VIDU 20-2-2

GETTING RID OF THE MOSQUITO MENACE - CARRIER OF MANY DISEASES

Mosquitoes have become a serious threat to man's health as they contribute to many diseases of epidemic scale. Dengue, Malaria, Filariasis and Japanese encephalitis are main diseases transmitted by mosquitoes, which have serious impacts both socially and economically. As a mosquito can fly a considerable distance from its place of birth, every person may expose themselves to these diseases creating a threat to public health.

Malaria has caused over one million deaths all over the world, while in Sri Lanka, 115 people have died in North and North East in 1998. There are two types of filariasis in Sri Lanka, namely urban and rural filariasis. There is a long history of filariasis in Sri Lanka, where it has been reported as far back as 339 A.D. At the moment there are over 120 million filariasis patients in the world. Out of these about 76 million patients do not show any symptom of filariasis. Japanese Encephalitis is an important health problem faced by people in many Asian countries. During the last decade this disease has spread in Thailand, Burma, India and Nepal. Annually about 50,000 people in the world acquire this disease and 15,000 people die from it. In Sri Lanka it is prevalent in the dry zone where paddy cultivation is done, and in the wet zone where pigs are reared. Until 1984 this has not been spread in dangerous level. In 1987 it became significant, killing 138 people out of 766 infected people. Encephalitis patients were reported at first in Anuradhapura and later in Polonnaruwa, Kurunegala and Puttalam districts. Dengue, during the year 2000, caused a grave health problem in Sri Lanka. During that year about 5000 patients were reported in the country of which about 400 patients were confirmed as having dengue. Out of the above mentioned diseases, dengue did the greatest damage to the people in our country.

A number of programmes were launched islandwide to control the diseases. Anti-dengue campaign week was announced. Shramadana campaigns to collect and remove refuse, empty tins, king coconut shells, polythene bags were launched at village level. Programmes launched to eradicate mosquito menace involved teachers, pupils and the local villagers where, school children played an important role, as they are more susceptible to Dengue. For the continuation of regular campaign to curtail mosquito menace, the following proposals have been made. Invention of efficient equipment sets, set aside a labour force to remove stagnant water from discarded gem pits, wells, canals and waterways, educate the general public of the danger of mosquito breeding places such as polythene bags, empty king coconut shells, discarded tyres, and lavatory pits. It is possible to reach an everlasting solution by devising schemes to control the mosquitoes before becoming an epidemic.

Prof. Karunaratne has pointed out that it is useful to consider the biological aspects in controlling mosquitoes, and all preventive measures should aim at personal care, house construction and its surrounding and the use of insecticides. Dr Maya Gunasekera has shown that before embarking to use suitable insecticides for the control of carrier mosquitoes in a selected area, it is better to recognize the mosquito varieties in that area and especially in control of malaria vector, DNA technique could be used. Mosquito related research is being conducted by various research institutes and universities in the island. National Science Foundation has granted financial aid for over 20 such research projects. Most of these researches are conducted in areas like mosquito species and their breeding places, recognition of various mosquito varieties, their classification, spreading of diseases and resistance to insecticides etc. It is timely and appropriate to aim most of these projects at controlling the mosquito breeding. Researchers should pay attention to this aspect.

Science Education Committee of National Science Foundation has issued this volume of VIDURAVA on "Mosquito Borne Diseases" thereby creating awareness among school children and the general public, which in turn helps to control the spread of diseases.

Editor

JAPANESE ENCEPHALITIS (J.E.)

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Introduction

Japanese Encephalitis (J.E.) is an infection of the central nervous system caused by a virus transmitted to man through mosquitoes. The Japanese encephalitis virus was first isolated in Japan in 1935 from the brain of a patient dying from encephalitis. The virus is transmitted in an enzootic cycle among mosquitoes and vertebrate amplifying hosts, chiefly domestic pigs and wading birds. Culex mosquitoes, primarily *Culex tritaeniorhynchus* and *Culex gelidus* are the principal vectors.

