

STUDY OF MALARIA AND ITS VECTOR IN RAWALPINDI/ISLAMABAD REGION

Ву

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## INTRODUCTION

One of the most vicious diseases of man is malaria. It has played a major role in shaping history, including the decline of civilizations. Human malaria is known to have contributed to the fall of ancient Greek and Roman empires. Troops in both the Civil War of U.S.A. and the Spanish-Americ War were severely incapacitated by this disease. More than one quarter of all hospital admissions in these wars were malaria patients. During World War-II, epidemics of malaria severely threatened both the Japanes and allied forces in the Far East. Malaria meaning "bad air" was so named because of association of the disease with odorous air of the swamps, particularly at night and fear of damp night air still exists even in United States.

The malarial parasite was discovered in the blood b Laveran in 1880. In 1898 Ross experimentally proved the mosquito transmission of the disease and worked out the detai in the case of bird malaria. 'Immediately afterwards Grassi and his pupils working independently described the cycle of human malaria in anopheles.

During last two decades a lot of work has been done on all aspects of malaria including morphology, biology, life cycle, transmission, epidemiology etc. and it is beyond the scope of this present work to review whole of the literature but anyhow some of the recent work is reviewed below. Ponnudurai <u>et al</u> (1982) obtained 13 isolates of <u>Plasmodium falciparum</u> from cases of malaria imported into the Netherlands and established in culture were tested for their sensitivity to chloroquine. Reproducibility of the test results depended on the exposure of a standardized number of parasites in culture to the drug. The maximum activity of chloroquine was obtained when medium with the drug was added to parasite cultures twice at 24 h intervals. The result of drug action over a period of 48 h was estimated best when parasites were counted 72 h after the commencement of the test.

Sensitivity to chloroquine could not provide a basis for the characterization of strains.

Herd and Jordan (1982) investigated malaria during pregnancy at the Harare Central Hospital in Salisbury, Zambabwe. 61 pregnant women were confirmed for malaria by microscopy. 21 of the 28 cases aborted and in 31 women 50% went into premature labour. 5 babies died in pre-natal period and 2 stillbirths occurred. Cerebral malaria was diagnosed in 8 mothers. Two received treatment with quinine. Both babies were born prematurely but lived.

Darlow et al (1981) described fansidar resistant falciparum malaria in Papua, New Guinea. They described a 7.4 kg baby aged 15 months with fifty thousand asexual parasites of <u>P. falciparum per mm<sup>3</sup></u>. He was treated with half a tablet of

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fansidar (12.5 mg Pyrimethamine with 250 mg sulphadoxine). The fever had subsided sixty hours later. The parasitaemia increased from 4,000 on day two to 6,7000 on day five. When the fever returned 70 mg chlorogine elixir was given on day six, seven and eight. The parasitaemia disappeared by day nine and did not recur over the following 28 days. Three other children aged between 3.5 and 5 years were treated similarly. All four children acquired malaria in Madang area.

Smalley and Brown (1981) reported <u>P</u>. <u>falciparum</u> malaria from six Gambian children aged 9 months to two years. They observed the combined role of lymphocytes and serum in increasing the production of gametocytes.

Smalley <u>et al</u> (1981) described <u>P</u>. <u>falciparum</u> in children aged between 4 month and 9 years. They studied the rate of production of gametocytes. Infections with mature gametocytes in the peripheral blood showed a greater proportion of infections.

Elamin (1981) reported 458 cases of cerebral malaria in adult Zambian Africans. The age range was 17-48 years. The authors concluded that adults are susceptible to severe malaria in Zambabwe.

Molineaux <u>et al</u> (1981) worked on the epidemiology and control of malaria in Garki district, Kano State, jointly by Government of Nigeria and WHO. Parasitaemia with

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<u>P.</u> <u>falciparum</u> and <u>P.</u> <u>malariae</u> was high and they concluded that there is a seasonal alternation in Prevalence between <u>P</u>. <u>falciparum</u> and <u>P.</u> <u>malariae</u>.

Molineaux and Gramiccia (1981) reported epidemiology and control of malaria in Garki district of Sudan Savanna of West Africa. Before spraying and drug distribution the incidence of Patent Parasitaemia was 100% <u>P</u>. <u>Falciparum</u> in young age groups, 80% <u>P</u>. <u>malariae</u> and 30% <u>P</u>. <u>ovale</u>. In 1973 after spraying parasite rate fell between 30-35% <u>P</u>. <u>Falciparum</u>, 13-16% <u>P</u>. <u>malariae</u>. The main anopheline vectors were <u>An</u>. <u>gambiae</u>, <u>An</u>. <u>funestus</u>, <u>A</u>. <u>arabiensis</u>. Sposozoite inoculation rate was estimated at 145 infections bites per year.

Juminer <u>et al</u> (1981) described the problems of malaria control in French Giana. They reported about 84% infections due to <u>P. falciparum</u> and 13.5% due to <u>P. vivax</u>. The main vector was <u>An</u>, <u>darlingi</u>, <u>An</u>, <u>aqualis</u> was of secondary importance.

Smalley <u>at al</u> (1981) studied the distribution of <u>P. falciparum</u> in peripheral blood and bone marrow of 22 malarious Gambian children aged 9 months to 5 years. They observed all the asexual stages and gametocytes in peripheral blood and bone marrow.

Rossan <u>et al</u> (1981) infected Panamian <u>Aotus</u> trivirgatus with Nigerian strain of <u>2</u>. <u>Talciparum</u>. A fluctuating patent parasitaemia lasted 190 days with 5 peaks.

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Tikasingh <u>et al</u> (1981) described on outbreak of malaria due to <u>P</u>. <u>malariae</u> on the Islanaddof Grenada. This outbreak was reported in 1978 although the island was declared free of malaria transmission in 1962. Two cases of <u>P</u>. <u>malariae</u> infection were detected in 1972-75 but it was unknown whether these; ere new infections or relapses of old cases of quartan malaria. In 1978 out break was well investigated by the PAHO/WHO Caribbean Epidemiological Centre which found 58 cases of <u>P</u>. <u>malariae</u> of which 57 were from the Wester Hall area of Grenada.

Lee <u>et al</u> (1980) conducted vector studies and epidemiology of malaria in Irian Jaya, Indonesia. They described human populations in the lowland littoral of south western Irian Jaya and found the spleen rate in children of ages 2 to 9 years in four villages ranged from 78% to 97% and the parasite prevalence was from 21% to 52%. The species of malarial parasite was <u>P. falciparum</u>. They also described three known vectors of malaria, <u>An. faranti</u>, <u>An. koliensis</u> and An. puntulatus, the later two being most abundant.

Magzoub (1980) described <u>P</u>. <u>falciparum</u> and <u>P</u>. <u>vivax</u> fection in Saudi Arabia and he surveyed the incidence of infection caused by <u>P</u>. <u>falciparum</u> and <u>P</u>. <u>vivax</u>. He identified five important anopheline vectors which were as under. <u>An</u>. <u>stephensi</u>, <u>An</u>. <u>surgent</u>, <u>An</u>. <u>gambiar</u>, <u>An</u>. <u>superpictus</u> and An. fluviatilis.

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Roy & Prasad (1980) reported falciparum malaria from some parts of Karnataka State of India. They treated the cases with chloroquine and found the parasite as sensitive to the drug

Shanmughan <u>et al</u> (1980) reported 6656 cases of <u>Plasmodium falciparum</u> from Tamil Nadu State of India and treated the cases with chloroquine. Adults were given a presumptive treatment of 600 mg and another 600 mg as radical treatment. Children received smaller amounts. At the time of radical treatment Primaquin 15 mg daily (adult dose) was also given for 5 consecutive days. Only one out of 6656 patients was positive on day 6 from the beginning of radical treatment.

Roy <u>et [al</u> (1980) described 674 patients with <u>Plasmodium vivax and 391 with P. falciparum</u> from Karnataka State of India. They treated the cases with chloroquine and performed blood tests at 7 - 10 days after treatment. Only 8 out of 674 P. vivax and 1 out of 391 P. falciparum were found positive.

Roy <u>et al</u> (1980) reported DDT and HCH resistance in <u>An. culifacies</u>. They concluded this resistance as a result of extensive use of pesticides.

Roy and Ghosh (1980) described the annual parasite incidence with child spleen and parasite rate from Karnataka State. The highest rates of spleen and parasite were 29.49 and

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14.53% respectively. There was no regular correlation between annual parasite index, spleen and parasite rates at different localities.

Chandraha <u>et al</u> (1980) reported density measurement of <u>An</u>. <u>culicifacies</u> during season of malaria transmission i.e. august to september in Pattokottai. A total of 26,228 mosquitoes belonging to 24 different species, including 19767 anopheles belonging to 8 species were recorded. A density of <u>An</u>. <u>culicifacies</u> was twice the number recorded during nontransmission season from the same area.

