adult emergence. Adult mosquitoes of both sexes were given 1% glucose solution on cotton pads for 24–36 hours. A cylindrical cage (12 cm long and 8 cm in diameter) made of 20 mesh galvanized wire screen

Accepted for publication: 23 June 1986.
[1]Malaria Research Centre (ICMR)
22-Sham Nath Marg
Delhi-110054, India.
[2]National Malaria Eradication Programme
22-Sham Nath Marg
Delhi-110054, India.

Table 1. Impact of fogging on mosquito populations in Delhi colonies

Date	Locality	Mosquito species	Nos. tested	No. of cages	Corrected mortality (%)
2.8.85	Ram Nagar Ext., Anarkali Rd, Rashid Mkt.	*Culex quinquefasciatus*	200	20	57.5
		A. subpictus	100		53.3
7.8.85	R.K. Puram, Sec. 1 and 4	*Culex quinquefasciatus*	180	25	2.2
		A. subpictus	195		15.2
8.8.85	NCERT, Katwaria, Saket	*Culex quinquefasciatus*	255	20	24.3
		A. subpictus	45		4.8
9.8.85	R.K. Puram, Sec. 5, 6, 8, 9 and 12	*Culex quinquefasciatus*	150	20	2.6
		A. subpictus	90		14.3
		A. stephensi	30		0.0
		A. culicifacies	30		23.3
10.8.85	Vasant Vihar	*Culex quinquefasciatus*	60	20	10.0
		A. stephensi	240		20.4
13.8.85	Malviya Nagar	*Culex quinquefasciatus*	90	13	0.0
		A. stephensi	105		0.0
14.8.85	Geeta Colony	*Culex quinquefasciatus*	60	22	15.0
		A. culicifacies	30		0.0
		A. stephensi	240		5.8
26.8.85	Tughlakabad	*A. stephensi*	150	14	0.66
		A. culicifacies	60		6.6
28.8.85	Mehrauli	*A. stephensi*	135	18	4.4
		A. culicifacies	135		10.3
29.8.85	Mehrauli	*A. stephensi*	150	17	43.3
		A. culicifacies	105		32.3
30.8.85	Lado Sarai, DDA Flats and Saket	*A. stephensi*	90	12	4.4
		A. culicifacies	90		1.1
31.8.85	Pushp Vihar	*A. stephensi*	150	20	3.3
		A. culicifacies	150		3.3

Table 2. Impact of fogging on different mosquito species in Delhi

Mosquito Species	Nos. tested	Percentage corrected mortality after 24 hours
1. *A. stephensi*	1290	10.33
2. *A. culicifacies*	600	9.66
3. *A. subpictus*	430	22.98
4. *Cx. quinquefasciatus*	995	18.61

was used for holding the mosquitoes for bio-assays. Adult mosquitoes were identified and transferred to the cages using a suction tube. Each cage carried a maximum of 25 adult

mosquitoes of both sexes. Mosquitoes were transferred to the field in cylindrical cages, well protected and covered with a wet towel. They were taken out at 1630 hours and placed at different sites by 1800 hours. A control batch was held at least 50–60 metres away from the fogging area. Cages were placed at a distance of 3 to 20 metres and also at different heights i.e., at ground level and second and third floors of the houses. All cages were collected 1 hour after fogging and transported to the laboratory and maintained at 28 ± 1°C and 70% RH. Mosquitoes were held in the same cage and given 1% glucose solution on cotton pads. Mortality of the mosquitoes was recorded after a lapse of 24 hours.

Results of bioassay tests are given in Table 1. Mortality of the mosquitoes exposed to the fog was very low and varied from 0 to 58%. The mortality in controls was 0 to 5% and rarely it was 10%. In areas that were highly congested and a good fogging was done as on 2nd and 29th August 1985, fogging killed 50% of the caged mosquitoes. In other localities where the mosquitoes were held at distances beyond 5 metres or on second or third floors, the mortality was very poor and at times negligible. Results of species-wise mortality are given in Table 2. The mortality of malaria vectors i.e., *Anopheles culicifacies* and *A. stephensi* was lowest (about 10%) and *Culex quinquefasciatus* and *Anopheles subpictus* about 20%. It was therefore concluded that fogging as practised by the New Delhi Municipal Corporation was not producing desirable mortality of malaria vectors and other mosquito species.

