CHARACTERIZATION OF THE EPIDEMIOLOGY OF URBAN MALARIA IN NAZARETH; ETHIOPIA

A Thesis

Presented to the

School of Graduate Studies

Addis Ababa University

In Partial Fulfillment of the

Requirements for the Degree of

Master of Science in Biology.

By

MEKONNEN YOHANNES

June, 1990.

ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

Characterization of the Epidemiology of Urban Malaria in the Town of Nazareth, Ethiopia

By

Mekonnen Yohannes

Approved by the Examining Board:

Name	Signature
Dr. Michael Smalley External Examiner	
Dr. Teferi Gemetchu Internal Examiner	
Dr. Beyene Petros Advisor	
Dr. Getachew Teferra Chairman	

ACKNOWLEDGEMENTS

I owe a dept of gratitude to the following:

The School of graduate Studies and the Department of Biology, Addis Ababa University, for research facilities.

Dr. Beyene Petros /Department of Biology/, my advisor, for his invaluable guidance, time and thought so generously given.

The National Programme for the Control of Malaria and other vector-Borne Diseases; Addis Ababa head office-Dr. Awash T/haimanot, AtoNegussie G!mariam, Ato Fessehaye Seilu, Ato Fekade Balcha, W/o Birkinesh Ameneshewa; staff, W/o Birkinesh Ameneshewa; Nazareth zone office-Ato Assefa Getachew and the staff, for helping me in many respects since my early days of research.
haile mariam Mamo hospital, Nazareth health center, for alloing me to use their Patient records. Nazareth City Council, the kebele associations and the People of the Study area for Providing me with information on Various aspects of the town and its Population as well as their Copperation in the Collection of Samples.
Institute of Agricultural Research/IAR/for their kindful offer of the meteorological data of the area.
National Institute for health and Research-W/o Senait Assefa and Ato Tesfaye Mengesha for material assistance.
W/t Wezenet Tewodros for allowing me to use her computer and W/o Azeb Mekasha for typing this paper.
Swedish Agency for Research Cooperation with Developing Countries /SAREC/ for financial support.

TABLE OF CONTENTS

Table of Cont. contd...

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Crossectional surveys were carried out to characterize the epidemiology of urban malaria in the town of Nazareth, located 90km southeast of Addis Ababa. The surveys principally investigated the major aspects of human malaria transmission associated with urban ecology. The study included determination of parasite rate, antimalarial antibody levels in the population by using the IFA test, the mosquito vector, the environmental conditions and population movement. Thick and thin blood films for parasite diagnosis and filter paper blood spots for antimalaria antibody detection by using *P. falciparum* antigens were collected from individuals residing in 6 representative kebeles of the town. The anopheline population was sampled (in Sep./Oct., Dec., 1988 & Oct. 1989) by indoor aspirator and pyrethrum space-spray collections. The mosquitos collected were used for salivary gland dissections as well as bloodmeal and sporozoite detection tests by using the ELISA technique. *A. arabiensis* was incriminated as the species responsible for the transmission of malaria as established by behavioral as well as oocyst-associated *P. falciparum* and salivary gland-associated *P. vivax* sporozoites antigen detection.

An overall parasite rate of 2.8% was observed among the 3890 individuals examined during the four surveys of 3 weeks average duration. *P. falciparum* was the dominant species (58.3%), *P. vivax* ranking next (41.7%). 22.9% of the 450 individuals, aged 10 yrs & over, examined both for parasites and antimalarial antibodies during the 2nd survey, were IFA positive. A marked seasonal variation both in the parasite rate and *A. arabiensis* populations was observed and the peak of both occurred in the months of Sep./Oct. Significant difference both in parasite prevalence rate and antimalaria antibody levels were observed between individuals residing in peripheral and central kebeles. Many of the blood smear and IFA positive individuals were found in the former indicating the relevance of focal factors to the transmission of malaria. There was no apparent decrease of parasite rates with age and the percentage of IFA positive individuals increased very slowly in the age group 15 years and above. These two features suggest a delayed acquired immunity typical of hypoendemic settings. In Nazareth malaria is seasonal, subject to epidemics and probably unstable and its control may be possible.

1. INTRODUCTION

Although the term malaria, derived from the Italian word for bad air (mal'aria), is indicative of a mistaken conception of the true causation of the disease, it is the term commonly used since the scientifically more appropriate term-Plasmodiosis- has never come to wider use (Wernsdorfer, 1980). Human malaria has been known to humanity from the dawn of civilization (Loban and Polozok, 1983) and the exact descriptions of malarial fever were given by Hippocrates in 400B.C. (Garnham, 1966).

Nowadays, over 100 species of malaria causative agents, members of the genus Plasmodium, in a wide range of vertebrates and exhibiting narrowly-defined host specificity, are known (WHO, 1987). Human malaria is caused by four Plasmodium species, namely, Plasmodium falciparum, P. vivax, P malariae and P. ovale. Human malaria parasites have a complex life cycle with Anopheles species as the arthropod host and man as the only natural vertebrate host, with the exception of P. malariae where higher non-human primates may be involved occasionally (Garnham, 1966). The parasites are naturally transmitted to man in the form of sporozoites through the bite of infected female anopheline mosquitos, during which about ten percent of the sporozoite burden may be introduced (WHO, 1987). The life cycle of human malaria parasites comprises an exogenous phase with multiplication (sporogony) in the mosquito and an endogenous asexual phase with multiplication (Schizogony) in the human host. The later phase includes invasion of liver cells by sporozoites, where they undergo exo-erythrocytie schizogony and the developmental phase in the blood corpuscles, the erthrocytie schizogony.

Erythrocytic schizonts rupture with a periodicity of 24-72 hours, characteristic for individual species, and each liberates 10-20 merozoites which attach to specific receptors on red cell membranes to initiate invasion (Cohen & Lambert, 1982). Upon invading new erythrocytes the merozoites can either initiate renewed blood schizogony or develop into sexual forms (macro-and microgametocytes (Garnham, 1966). The erythrocytic cycle is associated with clinical manifestations (paroxysms) of malaria, which depend on the presence of a definite quantum of parasites in the blood (WHO, 1963). In general, clinical symptoms develop within 8 to 30 days of inoculation of infective sporozoites. They consist typically of sequential chills, feve and sweating which may be preceded with a premonitory stage-headache, nausea, malaise (WHO, 1963).

The clinical symptoms of periodicity are not adequate to establish unequivocal diagnosis, since they can resemble that of many other diseases and variations in the classical clinical course are common, specially in children. This is particularly common in infections with P. vivax and P. falciparum, where multiple infections of individual erythrocytes can occur with subsequent establishment of two or more broods of parasites and quotidian fever pattern (Pinder, 1973). When one generation of the parasite prevails in the blood, fever typically has a regular intermittent pattern with the occurrence of paroxysms every 48 hours in cases of vivax and ovale (simple or benign tertian) malaria and every 72 hours with malariae (quartan) malaria (WHO, 1963). P. falciparum is invariably irregular in its pattern and the protean symptoms of falciparum malaria are misleading and may appear with dramatic suddenness and severity (Pinder, 1973). Parasitaemia can alone cause death or result in fatal cerebral, renal or pulmonary complications, particularly in non-immune individuals. Falciparum malaria is a significant cause of abortion and stilibirth, as well as of death in non-immune mothers (TDR, 1988).

Except in epidemics and apart from being a major cause of infant mortality in highly endemic area of falciparum malaria, the disease is insidious rather than dramatic. Malaria causes mainly chronic sufferings, results in an increased number of deaths from other causes, lowers life expectancy, and bring impaired growth in children. Over the centuries it has had a profound impact in curbing socio-economic development by reducing the productive potential of mankind wherever it persisted (Wernsdorfer, 1980; TDR, 1988).

Malaria was considered as one of the main health problems in the world and its control was actively undertaken after the Second World War. Impressive success has been obtained in a number of countries, mostly in the most developed ones. Nevertheless, malaria still remains prevalent throughout the tropics as well as the sub tropics and several hundreds of millions of people are at risk (Carnevale & Mouchet, 1987).

According to Tropical Diseases Research (TDR) reports (TDR, 1988), to date, the disease is endemic in 102 countries of the world, although only 61 report epidemiological data to World Health Organization (WHO). About 2700 million people (56% of the world's population) live in malarious areas-2266 million in countries where malaria control has neen or is still practised and 398 million in areas where no specific measures are being or have been undertaken. In these regions control efforts are mainly concentrated in certain urban areas only (Bruce-Chwatt, 1987). Only about 776 million people of the world (16%) live in areas where malaria has been eliminated over recent decades (TDR, 1988). Nevertheless, the persistence of endemic malaria in Africa, Asia and parts of Central and South America, makes the maintenance of the freedom from malaria in these areas, where "Anophelism without malaria" exists, increasingly difficult (Bruce-Chwatt, 1987).

Of the four main forms of malaria, vivax malaria is the most prevalent-covering many temperate, tropical and subtropical regions (TDR, 1988). The adaptability of P. vivax to the life in various climatic zones is made possible by the capacity of the parasite to survive for a long time in the liver of the host in a dormant state in the form of hypnozoites (Loban & Polozok, 1983). For example, in the northern latitudes, where the transmission period is short and seasonal, strains of P. vivax - owing to their long incubation period - remain in the host in the latent form for about 6-9 months, until the next transmission period (Wernsdorfer, 1980). In the tropical and subtropical zones, however, P. vivax populations are represented by sporozoites causing the development of the disease after a short incubation period (Garnham, 1987).

Thus, the natural populations of P. vivax (and P. ovale) seem to consist of sporozoites comprising a mixture of immediately developing and hypnozoite-destined ones (Garnham, 1987). This also explains the relapse phenomenon observed in P. vivax and P. ovale and the variations in incubation period and relapse patterns of P. vivax that occurs as a result of selection imposed by the local variations in transmission (WHO, 1987).

It is also known that ethnic genetic differences in the susceptibility to particular species play an important role in the distribution of P. vivax. This is reflected by the highest incidence of vivax malaria in Asia and its virtual absence from Central and West Africa, predominantly inhabited by people of negroid descent (Wernsdorfer, 1980). The Central and West African populations have a high frequency of the Duffy negative genotype (FyFy) - the resistance factor to P. vivax in blacks (Miller & Carter, 1976), both in their country of origin and after generations of residence in the USA (Pinder, 1973).

In Africa, P. vivax is common in North Africa, but in tropical Africa its distribution is limited to eastern and Southern regions, with Hamito-Semitic and Caucasian population groups (Wernsdorfer, 1980; Loban & Polozok, 1983). In Ethiopia, it shows a very distinct difference in Hamito-Semitic and nilotic populations (Armstrong, 1978), the latter exhibiting marked resistance to infection. In general, it shows a biphasic distribution with a preponderance in the north of the country but little in the South (Garnham, 1966).

Falciparum malaria is the most frequently occurring form throughout the tropics and subtropics (TDR, 1988), although it is inferior to P. vivax in terms of geographical distribution (Loban & Polozok, 1983). Regardless of its comparatively limited geographical area of incidence, P. falciparum is responsible for most cases of malaria and for the most severe and often fatal forms of the disease (TDR, 1984). This includes imported falciparum malaria in European countries and the USSR (Bruce-Chwatt, 1987), but the epidemiological danger associated with it is insignificant because the sub species of anopheline mosquitos prevalent in these areas are not susceptible to the tropical strains of P. falciparum from Africa (Loban & Polozok, 1983).

Malariae malaria is widely distributed, common in some places and absent in others, but less common than falciparum or vivax malaria (TDR, 1988). It is prevalent throughout the tropics, chiefly in the countries of Africa, Asia as well as Latin America. Ovale malaria is least prevalent of the four forms of human malaria and the geographical distribution of the parasite is quite peculiar. It occurs in tropical Africa and eastern Asia. Although its geographical distribution may suggest some relationship to ethnic factors and, at least, a partial explanation may be found in the receptivity of anopheline species, the causes underlying such peculiar and limited pattern of _P. ovale_ distribution are not fully understood (Wernsdorfer, 1980; WHO, 1987).

Over 400 species of anopheline mosquitos are known all over the world (WHO, 1987) of which around 67 were found to harbour sporozoites originating from natural infection. However, only about 27 of these anophelines are reported as potent vectors of human malaria under natural conditions due to their biting and other habits (Wernsdorfer, 1980).

The importance of an anopheline species as a vector of malaria is dependent on several characteristics, all of which may vary in degree, and, the complex interaction of which shapes the epidemiological significance of a malaria vector in a given area (WHO, 1975). The mosquito host must be susceptible to infection, breed in a suitable microhabitat, produce in sufficient numbers to maintain transmission, find and feed upon the human host present in sufficient numbers in infective state, survive long enough to allow the agent to develop or multiply and then bite a second time, in this case a susceptible individual (Russel etal., 1963; Macdonald, 1957).

It is not known for definite that if one of these qualities is below some given level, a particular anopheline species will not act as a vector, because the low level of one may be compensated for by the high level of others (Russel etal, 1963). Any factor of climate or environmental conditions, such as temperature, relative humidity, rainfall, vegetational features, etc. and seasonal changes that influence the density of a species, the behavior of the species, its longevity, or the development of Plasmodium parasite in its body, will modify the increase or decrease of the amount of malaria in an area (Beaver etal., 1984).

The main vectors of the Afro-tropical region include members of the Anopheles gambiae complex, the composite, A. funestus having a wider geographical distribution, and A. nili as well as A. moucheti having a distribution limited to more restricted areas. On the other hand, A. pharoensis, A. zeimanni, A. rufipes and others constitute vectors of secondary importance (Janssens and Wery, 1987). It is also realized that several anopheline species previously considered as belonging to a single species, based on classical morphological approaches are now found to be complexes of various sibling species, based on biochemical and cytogenetic methods of identification. For example, A. gambiae s.l. has been found to comprise six distinct species, namely, A. gambiae s.s., A. arabiensis, A. quadriannulatus, A. melas, A. merus, and A. bwambe.

A. gambiae s.s and A. arabiensis, the two most anthropophilic members of the complex, have the widest distribution and can occur together over extensive areas (Coluzzi, 1984) - perhaps existing in about 70 percent of Sub-Saharan Africa (White, 1974). These two members of the complex show differences in behaviour, levels of vectorial efficiency, variations in seasonal prevalence (Coluzzi, 1984), differential response to DDT and have specific distribution patterns that seem to be differentially determined by climatic factors (White, 1974). In general, A. gambiae s.s. predominates in the equatorial forest and humid situations, though, it also occurs in the Guinea and Sudan Savana, and even in the Sahel Savana, with a density related to rainfall. A. arabiensis is more successful in the drier zones, such as the Sudan Savana and further north to the Egyptian boarder, but also in the inland areas of East Africa during the dry season (White, 1974; Janssens & Wery, 1987). A. gambiae s.s. and A. arabiensis were also reported to have differences in the Human Blood Index (HBI), the former was found to be more anthropophagic (Molineaux & Gramiccia, 1980).

A. quadriannulatus has little or no direct medical interest owing to its marked zoophily (white, 1974). Nevertheless, since it can coexist with A. arabiensis the operational value of differentiating them is obvious in relation to the evaluation of vectorial capacity, of the effect of residual spraying and the spreading of insecticide resistance (Ooluzzi, 1984).

A. quadriannulatus shares similar larval ecology with A. arabiensis and A. gambiae s.s breeding in shallow, open sunlit freshwater pools (Coluzzi, 1984). Although, A. melas and A. merus larvae are able to develop in freshwater (White, 1974), both species thrive in brackish marshes and lagoons (Stevens, 1984) on the west and east African Coasts, respectively.

The composite A. funestus is one of the principal vectors in Central and Southern Africa (Stevens, 1984). It has a somewhat restricted distribution and occurs in degraded forests and Savanas and on mountain slopes. Although it readily feeds on man and does go indoors, it has a low sporozoite index (Janssens & Wery, 1987). It breeds in permanent vegetated waters including swamps, ponds, lake margins, streams, ditches, etc., (Stevens, 1984).

Although the role played by anopheline mosquitos in transmitting plasmodia is obvious, the epidemiology of human malaria is an intricate biological system in which the parasite, its vertebrate and arthropod hosts interact with one another and with the wider biological and physical environment (WHO, 1975, Wernsdorfer, 1980). The most important factor among these is the interaction of the vector population with man and his environment (WHO, 1975).

The source of infection is a malaria patient whose peripheral blood contains mature sexual forms (gametocytes). The infectivity of blood for mosquitos is influenced by several factors which include number, age, sex, distribution and quality of gametocytes (Beaver etal., 1984; WHO, 1975).

In all species of parasites there are inexplicable variations in infectivity which are sometimes attributed to varying maturity of gametocytes (Russel etal., 1963). While viable gametocytes are usually present in the blood at the time when the first clinical manifestations of malaria due to P. vivax, P. malariae and P. ovale appear, this is not the case with P. falciparum. Falciparum infections differ in the delay of gametocytes after the first appearance of parasitaemia, as they require relatively longer period (10-14 days) to reach maturity (Loban & Polozok, 1983; WHO, 1987).

Typically, infectivity increases with heavier gametocyte densities, although some carriers with less than ten gametocytes per mm^3 have been shown to infect more than half the mosquitos that fed on them (Burkot, 1988). These findings appear to disprove the old concept of a minimum value of gametocytes which could result in infection to mosquitos (WHO, 1987).

The relationship between the prevalence of gametocytes in the population and transmission is of paramount epidemiological significance (WHO, 1987). The comprehensive study of Muir head-Thomson (1957) of P. falciparum in Liberia has indicated that adults and adolescents form a reservoir of infection as did children aged 5 to 14 years and that both groups are only slightly less infective than the age-group 0 to 4 years. These findings had revealed that adults with low gametocyte load or near threshold density constitute a more serious problem. Thus asymptomatic low density gametocyte carriers are implicated as the main reservoir of P. falciparum in endemic area (WHO, 1987; Burkot, 1988). Likewise, the longer period of infections occurred at low gametocyte densities in the absence of clinical symptoms in non-immune subjects (WHO, 1987). Parasite carriers in endemic areas may serve as source of infection for a long time because malaria in such individuals is rarely identified and they receive no treatment (Loban & Polozok, 1983). Having escaped detection, they may also enter receptive areas, trigger subsequent transmission and thus be the cause of the introduction of malaria (Wernsdorfer, 1980).