Sharma <u>et dl</u> (1980) described the case of a man aged 40 years who had infection of <u>Plasmodium vivax</u> and was treated in december 1977. In april 1978 he was again admitted with fever, anaphylactic shock and <u>P. vivax</u> infection. Weekly chloroquine was advised and he remained well untill July 1978 when he stopped chloroquine because of his suspicion of eye toxicity. Fever, puffy face, hypotension and blood smear positive for P. vivax recurred 2 weeks later. Thereafter he took prophylactic chloroquine and remained free from symptoms.

Kalra (1980) diagnosed many cases of malaria in Greater Nicobars. The parasite in humans resembled <u>Plasmodium</u> <u>gynomolgi bastianelli</u>, of the 13 monkers i.e. <u>Macaca úmbrosús</u> and <u>M. fascicularis</u> were also found to be infected with this species. It was suggested that an ecological imbalance has resulted due to human activities that has changed the habits of vector speci <u>An</u>. <u>sundaicus</u>. This speci of mosquito is now exophilic and now bites both human and monkeys.

Campbell <u>et al</u>. (1980) reported the infection of <u>An</u>. <u>freeborni</u> with <u>P</u>. <u>falciparum</u> gametocytes. 9 days later at  $25^{\circ}$ C a single gut infection with a single oocyst was seen.

Kravchenko (1980) reported the incidence of malaria in the People Democratic Republic of Yeman. He described 263,658 cases during the period 1974-1976. Prevalence of the population was 18.2% on the average. <u>P. falciparum</u> was detected in 95% of the Positive cases, <u>P. malariae</u> in 4% and <u>P. vivax</u> in 1%. Most intensive malaria transmission occurred in Nov.-March and the Principal vector was An. gambiae.

Waren <u>et al</u> (1980) reported that <u>An</u>. <u>pseudopuntipennis</u> failed to develop oocysts of <u>P</u>. <u>falciparum</u> and <u>P</u>. <u>vivax</u> while <u>An</u>. <u>freeborni</u> developed sporozoites. y

Gysin <u>et al</u> (1980) infected Gynese Squirrel Monkeys with <u>P. falciparum</u>. Successful infections were obtained and parasite was maintained in these monkeys. All the stages except gametocytes were seen in the peripheral blood.

Haiti and Mukhopadhyay (1980) reported that in West Bengal during 1971-78 <u>P</u>. <u>malariae</u> was non-existant and <u>Flasmodium falciparum contributed 11.32%</u> of the cases and

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<u>An. philipiniensis</u> is claimed to be less common than it used to be.

Aderounmu <u>et al</u> (1980) reported 35 children aged 6 months to 6 years with falciparum malaria. They treated the cases with chloroquine 25 mg/kg over 3 days and observed for 7 days by examining the blood films. All the parasites had disappeared.

Collins <u>et al</u> (1980) reported that the Chesson strain of <u>P</u>. <u>vivax</u> when inoculated to <u>Notus trivirgatus</u> monkeys behaved similar as in man. Upon infecting the mosquitoes most susceptible was <u>An</u>. <u>freeborni</u> followed by <u>An</u>. <u>balabecensis</u>, <u>An</u>. <u>culicifacies</u>, <u>An</u>. <u>maculatus</u>, <u>An</u>. <u>atroparvus</u>, <u>An</u>. <u>stephensi</u>, An. quadrimaculatus, and An. glbimanus.

Collins <u>et al</u> (1980) maintained the Pakistan strain of <u>P</u>. <u>vivax</u> in 17 splenectomized Aotus monkeys of Colombian origin and studied its behaviour in <u>An</u>. <u>'albimanus</u> (Panama, El-Salvador and Haiti), <u>An</u>. <u>'freeborni</u> (California origin ) <u>An</u>. <u>maculatus</u> (Malaysia), <u>An</u>. <u>culicifacies</u> (Lahore) and <u>An</u>. <u>balabecensis</u> (Thailand). <u>An</u>. <u>freeborni</u> proved to be the best host in regard to oocyst production, the three asian species <u>An</u>. <u>maculatus</u>, <u>An</u>. <u>dulicifaciés</u> and <u>An</u>. <u>balabacensis</u> had higher infection in salivery glands. <u>An</u>. <u>'albimanus</u> was very poor host. Pasvol (1980) described the interaction between sickle haemoglobin and the malarial parasite <u>P. falciparum</u>. Blood containing synchronous late ring states of <u>P. falciparum</u> was obtained from infected patients in the Gambia, West Africa. The red cells were maintained in culture untill the parasites had reached a mature schizont stage: The Schizont infected cells were then concentrated and suspended to gather with normal non-infected red cells. The rate of invasion was expressed as the number of ring foci of the parasites present in hundred red cells. Cells from sickle cell train carriers showed a slight reduction in the rate of invasion under low oxygen tension as compared with aerobic conditions where as the opposite was observed with cells containing only normal adult haemoglobin.

Lundie (1980) described different problems with protean malaria and stated that in various erythrocytic forms, malaria of each plasmodia responsible for human malaria and their relationship to the forms encountered in the mosquito required long and painstaking work before descriptions. He mentioned that each speci has a vital role to play in therapy and the changes in normal appearances, immosuppression due to infection with babesia and thus leading to a false diagnosis of malaria, incorrect purely clinical diagnosis due to any reason and world wide travel are different problems encountered in protean malaria and therefore early and accurate diagnosis

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of malaria in non-malarial areas is needed.

Nakabayashi <u>et al</u>. (1980) reported that in Japan malaria has been completely eradicated 20 years ago but on the other hand imported malaria cases have increased recently. The number of malaria cases for seven years from 1972-1978 was 467. Most of the vivax cases were from S. Asia and Falciparm cases were mostly from Africa.

Mouchet et al. (1980) conducted malarial surveys in C ameroon, Zaire, Peoples Republic of Congo and Central African Republic. They recorded Plasmodic index below 50%. From entomological surveys they reported <u>An. gambiae</u>, <u>An. funastus</u>, <u>An. nili and An. moucheti</u>.

Hayes and Ferraroni (1980) reported that along the newly constructed roads in the Amazon (Brazil) malaria has been principal health problem. Infection rates vary from 11.4% to 67% of the population resulting in loss of work ranging from 14 to 42 days. Thirty percent of the total hospitalization at the tropical Diases Hospital in Manaus were due to malaria. <u>An. darlingi</u> is the main vector in the area and exhibits resistant behaviour to DDT. Chloroquine is distributed freely to the population which may be responsible for high prevalence of falciparum malaria.

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Sulzer (1980) described the studies which he had carried out from 1975-80 in the Eastern Andes valleys of Ecuador, Peru and in Bolivia. Malaria foci in which either <u>P. vivax</u> or mixed <u>P. vivex and P. malariae</u> were endemic. No evidence of presence of <u>P. falciparum</u> was found. He concluded that there is a pressing need to study parasitological, Serogical, entomological, anthropological and gentical aspects of the disease.

Rivera (1980) reported that out of 14.9 million population of Philipines under malaria risk, 41% are under attack, 17% under surveillance and 42% are under Premaintenance with no regular measures. Spraying limited to selected areas, inadequate financial support, unfilled position of malariologist, extensive population movement, drug resistence and lack of community participation are the main problems encountered in malaria eradication programme.

Nowell (1980) reported that Mariana Islands of Guam was known as "malaria free area" because of absence of anopheline mosquitoes. Anopheles was first discovered in 1948. Survey records indicate that anophelise were mostly distributed in the Island and was probably introduced sometime prior to survey date. History of malaria infections in Guam began with reports of relapsing infections in 1947 and 1948 in both military and civilian patients. Only single speci of Anophelas appeared in collections made in Guam until 1970. An additional

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eight species were reported during 1970-75 but only four of these have been confirmed. The increase in the number of vector mosquito species enhances the probability of future malaria infections on the island.

Abeyesundere (1980) studied recent trends in malaria morbidity and mortality in Sri-Lanka. He reported that climatic factors such as temperature and relative humidity are favourable for perennial transmission of malaria in Sri-Lanka. Draught conditions cause periodic epidemics of malaria at intervals of 3-5 years. Recent epidemic and resurgence of malaria has contributed to the loss of man days but it has not affected the mortality parameters. Since the resurgence of malaria in 1968, the malaria mortality has been low and general health rate too has been low. Present resurgence has been that of Plasmodium vivax.

Commey <u>et al</u>. (1980) reported that cerebral malarial is a common complication of falciparum malaria in Accra, Ghana, in older children who have acquired natural immunity. They suggested that such immunity could have been destroyed or delayed by chemoprophylaxis against malaria.

Horstmann and Dietrich (1980) studied thrombocytopenia in 12 patients with malaria. They concluded that platelet life span is shortened and production rates are increased. Macleod <u>et (al</u> (1980) detected, congenital <u>P. vivax</u> and <u>P. (malariae</u> in a 6½ weeks old female in San-francisco. The child developed fever, Lethargy and profuse sweating placentas of mother appeared normal, on physical examination the infant had a temperature of 39°C, heart rate of 180/min, and hepatosple-nomegaly. Haemoglobin was 9.5 g. Peripheral smear showed 1% parasitaemia with diagnostic mature Schizonts of <u>P. vivax</u> and <u>P. malariáe</u>, infant was treated with chloroquine 25 mg/kg in divided doses and remained healthy thereafter.