Results of a parallel study carried out by the Municipal Corporation of Bombay revealed that 5% malathion thermal fog killed less than 10% mosquitoes on the ground floor and there was no mortality at the upper floors in *Cx. quinquefasciatus* mosquitoes held in cages (P.B. Deobhankar, personal communication). The recommended procedure is that fogging machines should be operated at an output of 45 litres per hour to produce a dry fog with the vehicle moving at a speed of 8 kms or 5 miles per hour. The air temperature should be 18°C (65°F). Malathion 96% insecticide is used in diesel oil and a 5% malathion (w/v) fog is generated by constantly stirring the storage tank containing malathion and diesel oil mixture. The machine should be so adjusted as to give a maximum of 12 micron particle size. In order to achieve desirable results, thermal fogging should be carried out on weekly basis. The fogging should be done either in early morning or in the evening when wind velocity is low i.e., about 6 kms or 4 miles per hour. If fogging is carried out as per the above methods, it would kill adult mosquitoes in areas where the fog was suspended for at least 10–15 minutes. But in practice the prescribed procedures are rarely followed and fogging is carried out at 4 to 6 weeks or at even longer intervals, more as a goodwill rather than mosquito control measure.

The fog also does not penetrate the structures as people have the tendency to close doors, windows, etc., instead of leaving them open. As a result thermal fogging has very little value, if any, in the control of mosquitoes and malaria. It may be noted that thermal fogging costs are high i.e., about Rs. 1000 per hour covering about 5 kms × 100 metres area. Weekly fogging as a means to control mosquitoes would be a very expensive proposition and fogging at prolonged intervals would serve no useful purpose. Under the circumstances it is advisable to stop thermal fogging under the UMS. Instead, the concerned local bodies should depend on the recommended methods of anti-larval operations. There is need of adequate supervision under which all mosquito breeding places are identified, recorded and treated every week with suitable larvicides. The case detection and chemotherapeutic measures should also be strengthened.

REFERENCE

1. Pattanayak, S., R.G. Roy, K.G. Samnotra and M.S. Bendley (1981). Urban malaria scheme of the National Malaria Eradication Programme of India. *Indian J. Malariol.*, **18**: 21–27.

INDIAN JOURNAL OF MALARIOLOGY

Instructions to Authors

Editorial Policy

The Indian Journal of Malariology is devoted to the publication of original research which contributes significantly to the knowledge of any field of malariology. Papers of routine and repetitive nature dealing with gross observations may not be included. Articles will be published at the editor's discretion in the order accepted. Date of acceptance will be that date on which copy is accepted in final form for publication. Manuscripts should be submitted in triplicate to:

The Editor
Indian Journal of Malariology
Malaria Research Centre (ICMR)
22-Sham Nath Marg
Delhi — 110054, India.

Classes of Items Published

In addition to regular papers the journal publishes short notes and invited review articles. Book reviews may be published at the end of the journal.

Format

The matter should be arranged in the following order: Title, Name(s) of the author(s) with address of the Institute/University (as footnotes and numbered serially in superscript), Abstract, Introduction, Material and Methods, Results, Discussion, Acknowledgement and References. Authors should provide a short title to be used as running title, of not more than 5 words in case the title of the paper is long.

Preparation of Copy

Manuscripts should be typewritten in English on one side of the paper leaving 1½ inch left-hand margins. The entire matter should be typed double space including references, tables and captions. Abstract, tables, references and legends for illustrations should be typed on separate sheets of paper. Pages are to be numbered consecutively.

Tables should be placed singly on sheets of paper, along with relevant headings and footnotes. Tables should be self-explanatory and referred to in the text. Designation of tables must be with arabic numerals (e.g., 1, 2), avoid roman numerals (e.g., I, II). Do not use any horizontal or vertical lines in the body of the table.