The chain of transmission from humans to mosquitos and back to humans may be modified by various factors such as the species and the strain of Plasmodium, bionomics or habits of mosquitos; treatment of man by therapeutic and prophylactic drugs (Beaver etal; 1984). In the attempt to minimize or interupt transmission man may alter the physical, chemical or biological components of a given system. The measures may result in varying degrees of success or there may be a compensation reaction within the system tending to maintain the status quo-e.g. development of resistance of the parasite to drugs, of the vector to insecticides or of the human population to having their houses regularly sprayed (WHO, 1975).

Malaria transmission is a complex process and its control demands high quality epidemiological services that are capable of investigating and analysing epidemiological situations and developing appropriate control strategies and approaches. It may be necessary to update such plans continuously in accordance with the dynamics of malaria in the area concerned and this requires the monitoring of all major elements of malaria transmission (WHO, 1987).

The extent to which man becomes the victim of malaria depends, on the first place, on how his own habits affect his accessibility to vector feeding. For example, location and type of housing in reference to vector habits, night travels, outdoor sleeping, nomadism, immigration and local mobility of populations (WHO, 1975; Russel et al., 1963). Cultural attitudes toward mosquito (as well as cooperation in control and eradication programmes) and the effect of the quartet - poverty, ignorance, illiteracy, social deprivation-often referred to as the "vicious cycle of malaria" (Wessen, 1972), are no less important.

Human environmental activities, such as agricultural and engineering developments (e.g. dam sites, irrigation schemes, etc) and the uneven topographical concomitants or aftermaths of construction projects, more often enhance the production and longevity of malaria vectors by increasing surface waters, raising water tables, creating floods and seepages, as well as the consequent rise in relative humidity (WHO, 1975; Surtees, 1971; Wessen, 1972). These human induced mosquito breeding sites are most commonly associated with industrial operations or urban settings (Wessen, 1972).

If man is considered as a major ecological factor, urbanization is the major feature of the present day dynamic environment (Surtees, 1971). Urbanization is manifested in the rapid increase in populations of cities and towns everywhere, it is also manifested by the increasing penetration of modern "urban" ways into even the remotest countryside (Wessen, 1972). Ideally, urbanization should lead to a reduction in the number of mosquito vectors for such diseases as malaria with fewer natural breeding sites, greater control over water usage, and therefore reduced prevalence of mosquito-borne infections. Nevertheless, it has often led to an increase in these diseases (Surtees, 1971) and in many cases large epidemics have occurred (Youdeowei and Service, 1986).

A number of factors associated with urbanization affect the distribution and abundance of mosquitos. The layout of roads and grading of land for building, for example, frequently create roadside excavations which fill up with rain water and constitute suitable aquatic habitats for mosquitos which breed in shallow ground pools (Surtees, 1971), such as A. gambiae, the major malaria vector in Africa (White, 1974). Urban complexes grow from this developmental stage and a number of associated factors become limiting-type of water supply, water usage, inadequate sanitary waste or storm-water disposal systems, and others. The creation of numerous small bodies of water as a result of these factors and the ready availability of human hosts in high densities permits the rapid development of large mosquito populations (Ward, 1977; Surtees, 1971; Youdeowei & Service, 1986).

Other factors such as the transport of mosquitos of public health importance, constant movement of people from the villages to the towns or vice-versa (Bruce-Chwatt, 1987) and outdoor sleeping may affect malaria transmission in urban areas. Furthermore, as urbanization encroaches into natural habitats, the elimination of vertebrates may result in a change in the biting behavior of mosquitos from animals to man. Urbanization may also provide new resting sites which not only present better shelters but also are unsuited to the use of residual insecticides (Pinder, 1973).

Whatever, or wherever a malaria problem may be, each factor and aspect has to be considered in the light of local characteristics (WHO, 1975). In malariological practice a number of parameters are used to classify the condition of malaria in a given area, which permits a description of the epidemiological situation. For this purpose, cross-sectional surveys suffice in some cases, but in the majority of areas only longitudinal observations may yield the basis for such classifications (Wernsdorfer, 1980). The principal classical method is that of malariometric survey, which establishes spleen and parasite rates on the basis of screening the population for the presence of plasmodia in the blood and spleen enlargement (WHO, 1963).

Splenometry is less frequently used these days, since it is a non-specific physical finding and may be due to causes other than malaria (Janssens & Wery, 1987). Thus, microscopic examination has remained the mainstay of parasite detection in man (Bruce-Chwatt, 1985). It is generally accepted that about 100 fields of thick blood film be examined or 200 fields under low transmission (Bruce-Chwatt, 1985; Janssens and Wery, 1987). Thus the direct demonstration of parasites in blood films is still the method of choice to determine the occurrence of malaria and epidemiological studies of the disease are based primarily on these results (Voller and O'Neill, 1971). Normally, the limitations of this method, specially in groups of populations that have achieved a degree of immunity and in which parasitaemia is very scanty or periodical, are well known (Bruce-Chwatt, 1970). Unless several blood film surveys are carried out at different times of the year, single blood film surveys may reveal practically none of the transmission underway (Jeffery etal., 1975). This way it is not possible to predict with any degree of certainity, the amount and intensity of perennial malaria endemicity in a given area (Voller & O'Neill, 1971).

As a result, knowledge of the malaria experience of populations is nowadays being obtained by more sensitive and specific immunological methods based on the detection of antimalarial antibodies (Bruce-Chwatt, 1970). To date there are a number of serologic tests for determining antibody levels and these are now being used in serologic surveys and to a lesser extent in clinical diagnosis.

Among the several methods recommended for the routine detection and measurement of the antibodies to Plasmodium antigens the test most often used is the indirect fluorescent antibody (IFA) technique (WHO, 1974). The IFA test introduced to parasitology in the early 1960's (Nilson, 1978), is now very widely used both for the detection of asymptomatic infections in individuals and for a large-scale seroepidemiological surveys in endemic areas (WHO, 1987). The sensitivity, reproducibility and genetic specificity of the test have been widely recognized as a result of several serological surveys conducted in Africa (Collins etal., 1967, 1968, 1971, McGregor etal.; 1965, Molineaux & Gramiccia, 1980),and elsewhere in the world.

In the IFA method, visualization of the fluorescent markers, allows for the direct identification of the reacting parasite stage (Cohen & Lambert, 1982). Initial problems, related to the antigen, have been overcome by the use of the washed-cell thick films (Sulzer etal., 1969) and the development of short-term (Lopez-Antunano, 1974; Thomas & Ponnampalam, 1975) and continuous (Trager & Jenssen, 1976) in vitro cultures of P. falciparum, which provide a potential source of almost unlimited antigens. The use of blood, collected and dried on filter paper, further simplified the technique (Thomas & Chan, 1982).

In general, fluorescent antibodies become detectable within a few days of patent parasitaemia in non-immune people. In P. falciarum or P. vivax infections, the titer rapidly increases to reach a plateau for a few weeks then slowly declines to lower levels which can persist for months or even years (Collins etal., 1964, Voller & Bruce -Chwatt, 1968). Higher titers persist longer in P. malariae infections (Cohen & Lambert, 1982), but it does not necessarily reflect a high degree of naturally acquired immunity (WHO, 1987). The age-related variations in antibody titers have been well established in areas of high endemicity (McGregor etal., 1965).

Other serological techniques employed in immunodiagnosis include indirect haemagglutination (IHA) test (Kagan, 1972) and enzyme-linked immunosorbent assays (ELISA) (Voller, 1974).

Currently, immunological tests are also being used to assess the susceptibility of a given Anopheles strains to infection under natural conditions, which provide the two important indices- the "Oocyst" and "sporozoite" indices. In the past investigations were hampered by the difficulty in determining sporozoite rates by dissecting the salivary glands of individual mosquitos and the lack of morphological criteria to distinguish different species of sporozoites. The recent development of immunoradiometric (Colins etal., 1984) and ELISA (Burkot etal., 1984) methods for the detection, identification and quantification of sporozoites in mosquitos has facilitated large-scale investigations. The ELISA test has also been applied to detect and identify blood meals in mosquitos and proved to be both sensitive and specific (Service etal., 1986).

An overview of the complex life cycle of the parasites was required to establish the importance of each component part of the epidemiological puzzle. This was provided by the mathematical models of malaria by Ross in 1911 (Rogers, 1988). In the decades following Ross' earlier work, Macdonald (1950; 1952; 1956) developed a model that brought together for consideration all the main quantitative factors - entomological, parasitological and immunological - which constitute or limit malaria transmission. This was followed by several reports of epidemiological observations (Bekessy et al., 1976; Krafsur & Armstrong, 1978), that have confirmed the usefulness of Macdonald's formulae and which provided in-depth knowledge of the bionomics of the local vectors and transmission mechanisms.

One application of the Ross-Macdonald malaria models to malaria epidemiology is the evaluation of the vectorial capacity which attempts to measure the daily rate at which future cases arise from a currently infective case (Rogers, 1988). From measurements of epidemiological variables, in particular, the vectorial capacity, it was possible to specify the risk of malaria and to predict the impact of the various modes of intervention (Vercruysse etal., 1983). The various parameters have also been used alone or in various combinations inorder to obtain some sort of picture from the accumulated information about the endemicity in a given country or focus leading to a proposal of a number of classifications(Janssens & Wery, 1987).

According to the WHO (1963) classification, there are four levels of malaria endemicity, namely, hypo-, meso-, hyper-, and holoendemic malaria. The spleen index in children aged 2-9 years is a major criterion on which the classification is based. That is, about 10% in hypoendemic, varies from 11 to 50% in meso-, and is constantly over 50% in hyperendemic settings. In the hyperendemic type, adults also have high spleen index. In holoendemic conditions, the spleen index in age-matched children is always over 75%. The parasite index in young children is constantly over 75%. However, many workers believe that in the type of analysis restricted to parasitic, entomological and host-related factors, the only solid and useful system is the distinction between stable and unstable malaria, as proposed by Macdonald (Janssens & Wery, 1987). In actual fact, on close examination, the degree of variation is found to give a more valuable means of classification than does the severity of incidence (Russel etal., 1963). This is because, all grades of malaria severity are experienced in both sets of circumstances, the distinction between the stable and unstable malaria lying in the regularity with which transmission is maintaind over the years, rather than in the degree attained at any particular time (Macdonald, 1956). In stable malaria, there is very little variation from season to season. Epidemics would be very unlikely to occur and the community acquires a stable immunity.

Stable malaria is dependent on transmission by a vector highly susceptible to infection by the local parasites, with a frequent man-biting habit, a moderate to high longevity at favourable temperatures. The density of mosquitos is of lesser importance and an anopheline density resulting in an average of as few as 0.025 bites per person each night is sufficient to maintain stable transmission. Unstable malaria has characteristics which are the reverse of stable and its intermediate stages can be estimated by an entomological "Stability Index" (Macdonald, 1957. Russel etal., 1963).

On the other hand, based largely on climate, landscape, socio-cultural structures & human behaviour, some workers have investigated the malaria situation in Africa and delimited the eco-epidemiological diversity of malaria into various strata. These have distinguished nine strata based on transmission contingencies, i.e. 1) Perrennial, 2) seasonal, 3) Sporadic, 4) exceptional, 5) nomadic pastoralists, 6) modern irrigation schemes, 7) temporary development projects, 8) urban and suburban areas, and 9) non-immune visitors or temporary residents (Kouznetzov etal., cited by Janssens & Wery, 1987).

However, useful the stratification systems might be, the outstanding feature of malaria in most cases, is essentially that it is a localized problem (Janssens & Wery, 1987).

The malaria situation in Ethiopia has been reviewed by G/mariam (1988). Malaria is known as the principal cause of morbidity and mortality for long. As a result it has remained a bottleneck for socioeconomic development (Chand, 1965; Chang, 1962, Covell, 1957). For example, it has curbed the exploitation of the fertile and productive lowlands and as a result contributed its share to the over-population and consequent over cultivation of the highland plateaus (G/mariam, 1988). It is only a few decades since a new trend in population distribution can be identified. This is partly due to changes in economic policy which resulted in the development of commercial farming and urbanization in the Rift Valley and other low land areas (Schaller & Kuls, 1972; Kloos etal., 1988) and the launching of the malaria control programmes (G/mariam, 1988). This trend has intensified, in recent years, as a result of large-scale resettlement programmes of drought victims from eastern and northern provinces (Kloos etal., 1988).

Although research, on the epidemiology of malaria in Ethiopia is still in its infancy, it is generally believed that major variations in landscape and climate and complex human social forms create a wide range of macro-and microclimatic conditions and result in spatially varied incidence and distribution of malaria in the country (G/mariam, 1988). Recent estimates indicate that about 75% of the country, lying below 2000m in altitude, is malarious and over 64% of the total population inhabiting these regions is at risk (G/mariam, 1984).

Although the limiting altitude at which malaria transmission ceases cannot be fixed, because it varies to a considerable extent depending on such factors as the height of the reclaimed valleys (Janssens & Wery, 1987), it is generally considered non-existent at elevations exceeding 2000m (Covell, 1957; Schaller & Kuls, 1972). The temperate nature of the climate and seasonal distribution of rainfall ensure that man-biting densities and longevities of A. gambiae S.1. and A. funestus, under naturally prevailing conditions, are insufficient for stability of the disease (Krafsur, 1977). Where the microclimate supports vector life, as when A. gambiae populations shelter in warm huts in certain Ethiopian highlands (Turner, 1972), short-lived transmission occurs even at altitudes of 2400m (G/mariam, 1988). The brevity of the transmission season in most of these areas precludes the development of immunity and favours the incidence of periodic regional epidemics attended by a high mortality (Covell, 1957).

Due partly to the country's location between arid South-West Asia and humid central Africa, temperature, humidity and rainfall are strongly associated with altitude and relief (Kloos etal., 1988). The duration as well as the intensity of malaria in Ethiopia, as elsewhere in Africa (Bruce-Chwatt, 1984), in turn is closely related to the amount and distribution of rainfall throughout the year (G/mariam, 1988).

Owing to very limited rainfall, high evapo transpiration and absence of longstanding water bodies, the semi-arid and low-lying areas of the country are characterized by extremely short-lived malaria transmission (G/mariam, 1988). In the Danakil Depression and the Ogaden, anopheline mosquitos and other water-based vectors cannot survive away from the rivers, swamps and lakes, except in the irrigation systems, dams and water cisterns made for livestock and human use (Prothero, cited by kloos etal., 1988). In the proximity of permanent bodies of water malaria transmission is perennial and graded as meso-and hyperendemic in places below 1600m as opposed to hypoendemic zones which occur chiefly at elevations between 1600-2000m. Holoendemic settings occur primarily in the west around 1300m, where rainfall is present throughout most part of the year (Schaller & Kuls, 1972).

Seasonal variations in rainfall greatly influence the transmission of malaria in the country. The peak periods occur at the end of the "big" rains of June-September, when the inter tropical convergence zone brings southerly and southeasterly maritime air (Kloos etal., 1988), and around the short-lived showers of April-May (Covell, 1957; Schaller & Kuls, 1972). This is more or less similar throughout the country, except the Red Sea Coast, where all the rains fall in December and January (Kloos etal., 1988) resulting in a unique winter peak in malaria transmission (G/mariam, 1984; Schaller & Kuls 1972).

In general, except in relatively few lowlying localities along the course of permanent rivers or water bodies, malaria is markedly seasonal in incidence and of very low prevalence throughout the most densely inhabited regions of Ethiopia (Krafsur, 1977). The epidemic malaria that periodically scourges the highlands probably originates in more tropical lowland environment where it is stable and highly endemic. The causes for such major extensive epidemics, 1965, 1973 and 1981-82 epidemics, which occurred at intervals of 7 to 8 years, are considered to be climatological changes-such as exceptionally warm weather, prolonged rainfall or drought (Fountain etal., 1961, G/mariam, 1984).

The mosquito vector responsible for the occassional seasonal outbreaks as well as the periodic cyclical epidemics was A. gambiae s.l. (Fontain etal., 1961), which is the main malaria vector elsewhere in Africa (White, 1974), and an important vector of bancroftian filariasis (McConnel & Schmidt, 1973, McConnel etal., 1976) and Tataquine virus (Ota etal., 1976) in Ethiopia. Notorious for the rapidity of its population build-up even in short seasons, it is present at some or all times of the year over the whole of the country (O'Connor, 1967).

The A. gambiae complex, known to be composed of six member species in the Afrotropical Region (White, 1974), is so far represented in Ethiopia by two forms, as identified by cytogenetic and cross-breeding studies (Mekuria etal., 1982). These are A. arabiensis and the medically unimportant form, A. quadriannulatus. A. arabiensis is generally considered to be endophilic and anthropophilic (G/mariam, 1988), although an incipient tendency to feed indoors and rest elsewhere has been reported (Krafsur, 1977). In certain localities it has also been found to be exophagic (G/mariam, 1984).

The A. funestus group is widely distributed and is known to be the second important vector of malaria in the country (G/mariam, 1984), sometimes even dominating in certain areas of endemic malaria (Krafsur, 1971; Turner, 1972), owing to its tendency to breed in large, rather more shaded and permanent bodies of water (O'Connor, 1967).

Of the 42 anopheline species so far recorded in Ethiopia (G/mariam, 1988), A. nili and A. pharoensis are considered to have secondary importance in malaria transmission. A. nili, found localized in certain western and southwestern lowlands of the country (O'connor, 1967), has been found to be a potent vector of malaria in Gambella area, where it is known to exhibit endophagic and exophilic pattern of behaviour (Krafsur, 1977). A. pharoensis is of wide occurrence n Ethiopia and, breeding as it does like A. funestus (O'connor, 1967), it is regarded as a weak vector in tropical Africa, as a whole (krafsur, 1971), though it has naturally been found infected in Egypt, Nigeria and Kenya (DeMeillon, 1948; cited by O'connor, 1967) and so is claimed in Ethiopia at one time or another to be a vector though this remains to be established. Other anopheline species categorized as incidental vectors, at one time or another, include, A. coustani, A. zeimanni, A. paludis and A. d'thali. A. culicifacies adenensis has also been circumstantially incriminated as a vector in the southern Red Sea Coast (G/mariam, 1988).