History of the disease

Japanese Encephalitis has been reported in the South-East Asia and Western pacific regions for a long time with epidemics mainly in Japan, Korea and some Provinces of Taiwan. During the last decade, outbreaks of the disease have extended to some parts of Thailand, Burma, India, Nepal and Sri Lanka. For the first time, J.E. cases were reported from Australia in 1995.

It is a disease of public health importance in many Asian countries. Approximately 50,000 cases of J.E. with 15,000 deaths are estimated to occur annually. This disease primarily occurs in children. Approximately 25 percent of cases die and 50 percent develop permanent neurologic and psychiatric sequelae. The magnitude of the problem is even greater when it is considered that the disease often occurs in epidemics that are somewhat predictable and that J.E. is a vaccine preventable disease.

Clinical Features

The incubation period in man, following an infective mosquito bite, may range from 5 to 16 days. The course of the disease can be conveniently divided into three stages:

1. **A prodromal stage** occurs before the involvement of the Central Nervous System (CNS). Early symptoms of the prodromal state are; fever, rigors, headache, nausea and vomiting.

2. **An acute encephalitic stage** usually occurs by the third to fifth day. The symptoms are; altered sensorium (clouding of consciousness, excitement, confusion, disorientation, stupor and coma), convulsions, stiff neck, muscular rigidity, mask-like face, abnormal movements (coarse tremors, choreo-athetotic movements etc), dehydration and weight loss.

3. **A late stage** marked by recovery or the persistence of signs of CNS injury. Other signs and symptoms which also can be present at early or late stages are: increased deep tendon reflexes, thick and slow speech, aphasia and paresis.

Japanese Encephalitis (J.E.) in Sri Lanka

Japanese Encephalitis virus was first isolated in Sri Lanka in 1968. The isolation was done at the Medical Research Institute, Colombo. Since then J.E. cases have been identified from various parts of the country. The disease was considered as endemic in certain parts of the country. In the dry zone, the disease was reported mainly from areas where paddy cultivation is being done, while in the wet zone it was reported from the areas where pig breeding and coir products are made as cottage industries. The vector mosquitoes were also frequently identified in these areas.

In 1988, 409,888 doses of J.E. vaccine were administered to children 1-10 years of age in Anuradhapura, Polonnaruwa, Kurunegala and Puttalam RDHS divisions.