Chu (1980) reported 2404 cases of cerebral malaria in China in 1934-1978. Fatality was 25%. Death was due to cerebral edema associated with increased intracranial pressure, which resulted in respiratory and circulatory failure.

Marcelou Kinti (1980) reported 241 cases of malaria registered in Greece during the period January 1975 to December 1979. 80.9% of the cases were imported cases. 44.1% were infected with <u>P. falci, 37.9% with P. vivax</u> and 9.8% with <u>P. malarřa</u>. In 8.2% of the cases, Pararitaemia was not identified. No death due to malaria was reported.

Borrelly (1980) discussed socio-economic factors and malaria teams mission in developing countries. He concluded that malaria prevalence in a grographical area depends upon socio-economic factors and this knowledge might be used in planning epidemiological surveys. Ekanem (1980) reported malaria from Nigeria. Principal factors were <u>An. 'gambiae</u>, <u>An. 'arabiensis</u>, <u>An.</u> <u>funestus</u> and <u>An. melas.</u> 80% of the infections were due to <u>P. falciparum 15% P. malari'ae</u> and less than 5% <u>P. ovale</u>. Malaria is endemic and stable throughout Nigeria. Countrywide National Malaria control programme was initiated in 1975. Technical strategy of the programme is to employ several methods of malaria control.

Sharma (1980) worked on the epidemiology of malaria in Tarai Region, India. The area was hyperendemic for malaria during pre-DDT period and incidence of cerebral malaria was 50%-60%. Main vectors were <u>Ah. minimus</u> and <u>An. fluviatilis</u>. <u>An. culicifacies</u> played a supplementary role in malaria transmission during autumn. Reclamation of this region was achieved by DDT spraying and deforestation of about 3000 sq. km. belt.

Williams (1980) described results of WHO assisted national malaria prevalence cum vector survey in Sierra leone. He made comparison between the malaria prevalence in the vector control area and those elsewhere.

Verma (1980) reported parasitological, clinical and entomological aspects of malaria in Malumfashi district of N orthern Nigeria in 4061 children under 5 years. Parasite rate was 64.2%. The high rates occured in September. <u>P. falciparum</u> was 90%, <u>P</u>. <u>malariae</u> 8% and <u>P</u>. <u>ovale</u> 2%. In 245 School children of 5-9 years spleen rate was 63.3% at the end of wet season, 43.9% at the end of dry season. Highest catches of <u>An. gambiae</u> and <u>An. funestus</u> were recorded in October.

Al-Mashhadani (1980) studied the susceptibility of <u>An. gambiae</u> to malaria infection in Baghdad, Iraq. <u>An. gambiae</u> was 100% susceptible to <u>P. berghei</u> berghei (a rodent malaria) after 9 generations of selection. <u>P. yoelii</u> and <u>P. nigeriensis</u> gave similar results. Vector was also found to be susceptible to <u>P. vivax</u> and <u>P. falciparum</u>.

Pant and Gratz (1980) described the resurgence and global incidence of malaria over the past fourteen years due to resistance of anopheline vector to insictecides. Agriculture is often a major contributory cause of this resistance, through the wide-spread use of insecticides against crop pests.

Akhtar and Learmonth (1980) described annual malaria index of India covering the period 1965-77. The observations were based on annual malaria incidence or the number per 1000 population of confirmed cases of malaria microscopically during one year. The annual parasite index ranged from 0.99 to 50 per thousand. The authors also mentioned the possible role of various factors like rainfall, irrigation, migration etc. in the resurgence of malaria.

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Gad <u>et al</u>. (1980) described pathology of <u>Anopheles</u> <u>stephensi</u> after infection with <u>P</u>. <u>berghei</u> <u>berghei</u>. Mosquitoes fed on uninfected blood show a peak of mortality on 3rd or 4th day after feed. Mortality rate after a <u>P</u>. <u>berghei</u> <u>berghei</u> infected blood meal was higher. The effect of increased mortality was more pronounced at 25<sup>o</sup>C than at 21<sup>o</sup>C.

Galbraith <u>et al</u>. (1980) described placental tissues from ten Gambian women, either frozen in liquid nitrogen, or fixed in formalin or carnoys fixatives. Swears prepared from fresh tissue or blood films made from mother and chord were stained with Giemsa stain. Parasitaemia of <u>P</u>. <u>falciparum</u> was demonstrated.

Essien <u>et al</u>. (1980) described Plate let count in man during <u>P</u>. <u>falciparum</u> and after treatment. Mean platelet coart during infection was lower than that after treatment. The effect was also independent of degree of Parasitaemia or leucocytosis.

Batra <u>et al</u>. (1980) described Urban malaria vectors in salem, Tamil Nadu region of India. During one year study of mosquitoes feeding on man and cattle. They found that <u>An. subpictus</u> was most common spices biting man. <u>An. stephensi</u> being second. <u>An. subpictus</u> was more zoophilic than <u>An.</u> <u>stephensi</u> estimates of vectorial capacity of <u>An. stephensi</u> suggest that it is possible for malaria transmission to

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continue as a result of low level man-vector contact observed in the field.

Meira et al. (1980) reported number of malaria cases in Amazonia in 1976 and 1977 represented 89% and 94% of the total number of cases registered in the whole of Brazil. In August 1976, the authors initiated their study in Humaita, 409 individuals were included in the survey. 145 living on the margin of highway, 105 from villages on banks of madeira river and 113 from town of Humaita. The survey included clinical and epidemiological aspects. Clinical study included, clinical diagnosis, type of clinical picture and presumptive treatment. Epidiological study included previous history of attack, fever, spleonegaly, splenic index and previous treatment. Type of locality, age, sex and origin of sample was also recorded. Results indicated that differences exist in the incidence and severity of infections along the high ways, in localities situated along river madeira and in Urban Malaria. Problem appeared greater in people living by highways. Positive clinical diagnosis of malaria was made in 15.44 of the entire sample population. Spleen index in 2-9 years age group in river side, highway, and Urban samples was 36.5%, 20.83% and 5.88% respectively.

Dutt <u>et al</u>. (1980) report3d malaria two from west central states of India. Malaria control programme started in 1947 and a considerable resurgence of infection occured

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after 1966. They described the present trends of malaria control which has now replaced the former trends of eradication.

Ebisawa <u>et al</u>, (1980) described 93 cases of <u>Plasmodium falciparum</u> of which 9 were fatal and 3 maribund. They concluded that a good outcome can be expected if treatment is instituted within 5 days.

Miles and Davidson (1980) worked on the sibling species of <u>An</u>. <u>culicifacies</u> and reported that one of the species, either A or B is a vector of malaria in rural India and Sri-Lanka. Malathion and Fenitrothion resistance is present in field populations of this taxon.

Majer (1980) worked on the pathological aspects in <u>An. stephensi</u> superinfected with a becterium <u>Serratia</u> <u>warcescens</u>. At 25<sup>o</sup>C <u>P</u>. <u>berghei</u> infected <u>An</u>. <u>stephensi</u> die soon after the beginning of infection. These do not die so often at 21<sup>o</sup>C, the temperature needed for sporozoite production. He observed that Plasmodium infection alone was not responsible for mortality while another bacterium <u>Serratia</u> <u>warcescens</u> which grows at higher temperature was isolated and identified as responsible for increasing mortality.

Huang (1980) reported present status of malaria control in People Republic of China. He estimated that before founding of the Peoples Republic, more than 30 million cases of malaria occured annually and more than 70% of total number of counties were malaria infected. Anti-malarial compaigns were started in 1950's. During 1960's and 1970's substantial Progress has been made. Today 1/3 of population is living in malaria free areas, another 3rd with minimal malaria risk. <u>Pl. vivax</u> is chiefly found in the country, <u>P. falciparum</u> is chiefly found in southern border and <u>P. malariae</u> is rarely seen chief malaria vectors are An. sinensis of An. Balabacensis.

Jopling (1979) reported imported malaria into Britain and stated that 3/4 of the plasmodium infections recorded have been contracted in India where <u>P. vivax</u> is the predominating parasite. <u>P. falciparum</u> infections contracted in Africa are responsible for majority of remaining quarter and all 9 deaths were due to this parasite. The authors recommend the use of autimalarial tablets containing Pyrimethamines and a long acting sulpha compound.

Bray and Anderson (1979) described the increased prevalence and density of <u>P</u>. <u>falciparum</u> malaria during pregnancy. The increase was at a height relatively early in pregnancy and declines after mid term.

Hedman <u>et al</u>. (1979) described malaria control measures in Yekepa, Liberia. Between 1953 and 1961 this area was a part of WHO pilot project of malaria. They also conducte a malariae survey of the area and found spleen and parasite with respective rates of 95% and 67.5% for the surrounding areas.

Manson and Rajagopalan (1979) studied the natural mortality and breeding status of <u>An</u>. <u>stephensi in well in</u> Pondicheri, India. Breeding was found through the year with more positive wells during rainy season. The natural survival rate of immature stages varied from 1.9 percent in September to 10.4% in August.