Footnotes to the text should be avoided as far as possible, parenthetical insertions are preferable.

Illustrations should be sent in duplicate. All illustrations including figures, photographs, graphs and maps should be numbered consecutively in order of appearance in the text. Captions and legends should be typed separately and must not appear on the face of illustrations. Authors should identify each illustration on the reverse side with author's name, fig. no. and abbreviated captions. Line drawings should be clear and letters and numerals should be planned for legibility following reduction. Labelling should be neat and accurate. Photographs should be sharp, glossy, black and white prints, preferably mounted and covered with a transparent overlay for protection. Photographs should have allowance for reduction to 1/3 size. Size of art work for quarter page should be 24 × 21 cms; half page 45 × 24 cms and full page 57 · 45 cms approximately.

Data for tables, graphs etc., should be carefully verified. All statistics, percentages and other calculations should be checked thoroughly before submission of a communication. Once a paper is accepted for publication, data in it should be treated as final.

Nomenclature. Authors of scientific names should be omitted in abstract and title. Authors of scientific names of insects should be included at first instance in the body of the text.

Numbers less than one should have a zero set before the decimal point e.g., 0.1.

Measurements should follow the international system of units (SI Units). Kindly see WHO publication *The SI for the Health Professional*, WHO, Geneva. 1977. Use of the 24-hour time system (e.g., 0830 hours, not 8:30 A.M.) is preferable.

References should include only published references and papers in press. References in the text may be cited as Sharma *et al.* (1976) or (Mount *et al.*, 1972; Coombes *et al.*, 1973; Mount *et al.*, 1974). For references to a paper accepted for publication, the word 'in Press' should appear after the title of the periodical. Citations of unpublished work should be incorporated in the text itself (e.g., R.G. Roy, unpublished data or S. Pattanayak, personal communication). If the reference is to an article published without any authorship in a periodical, in place of author's name the term "Anonymous" should be used. Titles of Periodicals cited in the references are to be abbreviated as in the *World List of Scientific Periodicals.*

References to the literature, arranged alphabetically and numbered consecutively, must be placed at the end of the paper. The following style is acceptable for this journal:

Research Paper

Sharma, V.P. (1976). Elimination of aziridine residues from chemosterilised mosquitoes. *Nature*, **261** (5556): 135.

Books/Monographs

Rao, T. Ramachandra (1981). *The Anophelines of India.* (W.Q. Judge Press, Bangalore).

Landau, I. and Y. Boulard (1978). In *Rodent Malaria*, edited by R. Killick-Kendrick and W. Peters. (Academic Press Inc., London): 53–84.

Paper presented at Symposium/Conference

Subbarao, S.K. (1981). *Cytoplasmic incompatibility in mosquitoes.* Paper presented at the International symposium on recent developments in the genetics of insect disease vectors; Bellagio, Italy, 20–24 April.

Authors are requested to verify spelling, punctuation, titles and dates of all references. The address of the publisher should be given for books. References are attributable to authors, not to editors in case of compilations or contributory texts e.g.:

Killick-Kendrick, R. and W. Peters (1978). Ed. *Rodent Malaria.* (Academic Press Inc., London): 406. **(Incorrect).**

Landau, I. and Y. Boulard (1978). In *Rodent Malaria*, edited by R. Killick-Kendrick and W. Peters. (Academic Press Inc., London): 53–84. **(Correct).**

Correct references are the responsibility of the author.

Short notes should be prepared in a manner similar to the research papers and will have title, name(s) of author(s) with address of Institute/University as footnotes, acknowledgement and references.

Proofs

Page proofs of the articles will be sent to the authors for correction. Corrected proofs must be returned promptly to the editor or else the article may not be printed in the current issue or may be printed as it stands. Only minimal changes i.e., those that do not substantially alter the page make-up are allowable at the proof stage in exceptional cases. Alterations which are disallowed by the editor shall be deleted or charged to the author.

Reprints

Reprints will be mailed to the senior author.