All four species of human malaria parasites occur in Ethiopia. P. falciparum is the dominant species followed by P. vivax, P. malariae and P. ovale (G/mariam, 1984). P. ovale was reported first in 1938 and this was confirmed in 1969 in several parts of the country (Armstrong, 1969). It is claimed that the above precontrol pattern of encounter seems to be disrupted and a decrease in prevalence of the disease has been achieved (G/mariam, 1988). The condition, however, seems to be otherwise. In recent years the disease has taken an epidemic form and it is believed that most of the outbreaks were attended with heavy morbidity and mortality and the malaria parasite largely responsible was P. falciparum. In fact owing to the urgency of the problem which was aggravated by the 1972 & 1973 man made & natural calamities, a technical committee was assigned by the Ministry of Health to study the malaria situation (Report of Technical Committee on the Malaria situation in Ethiopia (RTCMSE) Oct. 1981 (Eth. c).

Although very limited studies of P. falciparum drug sensitivity tests have been made in the country so far (Dennis etal., 1974; Armstrong etal., 1976; G/mariam etal., 1982), the recent report of chloroquine-resistant strains from Southern and Western lowlands bordering Kenya, Somalia and Sudan (T/haimanot, 1986) coupled with the presence of drug-resistant malaria in the neighbouring countries, may indicate the risk of importation of resistant strains and the spread of resistant local strains

(G/mariam, 1988).

The history of organized control effort against malaria in Ethiopia dates back to the 1950's, when three Control Pilot Projects were launched with the aim of reducing or completely eliminating the disease from the fertile lowland areas (G/mariam, 1988). The projects have achieved an acceptable level of success in the interruption of transmission and also escaped the sequel of the 1958 epidemic (Fontain etal., 1961). Owing to the shock generated by the outcome of the epidemics of the time and inspired by successes attained in the Pilot Project areas and elsewhere in the world, a Malaria Eradication Service was established in 1959. In conjugation with this a Malaria Eradication Training Center was set up at Nazareth to train a staff of subprofessional workers. A classical eradication programme was launched in the most populated North and Central region in 1966. Nevertheless, later changes in emphasis on Malaria Eradication Programmes by WHO, has led to a change in concept and strategy and since 1972 the name of the Project has been changed to Malaria Control Programme (G/mariam, 1988), and recently to Malaria and other Vector Borne Diseases Programme.

The malaria service maintained its autonomy within the Ministry of Health and implemented a long-term antimalarial programme and at the moment control activities are carried out in most of the malarious areas of the country (G/mariam, 1984), excepting the war-stricken northern regions. Control measures include, drug distribution and the application of DDT of one or two spray rounds per year, depending on the endemicity of the area (G/mariam, 1984). It is also realized that in addition to DDT, which is believed to have been in use for over 20 years, other insecticides, such as Abate, Actellic and Ficam are being employed and that the effectiveness of DDT is becoming doubtful in some parts of the country (RTSCM, 1981E.C.). Despite the continuing intervention efforts and significanct success in many areas (G/mariam, 1988), perhaps owing to undercoverage and other technical and operational matters (RTSCM, 1981 E.C.), variable levels of endemicity persist and localized outbreaks are still a common occurrence (G/mariam, 1988) not only in places where residual spraying is withdrawn, but also in areas under operation (RTSCM, 1981). Further, factors contributing to the resurgence and spread of malaria are on the rise (G/mariam, 1988).

If one looks at the "Third World" in general, and Ethiopia in particular, one sees changes everywhere-deliberate changes in the environment by man, geared to alleviate the living conditions of the population. A substantial number of socio-economic development endeavours, notably large-scale irrigated farming, are currently underway and extensive areas have been identified for development in all major river basins (Kloos etal., 1988). Nevertheless, these far reaching ecological upsets brought about by development projects have frequently altered disease patterns and are well known in the dissemination of water-related and density dependent diseases, such as malaria (Youdeowei and service, 1986; Ward, 1977) and the indicated disease is a potentially serious health hazard in several Ethiopian lowland projects (Kloos etal., 1988).

The construction of irrigation schemes and the establishment of state farms, with the concomitant concentration of extensive labour force, having dissimilar social structures and customs (Youdeowei & Service, 1986), add new elements to the existing factors which affect the general pattern of malaria epidemiology, as are resettlement and villagization programmes, influencing the prevalence, severity and spread of the disease (G/mariam, 1988).

Another equally important factor involved in altering the ecology of malaria in Ethiopia, as in many parts of tropical Africa (TDR, 1988), is the rapid and unplanned urbanization - which is manifested in the increasing migration of people from villages to towns (Surtees, 1971). Available statistics suggest that urban population growth rates in Ethiopia have been consistently higher than those for the total population (Kloos etal., 1988) and malaria has become a feature of many urban centers in the country (G/mariam, 1988).

Malaria has been known to occur in several Ethiopian towns in undetermined magnitude. It has been described mostly in the lowlying towns of the country (WHO; cited by Bruce-Chwatt, 1985) and the urgency of the problem is currently being stressed (WHO, 1986; G/mariam, 1988).

The selection of a control strategy against malaria in urban settlements requires basic epidemiological information. Unfortunately, no epidemiological studies have been carried out in urban areas of Ethiopia, and only a few observations have been made in the small town of Gambella (Krafsur & Armstrong, 1978). Most of the existing information on the prevalence of malaria in some Ethiopian towns comes from the records of cases maintained by Malaria Control Programme Centers and other health services. Nevertheless, neither these types of sources nor other clinic-based epidemiological studies permit conclusions about either the distribution of disease or the utilization of health services among the representative urban population (Kloos etal., 1988).

To generate some information on the epidemiology of urban malaria in Ethiopia, cross-sectional surveys were carried out in the town of Naazreth, about 90km southeast of Addis Ababa. The study included parasitological, serological, entomological and environmental surveys. The aim was to,

-establish autochthonous transmission of malaria, the parasite species responsible, and the mosquito vectors involved;

-estimate the level of prevalence of the disease in the community;

-investigate factors associated with or contributing to the transmission of the disease in the urban ecology and identify feasible control measures.

2. MATERIALS AND METHODS

2.1. The Study Area

Founded around 1920, due to the building of a railroad line linking Djibouti and Addis Ababa, the town of Nazreth (formerly called Adama) is located in the Central region of the Rift Valley, about 90kms South East of Addis Ababa,the capital. Located in the Rift Valley, at an average altitude of 1600 (range 1550-1600), it has a typical arid climate and is constantly engulfed in dusty wind. (Fig. 1).

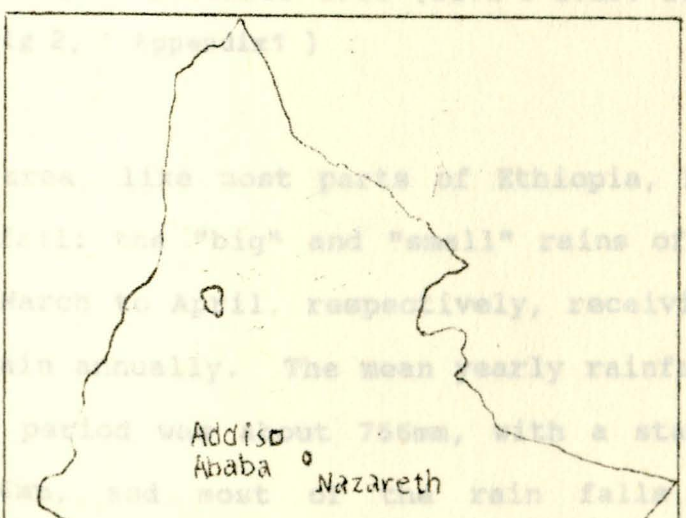

FIGURE 1

Map of Ethiopia with Location of Study Area

Past meteorological data (Institute of Agricultural Research Agro Meteorological Service-IARAMS) from the nearest town of Melkasa, 15kms south of the town, for the years 1977-1987 (Appendix 1) indicate that the area is characterized by relatively wide annual and diurnal ranges of temperature and a restricted rainfall with a clear single maximum. The yearly average maximum temperature of 30.4°C in May immediately precedes the main rainy season and a secondary peak of high temperature of 28.5°C in October occurs at its end. The mean daily maximum temperatures were 32.3°c in May and 28.3°C in October of 1988, while the mean daily minimum temperatures for these were 16.1°C and 11.8°C, respectively. The coldest months are November-January, with a minimum temperature of 7°C recorded in November 1988 (11.2°C-aver. for 1977-87) (fig 2, (Appendix1)

The area, like most parts of Ethiopia, has two periods of rainfall: the "big" and "small" rains of June to September and March to April, respectively, receiving about 500-900mm of rain annually. The mean yearly rainfall over an eleven-year period was about 756mm, with a standard deviation of 163.6mm, and most of the rain falls between June and October. (Appendix 1).

During the wet season there are innumerable temporary collections of surface waters of various sizes and durations.

Water may persist long after the rains in the burrow pits from which clay is extracted for building. Surrounding the town, specially along the South East and South West perimeter, there are several semi-permanent swamps and cultivable land. Private plots of land around human habitations within the town, particulrly in the peripheral administrative divisions (Kebeles), are also cultivated. The soils range from sandy to loamy and are intensively cultivated. The area grows teff, maize, millet, etc. and is also favourable for growing vegetables and fruits like onions, potatos, oranges, papaya, grapes and the like. With respect to vegetation, the region is characterized by spiny-leaved plants, dominated by Acacia, typical of arid areas.

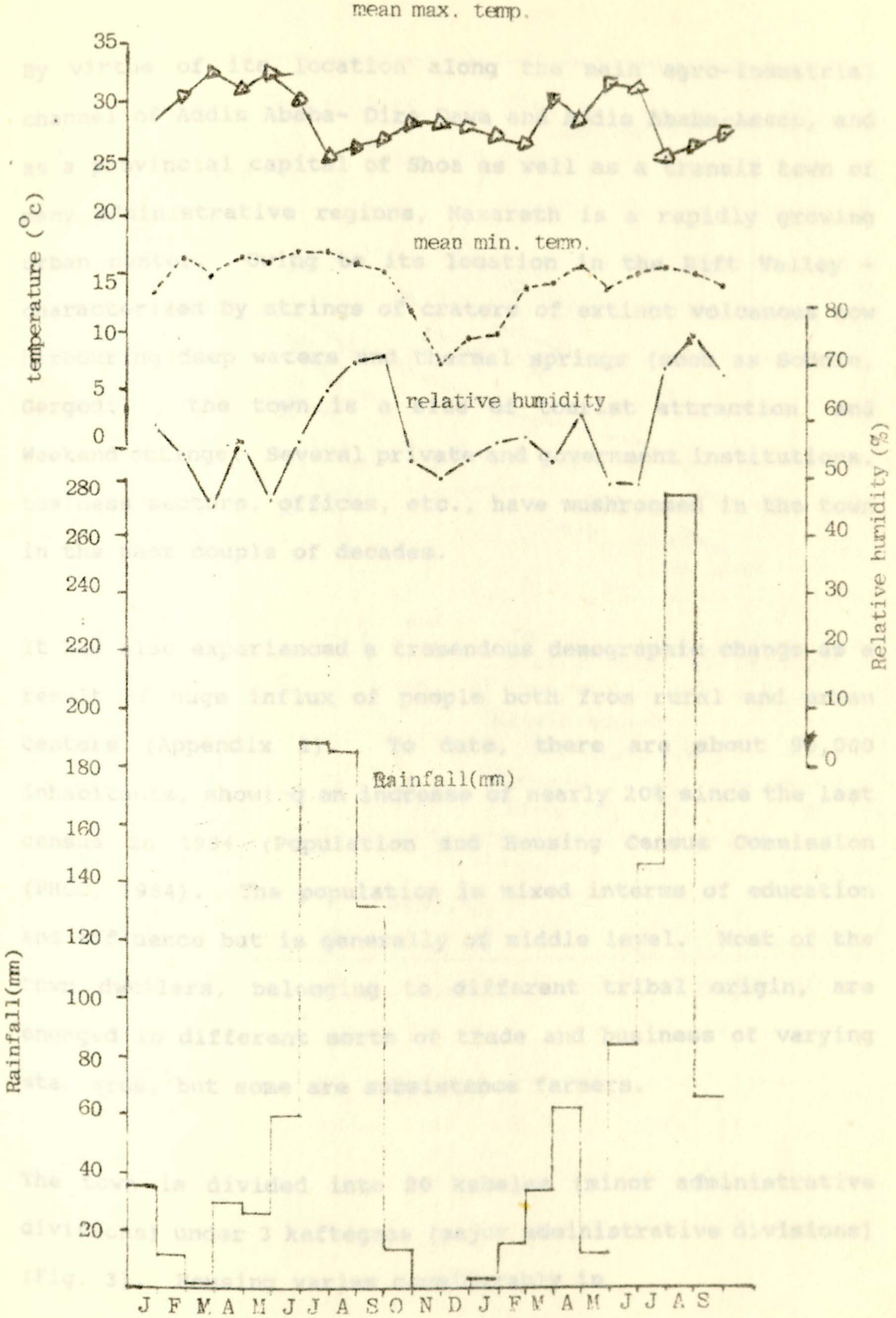

Fig. 2 The distribution of mean monthly, minimum and maximum temperature, rainfall as well as

By virtue of its location along the main agro-industrial channel of Addis Ababa- Dire Dawa and Addis Ababa-Assab, and as a provincial capital of Shoa as well as a transit town of many administrative regions, Nazareth is a rapidly growing urban center. Owing to its location in the Rift Valley - characterized by strings of craters of extinct volcanoes now harbouring deep waters and thermal springs (such as Sodore, Gergedi) , the town is a site of tourist attraction and Weekend outings. Several private and government institutions, business sectors, offices, etc., have mushroomed in the town in the past couple of decades.

It has also experienced a tremendous demographic change as a result of huge influx of people both from rural and urban centers (Appendix 2). To date, there are about 98,000 inhabitants, showing an increase of nearly 20% since the last census in 1984 (Population and Housing Census Commission (PHCC, 1984). The population is mixed interms of education and affluence but is generally of middle level. Most of the town dwellers, belonging to different tribal origin, are engaged in different sorts of trade and business of varying standards, but some are subsistence farmers.

The town is divided into 20 kebeles (minor administrative divisions) under 3 keftegnas (major administrative divisions) (Fig. 3). Housing varies considerably in

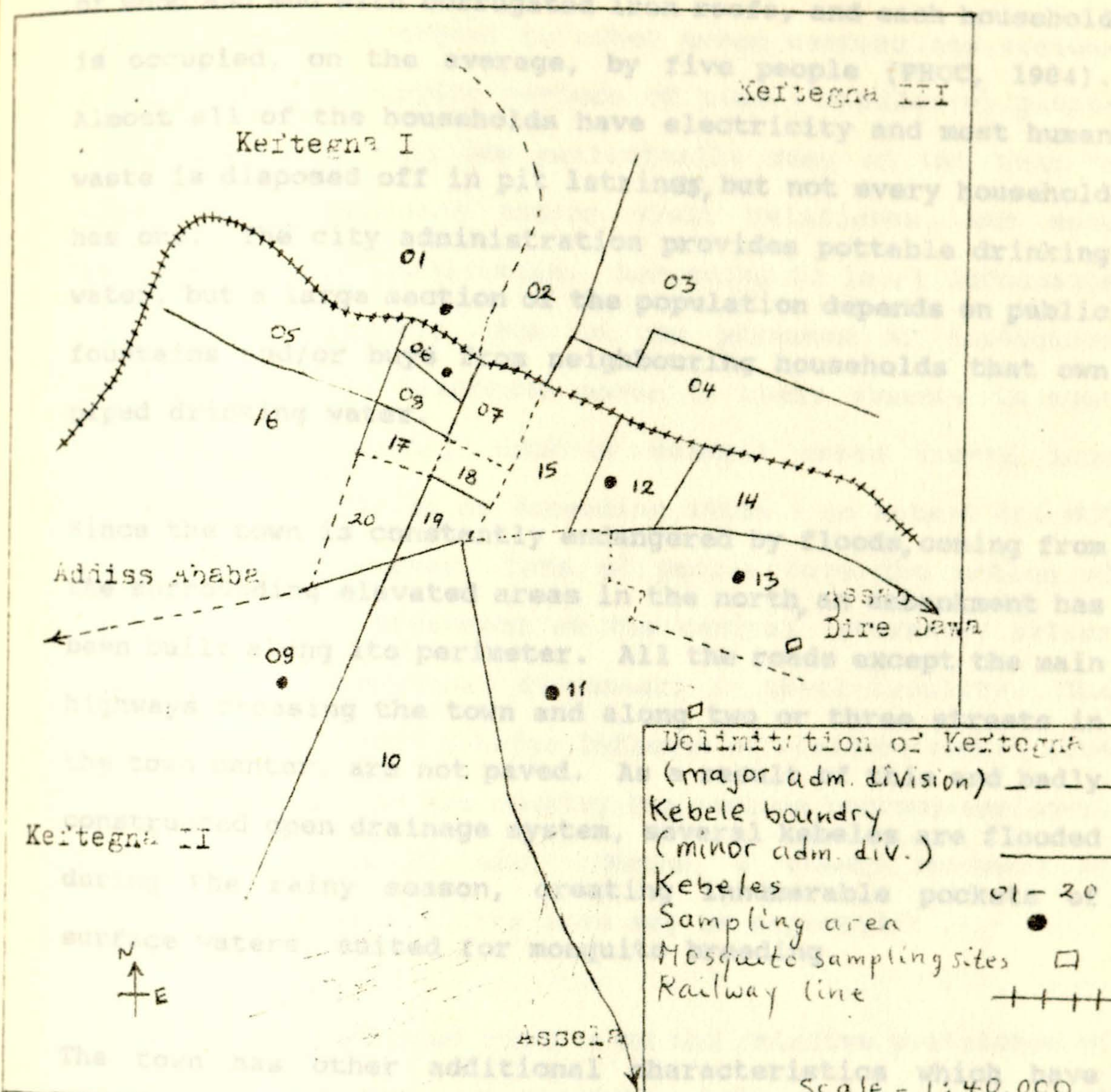

Fig.3. Asketch map of the town of Nazareth showing the study zones

quality. Most of the dwellings are rectangular, constructed of wood and mud with corrugated iron roofs, and each household is occupied, on the average, by five people (PHCC, 1984). Almost all of the households have electricity and most human waste is disposed off in pit latrines, but not every household has one. The city administration provides pottable drinking water, but a large section of the population depends on public fountains and/or buys from neighbouring households that own piped drinking water.