WHO expert committee (1979) described guide lines for planning, implementation and evaluation of malaria control programmes i.e. the national will, integration of malaria control into country health programme, feasibility and practicability of reducing malaria, Participation of community and institution of permanent malaria control measures etc.

Warren <u>et al</u>. (1979) described Phenotypes of <u>An</u>. <u>albimanus</u> and their susceptibility to <u>P</u>. <u>vivax</u> and <u>P.falciparun</u> Two malaria forms were more susceptible to <u>P</u>. <u>falciparum</u> than <u>P</u>. <u>vivax</u>. Green forms more susceptible to <u>P</u>. <u>vivax</u> than to either of the forms. Brown forms were more susceptible to P. falciparum.

Colline <u>et al</u>. (1979) described the pupal phenotypes of <u>An</u>. <u>frerborni</u> and their susceptibility to <u>P</u>. <u>falciparum</u> and <u>P</u>. <u>vivax</u>. All four strains were susceptible but differed in gut infection rate.

Collins <u>et al</u>. (1979) described the infection of Aotus monkey with Salvador strain of <u>P</u>. <u>vivax</u> and later on expond to <u>An</u>. <u>freeborni</u> infected with El-salvador Senta lucia strain of <u>P</u>. <u>falciparum</u>. Parasitaemia due to <u>P</u>. <u>falciparum</u> was higher than in monkeys which had not received <u>P</u>. <u>vivax</u>. They also observed that oocyst rate was high in those mosquitoes which had fed on monkeys already infected with <u>P</u>. vivax.

Schmidt (1978) described infection of owl monkeys with <u>P. falciparum</u> and <u>P. vivax</u>. Such infections were followe from day of in-noculation to death or self cure. Incidence of fatal infections ranged from 24.4-89.4% and 0.1-45.8% respectively for <u>P. falciparum</u> and <u>P. vivax</u>. Morbidity in both species was related to height of parasitaemia. However, at comparable level, symptoms with <u>P. vivax</u> were more severe than with <u>P. falciparum</u>.

Molineaux <u>et al</u>. (1978) described a malaria model for epidemiological evaluation of the disease the model comprised of both the parasitological and entomological observations and was tested with data from Garki district Kano state, Nigeria and Kisumu, Kenya.

Omer (1978) reported falciparum malaria in 90% of the people complaining fever in 3 villages in Gezira area of Sudan. Parasitaemia of 1000 mm<sup>3</sup> was treated with 25 mg/kg of chloroquine administered over two days. Falcipurm malaria was cured in 503 out of 506 patients, in another study he reported that chloroquine cleared falciparum parasitaemia by the 7th day in all 40 patients studied.

Bray <u>et al</u>. (1977) reported that <u>An</u>. <u>gambiae</u> is a probably worlds most efficient malaria vector. Adults bite man late at night and in early morning. When fed on <u>P</u>. <u>falciparum</u> gametocyte carriers both during day and night, oocysts were recovered but no difference was observed at these different times.

Monouchehri <u>et al</u>. (1977) reported that <u>An</u>. <u>culicifacies</u> had developed resistance to DDT in 1952 and Dieldrin in 1960 in Bandar Abas, Southern Iran. With WHO test malathion was also tested. Malathion which was expected to kill 100% with 1 hour exposure, killed only 3.2% of the mosquitoes.

Roy <u>et al</u>. (1976) described the prevalence of malaria in (Mysore) Karnataka state, in 1968 out of 102 <u>P. malariae</u> cases in India, 42 were from Karnataka state, and in 1972, 332 were from Karnataka state. Spread of infection occured between 1969 and 1972.

Pal (1976) described ABo blood groups of 133 malarial patients. Blood donors were compared to find out whether

malarial parasite shows some predilection to people of any particular blood group. No significant different was observed.

Bronm (1976) described the standpoint of world malaria eradication compaign. Untill 1956 DDT was the main weapon applied to the walls of the houses in interupting the transmission of malaria by shortening the cycle of Anopheles vector. The principal problems in Southern Asia, which is experiencing setbacks are, increasing in pesticides resistance in the vector and a decrease in available funds in face of increasing operational costs.

Hawking <u>et al</u>. (1971) reported that gametocytes of many malarial parasites are able to infect mosquitoes within only 6-10 hours. Infectivity occurs at night when the vectors feed and development of gametocytes from merozoites takes approximately six hours longer than the usually synchronized asexual cycle in the blood. <u>P. falciparum</u> gametocytes mature slowly in about 12 hours.

Shalaby (1966) determined age of 1910 females of <u>An. culicifacies</u> Giles in the Pauchmahals district of Gujrat state, India. He observed biparous age group upto 7 percent, uniparous 85 percent and nuliparous as 8 percent.

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Reisen <u>et al</u>. (1982) described the population ecology and reproductive behaviour of <u>An</u>, <u>culicifacies</u>. They placed laboratory reared pupae of <u>An</u>. <u>culicifacies</u> at a breeding site near a rural village in Punjab. Few pairings were observed at male swarms which formed at dusk indicating most mating occurred elsewhere. The number of marked adults collected resting indoor the first afternoon was less than numbers taken -in subsequent recapture attempts.

Reisen <u>et al</u>. (1982) described the seasonal trends in population size and survivorship of <u>An</u>. <u>culicifacies</u>, <u>An</u>. <u>stephensi</u> and <u>An</u>. <u>subpictus</u> in rural Punjab. They collected <u>An</u>. <u>stephensi</u> throughout the year and population increased during spring and post-monsoon season. <u>An</u>. <u>subpictus</u> and <u>An</u>. <u>culicifacies</u> increased at the onset of monsoon season and during post-monsoon season. <u>An</u>. <u>subpictus</u> declined with the onset of colder weather.

Reisen and Boreham (1982) described vectorial capacity of <u>An</u>. <u>culicifacies</u> and <u>An</u>. <u>stephensi</u> against . <u>P</u>. <u>falciparum</u> and <u>P</u>. <u>vivax</u> during monsoon malaria transmission season of 1978. They stated that infrequent feeding on humans coupled with reduced anopheline life expectancy contributed to low estimates of malaria vectorial capacity. Cattle and buffeloes interspersed throughout the village may have diverted host seeking females from human hosts. Vectorial capacity was less than  $1.01 \times 10^{-2}$  for <u>P</u>. <u>vivax</u> and less than 6.43 x  $10^{-3}$  for <u>P</u>. <u>falciparum</u> (for <u>An</u>. <u>culicifacius</u>) and less than 1.29 x  $10^{-4}$  for <u>P</u>. <u>vivax</u> and less than 1.09 x  $10^{-5}$  for <u>P</u>. <u>falciparum</u> (for <u>An</u>. <u>stephensi</u>).

Reisen <u>et al</u>. (1981) described the mating behaviour and competitiveness of chemosterilized males in nature. They released marked chemosterilized males in a shed near Kot Baghicha. Males behave similarly to unmarked males. However, estimates of mating competitiveness based on fertility of egg batches was less in released males.

Haq <u>et al</u>. (1981) described the effects of <u>Nosema</u> <u>algerae</u> on <u>An</u>. <u>stephensi</u> which decreased immature to adult survivorship, delayed developmental time, decreased female and male life expectancy at emergence, decreased net reproductive rate.

Mahmud and Reisen (1981) described the gonotrophic cycles of <u>Anopheles culicifacies</u> and <u>Anopheles stephensi</u> at  $16^{\circ}$ C to  $28^{\circ}$ C in an incubator and in nature using mark-capture methods. During February sexual maturity of released females was delayed, and imsemination rate of recaptured females reduced. Ovarian development beyond resting stage II and parity were delayed during winter, with <u>An. stephensi</u> maturing faster than <u>An. culicifacies</u>. Females lived longer and survivorship increased after the females had inhibited a blood meal.

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Reisen <u>et al</u>. (1981) described the dispersal, immigration and emigration rates, survivorship and absolute population size of <u>An</u>. <u>culicifacies</u>, <u>An</u>. <u>stephensi</u> and <u>An</u>. <u>subpictus</u> at a series of cattle sheds in rural Punjab during November, 1979 and May 1980 using capture-mark-releaserecapture and dissection methods. Population dispersal was temperature related and was more vagile during may longest detected flight was 1250 meters. Horizontal survivorship was greater during November and was less than vertical one. Survivorship during mulliparous period was greater than throughout total life.

Reisen and Mahmud (1981) described the behaviour of <u>An</u>. <u>culicifacies</u> and <u>An</u>. <u>stephousi</u> during spring and Monsoon seasons.

Rathore and Toqir (1981) and Rathore and Toqir (1980) reported malathion resistance in <u>An</u>. <u>stephensi</u>. They used 5% Malathion and with one hour exposure 68.95% individuals survived.

De-Zulueta <u>et al</u>. (1980) described malaria control and long-term periodicity of the disease in Pakistan. They reported that in Punjab epidemics occur regularly at an interval of approximately eight years. Against this background results of malaria control programme launched in 1975 are examined. The programme was equally supported by USAID and

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WHO. Malathion produced toxicity problems among spraying workers. Despite these difficulties an overall reduction of 76% in slide positivity rate was observed.