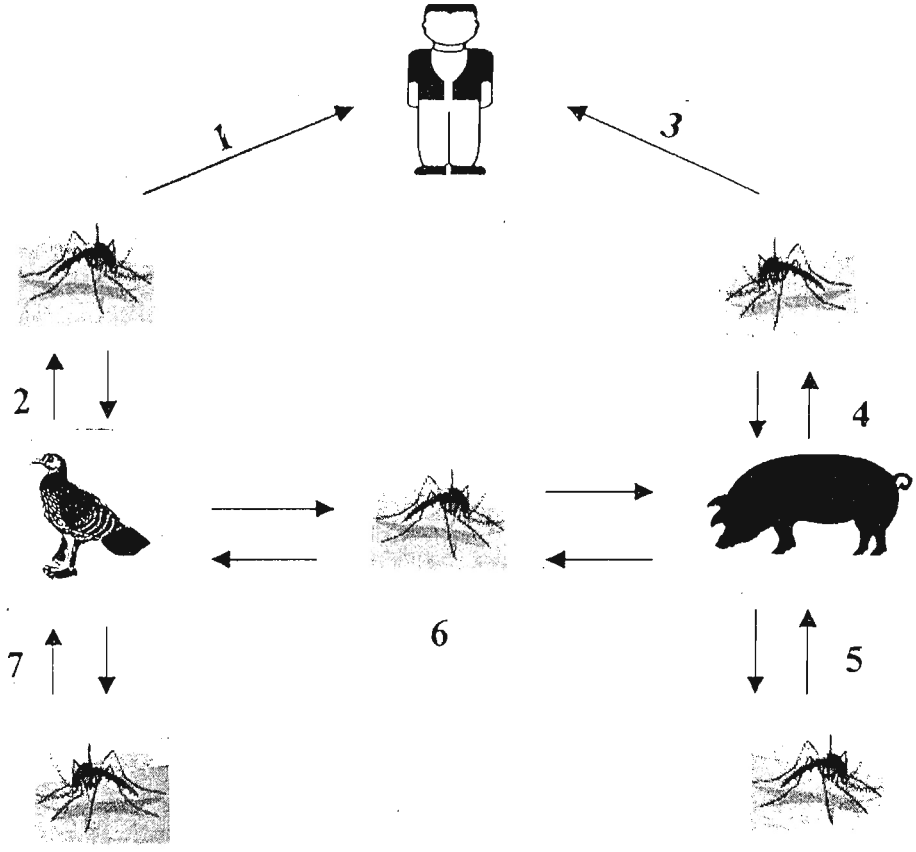

Figure 1: Life cycle of Japanese Encephalitis virus

1. **Man mosquito contact is high when adult mosquito densities build up to a maximum.**
2. **The cycle within birds.**
3. **No disease transmission from one human to another.**
4. **The virus develops in pigs.**
5. **Life cycle of the virus within pigs.**
6. **Mosquito breeds in water logged paddy fields and ground pools.**
7. **Mosquito prefers blood of poultry and animals**

The disease occurs throughout the year, and shows a marked increase with the North East monsoonal rains (November to February) as a result of increased mosquito breeding places, due to water logging of rice-fields and ground pools. Man mosquito contact is observed to be high, when adult insect densities build up to maximum, during this period.

Geographic distribution and incidence

Countries with proven epidemics of Japanese Encephalitis are India, Nepal, Sri Lanka, Burma, Laos, Thailand, Kampuchea, Vietnam, Malaysia, Singapore, Philippines, Indonesia, Saipan, China, maritime Siberia, Korea and Japan. Regular seasonal epidemics occur in northern South East Asia, China and Korea.

Epidemiological Patterns – Two patterns

(a) Sporadic cases - occasional cases widely distributed in time and space – seen in Japan, Taiwan, Korea and Sri Lanka.

(b) Regional Seasonal Outbreaks - Large number of cases occurring simultaneously at one time and place – seen in Thailand, Parts of India, Sri Lanka and Nepal.

Epidemic cycle

Japanese Encephalitis virus is transmitted by zoophilic mosquito vectors; consequently, wild and domesticated animals are the principal hosts. Man is considered to be a dead-end host for this virus due to the short duration and low titre of viremia in man and the relative preference of vector mosquitoes for animals over man.

Animal reservoirs

Pigs and some birds are the most important hosts for maintenance, amplification, and spread of the virus. Rodents appear to be unimportant hosts.

Bird-mosquito cycles are thought to be important in maintaining and amplifying Japanese Encephalitis virus in the environment. Viremia frequently follows infection of both wild and domestic birds with the virus.

Animals and mosquitoes infected with Japanese Encephalitis virus generally remain asymptomatic, though fatal Encephalitis occurs in horses and fetal wastage may occur in infected sows.

Vectors

The *Culex tritaeniorhynchus* mosquito is the main vector in the transmission of J.E. to humans in Asia.

This mosquito breeds in rice fields some distance from human dwellings but flies to peridomestic areas for blood meals. *Culex tritaeniorhynchus* mosquitoes can fly for a distance of up to 1.5 kilometers and have been found in treetops 43-50 feet above the ground, where virus could be spread among birds.

The *Culex gelidus* mosquito is also an important vector in the transmission of J.E.

Seasonality

Transmission of Japanese Encephalitis virus in the tropics may occur year round. Where seasonal epidemics occur, they generally begin during the rainy seasons when mosquito populations are maximal.