Since the town is constantly endangered by floods, coming from the surrounding elevated areas in the north, an embankment has been built along its perimeter. All the roads except the main highways crossing the town and along two or three streets in the town center, are not paved. As a result of this and badly constructed open drainage system, several kebeles are flooded during the rainy season, creating innumerable pockets of surface waters, suited for mosquito breeding.

The town has other additional characteristics which have malariologic implications. First, the major highways from Addis Ababa-Assab, Addis Ababa- Dire Dawa, Nazareth-Assela, result in the ingress of temporary residents (truck drivers, labourers, merchants, etc.) returning from areas known to be endemic for malaria. In general, there appears to be a constant stream of people in and out of the town of Nazareth.

The town dwellers travel to other urban centers and various rural areas for varying periods of time. Similarly, people of the outlying villages periodically stay in the town to market their products and/or visit relatioves, but more important to seek medication. According to local informants and personal inquiry, due to the presence of inadequate permanent malaria treatment posts or their absence in most parts of the region, lots of malaria cases travel long distances - as far as or exceeding 25kms - to attend the MCC in the town. Moreover, lots of people have the notion of obtaining better treatment at the central laboratory rather than a one-or-two-manned dispensary in their locality. The town is also subject to large influxes of visitors coming from different corners of the country for various reasons-seminars, business, excursions, etc. Hence, a steady movement of carriers of malaria in the town may be expected.

There are no documented reports on the relative prevalence of malaria in the town of Nazareth, but according to local informants and the records of health facilities in the town, there is a moderately high prevalence of malaria- P-falciparum being the dominant species. Statistical reports for the peak transmission periods (Sept.-Nov.) of 1985-86, 1986-87, and 1987-88 show over 2000, 3500, and 4300 cases admitted to the local Malaria Control Programme Center, H/mariam Mammo Hospital and a government health center.

Medical care for malaria patients is provided by the local Malaria Control Programme Center, which is a primary reference center, as well as by H/mariam Mammo Hospital, one health center and several private dispensaries. No systematic antimosquito household spraying programme has been undertaken in Nazareth in recent years, except in selected parts of the town around 1981/82 when an epidemic flared up in the area. The principal control measures at the time of the survey consisted of environmental management (drainage, filling, etc), periodic application of oil (rarely Abate) to larval habitats and chloroquine and/or primaquine distribution to confirmed cases, but sometimes for presumptive treatment also. During the peak transmission season, chloroquine was being distributed en mass for those attending the center (with or without taking blood samples) and to febrile cases by canvassing from house to house. Occassionally, Fansidar was being prescribed for refractory cases. Inspite of the antimosquito control measures, coupled with drug distribution, malaria transmission appears to be unabated. Infact, as indicated earlier, there is an increasing trend in malaria prevalence and there is no documented thing on any effort made to study the cause and effect relationship of malaria so as to implement situation-specific control measures.

2.2 The study population

The surveys were carried out from July 1988 to May 1989 in six kebeles, two from each keftegna, by purposive sampling (Fig. 3). This was done by a house-to-house visit with a view to collecting blood samples and gathering demographic information from the members of the households selected with the help of a questionnaire (Appendix 3). The description of the selected Kebeles is presented in Table 1.

For calculating the sample size the method of Swaroop (1966) was adopted. Briefly, the desired sample size was determined visually from tables containing a series of confidence intervals at a specified probability levels (95%, 99%) corresponding to varying sample sizes and estimates of the level of prevalence. For example, if the percentage has been estimated to be 20, the column head 20% is consulted for the confidence limit corresponding to tentative sample sizes. Accordingly, it was aimed to take 1000 individuals (corresponding to 3-7 confidence interval with a 99% probability level), plus an allowance of 10% for non-response, in the population. This was based on the 3.39% estimated prevalence rate of the disease in the town (obtained from MCP head quarters), which was raised to 5%

Table 1. Description of the six kebeles (zones)

	Kebeles selected					
	01	06	09	11	12	13
- Area in hectars	159	17.5	390.4	517.5	35.9	84.5
- Population (estimated)	3339	4341	6956	10433	4881	3504
- Elevation (relative)	High	High	Low	Low	High	low
- Cultivated land in proximity to or around human habitations	+	-	++	++	-	++
- Vegetation around human habitations	+	-	++	++	-	++
- Permanent of semi-permanent water collections	-	-	++	++	-	++
- Public fountains	+	+	+	+	+	+
- Large scale construction projects institutions or newly constructed houses	±	±	+	++	-	+
- Paved roads	-	-	-	-	-	-

Key - absent
± may or may not occur
\+ few
++ abundant

considering those cases which seek medication elsewhere than the local MCP center.

The households to be sampled from each kebele were systematically drawn, taking every 20th household from a random start. The study enrolled all the consenting members of the households present at the moment. For every individual included in the sample, various personal and socio-economic information were sought-listed on the questionnaire (Appendix 3).

2.3. Parasitological Survey

Parasite prevalence surveys were carried out in Jul./Aug., 1988; Sept./Oct., 1988; Nov./Dec., 1988; and Apr./May, 1989. Surveys were made by a house-to-house visit, without a second visit to the homes of absentees. At each survey, thick and thin blood films, made on the same slide, were collected, stained with Giemsa, (in the field and in lab. in Addis Ababa) and examined for parasites.

For this the finger was cleansed with an alcohol-moistened swab, dried with a piece of dry cotton, punctured with a disposable blood lancet and after wiping off the first drop of blood, thick and thin films were made. The blood smears were air-dried in a horrizontal position and identity numbers marked on the thin films. The thin films were then fixed in 100% methanol for 30 seconds, and the smears stained with 3% Giemsa for 20 minutes and examined for parasites. The thick films were scanned for the presence of parasites and, when present, the species was identified by examining the thin film. Smears with no parasites detected in 200 oil immersion fields were classified as negative. For those found positive, the number of fields containing parasites was recorded.

Test for homogeneity in malaria prevalence in the four surveys undertaken and between the kebeles was undertaken by the chi-square analysis. Chi-square analysis was also used to assess any significant difference in malaria prevalence between the different age-groups.

2.4. Serological Survey

2.4.1. Collection of Blood Samples on Filter Paper

Blood samples for antimalarial antibodies were collected from a subsample of individuals, aged ten and above, included in the second parasitological survey, without any distinction between infected and uninfected ones. This was done by finger-pricking on Whatman no. 3 filter paper as described by Mathews (1978). In brief, the filter papers were cut into strips and imprinted with two circles about

12mm in diameter. From the same prick made on the individual for the preparation of blood films, blood was let to flow into the paper, held with the printed circles uppermost. The blood was allowed to soak in and spread out on its own until it filled the inscribed circles. Each set, taken from the same subject, was then marked with reference number on the space available at one end of the strip. The filter papers were protected from dirt and flies by standing them on their sides inside a slide box, and allowed to dry completely. After thorough drying, the papers were bundled in groups of ten arranged in such a way that the blood spots of one filter paper lied adjacent to the free long end of another. They were then sealed in the self-sealing polythene bags and stored at 4°C in the field until it could be stored in the deep freeze (-20°C), at the Department of Biology, Addis Ababa University.

2.4.2. Preparation of Plasmodium falciparum Antigen Slides

P. falciparum antigen slides were prepared according to Sulzer etal. (1969). Briefly venous blood was obtained from patent infections of P. falciparum from individuals who attended the Malaria and other Vector Borne Diseases Center in Addis Ababa. The parasitized blood sample was cultured in vitro in flat-bottomed tissue culture petridishes by using the Candle-Jar method of Trager and Jenssen (1976), for the maturation of parasites.

After 26 hr of incubation at 37°C, by which time most of the ring-stage parasites would mature into schizonts (established by repeated thick-smear examinations), the blood containing schizonts was removed from the wells of the petridishes by centrifuging at 1000xg for 10 min and discarding the supernatant. The cells were resuspended in phosphate-buffered saline (PBS), centrifuged and the buffer discarded. Resuspension and centrifugation was repeated three times. The erythrocytes were then resuspended in PBS and parasite concentration per drop of suspension adjusted to about 20 per oil immersion in a thick smear preparation. Eight antigen spots were made on each slide by dispensing about 10 microliters of the infected red blood cell suspension to each antigen spot. The smears were allowed to dry at room temperature, wrapped in tissue paper, placed in groups of 10 in self-sealing polythene bags with a dessicant (calcium chloride) and stored at -20°C until used.

2.4.3. Filter Paper Elution

Blood samples absorbed on filter papers were eluted in PBS-Tween 20(8g Nacl; 0.2g KH_2PO_4;2.9g $Na_2HPO_4 \cdot 12H_2O$;0.2gKCl;0.5ml Tween 20;0.2g NaN_3;1l Dist.H_2O-PH 7.4) according to Evengard etal, (1988). Just before the test the plastic bags containing the filter papers were removed from the freezer and allowed to warm to room temperature. Discs of about 10mm in diameter of the blood soaked circle were cut from the filter papers with scissors, placed singly in glass tubes, and 0.4ml PBS (pH7.4) containing 0.05% Tween-20 was added. The discs were pressed into the buffer with a wooden applicator stick and allowed to elute overnight at 4°C. The discs were then squeezed with the applicator against the tube wall before being discarded. From the dark brown eluate recovered, which corresponds to a dilution of about 1:16 (Bruce-Chwatt, 1985; Kagan, 1972) further 4-fold dilutions were made.

2.4.4. Performance of the Indirect Fluorescent Antibody (IFA) Test.

The assay for malaria-specific antibodies using P. falciparum antigens was performed by the IFA test according to WHO (1974). On the day of testing, the antigen slides were removed from the deep freezer and allowed to warm to room temperature. The slides were labelled and the antigen spots delineated using nail-polish. They were then placed on wet tissue paper spread in a shallow enamel pan, with the smear side up. About 30 microliters of 1:16 and 1:64 dilutions of the test sera in PBS (PH7.4) were dispensed on the labelled antigen mounts. The slides were then covered and incubated for 30 min at 37°C .

After incubation, the slides were rinsed with PBS and placed in slide chambers in PBS on slowly moving rotator for 15 min. They were then removed from the washing buffer and the excess buffer poured off and the slides shaken dry. Fluorescein isothiocyanate (FITC)-tagged anti-human globulin (Goat antihuman IgG, Sigma, USA) diluted 1:32 and containing 0.1 percent Evan's blue, was added in 30 microliter volumes over all antigen spots. The slides were placed on wet filter papers, covered and incubated at 37°C for 30 min. The preparation was then rinsed and washed with PBS by rocking for 15 min, air-dried at room temperature and mounted in 10 percent glycerol in PBS (PH9.0). Cover slip was placed over the preparation and the slides read by using a fluorescence microscope, Model BH_2-RFL mounted on

Olympus microscope BHS (Olympus Optical Co. Ltd., Tokyo, Japan). Sera positive at 1:64 dilution were further diluted to determine the endpoint. The titer of each test serum that showed difinite fluorescence was taken as the highest dilution. A titer of $\geq$1:16 was used as a criterion of positivity (Bruce-Chwatt, 1985; Sulzer etal., 1969). The geometric mean of the reciprocal titer was determined by using the formula GMRT = antilog $\sum f(\log x)/N$, where x is the reciprocal titer; N-number of sera tested, f-number of sera with a given titer. The reciprocal of this number is presented as the mean FA response and roughly indicates the malaria experience of the population (Collins etal., 1968).

Chi-square values were calculated to determine possible differences in serological responses between individual residents in different Kebeles, and age-groups.

2.5. Entomological Survey

Indoor resting mosquitos were collected by aspiration and pyrethrum-spray ("Knockdown" collection) in the morning hours (05.30-7.00A.M.). For this, malaria-positive households were identified in areas bordering on breeding sites inorder to obtain adequate numbers of mosquitos for salivary gland dissection and for immunoassaying. Collections were made in Sept./Oct./ Dec. 1988 and October, 1989 - The presumed time of main transmission season immediately after the peak mosquito density. The methods employed are those recommended by WHO (1975) for studies of short duration, as the present one. The households consisted of native huts (Tukuls) and rectangular houses of wood and mud walls and corrugated iron roofs. The tukuls were rounded in shape and of varying diameters (2-4m), with walls consisting of a framework of posts and closely packed twigs - plastered from inside with mud, an earth floor, and a conical roof of thatch. ~~mud, an earth floor, and a conical roof thatch~~. Most of the people in the area owned cattle, goats, etc., usually kept in open enclosures in proximity to the house or inside a small section of human habitations separated by loosely fit sticks.

2.5.1. Aspirator Collection and dissection for sporozoites. Anopheline mosquitos were searched on the walls and objects inside the house by using flashlights and aspirated. The collected mosquitos were placed in paper cups and delivered to the temporary examination post in the field. Salivary gland dissections were performed on the day of collection. For this mosquitos were killed with chloroformtransferred to plastic petridishes disected under a dissecting microscope, coverslips applied on the extracted salivary glands and examined under high power microscope. A number of mosquitoes ~~under a dissecting microscope~~ comprising of freshly fed and half-gravid individuals were kept in paper cups, humidity maintained by applying wet cotton pads over the cups, in order to estimate the ovipositional interval.

2.5.2. Pyrethrum Space-Spray Collection

Indoor-resting anophelines were collected from native huts (Tukuls) and rectangular houses by spreading white sheets on the floor and horrizontal objects and fogging with 1% pyrethrum solution in Kerosene, dispensed from a hand pump. Tukuls were fogged first from the outside to minimize mosquito escape. The specimens were collected 10 min after spraying using entomological forceps and kept in labelled pill boxes lined with moist paper, for later processing.

Mosquitos captured by both methods were used as a measure of mosquito density and for the determination of salivary gland infections as well as for bloodmeal identification. The anophelines collected were identified morphologically by using the key prepared by Verrone (1962) and inorder to estimate quantitatively man-vector contact from the averaged values of house or hut density, note was made of the abdominal condition (unfed, fed, half-gravid, gravid, (WHO, 1975).

2.5.3. Human bloodmeal identification and detection of Plasmodium falciparum and P. vivax infections in mosquitos by Enzyme-linked Immunosorbent Assay (ELISA).

Few specimens of A. gambiae s.l. and A. pharoensis which could not be dissected for salivary gland sporozoites were used in the test. The mosquitos were stored in pill boxes at room temperature in several layers of tissue paper until used.

2.5.3.1. Preparation of mosquitos for Bloodmeal and Sporozoite ELISA

Mosquitos were first prepared by cutting them transversely at the junction of the thorax and abdomen, after removal of their appendages, under a dissecting microscope. The "head-thorax" and "abdomen" portions of these mosquitos were prepared and tested separately- the former by "Sandwich sporozoite ELISA" and the latter both by sporozoite ELISA, to detect Oocyst-associated sporozoites, and by the direct bloodmeal ELISA. For this, the head-thorax portions of individual mosquitos were placed in the wells of a 12-welled white porcelain microtissue grinders, to which 50 microliters blocking buffer (BB) (0.01M PBS—8g NaCl; 0.2g KH_2PO_4;2.9g $Na_2HPO_4.12H_2O$;0.2gKCl;0.5ml Tween20; 1l dist. H_2O-), $PH_{7.4}$, with 1% Bovine serum albumin (BSA), 0.5% boiled casein, 0.01% thimersol, and 0.002% phenol red) containing 0.5% Nonidet P-40 (NP-40) (all reagents from Sigma, USA), was added. The triturates were transferred to 0.5ml microcentrifuge vials, and 250 microliters BB, without NP-40, was added.

The abdomen portions were first ground in 50 microliters PBS; 10-microliter aliquots, further diluted in PBS (1:50), were used for the bloodmeal ELISA and the remaining 40 microliters were used in the sporozoite ELISA. To the 40 microliter triturates 60 microliter BB containing NP-

40 was added initially and 200 microliters BB without NP-40 was subsequently added to bring the final volume to 300 microliters. The ensuing assay procedures of direct bloodmeal and Sandwich sporozoite ELISA's were based on that of Beier etal. (1988) and Wirtz (unpublished document), respectively.

2.5.3.2. The Sandwich Sporozoite ELISA

Disposable polyvinyl chloride, U-shaped, immulon 2, 96-well, microtiter plates (Dynatech Labs., USA) were coated with 50 microliters of the appropriate monoclonal antibody (MAB) solution. For P. falciparum ELISA, 2 microgram MAB 2A10 (Kirkegaard and Perry Labs. USA) per milliliter PBS was used; and for P. vivax, 1.0 microgram MAB NSV No3 (Naval Medical Research Unit, Bethesda) per ml PBS was used. The plates were covered and incubated overnight at room temperature (All incubations described hereafter were made at room temperature). The well contents were emptied and the wells filled with BB and incubated for 1hr. After removal of the buffer, a 50 microtiter aliquot of each mosquito triturate was added to wells on both P. falciparum and P. vivax test plates, in duplicates. The plates were covered and incubated for 2hr, then aspirated and washed 2 times with PBS, containing 0.05% Tween-20 (PBST) using a multiple dispenser. Then, 50 microliter aliquots of the homologous horseradish peroxidase-conjugated MAB (0.05 microgram/50 microliters BB/well (Kirkegaard & Perry labs.) were dispensed to each well of the respective plates. Following 1hr incubation, the plates were emptied and after 3 washes with PBST, 100 microliters of Orthophenylene diamine (OPD), a peroxidase substrate was added to each well. The plates were covered, placed in the dark and left for 30 min, after which time 25 microliters of 8N H_2SO_4 was added to each well to stop the reaction. Absorbances at 492nm were then read in a through-the-plate ELISA microtiter plate reader (Cambridge Life Sciences, England).

One positive and seven negative controls were included on every ELISA test plate. Negative controls consisted of seven male A. gambiae specimens prepared in the same way as the test samples. Positive controls consisted of recombinant $R_{32}tet_{32}$ P. falciparum circumsporozoite (cs) proteins ( ) and synthetic P. vivax CS peptides. Samples were considered positive if absorbance values exceeded two times the mean absorbance of seven negative controls.