Collins <u>et al</u>. (1980) described <u>P. vivax</u> (FVO strain) in <u>Aotus trivirgatus</u> monkeys. Parasitaemia in intact and splenectomized animals was similar to that reported for this strain in man. Comparative infectivity studies with mosquitoes fed with infected monkeys indicated that the most susceptible was <u>An</u>. <u>freeborni</u> followed by <u>An</u>. <u>balabecensis</u>, <u>An</u>. <u>culicifacies</u>, <u>An</u>. <u>maculatus</u>, <u>An</u>. <u>atroparvus</u>, <u>An</u>. <u>stephensi</u>, <u>An</u>. <u>quadrimaculatus</u>, <u>An</u>. <u>albimanus</u>. Transmission was sporozoites from <u>An</u>. <u>maculatus</u> was demonstrated two times.

Collins <u>et al</u>. (1980) described the effect of West Pakistan strain of <u>P</u>. <u>vivax</u> in <u>An</u>. <u>freeborni</u> (of California origin), <u>An</u>. <u>maculatus</u> (Malaysol), <u>An</u>. <u>culicifacies</u> (Lahore, Pakistan) and <u>An</u>. <u>balabecensis</u> (Thailand). The parasite was maintained in 17 splehectomized Aotus monkeys of Colombia origin and a degree of immunity was detected due to reduced parasitaemia. <u>An</u>. <u>freeborni</u> proved to be the best host in regard to oocyst production. The three asian species, <u>An</u>. <u>maculatus</u>, <u>An</u>. <u>culicifacies</u>, and <u>An</u>. <u>balabecesis</u> had higher infection in salivary glands.

Reisen and Mahmud (1980) described the life tables of An. culicifacies and An. stephensi. Life expectancy at

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emergence for female <u>An</u>. <u>culicifacies</u> was 12.2 days significantly longer than for <u>An</u>. <u>Stephensi</u> which was 8.1 days. <u>An</u>. <u>culicifacies</u> males were short lived (6.8 days) than <u>An</u>. <u>stephensi</u> males (8.0 days). The net reproductive rates, mean generation times and instantaneous rates of increase were respectively, 51.2, 25.6 and 0.153 for <u>An</u>. culicifacies and 45.2, 19.9 and 0.190 for An. stephensi.

Rathore and Toqir (1980) reported DDT resistance in <u>An</u>. <u>culicifacies</u> and Rathore <u>et al</u>. (1980) reported DDT resistance in <u>An</u>. <u>culicifacies</u>, <u>An</u>. <u>stephensi</u> and <u>An</u>. <u>subpictus</u> but <u>An</u>. <u>nigerimus</u> and <u>An</u>. <u>pulcherimus</u> were found to be susceptible. Dieldrin resistance was present in all these species. <u>An</u>. <u>stephensi</u> showed Malathion resistance. An. nigerimus and An. subpictus showed resistance to fenthion.

Baker and Sakai (1980) worked on the genetics and cytogenetics of malaria voctor An. culicifacies.

Taylor and Siddiqui (1979) described owl monkeys (<u>Aotus trivirgatus</u>) from South and Central America as hosts for <u>P. falciparum</u> (Asian FVO strain). All 10 Panamian monkeys infected with the FVO strain were bled out with parasitaemias above 25%. One survived and two were killed with paeasitaemia 40%.

Reisen (1978) described the bionomies, sampling methodology and the effects of insecticides on <u>An</u>. <u>culcifacies</u>

<u>An</u>. <u>stephensi</u> and <u>An</u>, <u>superpictus</u>. Bovid baits provided highest collections while light traps provided lowest number of collections. Most species exhibited peaks in late spring and after monsoon rains. Spray of organophosphorus insciticides, was effective in controlling vectors.

Suleman <u>et al</u>. (1977) described the time of attraction of <u>An</u>. <u>annularis</u>, <u>An</u>. <u>culicifacies</u> and <u>An</u>. <u>stepensis</u> to light traps. The traps were hung over bovid feed troughs and were thus near the primary blood meal source and resting sites of endophilic species. Majority of collected females were freshly fed. Over 83% of the females were collected during first half of the night with most of these comming between dusk and 2000 hours.

Ainsley (1976) successfully colonized <u>An</u>. culicifacies in ware cages measuring 60 cm on each side.

Reisen and Aslam Khan (1976) described the swarming and mating behaviour of <u>An</u>. <u>culicifacies</u> in nature during December at a cattle shed near Sattoki, Lahore. Swarming commenced 20.9 minutes before and ended 20 minutes after sunset. Mating occurred 6.1 minutes before and 15.8 minutes after sunset. The swarms were principally composed of males with females only entering for mating. Copulation lasted 27.2 seconds and was completed in flight. Reisen (1975) described intraspecific competition in <u>An</u>. <u>stephensi</u>. He reared this mosquito in food and space limited environments and observed that it took longer to mature, had increased larval and pupal maturity, produced adults which were smaller in size, had proportionately fewer males in the population and produced females which imbibed less blood and layed fewer eggs.

Aslam Khan <u>et al</u>. (1972) described fifty genetical and morphological variation in natural population of malaria mosquito, Anopheles stephensi, from Karachi, Pakistan.

Aslam Khan (1972) reported <u>An</u>. <u>barianesis</u>, <u>An</u>. <u>pulcherimus</u> of <u>An</u>. <u>metaboles</u>, from Punjab.

Aslam Khan and Salmon (1969) described the bionomics of <u>An. annularis</u>, <u>An. stephensi</u>, <u>An. pulcherimus</u>, <u>An.</u> <u>culicifacies</u>, <u>An. subpictus</u> and <u>An. nigerimus</u>. <u>An.culicifacies</u> and <u>An.stephensi</u> were found resting indoor. Biting activity of <u>An. nigerimus</u> starts just at sunset. Peak biting activity of An. pulcherimus starts one hour after sunset.

An integrated malaria eradication programme was suggested in 1950 and by 1955 this programme was accepted by World Health Organization. By 1958 seventy six countries were planning, carrying out or had completed the eradication of malaria. In Pakistan nationwide malaria eradication programme was launched in 1961 under the auspices of World Health Organization and with the support of UNISEF and USAID. In Rawalpindi/Islamabad region malaria activities were started in 1964 under the same compaign. In 1965 DDT at the rate of 1 gm/sq.m (one round) was sprayed for the control of anopheline malaria vector. From 1966-67 one round of DDT and a second selective of the same was sprayed. Upto 1970 only selected areas where mosquito population and parasite rate was high were sprayed. During 1971-76 DDT and BHC was sprayed in selected areas. During 1977 to 1979 two rounds of BHC and in 1980 Malathion was sprayed for vector control. In 1981 Islamabad was made a separate district therefore Rawalpindi Communicable Disease Control Department stopped their activities in this region. Rawalpindi region has been selectively sprayed with Malathion in June - July 1982 but Islamabad district has not been sprayed so far.

In other countries a bulk of research work is being carried out on malaria but so far very little attention has been paid in this regard in this region. Main objectives for the present study were to find out parasite rate, parasite species actually responsible for causing the disease, role of anopheline vectors in disease transmission, behaviour of the vector, types of breeding places and the types of preventive or control measures against vector species in the area under study.

#### METHODS AND MATERIALS

4000 Persons were surveyed for malarial parasite from 10 villages and localities around Rawalpindi/Islamabad during September-November, 1982. From each village a sample of at least 400 persons was taken. The particulars of each person, and locality were noted in a separate proforma having the following information. Name of locality, name of person, age, sex, physical appearance, previous attack of malaria if any and spleen size.

<u>Preparation of smears</u>: Blood smears were prepared by finger pricks with the help of pricking needles. Finger tip was cleaned with methylated spirit soaked cotton to avoid contamination. Pricking needle was also cleaned with methylated spirit before and after pricking. Both thick and thin smears were prepared on the same slide.

1. <u>Thin smears</u>: A drop of blood was placed at about middle of the slide, a thin smear was prepared and dried.

2. <u>Thick smears</u>: Three or four drops of blood were placed at the other end of slide and a thick smear was made which later on dried. After smear preparation these were labelled with glass pencil, wraped in a clean paper and brought to the laboratory for staining. Before staining thin smears were fixed in methyl alcohol and stained with Giemsa stain. <u>Staining procedure</u>: Giemsa stain solution was prepared with the ratio of 3 drops of stain with 1 ml of buffered distilled water. Slides were placed in coplin jars and stained for 30 minutes after which these were washed with distilled water and dried.

<u>Parasitological examination</u>: First of all thick smears were examined for 10 minutes each, under oil immersion. If a thick smear was found positive for malarial parasite, the thin smear was then examined for specific identification. In the case of thin smears at least 200 fields were examined under oil immersion.

<u>Vector survey</u>: For entomological survey mosquito collections were made in the houses, sheds, toilets, stores etc. of the surveyed localities.