Twenty-five reprints will be supplied free of charge to single authors and thirty for joint authors to be distributed on an equal basis. Additional reprints may be ordered on payment when the proofs are returned. A reprint order form will be supplied with the proofs.

Statement about ownership and other particulars of Indian Journal of Malariology

FORM IV

(See Rule 8)

1.	Place of Publication	Malaria Research Centre (ICMR) 22-Sham Nath Marg Delhi—110054
2.	Periodicity of its publication	Half yearly
3.	Printer's Name Nationality Address	V.P. Sharma Indian Malaria Research Centre 22-Sham Nath Marg Delhi—110054
4.	Publisher's Name Nationality Address	Dr. V.P. Sharma Indian Malaria Research Centre 22-Sham Nath Marg Delhi—110054
5.	Editor's Name Nationality Address	Dr. V.P. Sharma Indian Malaria Research Centre 22-Sham Nath Marg Delhi—110054
6.	Names and addresses of individual who own the newspaper and partners or shareholders holding more than one percent of the total capital	Malaria Research Centre (ICMR)

I, V.P. Sharma, hereby declare that the particulars given above are true to the best of my knowledge and belief.

Sd/-
(V.P. SHARMA)
Signature of publisher

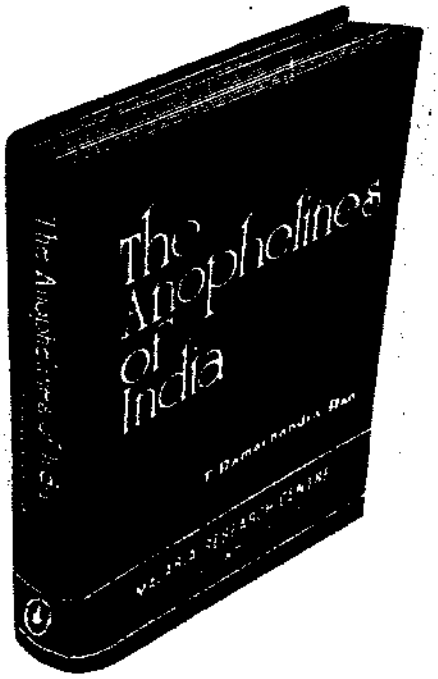
16 May 1986

THE ANOPHELINES OF INDIA

Revised Edition 1984

by

T. Ramachandra Rao



An authoritative and comprehensive publication which brings together all that is known about distribution, bionomics, ecology, relation to disease and basic principles of control of anopheline mosquitoes.

Size CROWN 4 TO pp 538 (Hardbound)
Rs. 150.00 (India) US $45.00 (Other countries)
25% Discount per copy.

The monograph can be obtained by sending M.O./bank draft/postal order/cheque crossed and marked payable to Malaria Research Centre (ICMR), 22-Sham Nath Marg, Delhi-110054, INDIA.

MALARIA RESEARCH CENTRE

PRICED PUBLICATIONS

The Anophelines of India (Rev. ed.) 1984 by T. Ramachandra Rao

Price: India Rs. 150/- ; Foreign US $45/- } 25% Discount

Indian Journal of Malariology

Volume 18 Nos. 1 & 2 (1981)
Volume 19 Nos. 1 & 2 (1982)
Volume 20 Nos. 1 & 2 (1983)
Volume 21 Nos. 1 & 2 (1984)
Volume 22 Nos. 1 & 2 (1985)
Volume 23 Nos. 1 & 2* (1986)

*To be released in December.

Annual Subscriptions

India Rs. 30/- ●

Foreign US $10/- (Including Airmail postage)

● (50% Discount to Individual subscriber)

These publications are in stock.

The Editor
Indian Journal of Malariology
Malaria Research Centre
22-Sham Nath Marg
Delhi-110054 (India)

Sir,

I enclose herewith a bank draft/postal order No/s..... for $/Rs..... (in the name of Malaria Research Centre, Delhi 110054) towards subscription/payment for Indian Journal of Malariology for the year..... (2 Nos.)/The Anophelines of India (Revised edition) 1984. The journals/publication should be mailed to me/my client at the following address.

.....

.....

.....

.....