2.5.3.3. The Bloodmeal ELISA

Fifty microliter mosquito abdominal triturate of each diluted sample, prepared as described earlier, was dispensed into separate wells of duplicate microtiter plates; 50 microliter of control positive material consisting of human serum was placed in one well of each strip. Seven control negative wells contained abdominal triturate of seven male A. gambiae s.l, prepared as test samples. The plates were covered and kept overnight at room temperature. The well contents were then aspirated, washed 2 times with PBST, and 50 microliter of peroxidase-labelled antihuman IgG, diluted 1:2000 in 0.5% boiled casein, containing 0.025% Tween 20, was added to each well. After 1hr wells were emptied, washed 3 times with PBST, and then 100 microliters of OPD, a peroxidase substrate, was dispensed to each well. The plates were covered, placed in the dark and the reaction stopped after 30 min by the addition of 25 microliter 8N H_2SO_4 to each well. The absorbance of the contents of the wells at 492nm was determined with an ELISA reader. All sample wells with absorbance exceeding the mean plus 3 times the standard deviation of seven negative control wells, were considered as positive. A visual assessment of the plates was also made and any wells with more yellow to orange-brown colour than the control negative wells were considered as positive.

.6. Environmental Parameters

A number of surveys were carried out to locate and assess possible vector breeding sites in the sampling areas selected. Some of the larvae and pupae collected at such surveys, and during searches made along with the parasitological surveys, were transported to the laboratory in the Department of Biology, and the adults identified after emergence.

Information regarding water supply, its uses and distribution were obtained from records of the city council, by direct observation and interview of the members of the households sampled. Antimalarial practices were also ascertained by direct questioning on the use of anti-mosquito practices and antimalarial drugs. The interviews were based on a questionnaire designed for the study (Appendix 3).

3. RESULTS

3.1. The Study Population

A total of 3890 (approx. 4% of the total population) persons were enrolled in the study from 695 households visited. This was achieved by enrolling 903 persons from 168 households during the first survey, 1018 individuals from 169 households in the second, 1038 persons from 179 households, in the third, and 931 individuals from 179 households in the fourth survey. The distribution of the households and individuals sampled in relation to the sampling areas as well as the population composition by age and sex is presented in Table 1.

The population composition and sex ratio observed in the study population was, in general, similar to the 1985/86 census for the town (obtained from the City Council) and also similar to the reports for various towns in Ethiopia (HPCC, 1984). The population was characterized by relatively young individuals (58% under 15 years) and a very small number of older age groups (1.2% 65 years and older). The sex ratio for all ages in the six kebeles combined was 1:1.6 males to famales.

Table 2 Description of the study population

Kebeke	Aver. No. of house-holds/ survey	No. of people enrolled Aver./Survey Male	Female	Aver./ household	No. of individuals sampled in age grou <1yr Aver./ survey	2-4 Aver./ survey	5-9 Aver./ survey	10-14 Aver./ survey	>15 Aver./ survey
01	31	68.25	100.75	5.5	7.75	23.5	37.75	34.75	65.25
06	24.75	57.5	86	5.8	8.75	16.5	34.25	32	52
09	35.5	81.5	128.5	5.9	13	27.75	39.5	40.25	89.5
11	51.5	96.25	164.5	5.1	10.75	31	60.75	44.5	113.75
12	16	37.5	54.3	5.7	3.5	11.75	20.25	16	40.25
13	15	37	60.5	6.5	4.75	9.75	17.75	16.75	48.5
Total	173.75	378	594.5	5.6	48.5	120.25	210.25	184.25	409.25

3.1.1. Malaria Experience of the Study Population

Malaria was experienced by individuals from all the six kebeles, however, most of them belonged to ~~the~~ ~~were~~ the kebeles 11,09, and 13, in that order. A total of 733 individuals reported to have had malaria in the past four years, 560 of which claimed to have contracted the disease in 1988/89, period of the present survey. Of the 560 individuals, 36.7 percent reported to have had a malaria attack or febrile episodes within the previous two months and 24.5 percent within the previous two weeks. Most of the people with a history of malaria (68.1%) said that they obtained treatment from the local Malaria Control Center (MCC), 11 percent from other government dispensaries and 20.9 percent from private practitioners. The responses obtained as to why people buy antimalarial drugs from Pharmacies or visit private practitioners when they could get the same drug from MCC free of charge or from other government dispensaries at a cheaper cost were varied - these included the long hours of waiting on long queues and the fact that the MCC people often miss the infection when private practitioners diagnose malaria most of the time - particularly during the peak transmission season.

Furthermore, under emergency situations private dispensaries were more convenient as the government health services are more often closed.

With regard to self protection against malaria, none of the houses selected had mosquito proofing and no one used bednets or other mosquito protective materials. Nevertheless, some respondents reported to have used fumigants to avoid biting nuisance. Only 25 individuals reported to have taken antimalarial drugs for prophylaxis in the past two years or so.

3.2. Parasitological Survey

A total of 3890 blood smears collected, from individuals of six kebeles and comprising all age groups, during the period July/August, Sept./Oct., Nov./Dec. 1988 and Apr./May, 1989 were examined. Only 108 (2.8%) individuals were found to be infected with malaria parasites. The parasite species detected were P. falciparum and P. vivax (Table 3). Some of the infections could not be identified definitely owing to the intake of antimalarial drugs by the infected individuals. Most of these individuals reported to have been treated at the local MCC, although confirmation of the reports were not possible as the MCC was unwilling to provide access to its patient records.

Slide positivity was highly associated with fever as most infected individuals were more frequently febrile (81.5%) than uninfected ones (X^2 0 1569.9 P<0.05). The background of non-malarious fever, however, was also high (48.5%) and also some blood smear-positive individuals reported no fever (18.5%). Among the 171 individuals with fever, 51.5 percent had parasitaemia. Twenty five percent of the afebrile parasite carriers were infected with P. vivax while the remaining (75%) constituted P. falicparum infections. 72.2 percent of the afebrile carriers had only P. falciparum gametocytes in their peripheral blood and these were above the age of 5 years. 90% of the afebrile positives were permanent residents and among these 30 percent either have had a full course of chloroquine treatment or had taken some doses of antimalarial drugs in the recent past. Of the total infected individuals, however, only 8.3% were recent arrivals and no significant differences (x^2 = 0.005 P = 0.05) in parasite rates were observed between residents and non-residents.

Table 3. Cumulative parasite prevalence rate in the study population in Nazareth (N o 3890)

Sex.	No. exam'd	Infected (%)	Plasmodium Infection Falciparum (%)	Vivax (%)
Male	1512	4 6(3.0)	23(50)	23(50)
Female	2378	62(2.6)	40(64.5)	22(35.5)
Total	3890	108(2.8)	63(58.3)	45(41.7)

Table 4. Infection with malaria parasites and residential status of the population in Nazareth.

*Residence status	Infected (%)	Not infected (%)	Total
Resident	97(2.8)	3389(97.2)	3486
Non-resident	11(2.7)	393(97.3)	404
Total	108(2.8)	3782(97.2)	3890

*Resident > 1yr

Results of the study of prevalence of malaria infection in individuals residing in different kebeles identified positive cases from all the six kebeles in at least 2 of the surveys (Tables). However, more cases (4%) (x^2= 28.7, p= o.o5) were detected from the kebeles located peripherally at close proximity to the semi-permanent marshes and water pools formed during the rainy seasons (Table 6). Located in lowlying planes of the town, these kebeles are flooded by waters coming from the town's center as well as the surrounding elevated areas. Water flowing in or overflowing the open drainage and the trench encircling the town and bissecting these kebeles, floods the kebeles. Differences in parasite prevalence rates were also observed within these and among the other three kebeles - 01, 06 and 12 which are relatively central and situated at elevated parts of the town. Nevertheless, the observed differences were not statistically significant (X^2= 4.57 - peripheral; 3.44 - central, P= 0.05).

ble 5. Parasite prevalence rates among inhabitants of the six kebeles in Nazareth during the four sampling periods.

bele	Jul/Aug 1988 Parasite rate (%)	Sept/Oct 1988 Parasite rate (%)	Nov/Dec 1988 parasite rate(%)	Apr/May 1988 Parasite rate(%)
01	182(1.7)	174(3.5)	160(1.3)	160(-)
06	133(-)	175(1.1)	166(0.6)	108(-)
09	119(2.5)	242(6.6)	252(3.2)	227(0.4)
11	262(2.3)	256(11.3)	254(5.5)	271(0.7)
12	93(1.1)	93(2.5)	97(1.0)	84(-)
13	114(1.8)	78(5.1)	109(4.6)	89(-)
tal	903(1.7)	1018(5.8)	1038(3.0)	931(0.3)

Table 6. Relationship of prevalence of malaria infection to geographical location of the kebeles.

Location (Kebele)	Infected (%)	Not Infected (%)	Total exam'd
Peripheral (09,11,13)	90(4)	2183(96)	2273
Central (01,06,12)	18(1.1)	1599(98.9)	1617

Of the total infected individuals, 8.3 percent had visited malarious areas outside Nazareth, for a limitd period of time, a few days before the blood smears were taken. Out of the total population, however, only 153(3.9%) have travelled outside Nazareth. Of these 91(59.5%) individuals were from the peripheral kebeles and the remaining 62(40.5%) belonged to the central kebeles (Table 7). Nine (5.9%) of the 153 individuals with a travelling history were found positive of malaria parasites — 5(3.3%) from the peripheral and 4(2.6%) from the central kebeles, but difference was not statistically significant ($X^2 = 0.011$, $P = 0.05$). Comparison of the travelled-infected vs untravelled infected among the central and within the peripheral kebeles have also shown no statistically significant difference (the differences between the rates 5.6% - central and 1.59%-peripheral, were less than twice the standard error (S.E) of the differences, 6.2% and 4.84%, respectively). The overall difference of infection rate between travelled and untravelled ones was also not statistically significant (the difference of the rats, 3.23% < 2 SE of the difference, 3.65%). Nevertheless, comparison of the untravelled infected between the central and peripheral kebeles showed statistically significant differences(1.9>2sE, 1.03).

Table 7. Prevalence of infection with malaria in individuals with and without history of travel within the period of one month at the time of interview.

Status	Infected (%)	Not Infected (%)	Total exam'd
Travelled	9(5.9)	144(94.1))	153
Peripheral	5(5.5)	86(94.5)	91
Central	4(6.5)	58(93.5)	62
Not Travelled	99(2.6)	3638(97.4)	3737
Peripheral	85(3.9)	20 97(96.1)	2182
Central	14(0.9)	1541(99.1)	1555

The four cross-sectional blood examination surveys (3 weeks average duration), of clusters of population of all age groups, permitted the evaluation of the level of prevalence of the disease and the determination of the parasite species involved (Table 8). The cumulative parasite rates observed during the 4 surveys showed significant variation (X^2=59.5 P =0.05).

Parasite species distribution has also varied between the four surveys (Table-8). During the first survey (Jul./Aug.), 33.3 percent and 66.7 percent of the infections were due to P.

falciparum and P. vivax respectively. During the second and third surveys (Sept./ Oct.; Nov./ Dec.) a decrease in P. vivax prevalence and a striking increase in P. falciparum was noticed. In the last survey (Apr./May.,1989), although the parasite rate was low, P. falciparum infections were recorded. The trophozoite and gametocyte rates of P. falciparum followed the same trend starting to build up during the rainy months of Jul./Aug., and reaching a maximum around the end of the dry season.

Table 8. Variations in parasite species prevalence in all age groups in Nazreth

Period	Parasite rate (%)	Plasmodium species prevalence falciparum (%)	vivax (%)
Jul./Aug.1988	1.7	33.3(5)	66.7(10)
Sep./Oct.1988	5.8	57.6(34)	42.4(25)
Nov./Dec.1988	3.0	74.2(23)	25.8(8)
Apr./May 1989	0.3	33.3(1)	66.7(2)
Total	2.8	58.3(63)	41.7(45)

N.B. Values in bracket are number of infected individuals

was noticed. In the last survey (Apr./May.,1989), although the parasite rate was low, P. falciparum infections were recorded. The trophozoite and gametocyte rates of P. falciparum followed the same trend starting to build up during the rainy months of Jul./Aug., and reaching a maximum around the end of the dry season.

Table 9. Cumulative age-specific malaria infection prevalence among inhabitants of Nazareth.

Age group (in years)	Infected	Not infected	Parasite rate (%)
0-1	5	189	2.6
2-4	11	470	2.3
5-9	22	819	2.6
10-14	10	727	1.4
15-19	22	507	4.2
20-30	16	462	3.3
31-40	12	250	4.6
41-50	6	153	3.9
≥51	4	205	1.9
Total	108	3782	2.8

The prevalence of malaria infection in all age groups was significantly highest during the months of Sep./Oct. (Table 8.) In general, the age group most affected was the 15 to 50 years and over, although the difference was not statistically significant ($X^2 = 13.14$ P=0.05) (Table 9). The P. falciparum gametocyte rate was higher in those aged 10 and above (0.3%) than in under 9 years of age (0.1%).

3.3. Serological Survey

Serum samples obtained in Sep./Oct., 1988 from 450 individuals aged 10 and over who lived in the six kebeles were assayed for anti- P. falciparum antibody. Thick blood films taken from the finger prick had been examined for malaria parasites and slide positivity rates for P. falciparum and P. vivax were obtained (68% and 32%, respectively). Peak malaria transmission, with 3.3 and 2.5 percent infection due to P. falciparum and P. vivax, respectively, occurred during the period of serum collection.

IFA based serological tests and the parallel blood smear examination of 450 samples revealed 6.2 percent infection and an overall seropositivity of 22.9 percent (Table 10). Twenty one (4.7%) of the serum samples were both IFA and slide positive. Of these 17 (81%) were P. falciparum infections and the rest (19%) were due to P. vivax. A total of 5(55.6%) individuals demonstrating P. vivax peripheral blood parasitaemia failed to have a positive reaction in response to P. falciparum antigens, although 2(10.5%) falciparum patent infections also turned out to be IFA negative.

After age-stratification, moderate increase in serologic responses with age was apparent (table 10 and Fig. 4). The malaria antibody prevalence rates were closely related to the

10. Comparison of malaria parasite prevalence rate and antimalaria antibody response of samples collected in Sep./Oct., 1988 (n=450)

group	No exam'd	Parasite rate (%)	IFA Serum dilution end points 1:16	1:64	≥1:256	% positive ≥1:16	GMRT	IFA paras posit (%
-14	125	4.8	10	3	2	12	1:2	4.8
-19	93	7.5	14	7	3	25.8	1:2.5	7.5
-30	91	7.7	14	3	7	26.4	1:2.7	3.3
-40	60	8.3	9	9	2	33.3	1:3.4	3.3
-50	42	4.8	3	4	3	23.8	1:2.7	4.8
51	39	2.6	4	6	0	25.6	1:2.5	2.6
tal	450	6.2	54	32	17	22.9	1:2.3	4.7

GMRT = Geometric mean reciprocal titer

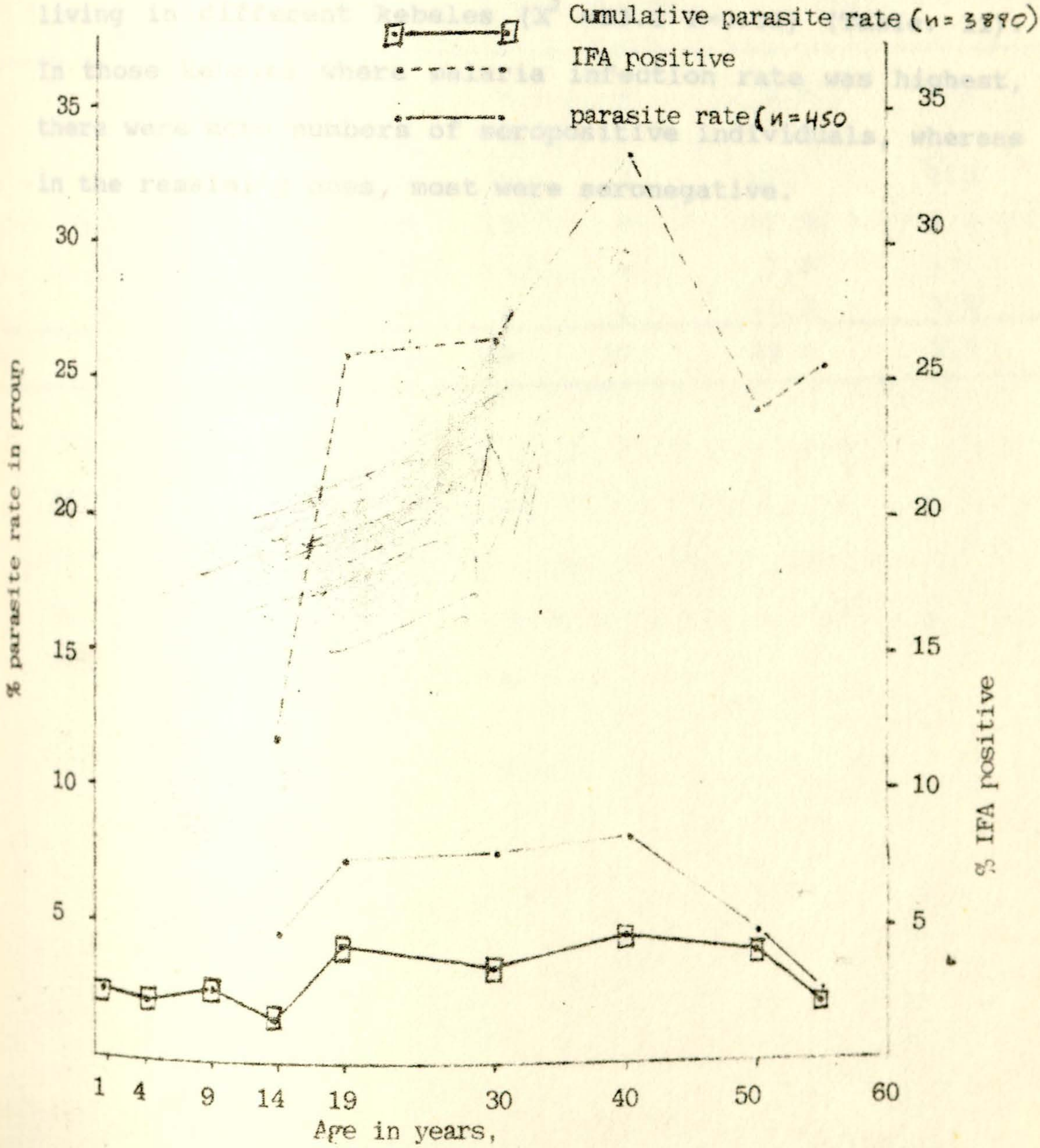

Fig. 4 ... and percent

parasite prevalence rates but run at a higher level. The parasite rates showed a fall with age . However, the parasite rate didnot decrease after the age of 15, but at a later age.