Japanese Encephalitis Prevention and Control Measures

Japanese Encephalitis could theoretically be prevented by a combination of the following:

1. Surveillance
2 Treatment and management of patient
3. Vector control
4. Vector avoidance/prevention of mosquito bites
5. Immunization of susceptible persons
6. Protection of animal reservoirs
7. Immunization of amplifying host

Surveillance

Surveillance aims at building up an early warning system in known infected zones as well as in those where the virus is not known to occur but where ecological conditions are favourable to the introduction of the virus.

Surveillance is mainly based on early recognition of first suspected case(s) and their prompt reporting to the health authorities.

Vector Control

The use of pesticides to control Japanese Encephalitis virus vectors has generally been effective only in some areas for a certain period of time and at great cost.

Prevention of mosquito bites

1. Keeping people away from mosquitoes by distancing houses from rice fields and pig sites. Use of netting also can help but is impractical.

2. Laws requiring that pigs be grown only in localized pig pens, may be away from human habitation will help in disease control.

Immunization against Japanese Encephalitis (J.E.)

Sri Lanka adopted immunization against J.E. as a major strategy for the prevention and control of the disease in high risk areas in 1988. The target population is children between the ages of 1 and 10 years. This age group was selected considering the age distribution of patients, immunity levels and the cost of vaccine, to achieve maximum long term results making the programme cost-effective.

The vaccination programme was first conducted in Anuradhapura, Polonnaruwa and Puttalam districts and later it was extended to other districts based on the epidemiological situation of the disease in these areas.

Over 10 million doses of J.E. vaccine were administered to children in Sri Lanka from 1988-1999. According to the data available there is a general reduction in incidence of J.E. in areas where immunization against J.E. has been carried out and it has not been reported in children who were immunized against J.E.

Adverse reactions following immunization with J.E. vaccine were monitored from the beginning of the programme. No major side effects were reported.

In 1997, immunization programme was extended to Colombo, Kalutara, Matara, Hambantota, Ampara and Galle districts based on the epidemiological situation of J.E. in these areas.

Japanese Encephalitis Vaccine J.P. (Beijing strain)

Japanese Encephalitis Vaccine "SEIKEN" is a colourless to slightly turbid liquid preparation containing inactivated Japanese Encephalitis virus.

Administration and Dosage

Primary Immunization: In general usage, two subcutaneous injections of 0.5 ml (under 3 years of age, 0.25 ml) are given at 1-4 week interval.

Booster Immunization: A single subcutaneous injection of 0.5 ml (under 3 years of age, 0.25 ml) is typically administered about 1 year after the completion of the primary immunization. Further booster immunizations are given at the first sign of a local epidemic or every 4-5 years in order to maintain effective immune levels.

Contraindications

Fever, a serious disease, experience of anaphylactic shock to any of the vaccine components in the past and considered unsuitable for vaccination for any reason other than those given below.

- Fever or allergic reaction, such as rash etc., within two days of previous vaccination.
- A convulsion in the past (one year)
- Diagnosed with a form of immunodeficiency in the past.
- May be likely to experience an allergic reaction of any of the vaccine components.

Clinical side effects

Local oedema, swelling, pain and such general reactions as fever, chills, headache and fatigue may be experienced but those usually diminish within a few days.

Comparison of effects of disease and vaccine

a) Disease

Japanese Encephalitis

Caused by an arthropode-borne flavivirus (J E virus). J.E. spread by the bite of infective mosquitoes. After incubating for 1-2 weeks, the infection causes an acute brain syndrome characterized by reduced consciousness, generalized spasticity, focal neurological signs and fits.

b) Effects of disease

There is no specific treatment, and the prognosis is poor, especially in cases in which consciousness is impaired. The case fatality rate of encephalitis is 25%, and 30% have neuropsychiatric sequelae.

c) Side effects of vaccination

Local indurations, redness and tenderness are common. Systemic adverse sections such as fever, headache, malaise, rash, dizziness, myalgia, nausea and vomiting have been reported in 10% of recipients. Serious reactions reported in 62.4 per 10,000.