The mosquitoes were collected with the help of mouth aspirator in flash light. Outdoor sweeping collections were done during different hours of the day and in early evening when mosquitoes started flying in swarms in air upon human heads and animals. Behaviour of mosquitoes like resting, biting and breeding habits was also noted. Only anopheline mosquitoes were taken into account and were identified with the help of identification key (personal communication with Pakistan Medical Research Centre, Lahore). Diagrams for the specific identification of mosquitoes were made with the help of camera lucida.

<u>Dissection of mosquitoes</u>: Female anopheline mosquitoes were dissected for the presence of sporozoites and oocysts in salivary glands and stomach wall respectively.

1. <u>Dissection of stomach</u>: For stomach dissection female mosquito was placed on a microscope slide with apex of abdomen to the right. Abdomen was separated from the thorax leaving only the metanotum attacked to abdomen. Left dissecting needle was fixed in metanotum and with the help of right needle, integument on 7th abdominal segment was cut, a small drop of physiological saline was placed at the tip of abdomen and stomach was drawn out. The stomach was separated and transferred to a clean side of the slide, another drop of physiological saline added and stomach was then covered with coverslide. Examination of the removed stomach was then started from its posterior part field by field as with blood slide under 40 X objectives.

2. <u>Dissection of salivary glands</u>: Female was placed on microscope slide with head pointing right. Neck was removed, a drop of physiological saline was placed close to it, thorax was pressed a little above left hand needle for the traction of salivary glands. These were then covered with a coverslide and examined under 40 X objective.

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<u>Washing of slides</u>: Slides were washed in detergent (surf). About 50 gm of detergent powder was put in medium sized bucketful water and slides were thoroughly washed to remove dirt and grease. The slides were later on rinsed in running tap water for about half an hour and were then stored in 70 percent alcohol.

#### Preparation of stains and solutions:

1. <u>Giemsa stain</u>: One gram of Giemsa stain powder was groun together with 50 ml each of methanol and glycerol in a morta for 15 minutes by adding small amounts of glycerol and alcohol. This was then transferred to a stoppered bottle, kept for 24 hours by shaking at intervals and then filtered.

2. <u>Buffered solution</u>: The stain was diluted with buffered distilled water having pH 7 - 7.2. This solution consisted of:

Disodium hydrogen phosphate 20 gms. Potassium dihydrogen phosphate 0.4 gms. Distilled water to make 1,000 CC.

#### RESULTS

Parasite Survey: Out of 4,000 individuals examined at ten different locations (400 from each location), <u>152</u> cases were found as feverish. Spleen of five cases was swollen and enlarged. Only 14 of the thick swears were diagnosed as positive. These were 3 at Noorpur Shahan, 2 each at Barakao, Malpur, Morgah and Tarnol and one each at Koh-i-Noor colony, Rawal Dam colony and Taxila. 3 cases one each at Barakao, Noorpur Shahan and Tarnol were observed with chills, head partially sweating, high body temperature and pale colour. Patients complained of severe headache, backache and fever. Upon examining the swears in laboratory they were found as positive.

In thick swears parasites appeared concentrated, interpretation of specific results was difficult, therefore thin swears observed for identification of plasmodium species involved in the infection. Ten cases were identified as <u>Plasmodium vivax (Table 1)</u>. These are one each from Koh-i-Noor Colony and Taxila and 2 each from Noorpur Shahan, Mulpur, Morgah and Tarnol.

In all <u>P</u>. <u>vivax</u> cases ring, trophozoite and schizont stages were seen. Red blood corpuscles had enlarged in size and their colour had changed from red to light yellow. Chromatin dots of ring stage, pink red in colour were seen on the sides as well as within the corpuscles. Within corpuscles small red dots were the shuffner's dots. Different amoeboid trophozoites which were in the process of development were seen. Blue cytoplasm of parasites and chromatin dots, both scattered throughout the corpuscle were clearly visible. In one older amoeboid trophozoite cytoplusm appeared in the form of a circle with chromatin dot on one side of periphery. Mature trophozoites were also seen. Schizonts were seen in the process of division containing red segments. Both the trophozoites and schizonts were lying scattered irregularly, nearly filling the corpuscle. Schizonts were seen -in different stages of development. Segments of chromatin were observed lying irregularly in the corpuscle. All trophozoites, schizonts and segments were large enough in size. No gametocyte was seen in any of the swears.

Two cases both from Barakao were identified as <u>P. falciparum</u> because the corpuscle had not enlarged and the trophozoites and schizonts seen were small or medium sized. Trophozoites in initial stages were seen. Schizont appeared compact and round. Chromatin dots in division process were clearly visible which were pink in colour. Cytoplasm was light blue. Chromatin dots of ring stage were seen on the sides as well as within the corpuscle but both the dots and cytoplasm was not clear. Infection in both

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P. falciparum cases was single.

In two cases, one each from Noorpur Shahan and Rawal Dam Colony, thick swears were diagnosed as positive but thin swears got damaged during staining process. Therefore, these two cases could not be identified upto species level. An overall parasite rate was observed as 0.35%.

<u>Vector Survey</u>: From September to November 1982, i.e. Post monsoon season a total of 2924 anopheline females were collected. All theo species were found mostly endophilic in their habit. From underside of bridges, drainage pipes and evening sweepings, very few anopheline females were collected. Males were dominating in outdoor collections and vice versa. From grass sweepings few females were collected. The species collected and identified were <u>An. culicifacies, An. stephensi, An. subpictus, An. annularis, An. fluviatilis, An. pulcherimus</u> and <u>An. nigerimus</u>. From bed rooms female population was low while that from cattle and buffalo sheds was high. Anopheline females feed both during day and night but peak feeding in <u>An. culicifacies</u> and An. stephensi was observed in late hours of the night.

More females were collected from sheds/rooms with zink sheet ceiling as compared to that of tree branch ceiling. Adults hide themselves in tree branches. Females were found resting indoor in humid, darkened and sheltered sites like

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chicken barns, outbuildings, garages, stores, toilets, human and animal dwellings. In animal sheds mosquitoes rest in and near ceilings. In bed rooms, stores etc. in and near the ceilings as well as hidden under beds, chairs etc.

Upon locating the breeding places, larvae were seen in street drains, where drains were blocked due to accumulation of soil, stones, grasses etc. stagnant rain or drain water ditches, stagnant water on the sides of the streams, broken pottery and even carelessly thrown used tins and motor tyres. Larvae were found crowded on the sides of water ditches and containers etc. when collected, these swim away and gather at some other place. Anopheline larvae lie straight on water surface and have no pronounced siphon on last abdominal segment while culicines were found hanging in water with pronounced siphon on last abdominal sigment. If anopheline larvae were dominating, culicines were less and vice versa. Anopheline larvae were found mostly in shallow water while culicines in deep water. Larvae protect themselves by sinking when collecting cup was lowered for collection and reappeared at some other place.

Maxillary palpi of anopheline adult mosquitoes are as long as proboscis. In <u>An</u>. <u>annularis</u> hind tarsi are all white, both body and wings are very dark in colour. <u>An</u>. pulcherimus also has got white hind tarsi but body

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densly covered with white and grey scales. An. culicifacies, An. fluviatilis, An. stephensi and An. subpictus wings have 4 black spots on anterior margin. An. nigerimus has got less than 4 black spots at anterior margin and base of front femor is enlarged. Both An. stephensi and An. subpictus have white rings on tarsal sigments but An. culicifacies does not have them. In An. stephensi there are white scales on femor and tibia but in An. subpictus there are no such white scales. An. fluviatilis differs from An. culicifacies in having 3rd longitudinal wing vein as white with dark distal and basal patches and wing margin with 5 white spots at the ends of veins. Average temperature during September and October was 16 - 37°C and 9 - 35°C respectively. Humidity at 0800 A.M. and 0500 P.M. was 58 and 40% (September) and 52 and 34% (October), which is favourable for oviposition, feeding and survivorship etc.

During salivary gland and stomach dissections no sporozoite or oocysts were seen. Since cattle/buffaloe shed's collection was high it appeared that man-mosquito contact was less and secondly the success of parasite within female mosquito depends upon -breeding and feeding habits of the species.

# Key to the mosquitoes collected in Rawalpindi/Islamabad region

- a- Palpi same length as proboscis-----Anophelines 2
   b- Palpi shorter than proboscis-----Culicine.
- 2. a- Several hind tarsi all white-----3
  - b- Hind tarsi not completely white, may be ringed with white-----4
- a- Body densely covered with white and grey scales
   -----An.pulcherimus.

b- Body and wings black-----An. annularis.

- a- Wings with 4 blacks spots at anterior margin,
   base of the front femour not enlarged-----5
  - b- Wings less than 4 black spots at anterior margin, base of the front femor enlarged-----An.nigerimus.
  - 5. a- Tarsal segments ringed with white-----7
    b- Tarsel segments all black without white rings----6

6. a- Third longitudinal wing vein black,

wing margin with 2 white spots----An.culicifacies.