As with the parasitological results, the occurrence of seropositivity showed significant variability in individuals living in different kebeles (X^2 =53.1 P=0.05) (Table. 11). In those kebeles where malaria infection rate was highest, there were more numbers of seropositive individuals, whereas in the remaining ones, most were seronegative.

Table 11. Anti-malaria cntibody response of individuals living in six different kebeles in Nazareth (n = 450).

No exam'd	Parasite rate (%)	IFA Serum dilution end points			% positive ≥1:16	GMRT	IFA an Parasi positi (%)
		1:16	1:64	≥1:256			
39	2.6	1	-	-	2.6	1:1	2.6
52	-	-	-	-	-	-	-
157	5.1	31	13	7	32.5	1:3	3.8
103	14.6	15	15	9	37.9	1:5	11.7
40	2.5	3	-		7.5	1:1	2.5
59	5.1	4	4	1	15.3	1:2	5.1
450	6.2	54	32	17	22.9	1:2.3	4.7

3.4. Entomological Survey

Two species of Anopheles, A. gambiae s.l. and A. pharoensis, were collected indoors. Frequently more A. gambiae s.1 was found indoors than A. pharoensis. Density of A. gambiae s.1 was highest in Sept./ Oct. High density of this species persisted until mid-October, (Table 12) when mosquitos bred in drying up residual pools, in water accumulated in rubber tyres, disposed receptacles or barrels, cement-built troughs for watering cattle and other items. Thereafter A. gambiae s.1 populations diminished until only two specimens were recovered from ten houses in Dec. 1988.

Observations on the resting behaviour of A. gambiae s.1 populations showed that most of the mosquitos generally rested below 2 meters, in dark corners of the rooms on walls, hanging objects, and below 1 meter near sleeping quarters of the people. A total of 62 rooms or houses were visited and the summary of the results is shown in Table 12.

Table 12 Indoor resting collections of Anopheline mosquito by aspirator and pyrethrum space-spray.

hod of ction	Period	Habitat investigated (Tukuls & Rectangular houses)				*A. gambiae* s.l
		No. of rooms exam'd	No. of rooms positive	Aver. No. of occupants	Total No. of females	Aver. room density
tor	Sep./Oct. 1988	17	17	3.8	176	10.4
			*5		*13	*0.76
rum	Oct., 1988	9	9	4	209	23.2
-Spray	Dec. 1988	10	1	3.58	2	0.20
	Oct. 1989	26	20	3.6	144	5.54
		45	30	3.7	355	7.9
l		62	47	3.8	531	8.6

* refers to *A. pharoensis*

Anopheline density expressed as the average number of species per house was estimated both by aspirator and pyrethrum knockdown space-spraying collections. Average density of mosquitos per room varied from 23.2 in October to 0.2 in December in knock-down collections and the values obtained for aspirator collections were 10.5 in Sept./ Oct , 1988.

In order to estimate man-vector contact from averaged values of mosquito density per house, note was made of the abdominal conditions of the mosquitos (Table 13). The overall average of bites per man per day was estimated at 0.20 (Table 14). Collections made both by aspirator and space-spraying were characterized by a high proportion of gravid over fed ones suggesting a marked tendency to endophily. In aspirator collections, relatively higher values of fed to gravid ratio were obtained in samples collected in late September, because gravid females tend to hide in dark and inaccessible areas where aspirator collection would be more difficult. Freshly fed females donot tend to fly far away from the site of feeding. The overall proportion observed for half-gravid A. gambiae s.1 populations was 58%.

Table 13. Abdominal conditions of indoor-resting samples of A. gambiae s.l Sept./Oct. & Dec., 1988; Oct. 1989

Period	Abdominal Condition					
	Unfed	Fed	Half Gravid	Gravid	Total	Fed/ Gravid
Sep.Oct. 1988	34 (19)	11 (6)	97 (55)	34 (19)	176	0.32
Oct. 1988	10 (4.8)	4 (1.9)	143 (68.4)	52 (24.9)	209	0.02
Dec. 1988	-	-	-	-	2 (100)	-
Oct. (1989)	3 (2.1)	31 (21.5)	68 (47.2)	42 (29.2)	144	0.74
	13 (3.7)	35 (9.9)	211 (59.4)	96 (27)	355	0.4
	47 (8.9)	46 (8.7)	308 (58)	130 (24.5)	531	0.4

N.B. Values in bracket are the percentages of each category

Infection rates and human blood Index (HBI).

Out of 266 A. gambiae s.1 dissections for salivary gland infections, none were found positive. However, the ELISA test performed on 74 A. gambiae s.1 specimens, divided between thorax and abdomen, detected one P. falciparum (1.35%) and another P. vivax (1.35%) infections. Of the two infected mosquitos, one was positive for P. falciparum in the posterior portion only, and the other for P. vivax in the anterior portion only.

Trophic preferences of A. gambiae s.1 were also evaluated on a total of 60 specimens by using the ELISA test. Human blood was detected in 52 of the specimens tested, indicating a human blood index of 0.87. The result shows that A. gambiae s.1 is predominantly anthropophilic in Nazareth, as only a few percentage of samples did not react against human antisera.

The result of the ELISA test were read spectrophotometrically by using an ELISA reader and the absorbance values ranged from 0.07 to 0.17 at 492nm. In general, the readings were low as compared to reports of other workers (Beier etal., 1988).

Table 14. Average man-biting rates of A. gambiae s.l collected by aspriation and pyrethrum space-spray Sep./Oct. 1988; Oct., 1989

Method of collection	Proportion fed	Average number of occupants	Average room density	Average* bites/man/day
Aspirator Sept./Oct.1988	0.06	3.8	10.4	0.16
Pyrethrum space-spray Oct. 1988	0.02	4	23.2	0.12
Oct.1989	0.22	3.6	5.54	0.34
Average	0.09	3.8	8.5	0.2

* Calculated by: Aver. room density x Proportion freshly fed ÷ Aver. no. of human occupants/house

Quantitative Analysis

Although the entomological data collected do not permit detailed quantitative analysis of the dynamics of malaria transmission, a rough estimate of some of the parameters can be made of it. Thus, inorder to estimate quantitatively the role of A. gambiae s.l in malaria transmission in Nazareth, the entomological inoculation rate was calculated. This was based on the indoor resting A. gambiae s.l populations by first multiplying average house density by the proportion of freshly-fed and dividing the product by the average number of human occupants per house (Table- 14). Accordingly by multiplying the obtained figure with the sporozoite rate, a value of 0.002 was obtained from the knock-down collection for Oct. 1988. In the present study, the gonotrophic cycle was estimated to be 2.5-3 days, but further studies are needed to confirm it. Freshly fed and late fed A. gambiae s.l. mosquitos collected by aspiration and kept in paper cups all became gravid from 45-60hrs.

3.5. Environmental Survey

The inhabitants of Nazareth obtain pottable drinking water piped to the town from a source- Awash River- about 20 kms south of the town. The main pipeline reaches all 20 kebeles but water is not uniformly distributed in the kebeles. As a result a sizable part of the population depends on public fountains and/or buys from the neighbouring households with piped water. Due to the absence of adequately piped water, the inhabitants store water for a variable period of time. Most of them use barrels fitted with lids both for delivery and temporary storage. Although these storage devices appeared not to be suitable for mosquito breeding, container larval habitats cannot be totally ruledout, for water is also stored in open mouthed barrels and other receptacles for construction and other purposes.

Anopheline larvae were isolated from a variety of collections of water both natural and man made. These included: Cement-built water reservoirs and troughs collected for construction and for watering cattle; bird fountains or small collections of water from dripping taps, burrow pits or sites dug for damping garbage, pools-dug for local brickmaking, at construction sites for tree-planting; ditches, lowlying areas, installation of municipality water supply wthout disposal of waste water; open and blocked drains; water in natural depressions, discarded tanks or barrels and a number of disposed receptacles, tyres, etc.

Of the artificial container habitats, tyres were most numerous and were often found with tremendous numbers of anopheline larvae contributing to the overall mosquito problem. Large-scale tyre accumulations were observed in some of the sampling areas, notably kebele 11 – especially at the Relief and Rehabilitation Center (RRC) and other government institutions nearby. Tyres were also present outdoors in service stations, tyre dealer and behind a number of private residents, often with innumerable anopheline larvae. Water-filled containers were generally available for mosquito-breeding during the rainy season and at the beginning of the dry season. Most of these dried up during the dry season.

Rainpools in ditches, pits, were most abundant during the rainy season, with specific durability depending on size and location. They dried up during the dry season but the semi-permanent swamps in some of the kebeles retained water until the month of November. A. gambiae s.1 populations appeared to have favoured the shallow, turbid and often sunlit pools especially those dug for brick making and at construction sites, for most of them contained innumerable number of larvae of this species. Although positive breeding sites were encountered in all the kebeles sampled, lots of them were found in kebeles 11, 09 and 13 the low-lying peripheral kebeles.

The peripheral kebeles had also other factors with malariologic implications in addition to the presence of abundant temporary and semi-permanent collections of water. Cultivable land and vegetation were abundant in these kebeles (Table 1). Owing to the growth of the town along the east-south- and south west perimeter, where these kebeles are located, new construction sites are plenty and lots of private and government institutions have flourished the area in the recent past and others are on the way. In addition to creating abundant mosquito breeding sites, Institutions such as the RRC in kebele 11, invite a lot of people thereby making the area more accessible to people. According to local informants, before a couple of years, the RRC was said to have been distributing food aid to the drought victims of the surrounding malarious area in the town - the people, staying for a variable period of time until they get their share. The Military Camp in Kebele 09 provides another example. Troops coming from different corners of the country stay for a couple of months until dispatched to some other place.

4. DISCUSSION

The present study revealed P. falciparum and P. vivax to be the species responsible for malaria in the population and A. gambiae s.1 to be the principal vector of malaria in the town of Nazreth. The other species of mosquito, A. pharoensis was found in small numbers indoors, only occassionally. Since A. pharoensis is considered to be of no or little importance in the transmission of malaria in Ethiopia (Krafsur, 1971), A. gambiae s.1 can be considered to be the principal vector. The detection of these two species in the region, is in line with what was reported by Verrone (1962) and O'connor (1967). Furthermore, it is well established that A. gambiae s.1 is the most abundant man-biting anopheline mosquito, in the town, and has long been incriminated as the main vector of malaria in Ethiopia (Covell, 1957). However, only very few infectd specimens of A. gambiae s.1 have been reported in the country, in general, and none in the town of Nazareth and its vicinity. In the present study, two specimens, one with P. vivax salivary gland infection and the other with oocyst-associated P. falciparum sporozoites were detected by the monoclonal antibody-based ELISA technique. This finding more definitely incriminates A. gambiae s.1 as the main vector of malaria in the town of Nazareth. Since A. gambiae s.s has never been reported in the county, so far, the present sibling species is probably A. arabiensis. This is because, A. arabiensis has been described from various parts of the country, including Showa administrative region, and the Awash Valley (Mekuria etal., 1982), and hence is considered to be the only important vector representing the A. gambiae complex in Ehiopia.

The detection of infected mosquitos coupled with the infection in infants and the relatively high anti- P. falciparum antibody levels in the general population is a definitive proof of an autochthonous transmission of malaria in the town of Nazareth. The study has also shown that in spite of intensive mosquito control measures, accompanied by antimalarial drug administration, malaria transmission has been taking place both during the peak transmission season (September-November) and following the small rains (April-May). As might be expected, the transmission intensity was accentuated during the months of September-October.

The moderately high mean annual minimum (14.2°c) and maximum (28.9°c) temperature, together with numerous collections of water of various sizes and durations appeared to have favoured the breeding of a large number of A. arabiensis populations (Fig. 2). Such environmental conditions are favourable to A. arabiensis as it can breed in transient pools as well as in large bodies of flood pools. The highest mosquito density in Sep./Oct. is the result of intense breeding of A. arabiensis populations favoured by standing waters in ditches and pools dug out for brick-making and tree-planting, etc. together with the moderately high mean maximum monthly temperature of 26.7°c in October, as well as humidity reaching as high as 70 percent. However, mosquito breeding was found to continue beyond October in the collection of surface water of various sizes and durations such as in the semi-permanent swamps formed during the rainy months and persisted until November in some kebeles studied. As the water for construction, watering cattle, etc., was being delivered from the Awash River and kept in barrels and cement-built water reservoires for a variable period of time, and this may act as a potential source of A. gambiae s.1 introduction into the town in the dry season, as are discarded rubber tyres often found positive for anopheline larvae. During the months of Apr./May, 11.2% of the households visited were positive for anopheline larvae and most of these were in discarded rubber tyres. Large-scale deposits of rubber tyres were observed in the town serving as breeding sites for A. arabiensis populations. The improperly discarded tyres' problem is magnified by growing evidence that tyres promote rapid development of mosquitos and enhance their vectorial ability, since the nutritionally deprived emerging mosquitos tend to be more aggressive and efficient in feeding on humans (Baumgartner, 1988). The persistence of man made breeding sites, such as discarded tyres in the dry season, has been shown to result in increased population of A. arabiensis, and is known to have led to a number of epidemics in many urban areas. For example, in 1973, an estimated 25 percent of the population of Kinshasa was reported to have been affected (YoundeOwei and Service, 1986).

Fluctuation in entomological and parasitological variables followed a similar pattern and significant variations (X^2 =59.5, p = 0.05) were observed in parasite prevalence during the four surveys undertaken. This might be explained by the seasonal changes in the rates of mosquito emergence which are known to produce annual cycles in mosquito density, in the number of infected mosquitos and in malaria prevalence in both the mosquito and human populations (Aron and May, 1982). Variations in parasite species distribution has also been observed during the four surveys, P. vivax predominating during the rainy months of Jul./Aug.. P. falciparum dominated during the beginning of the dry season all through Nov./Dec.. This may be due to differences in the incubation interval between the two species, owing to the slightly longer period of the extrinsic cycle and the prolonged interval before infective gametocytes appear in the peripheral blood in falciparum infections. The prevalence of malaria infection showed a close concordance with the abundance of A. arabiensis and the maximum prevalence was observed at the beginning of the dry season (Sept./Oct). However, as the dry season progressed, (Oct. - Dec.) a marked fall both in the density of A. arabiensis populations and parasite prevalence was observed. A number of interacting factors could have brought this change, but it could also be attributed to factors which included DDT spraying the drying up of breeding sites and the cold period that followed (mean monthly minimum temperature of 7°c recorded in November and 9.4°c in December), as well as the low relative humidity recorded (Fig. 2). The dry period in Nazareth, however, appeared not to be sufficiently long to completely stop malaria transmission.

Thus, as far as artificial breeding conditions are created by man and in the presence of an efficient vector as A. arabiensis - notorious for its survival over long dry periods in partial hibernation, has the ability to build up its population in small transient collections of water (Omer and Cloudsley - Thompson, 1970) - it is not surprising that interruption of malaria transmission was not possible by the control measures currently in use in the town.

Among other urban factors, that might influence the epidemiology of malaria in Nazareth, deserving attention is the rural-urban and urban-rural pattern of population movements and potential large-scale importation of parasites. Malaria cases coming from the outlying malarious villages, seeking medication, are especially important in this respect. High in-and-out-migration in peri-urban (eg. subsistence farmers) and urban populations may help maintain malaria prevalence in the fringe areas and promote inter-urban transmission. Migrant labor force coming from malarious areas during the harvesting season, and often sleeping outdoors in the suburbs, could also influence the epidemiology of malaria, increasing the potential of infective reservoir to A. arabiensis populations. On the other hand, other factors associated with the urban environment, such as high population density and easy access to treatment as well as control measures applied could be expected to reduce infection rates and may have led to the underestimation of the situation.

Differences in prevalence observed between different parts of the town- inter kebele as well as peripheral versus central location of the kebeles - indicated that malaria transmission is a focal phenomenon and that it is influenced by microecological conditions existing even in two adjacent localities of the town. The observed variations in prevalence are probably due partly to the presence or the absence of permanent or transient mosquito breeding sites, cultivable land and/or vegetation - in reference to mosquito resting sites - in or around human habitations. Topography, accessability of the area to people, differences in the socio-economic status, and the like, could also contribute to the variation.

The standard characterization of the epidemiology of malaria is based on an age-prevalence curve or on age-stratification, showing the proportion of each age group with malaria parasite positive blood smears (Aron & May, 1982). In hyper-or holoendemic conditions prevalence of infection peaks at an early age with an increase upto the age of 9 years and shows a sudden fall in the age-group of 10 to 15 years and then slowly declines with age (WHO, 1963). The result of the present study, however, did not reveal such a pattern (Table 9). Instead, although relatively high prevalence rates were observed in infants and children individuals in the

the difference was not statistically significant (X^2=14.7 P=0.05). The picture of the prevalence rate together with the gametocyte rate, which was also higher in those aged 10 years and over, was suggestive of a slow development of immunity to the blood forms of malaria parasites. Such low immunogenic stimulation, may be due either to abbreviated parasitaemias owing to the consumption of antimalarial drugs or a low transmission level, brought about by control measures. It may also be a "post eradication" phenomenon, in that the withdrawal of residual insecticide spraying might have led to reduced immunity in the population contributing to the resurgence of increased malaria transmission in the town in particular , and in the country, in general. Similar explanations, have been suggested by some workers (Vercruysse etal., 1983; Gardiner etal., 1984) for similar conditions in a number of urban areas in tropical Africa.

The occurrence of malaria infection in infants or children in stable communities confirm continuing local transmission, while a higher rate of malaria in recent arrivals may suggest that the residents have developed some protective immunity (Russel etal., 1963). In this study (Table 4), no statistical differences in parasite rates were observed between permanent residents and recent arrivals (X^2 = 0.05 P = 0.05), suggesting that the population enjoys variable levels of immunity & that importation of malaria from other malarious regions is not a prominent feature of the disease epidemiology in the town of Nazareth. The absence of statistically significant difference in infection rates between individuals with and without travelling history may also support this view.

As immunity in the inhabitants of Nazareth appeared to develop slowly and since individuals aged 10 and over make the bulk of the population, they probably constitute the main reservoir of infection in the town. It is to be expected that, in places like Nazareth, where the large part of the population is actively engaged in trade or business, the indicated group is more affected than children under 10 years, for it constitutes the highly mobile elements of the population both within and outside the town, making them more vulnerable to mosquito attacks. This is supported by the fact that most of the asymptomatic gametocyte carriers, which are considered to be the main source of infection (Muirrhead-Thomson, 1957; Burkot et al., 1988), detected in Nazareth were aged 10 years and above.

As shown by the WHO (1975), of value in assessing the potential infectivity to mosquitos of a given population is the point prevalence of gametocyte carriers among a representative sample of the population. In this regard the average P. falciparum gametocyte rate of 0.5 percent detected in the first three surveys (July-Dec. 1988) indicates that one out of 200 susceptible mosquitos biting any given person could potentially become infected with P. falciparum. Though the gametocyte rate observed was low, probably due to easy access to antimalarial drugs and prompt treatment, that of P. vivax included, the gametocyte rate for both species may not be too low not to sustain the infection of A. arabiensis in Nazreth. Thus, the relatively high sporozoite rate recorded for P. vivax (1.35%) (95% confidence limits, 0.02-5.45) during the study could be due to high anthropophily of A. arabiensis. In addition, the paucity of animals and the high density of human population in the town may have led to an intensive feeding of A. arabiensis. In an urban area of Senegal, where the situation is hypoendemic Vercruysse & Janclose (1981, cited by Goriup & Van der kaay, 1984) reported similar finding - a sporozoite rate of 1.35% in highly anthrophilic A. arabiensis populations, and the number of infective bites per year was estimated to be 43.

ELISA technique for bloodmeal source identification revealed a HBI of 0.87, showing A. arabiensis to be predominantly anthropophilic. The few A. arabiensis specimens that turned out to be negative for human blood might have fed on the blood of other hosts. This is possible because, most of the people, from whose houses the mosquitos were collected, owned Cattle, Sheep, etc., and in most cases the animals were kept inside human habitations or in enclosures in close proximity to the dwellings. It may also be due partly to the digestion or breaking of blood proteins since half gravid specimens were also included in the test, and the dried specimens were stored without a dessicant. The same argument may hold true for the low ELISA readings obtained, as compared to reports of other workers (Beier etal., 1988). In view of the limited biotype from which the material was collected, the true rate of anthropophily may be higher than the determined value of 87 percent, a high figure characteristic of urban areas (Vercruysse, etal., 1983).

Although, owing to the economy of time and cost, the entomological observations were limited in time and scope to permit the quantitation of all relevant parameters, a preliminary estimate of the entomological inoculation rate was computed (0.002 infective bites/man/day , for the indicated method earlier). This shows that on the average each individual is bitten by about one infected mosquito in a year. The entomological inoculation rate was based only on the knock-down collection, a method when used alone could lead to an understimation of the average room density of mosquitos as compared to night bite collection and exit-trap plus pyrethrum space spray collections. The average room or house density may further have been underestimated owing to possible mosquito escape through openings during and immediately after spraying, the failure to recover all the knocked-down specimens-due to the presence of household furniture and other items,which the inhabitants were unwilling to empty. Because of these factors the entomological inoculation rate most likely is an underestimate. Nevertheless, in general, entomological inoculation rate by far exceed the inoculation rate derived from infant parasite rates, in a longitudinal study of infants (WHO, 1975).

The usefulness of the IFA test in studies in the epidemiology of malaria is well recognized (Collins etal., 1968; Kagan, 1972). The occurrence of positive serological responses give a clearer indication of the number of individuals in the population who have actually experienced malaria. The results of the present study, on 450 serum samples obtained from individuals aged 10 years and above, revealed a parasite rate of 6.2 percent and an overall seropositivity of 22.9 percent with 1:2.3 geometric mean reciprocal titer (GMRT).

Agreement between slide diagnosis and IFA response for 19 sera with slide-proven P. falciparum and 9 with slide proven P. vivax was found to be 89.5 and 44.4 percent, respectively. All in all, out of 28 individuals with patent infections, 75 percent showed positive IFA responses against the P. falciparum antigen used in the study. In a study by Gleason and Colleagues (1971), 76.7 percent agreement was found between 120 P. vivax and 39 P. falciparum patent infections and IFA response. Similarly, Sulzer etal., (1969) have reported 82.8 percent of P. vivax serum samples to have cross-reacted with P. falciparum antigen slides, although the heterologous titers were lower than the homologous ones. Such evidence indicates that the serological response of the population in Nazareth would be higher had the P. vivax antigen slides been included in the study.

In general, the malaria antibody prevalence rate observed was closely related to the parasite prevalence rates but run at a higher level as it perhaps reflects the cumulative experience of the individuals to the disease. Findings from other studies have shown the proportion of IFA positive response in a population of endemic areas to increase more rapidly if the transmission is more active and the relationship between the GMRT and percent positive to be close to linear (Kagan, 1972). For example, in Gambella, where there is intense malaria transmission, over 75 percent of the population tested was found to be IFA positive (Collins & Skinner, 1972). Likewise in Garki (Nigeria), where malaria transmission is more intense, 100 percent of the individuals, aged 1 year and above showed a positive IFA response and the antibody titer increased rapidly with age reaching a plateau in the early adolescent group (Mollineaux and Gramiccia, 1980). The increasing antibody titer with age is accompanied by rare and discrete parasitaemia in adults in this type of conditions.

The parasite rate in the town of Nazareth, however, did not decrease immediately after the age of 15 and the percentage of antibody carriers increased in the group 15 and above to reach a plateau at a later age. This may indicate a delayed development of acquired immunity in the town, typical of hypoendemic settings (Jeffery et al., 1975). Similar observations were made by Ngimbi etal. (1980) in a study carried out in the city of Kinshasa, Zaire.

Furthermore, the increasing GMRT with age probably indicates the accumulated experience with malaria at the older age group. Nevertheless, the GMRT appeared to be low and such low serological profiles are commonly encountered in low malaria incidence areas (Warren etal., 1975; Ray etal., 1988).

It is also known that the level of response, that is the IFA titer achieved and maintained is directly associated with the persistence of parasitaemia and that there is a boosting effect of renewed parasitaemias either through relapse or reinfection (Collins etal.,1964; Voller and Bruce-Chwatt, 1968). Thus, in areas like Nazareth, where early and prompt treatment against the disease is available, and where there is a less likelihood of reinfection, the initial titers resulting from the infection may be of a low order and may decay relatively rapidly (Jeffery etal., 1975). Collins etal (1975) have reported the disappearance of detectable antibody in 50 percent of P. vivax infected volunteers, 121-180 days after the first appearance of parasites in the peripheral blood (with a mean of 7.9 days duration of parasitaemia). Decay of antibodies, of even shorter duration has been documented (Kagan, 1972).

The significant difference ($X^2 = 41.2$ $P=0.05$) in IFA response, as with the parasitological findings observed, between individuals living in the peripheral kebeles and the relatively central ones is an indication that malaria is of very low prevalence in the centrally located kebeles and that its transmission in Nazareth varied from one locality to another, as reported by Waren etal. (1975).

The present study has also revealed that both children and adult residents in the town may have had little experience of malaria and suggested a very high susceptibility to infection leading to a high risk of severe disease. With the likelihood of the right combination of rain, temperature and other factors that prevail in Nazareth favoring mosquito breeding, slackening of the control efforts would lead to epidemic situations of malaria. From the available evidence, including fluctuations in climatic conditions (Appendix 1) and mosquito density observed in Oct., 1988/89 (Table 12), malaria, in Nazreth, appears to be of an epidemic type, characteristic of hypoendemicity (Macdonald, 1957).

5. CONCLUSION AND RECOMMENDATIONS

The intricate relationships existing between the numerous factors determining the epidemiological situation of urban malaria has required generation of information on the parasitological, entomological and sociological aspects of the town of Nazareth. The malaria epidemiological situation in Nazareth is the result of human intervention in the environment in the form of urbanization processes that, in addition to some pockets of natural mosquito breeding foci, created many types of artificial breeding sites and conditions. Extensive population movements and antimalaria control measures instituted have rendered a unique epidemiological picture.

Thus, the present study has been undertaken amid intensive mosquito control measures coupled with drug administration. As a result, it has not been possible to generate adequate data on all parameters needed to undertake a detailed mathematical analysis of the dynamics of malaria transmission, following the classical standard approach. However, based on the findings of the study, the malaria situation in the town of Nazareth can be characterized in the following manner:

(i) the malaria parasite species P. falciparum and P. vivax are involved.

(ii) parasite rates are proportionally higher in the age group 10 tö 20 years and tended to decrease with increasing age, that is after around 30 to 40 years.

(iii) there is marked seasonal variation in prevalence and the peak occurred in the months of Sept./Oct.

(iv) Both behavioural (bionomic) and the finding of natural infections in the stomach (midgut) as well as in the salivary glands confirm the role of A. arabiensis as a primary vector involved in the transmission and maintenance of malaria in the town. Based on this study, very little can be said about the vectorial status of the other anopheline mosquito - A. pharoensis - in the town. The HBI and fed to gravid ratio determinations have indicated that A. arabiensis populations are highly anthropophilic and endophilic.

(v) A. arabiensis breeding may take place throughout the major part of the year. Both intradomestic and peridomestic (outside a compound) breeding of this species was encountered. These included: disposed rubber tyres, barrels, and other receptacles, ditches, blocked open drains, borrow pits or those dug for construction and local bricking-making, etc. The detection of the aquatic stages of A. arabiensis in small collections of water formed even under-dripping water taps, in the dry season, suggested a year round cycle of breeding.

(vi) Inter-urban variations in prevalence are evident. Autochthonous transmission takes place in most parts of the twon. However, it is more intense in the peripheral regions. This was established both by parasitological and serological surveys.

(vii) These surveys have also revealed that the disease in the town is markedly seasonal, unstable and subject to epidemics. This instability suggests that it may be possible to control the malaria transmission cycle in the town of Nazareth.

(viii) The increase in the extent of malaria transmission in Nazareth in particular and in Ethiopia, in general, appears to be a "post eradication" phenomenon. Chief among the factors responsible for this exasperating situation is the withdrawal or total abandonment of residual insecticide spraying in many parts of the country. This appears to have led to reduced immunity in the population, thus escalating the resurgence of malaria transmission with the consequent heavy morbidity and mortality inflicted upon the populations.

(ix) Malaria in the town of Nazareth and those reported in other Ethiopian towns may also be an indication of the development of deteriorating quality of the urban habitats.

The present study was an initial step in the characterization of the epidemiology of urban malaria in Nazareth. Based on the present findings a few considerations with regard to tackling the malaria problem in the town of Nazareth may be suggested:

1. Inorder to tackle the malaria problem on the long-term basis, the bioenvironmental approach appears to be a more feasible one. This would involve vector control through source reduction method through a "self-help" strategy. For this the existing infrastructures such as the city council and the kebeles must be used.

The community should be motivated through health education to participate in filling, leveling or draining ditches, burrow pits, and other small depressions, installation of their own soakage pits, cleaning of drains and all vector control activities such as drying of pools and puddles around their houses, provision for drainage of splashed water at backyard water taps, control of breeding inside rubber tyres by indoor or outdoor storage under a roof, vertical stacking or by boring large drainage holes to prevent water accumulation in it, just before or during the rainy months.

- Similarly, systematic surveys may be organized by the community to search and destroy intradomestic and peridomestic breeding. The remaining environmental control measures for vector source reduction would require engineering measures so as to drain the semi-permanent marshes and the drainage system on periphery to the town.

- Reduced contact between man and mosquito may also be achieved by introducing and popularizing mosquito nets and screening windows and doors. It may also be useful to introduce legislation forbidding cultivation of urban land and storage of domestic water in the open.

- Intensification of surveillance for the detection and treatment of cases and also the application of focal residual spraying in the peripheral kebeles before the peak transmission season as the need arises. In the mean time, insecticide susceptibility tests should be run. Moreover, programmes should be organized for assessing and monitoring the status of the sensitivity of P. falciparum to chloroquine.

- The risk of importation of parasites may be minimized by establishing new permanent treatment posts or by utilizing the existing health services, in as many localities as possible in the outlying villages. Increasing the number of microscopists, may also unburden the existing few and thus reduce the chance of missing low density infections by the microscopists owing to over working. It may also help reduce the number of people visiting private dispensaries thereby avoiding misuse of antimalarial drugs.

5. Realistic control programmes require intersectoral cooperation, community participation and above all trained personnel at all levels. Owing to low salaries, meagre future prospects and per diem often working on their free time without being paid for, most of the Malaria Control Program staff may not be expected to work efficiently or with zeal. Hence these conditions should be rectified. Chances of obtaining higher posts, refresher programmes, opportunities for graduate, post-graduate studies should be sought. Possibly of most importance is the collation, correlation and dissemination of information on current problems and research effort for research bodies within the country (and abroad) and working in coordination for a common goal-effective control of malaria.

All in all, although the results of the present study are somewhat illuminating as to the level of malaria and in general about the characteristics of the epidemiology of malaria in the town of Nazareth, it still falls short of providing a definitive quantitative evaluation of malaria transmission in the town. Hence, inorder to have a more complete picture of malaria epidemiology in the town, it should be augmented by further research which include:-

- Vector- distribution or extent of dominance and quantitative assessment of breeding.
  - bionomics including biting habits in relation to human activity, age composition and trends in density, infectivity and parity throughout the year at monthly intervals or in general longitudinal study of vector population dynamics, estimation of the gonotrophic and extrinsic cycles, susceptibility level of vectors,
- Parasitology - longitudinal study of infants or children and adults if possible
  - drug sensitivity tests of *P. falciparum* to chloroquine.

REFERENCES

Armstrong, J.C. 1969. Plasmodium ovale endemic in Ethiopia. Trans, R. Soc. Trop. Med. Hyg., 63:287-288.

Armstrong,J.C. 1978. Susceptibility to vivax malaria in Ethiopia. Trans. R. Soc. Trop. Med. Hyg., 72:342-344.

Armstrong, J.C. Asfaha, W. and Palmer, T.T. 1976. Chloroquine Sensitivity of Plasmodim falciparum in Ethiopia. I. Results of an in vivo test. Am.J. Trop. Med. Hyg., 24:5-9.

Aron, J.L. and May, R.M. 1982. The population dynamics of malaria. In: R.M. Anderson (ed.) 1982. The population dynamics of Infectious Diseases: Theory and Applications. Champman and Hall, London and New York.

Baumgartner, D.L. 1988. Suburban accumulation of discarded tyre in North Illinois and their associated mosquitos. J. Am. Mosq. Ass., 4(4): 500-508.

Beaver, P.C., Jung, R.F. and Cupp, E.W, 1984. Clinical Parasitology 9th ed., Lea and Phebiger, Philadelphia, USA.

Beier, J.C. etal., 1988. Bloodmeal identification by direct Enzyme-linked Immunosorbent Assay (ELISA), tested on Anopheles (Diptera:

Culicidae) in Kenya. J. Med. Entomol., 25(1): 9-16.

Bekessy, A., Molineaux, L. and Storey, J. 1976. Estimation of incidence and recovery rates of Plasmodium falciparum parasitaemia from a longitudinal data. Bull. Wld. Hlth. Org., 54:685-693.

Bruce-Chwatt, L.J. 1970. Seroepidemiology of malaria. J. Parasitol., 56(3): 552-555.

Bruce-Chwatt, L.J. 1984. Lessons learned from applied field research activities in Africa during the malaria eradication era. Bull. Wld. Hlth. Org., 62(suppl.): 19-29.

Bruce-Chwatt, L.J. 1985. Essential Malariology. William Heinemann, London.

Bruce-Chwatt, L.J. 1987. Un de Venis Viator et quo Vadis? Ann. Trop. Med. Parasitol., 8(15): 471-486.

Burkot, T.R. 1988. Non-random Host Selection by Anopheline mosquitos. Parasitology Today, 4(6).

Carnevale, P. and Mouchet, J. 1987. Prospects for malaria control. Inter. J. Parasitol., 17(1): 181-187.

Chand, D. 1965. Malaria problems in Ethiopia. Ethiop. Med. J., 3(1): 27-34.

Chang, W.P. 1962. General review of health and medical problems in Ethiopia. Ethiop. Med. J., 1(1): 9-27.

Cohen, S. and Lambert, P.H. 1982. Malaria. In: S. Cohen and K.S. Warren, (eds.) 1982. Immunology of Parasitic Infections. 2nd ed. Oxford, Blackwell, pp. 422-474.

Collins, W.E., Jeffery, G.M. and Skinner, J.C. 1964. Fluorescent antibody studies in human malaria. II Development and persistence of antibodies to Plasmodium falciparum. Am. J. Trop. Med. Hyg., 13:256-260.

Collins, W.E., Skinner, J.C. and Coifman, R.E. 1967. Fluorescent antibody studies in human malaria. V. Response of sera from Nigerians to five Plasmodium antigens. Am. J. Trop. Med. Hyg., 16:568-571.

Collins, W.E., Lunde, M.N,, and Skinner, J.C. 1975. Development of antibodies to Plasmodium vivax as measured by two different serologic techniques. Am. J. Trop. Med. Hyg., 24:412-416.

Collins, W.E. etal. 1968, Studies on the relationship between fluorescent antibody response and ecology of malaria in Malaysia. Bull. Wld. Hlth. Org., 39:451-463.

Collins, W.E., Warren, McW., Skinner, J.C. 1971. Serological malaria survey in the Ethiopian highlands. Am. J. Trop. Med. Hyg., 20:199-205.

Collins, W.E. and Skinner, J.C. 1972. The indirect fluorescent antibody (IFA) test for malaria. Am. J. Trop. Med. Hyg., 21(5): 690-695.

Collins, F.H. etal. 1984. First field trial of an immunoradiometric assay for the detection of malaria sporozoites in mosquitos. Am. J. Trop. Med. Hyg., 35:538-543.

Coluzzi, M. 1984. Heterogeneities of the malaria Vectorial system in tropical Africa and their significance in malaria epidemiology and control. Bull. Wld. Hlth. Org., 62(Suppl.): 107-113.