- b- Third longitudinal wing vein white with basal and distal black patches wing margin with 5 white spots at the ends of veins-----An. fluviatilis.
- 7. a- Femor, tibia and portions of palpi with white specks-----d-----<u>An</u>. <u>stephensi</u>.

b- Femor, tibia and palpi without specks --- An. subpictus,

-: 42 :-

## Table - 1

-: 43 :-

# Number of Positive Cases of <u>P</u>. <u>vivax</u> and <u>P</u>. <u>faliciparum</u> in different localities of Rawalpindi/Islamabad.

Locality.	Number of persons examined.	Positive Cases	<u>P.vivax</u>	P.falciparum	Plasmodium.sp. (unidentified)
Barakao	400	2	-	2	-
Chak Shadad	400	-	-	-	1911 - J. 1914 P
Dhamial	400	-	-	-	
Koh-i-Noor colony	400	1	1	-	
Malpur	400	. 2	2	-	-
Morgah	400	2	2	-	-
Noor Pur Shahan	400	3	2	-	1
Rawal Dam colony	400	1	-	-	1
Tarnol	400	2	2	-	
Taxila	400	1	1	-	-
Total	4,000	14	10	2	2

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# Table - 2

# Number of Positive Cases with ages according to sex.

	Males			Females			
Locality	No.examined	No. positive	Ages of <u>positive(years</u> )	No. examined.	No. positive	Ages of positive(years)	
Malpur	396	2	6 , 46	4	-		
Barakao	367	1	14	33	1	14	
Koh-i-Noor colony	307	1	26	93	-		
Tarnol	390	2	15 , 16	10	-		
Dhamial	395		_	5	-		
Morgah	299	2	7 , 13	101	-		
Noor Pur Shahan	383	2	27 , 46	17	1	6	
Chak Shazad	367	-	-	33	-		
Rawal Dam colony	364	1	11	36	-		
Taxila	327	1	45	73	-		
			11111111111111111111111111111111111111				
Total	3595	12		405	2		

#### Table - 3

Anopheline females collecte	d during September to November 1982.
Species.	Number collected & examined
An. culicifacies.	1579
<u>An</u> . <u>fluviatilis</u> .	149
<u>An. stephensi.</u>	756
<u>An</u> . <u>subpictus</u> .	181
<u>An. nigerimus.</u>	95
<u>An. pulcherimus.</u>	73
An. annularis.	191

## Anopheline mosquitoes

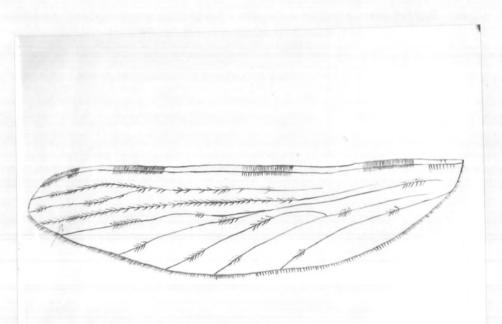
-: 46 :-

Palpi is always as long as proboscis.



Anopheles culicifacies

Third longitudinal wing vein is completely black.

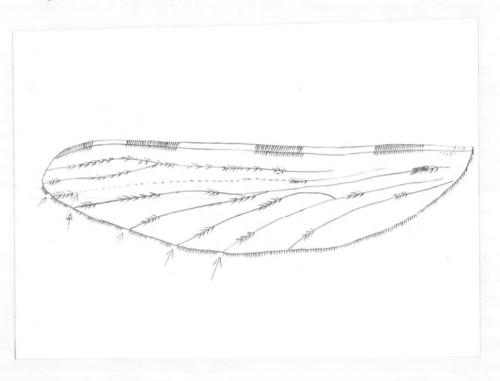


-: 47 :-

## Anopheles fluviatilis

-: 48 :-

Third longitudinal wing vein is white with dark distal and basal patches. There are 5 white spots at the ends of veins.



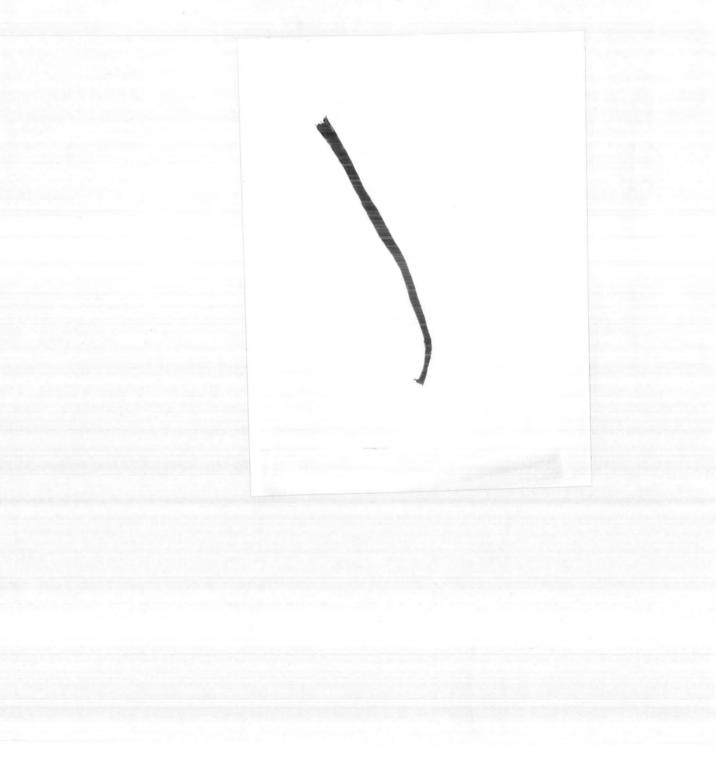
Anopheles culicifacies

-: 49 :-

and

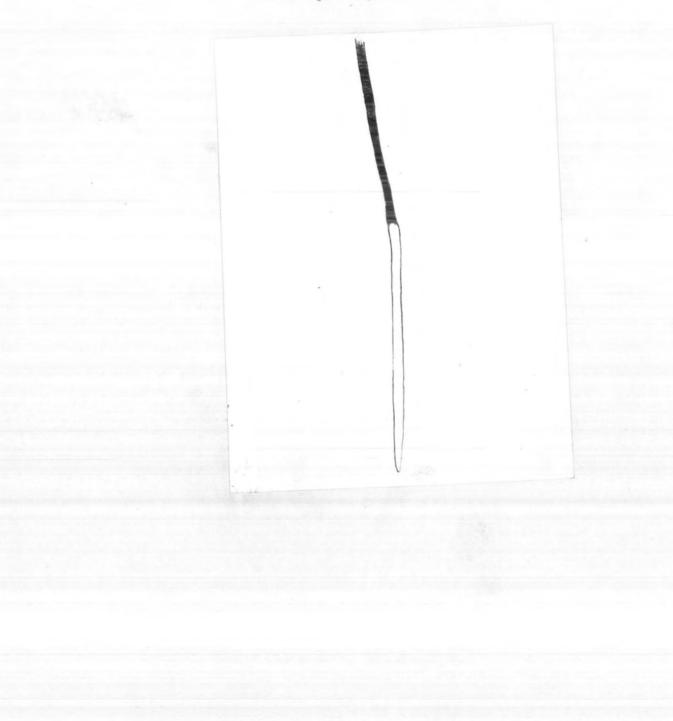
# Anopheles fluviatilis.

Black tarsel segments without white rings.



## Anopheles annularis

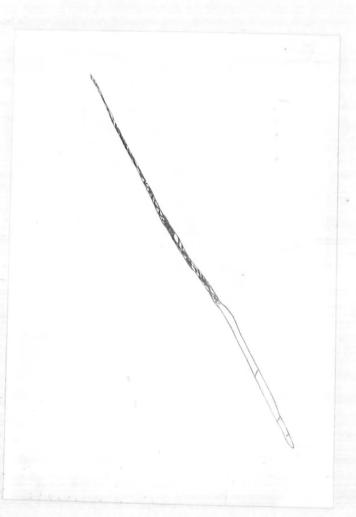
Hind tarsi all white. No white specks on legs (body is also black without white specks).



## Anopheles pulcherimus

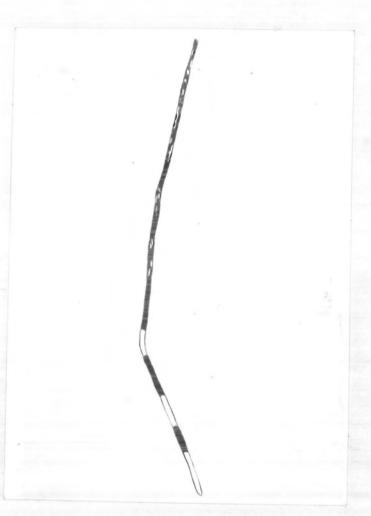
-: 51 :-

Hind tarsi all white but legs and body is densely covered with white specks.



## Anopheles stephensi

Tarsel segments ringed with white. Tibia and tarsi with white specks.



Anopheles stephensi

-: 53 :-

Palpi with white speckling.