Covell, G. 1957. Malaria in Ethiopia. J. Trop. Med. Hyg., 60(1):7-16.

Dennis, D.T. etal., 1974. Chloroquine tolerance of Ethiopian strains of Plasmodium falciparum Trans. R. Soc. Trop. Med. Hyg., 68(3): 241-245.

Evengard, B., Linder, E. and Lundbergh, P. 1988. Standardization of a filter paper technique for blood sampling. Ann. Trop. Med. Parasitol., 82(3):295-303.

Fontain, R.E., Najjar, A.E. and Prince, J.S. 1961. The 1958 malaria epidemic in Ethiopia. Am. J. Trop. Med. Hyg., 10(6): 795-803.

Gardiner, C. etal., 1984. Malaria in urban and rural areas of Southern Ghana: A survey of parasitaemia, antibodies and antimalaria practices. Bull. Wld. Hlth. Org., 62(4), 607-613.

Garnham, P.C.C. 1966. Malaria parasites and other haemosporidia. Oxford, Blackwell.

Garnham, P.C.C. 1987. The liver in malaria with special reference to the exo-erythrocytic phase. Ann. Trop. Med. Parasitol., 81(5): 531-537.

Gebremariam, N. 1984. Highlights of Malaria situation in Ethiopia. In: Proceedngs of the workshop on the promotion and strengthening of malaria control through a primary health care. Addis Ababa, 1984, pp. 5-17.

Gebremariam, N. 1988. Malaria. In: Z.A. Zein and H. Kloos (eds.), 1988. The ecology of health and disease in Ethiopia. Ministry of Health, Addis Ababa, pp. 136-139.

Gebremariam, N., Abdulah, Y and Mebrate, A. 1982. Preliminary studies of the response of Plasmodium falciparum to chloroquine in Nazareth town, Central Ethiopia. Ethiop. Med. J. 20:1-7.

Gleason, N.N. etal. 1971. Agreement between microscopic diagnosis and the indirect immunofluorescent antibody (IFA) test in Plasmodium vivax and P. falciparum infections. Am. J. Trop. Med. Hyg., 19:209-214.

Goriup, S. and Van der Kaay, H.J. 1984. Lessons learned from applied field research activities in Africa during the malaria eradication era. Bull. Wld. Hlth. Org., 62(Suppl.), 31-39.

Janssens, P.G. and Wery, M. 1987. Malaria in Africa South of the Sahara. Ann. Trop. Med. Parasitol,, 81(5): 487-498.

Jeffery, G.M. etal. 1975. Application of the indirect fluorescent antibody (IFA) method in a study of malaria endemicity in Mato Grasso, Brazil. Am. J. Trop. Med. Hyg., 24(3): 402-411.

Kagan, I.G. 1972. Evaluation of the indirect heamagglutination (IHA) test as an epidemiologic technique for malaria Am. J. Trop. Med. Hyg., Ibid., 21(5): 683-689.

Kloos, H., Adugna, A. and Sahlu, E. 1988. The physical, Biotic and Human Environment in Ethiopia: The perspective of Medical Geography. In: Z.A. Zein and H. Kloos (eds.). 1988. The ecology of health and disease in Ethiopia. Ministry of Health, pp. 18.42.

Krafsur, E.S. 1971. Malaria transmission in Gambella, Illubabor Province. Ethiop. Med. J., 9:75-94.

Krafsur, E.S. 1977. The bionomics and relative prevalence of Anopheles species with respect to the transmission of Plasmodium to man in Western Ethiopia. J. Med. Entomol., 14(2): 180-194.

Krafsur, E.S. and Armstrong, J.C. 1978. An integrated view of entomological and parasitological observations on falciparum malaria in Gambella, Western Ethiopian lowlands. Trans. R. Soc. Trop. Med. Hyg., 72:348-356.

Loban, K.M. and Polozok, G.S. 1983. Malaria. Englsih translation (Alexander Shelepin), Mir Publishers, 1985. Moscow, USSR.

Lopez-Antunano, F.J. 1974. Falciparum malaria antigen slides for indirect immunofluorescent test made from in vitro culture (correspondence). Trans. R. Soc. Trop. Med. Hyg., 68(3), 257.

Macdonald, G. 1950. The analysis of infection rates in diseases in which superinfection occurs. Trop. Dis. Bull., 47:907-915.

Macdonald, G. 1952. The analysis of equilibrium in malaria. Trop. Dis. Bill., 49:813-829.

Macdonald, G. 1956, Epidemiological basis of malaria control.

Bull. Wld. Hlth. Org., 15:613-626.

Macdonald, G. 1957. The epidemiology and control of malaria. Oxford, University Press, London.

Mathews, H.M. 1978. Guide to collection, storage and processing of blood specimens on filter paper. Malaria Lab., General Parasitology Branch Center for Disease Control. Atlanta, Georgia.

McConnel, E. Asfaha, W. and Dennis, D.T. 1976. A survey for Wuchereria bancrofti in Ethiopia. Ethiop. Med. J., 14:41-36.

McConnel, E. and Schmidt, M.L. 1973. Bancroftian filariasis in Gambella, Illubabor Province, Ethiopia. Trop. Geor. Med., 25(3):300-303.

McGregor, 1.A.etal. 1965. Immunofluorescence and the measurement of immune response to hyperendemic malaria. Trans, R. Soc. Trop. Med. Hyg., 59:395-414.

Mekuria, Y., Petrarca, V. and Tesfamariam, T. 1982. Cytogenetic studies on the malaria vector mosquito, Anopheles arabiensis patton in the Awash Valley, Ethiopia. Parasitologia, 24(2-3): 237-243.

Miller, L.H. and Carter, R. 1976. Innate resistance in malaria Exp. Parasitol., 40:132-146.

Molineaux, L. and Gramiccia, G. 1980. The Garki Project. Geneva, WHO.

Muirhead-Thompson, R.C. 1957. The malaria infectivity of an African village population to mosquitoes (A. gambiae). Am. J. Trop. Med. Hyg., 6:971-979.

Ngimbi, N.P., beckers, A. and Wery, M. 1980. A percu de la situation epidemiologique du paludisme a Kinshasa (Republiqe du Zaire) en 1980. Ann. Soc. Belge. Med. Trop., 62:121-137.

Nilson, L.A. 1979. Technical aspects of serodiagnostic tests. In: Immunodiagnostic techniques in Malaria-Transactions of th 3rd meeting of the Scientific Working Group on the Immunology of Malaria, Panama, June 1979 pp. 71-92. Geneva: UNDP/World Bank/ WHO.

O'Connor, C.T. 1967. The distribution of anopheline mosquitos in Ethiopia. Mosq. News, 27(1):42-54.

Omer, S.M. and Cloudsley-Thomspson, J.L. 1970. Survival of female Anopheles gambiae Gilles theough a 9-month dry season. Bull. Wld. Hlth. Org., 42: 319-330.

Ota, W.K. etal. 1976. Arbovirus recoveries from mosquitoes collected in Gambella, Illubabor province, Ethiopia. J. Med.

Entomol., 13(2): 173-178.

Pinder, R.M. 1973. Malaria: The design, use, and mode of action of chemotherapeutic agents. Bristol: Scientechnica (Publishers) Ltd.

Population and Housing Census Commission. 1984. Population and Housing census Preliminary report V.1. No. 1 Prepared & Printed by Central statistical office.

Ray, K. etal. 1988. Evaluation of serology as a tool for malaria surveillance in East Shamparan District of Bihar, India. Ann. Trop. Med. Parasitol., 82(3): 225-228.

Report of Technical Study Committee on the Malaria Situation in Ethiopia. October, 1981 (Eth. C.) 15pp.

Rogers, D.J. 1988. The dynamics of vector-transmitted diseases in human communities. Phil. Trans. R. Soc. Lond. B 321: 513-539.

Russel, P.F. etal. 1963 Practical Malariology. 2nd ed. Oxford University Press, London.

Schaller, K.F. and Kuls, W.. 1972. Ethiopia: A Geomedical Monograph. Springer-Verlag. Berlin, Heidelberg, New York, pp 98-101.

Service, M.W., Voller, A. and Bidwell, D.E. 1986. The enzyme-linked immunosorbent Assay (ELISA) test for the identification of bloodmeals of haematophagus insects. Bull. Entomol, Res., 76:321-330.

Stevens, P.A. 1984. Environemtnal management activities in malaria control in Africa. Bull. Wld. Hlth. Org., 62(Suppl.): 77-80.

Sulzer, A.J., Wilson, M. and Hall, E.C. 1969. Indirect flucrescent antibody (IFA) tests for parasitic diseases. V. An evaluation of a thick smear antigen in the IFA test for malaria antibodies, Am. J. Trop. Med. Hyg., 18(2): 199-205.

Surtees, G. 1971. Urbanization and the Epidemiology of Mosquito Borne Diseases, Abst. Hyg., 46(2): 121-134.

Swaroop, S. 1966. Statistical methods in malaria eradication. WHO Monograph series, No. 51.

Teklehaimanot, A. 1986. Chloroquine resistant <u>Plasmodium falciparum</u> in Ethiopia. Lancet, ii: 127-129.

Thomas, V. and Chan, W.C. 1982. Evaluation of filter paper eluates for seroepidemiological studies on malaria in Peninsular Malaysia. Trop. Geor. Med., 34: 145-149.

Thomas, V. and Pinnampalam, J.T. 1975. Thick smear Plasmodium falciparum antigen from in vitro cultures for the indirect immunofluorescent antibody test. Bull. Wld. Hlth. Org., 52(1):33-37.

Trager, W. and Jansen, J.B. 1976. Human malaria parasites in continuous culture. Science, 193:673-675.

Tropical diseases research (TDR). 1984. Seventh Programme Report Jan. 1983-31 Dec. 1984. UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases. WHO, Geneva, 1985 p.3-66.

TDR. 1988. Eighth Programme Report of the UNDP/World Bank/ WHO Special Programme for Research and Training in Tropical Diseases, WhO; Geneva, pp. 17-48.

Turner, R.L. 1972. Malaria epidemiological observations from unsprayed study districts in Ethiopia. Mosq. News, 32(4):608-613.

Vercruysse, J.Jancloes, M. and Van de Veldon, L. 1983. Epidemiology of seasonal falciparum malaria in an urban area of Senegal. Bull. WHO, 61:821-831.

Verrone, G. 1962. Outline for the determination of malarial mosquitos in Ethiopia. Part-I adult female anophelines. Mosq.

News, 22(1)37-49. Part II. Anopheline larvae. Mosq. News, 22(4):394-401.

Voller, A. and Bruce-Chwatt, L.J. 1968. Seroepidemiological Survey in Nigeria. Bull. Wld. Hlth. Org., 39:883-897.

Voller, A. and O'Neill, P. 1971. Immunofluorescent method suitable for large-scale application, Bull. Wld. Hlth. Org., 45:524-529.

Voller, A. etal. 1974. A microplate method of enzyme-linked immunosorbent assay (ELISA) and its applications to malaria. Bull. Wld. Hlth. Org., 51:209.

Ward, R.A. 1977. Recent changes in the epidemiology of malaria relating to Human ecology. Proc. 15th Intern. Congr. Entomol. pp. 523-529.

Warren, McW. etal. 1975. The seroepidemiology of malaria in Middle America. Am. J. Trop. Med. Hyg., 24: 740-748.

Wernsdorfer, W.H. 1980. The importance of malaria in the world. In: J.P. Kreier (ed). Epidemiology, Chemotherapy, Morphology and Metabolism. Academic Press, Inc. Vol. 1 p. 1-93.

Wessen, A.F. 1972. Human ecology and malaria. Am. J. Trop. Med. Hyg., 21(5): 658-662.

White G.B. 1974. Anopheles gambiae complex and disease transmission in Africa. Trans. R. Soc. Trop. Med. Hyg., 68:278-298.

Wirtz, R.A. 1988. ELISA kits for detection of plasmodium falciparum and P. vivax sporozoites in infected mosquitos (unpublished document).

WHO, 1963. "Terminlogy of Malaria and Malaria Eradication". WHO, Geneva.

WHO, 1974. Serological testing in malaria (Memoranda). Bull. Wld. Hlth. Org., 50(6): 527-535.

WHO, 1975. Manual on practical Entomology in Malaria. Geneva, WHO offset publications, No. 13 part 1 and 11 351 pp.

WHO Malaria Action Programme, 1986. Guidelines to the management of severe and complicated malaria in Ethiopia. National Programme for the Control of Malaria and Other Vector-Borne Diseases. Ministry of Health, 1987.

WHO. 1987. The Biology of malaria parasites. Report of a WHO Scientific Working Group. Wld. Hlth. Org. techn. Rep. Ser. 743, 229pp.

Youdeowei, A and Service, M.W. 1986. Pest and Vector Management in the Tropics. Longman Group Ltd., England.

Appendix - 2 Population of Nazreth, 1970-1981 (E.C.)

SEX	YEAR OF CENSUS											
	1970	1971	1972	1973	1974	1975	1976	1977	1978	1979	1980	1981
Male	34549	34902	35475	35752	34830	35939	35969	36743	41460	41974	43028	46260
Female	34981	35824	35989	37697	40193	40343	42032	43550	41153	41470	41269	43135
Total	69530	70720	71464	73449	75013	76282	78001	80293	82613	83444	84297	89395
												*98408

* source-Nazreth city council; * estimate of MCC

Appendix. 1 Meteorological conditions prevailing in Nazareth area, 1977-1989.

	Rainfall (MM)			Temperature (°c)						MEAN			Relative humidity (%)		
	1977-87	1988	1989	1988 Max	1977-88 Max	1989 Max	1977-88 Min	1988 Min	1989 Min.	1977-88	1988	1989	1977-88	1988	1989
Jan	16.3	35.4	00	28.4	27.4	27.2	12.1	13.7	9.8	19.8	21.05	18.5	49	59	56
Feb	31.6	12.2	15.7	30.3	28.4	26.5	13.7	16.3	14.2	21.1	23.3	20.4	51	54	57
Mar	46.3	2.4	34.0	32.3	29.7	30.4	15.7	15.2	14.5	22.7	23.3	22.5	30	45	53
Apr.	50.0	29.9	61.6	31.1	29.8	23.5	15.7	16.4	15.9	22.8	23.8	22.2	32	56	61
May	74.6	26.2	1.2	32.3	30.3	31.7	15.9	16.1	14.2	23.1	23.7	23	56	46	49
June	86.3	59.3	83.5	30.3	29.7	31.5	16.3	16.9	15,5	23.0	23.6	23.5	56	56	49
Jul	144.3	188.7	147.4	25.2	26.5	26.4	15.3	16.8	16.0	20.9	21.0	21.2	65	65	69
Aug.	164.6	186.2	273.2	26.1	26.0	26.3	15.4	15.8	15.4	20.7	21.0	20.9	70	70	75
Sept.	84.2	132.1	66.2	26.7	27.2	27.5	14.5	15.4	14.4	20.9	21.1	21.0	69	71	68
Oct.	43.4	14.0		28.3	28.5		12.1	11.8		20.3	20.1		51	53	
Nov.	10.8	00		28.3	28.1		11.6	7.0		19.9	17.7		33	50	
Dec.	6.8	4.5		27.8	27.1		11.2	9.4		19.2	18.6		48	53	

Appendix 3.

A. Area Identification

1. Keftegna_____ 2. Kebele_____ 3. House hold No. L_____

B. Particulars of the sample member

1. Name __________ 2. Xex_______ 3. Age

4. Occupation__________ 5. Average monthly income_____

6. Educational status

6.1. Are you literate Yes No, If yes level of Educ._____

6.2. If yes, Now attending

6.2.1. School __________ Grade ______ Sect.____

6.2.2. Have you ever been absent from school? Yes Ne

6.2.3. If yes reason for absence __________

7. Have you ever had a history of malaria or fever? Yes No.

8. If yes, have you ever used medicine? Yes No

9. If yes, how did you obtain the medicine you used?

____

10. What was the brand of medicine used?

(Chloroquine, Quinine, Pirmaquine, Fansidèr, Others (name)

____

11. Have you ever used medicines for preventive purposes against malaria? Yes No

12, If yes, how did you obtain the medicine used?

____

13. What was the brand of medicine used? (Chloroquine, Quinine, Primaquine, Fansider, others (name)__________

____

14. If the answer for No. 11 is yes, have you ever been to a malarious area? Yes No

17. If your answer for No. 16 is less than 2 years, where was your place of previous residence?

Region ________________ Awraja ____________ Wereda ________
Town or Village ____________

18. Have you ever travelled for another place in the past? Yes No

19. If yes, when and where?

Date	Region	Awraja	Wereda	Town Vill
a ______	______	______	______	______
b. ______	______	______	______	______
c. ______	______	______	______	______
d- ______	______	______	______	______
e ______	______	______	______	______

Information on housing unit

1. What is the type of housing unit?
   a. Conventional (mud,brick, stone, other).
   b. Improvised
   c. other (specify)

2. What is the type of building in which the housing unit is found?
   a. Non-storeyed detached
   b. Non-storeyed 2 or more units attached
   c. One or more storeyed detached
   d. One or more storeyed detached

3. What is the purpose of the housing unit?
   a. Residential only
   b. Residential and establishment

4. What is the major material used for construction of wall of housing unit?
   a. Bricks/stone and mud/cement
   b. Wood and mud
   c. Bamboo and Mud/Reed and Mud
   d. Corrugated iron
   e. Grass, straws etc.

5. What is the major material used for construction of roof of housing unit?

a. Corrugated iron

b. Concrete or cement

c. Wood and mud

d. Tbatch, Thatch and mud, reed/Bamboo

6. What is the number of rooms in the housing unit?____________

7. Were does the housing unit get drinking water from?__________

a. Tap inside the house

b. Tap in compound private/shared

c. Outside compound, public tap

d. Protected well or spring

e. Unprotected well or spring

f. River/pond

8. What type of toilet facility does the housing unit have?

a. None

b. Frush, toilet, private/shared

c. Pit, private/shared

DECLARATION

I, the undersigned, declare that this thesis is my work and tht all sources of material used for this thesis have been duly acknowleded.

Name Mekonnen Yohannes

Signature:

Place: Department of Biology
Addis Ababa university

Date of Submission: June, 1990