Anopheles subpictus

54

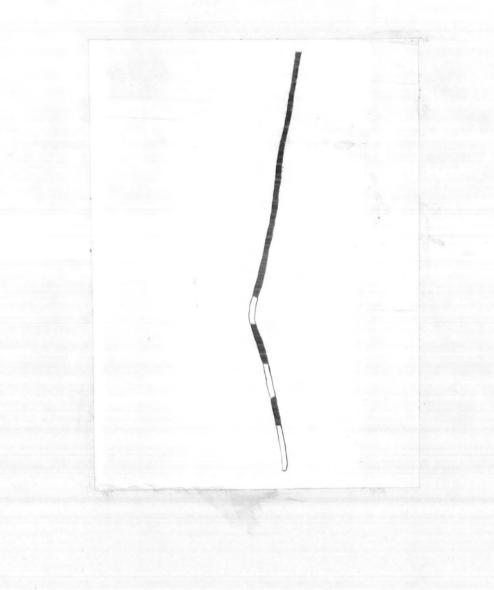
Palpi without white specks.



Anopheles subpictus

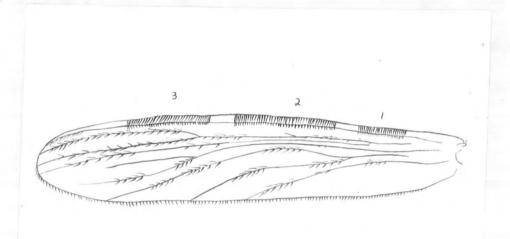
-: 55 :-

Tarsel segments ringed with white. Tibia and tarsi without white specks.



Anopheles nigerimus

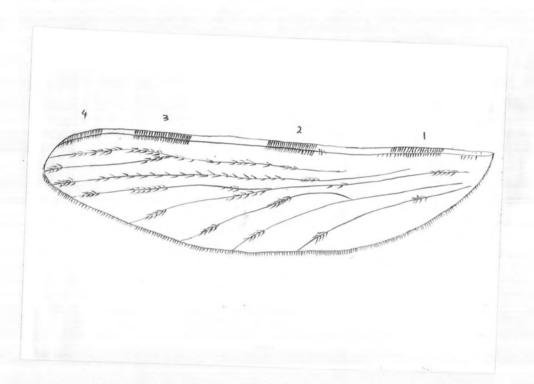
Wing with less than 4 black spots at anterior margin.



-: 56 :-

-: 57 :-Anopheles nigerimus Base of front femor enlarged. <u>An</u>. <u>culicifacies</u>, <u>An</u>. <u>fluviatilis</u>, <u>An</u>. <u>stephensi</u> and <u>An</u>. <u>subpictus</u>

Wing with 4 black spots at the anterior margin.



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#### Discussion

During malarial parasite survey 14 cases were diagnosed as positive out of total of 4,000 individuals examined from Rawalpindi/Islamabad region. Ten cases were identified as P. vivax, two as P. falciparum while 2 of the cases could not be identified upto species level. These studies were conducted during post-monsoon season, i.e. September to November 1982 when vector population is high. An overall parasite rate of 0.35 percent was observed during the studies. De-Zulueta et al. (1980) described malaria control and long term periodicity in this country. They compared the results of malaria control programmes for the year 1970-77. In November to December 1976 malaiometric surveys were conducted under the direct supervision of Directorate of Malaria Control to assess the results of surveillance in the country. They concluded the slide positivity rate as high during monsoon and post-monsoon In March-April 1981 malarial control department season. examined 1079 blood films. 24 cases were positive, 23 for P, vivax and 1 for P. falciparum. Parasite rate in Punjab and NWFP was 5.28 and 2.06% in 1976 and 2.13 and 0.35% in 197' respectively. In Islamabad region parasite rate in March-April 1981 was 2.22 percent and the present parasite rate of Islamabad region has been observed as 0.4 percent and

that of Rawalpindi as 0.3 percent. The parasite rate of Islamabad region appears a little higher than that of Rawalpindi because of no malaria control activities while Rawalpindi region is occasionally sprayed by the district malaria control department. Since in urban area of Rawalpindi/Islamabad drainage system and preventive measures are comparatively better than rural populations, therefore parasite rate is expected to be higher in rural populations of Rawalpindi and Islamabad. The parasite rate of India was reported 0.99 to 50 per thousand by Akhtar and Learmonth (1980). Roy and Ghosh (1980) reported parasite rate of only Karnataka State as high (14.53%).

Dissection of the salivary glands and stomach of anopheline female mosquitoes did not reveal any of the sporozoites and oocysts respectively. It is due to the reason that most of the mosquitoes were collected from cattle sheds where they were resting in large numbers than human dwellings.etc. It therefore points to the conclusion that since the man-vector contact is low, the rate of parasite also appeared low. Reisen and boreham (1982) observed very low vectorial capacity due to interspersed cattle and buffaloes through the village of Khano-Harni, Punjab Province during monsoon malaria transmission season. Very small populations of human feed were detected in <u>An</u>. culicifacies and An. stephensi but not in An. subpictus.

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They reported host selection pattern of indoor resting females during 1978, i.e. September, October and November 2, 1, 4 (human) and 16, 17, 82 (bovid) for An.culicifacies, 0, 0, 2 (human) and 47, 49, 78 (bovid) for An.stephensi and 0, 0, 0 (human) and 28, 2, 89 (bovid) for An. subpictus. Reisen et al. (1982) detected low incidence of human feeds during November and December when female population of An.stephensi was high. Molineaux and Gramiccia (1981) worked on epidemiology of malaria in West Africa and reported sporozoite inocullation rate as 145 infectious bites per year. Batra et al. (1980) worked on malaria vectors in Tamil Nadu State of India and concluded that it is possible for malaria transmission to continue as a result of low level man-vector contact observed in the field. Campbell et al. (1980) infected An. freeborni with plasmodium falciparum gametocytes at 25°C and after 9 days a single gut infection with a single oocyst was seen.

During the present survey 7 anopheline species have been identified from Rawalpindi/Islamabad region which are as follows: <u>An. culicifacies</u>, <u>An. fluviatilis</u>, <u>An</u>. <u>stephensi</u>, <u>An. subpictus</u>, <u>An. annularis</u>, <u>An. pulcherimus</u> and <u>An. nigerimus</u>. Communicable Disease Control Department of Rawalpindi district, during malariometric survey of March-April, 1981 identified 5 anopheline species, i.e. <u>An</u>. <u>culicifacies</u>, <u>An. fluviatilis</u>, <u>An. stephensi</u>, <u>An. subpictus</u> and <u>An</u>. <u>annularis</u> from this region. In the present survey two more species, i.e. <u>An</u>. <u>nigerimus</u> and <u>An</u>. <u>pulcherimus</u> has been recorded which is a new record from this area. Aslam Khan (1972) reported An. pulcherimus from Lahore.

Indoor collections of female population is high when compared to that of outdoor one which is in accordance with the earlier observations of Reisen (1978) who reported that female <u>Anopheles culicifacies</u> and <u>An</u>. <u>stephensi</u> are endophilic. Although seasonal variatiobs have not been worked out during the present studies but it appears that the population of anopheline mosquitoes is higher during September, October and November which is supporting the observations of Reisen <u>et al</u>. (1982) who reported that female and male resting indices increased in September, were low in October and then again increased in November-December.

From the present studies it has become apparent that there is no substantial difference in malaria cases between suburban areas of Rawalpindi and Islamabad. Although a very slight increase in malaria cases has been observed in Islamabad district but it is to be pointed out that this district has not been sprayed since 1981. It is evident that mosquito breeding places are uniformly spread in the whole area of Rawalpindi-Islamabad. It is therefore proposed that there is a need to improve the drainage system in urban

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and suburban areas so that water does not remain stagnant for mosquito breeding. Insecticides should be sprayed to kill adults as well as larvae. A concerted vigilance is also required to detect malaria cases in all the rural areas and to test the sensitivity of drugs being commonly used.

#### SUMMARY

Malarial parasites and vector survey was conducted during post Monsoon season of 1982 when the vector population was sufficiently high. A total of 4,000 randomnly selected persons were examined with age and sex differences at Rawalpindi/Islamabad and the adjacent areas. 14 cases were diagnosed as positive for malarial parasite. Ten cases were identified as <u>Plasmodium vivax</u>. Two as <u>Plasmodium falciparum</u> and two of the cases could not be identified. The parasite rate was observed as 0.35%.

Vector survey was conducted to find out the anopheline species available in the area, and to note their behaviour and their role in transmitting the malarial parasite to human beings. A total of 2924 anopheles females were collected. Seven species were identified and dissected to find out sporozoites/oocysts in the salivary glands/stomach wall respectively. All the dissection, did not reveal any parasite. The females collected were from cattle/buffalo sheds. The slide positivity rate was also very low. It was therefore concluded that cattle/buffaloes were interspersed in the area and so the host seeking females had diverted from human hosts.

The species collected were An. annularis,

<u>An. pulcherimus, An. culicifacies, An. fluviatilis, An.</u> <u>stephensi, An. subpictus and An. nigerimus</u>. All the anophelines were mostly resting indoor and behaved as late night feeders.

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