Notes for Parents

1. If your child has a fever (temperature) after the vaccination.

If your child has a fever, give paracetamol (dose based on weight). Give no more than 6 doses in 24 hours. If fever persists, consult your doctor.

2. Advice to parents on reactions to vaccination

Many vaccine infections may result in soreness, redness, itching, swelling or burning at the infection site for 1 to 2 days. A cold, wet cloth will help to relieve this. Sometimes a small lump may persist for some weeks or longer. If adverse events like urticaria, fits occur consult your doctor immediately.

LYMPHATIC FILARIASIS

Dr C.H. Gauthamadasa
Anti-Filariasis Campaign

Lymphatic filariasis is a mosquito borne disease having a very ancient history. It is caused by a nematode parasite transmitted by a mosquito.

Sri Lanka has had two forms of human filariasis caused by the parasite *Brugia malayi* transmitted by *Mansonia* mosquitoes and *Wuchereria bancrofti* transmitted by the mosquito *Culex quinquefasciatus*.

Since 1965 no cases of Brugian filariasis have been reported from Sri Lanka. Present problem is due to Bancroftian filariasis (urban) and the endemic area is confirmed to three provinces namely Western, Southern and North Western exposing a population of 9.5 million at risk.

Earliest reference to this disease was made in the 6th century B.C. in the Vinaya Pitaka, which contain rules for ordination of Buddhist monks. Earliest scientific information regarding the disease was available only in 2879 A.D. Historical evidence supports the view that Brugian filariasis was introduced in the 12th century A.D. by the Malays and Bancroftian filariasis in the 15th century A.D. by the Chinese both as a result of foreign invasions.

The prevalence and distribution of filariasis was known only after 1914, subsequent to the survey carried out by Manson Bahr. The distribution on an island wide basis was not known until 1939 when Dassanayaka completed his comprehensive survey. This survey revealed the presence of *B.malayi* infection in several foci in five provinces and *W. bancrofti* infection in the towns of Galle and Matara. In 1947 a special campaign was established to deal with this problem. Subsequent to control measures adopted against the parasite and the vector the disease was brought under control after two years and since 1965 no cases of brugian filariasis have been reported. Since then the problem is due to bancroftian or urban filariasis.

During the early stages of the disease the patients are asymptomatic and are a source of danger to the society as they are capable of transmitting the disease. These asymptomatic carriers often found to have damaged lymphatic and renal systems.

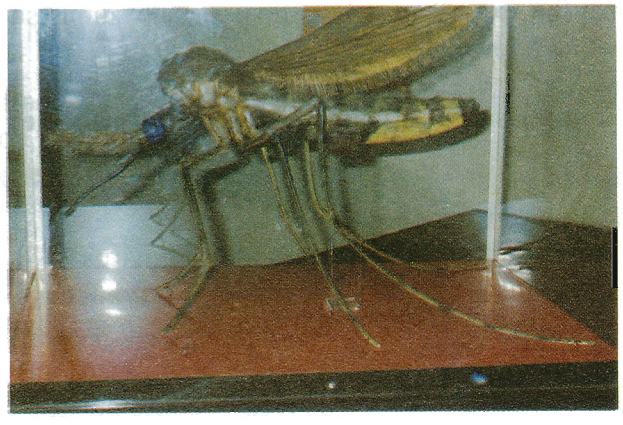

Figure 1: The mosquito vector (*Culex quinquefasciatus*) responsible for transmission of filariasis.

When an infected mosquito bites a human being the infective larvae enter the lymphatic system of man and develop into adult worms during a period of 9-12 months. The life span of an adult worm is about 5-6 years. During this life span a female worm produces millions of microfilariae, which find their way into the peripheral circulation at night.

Microfilariae live for a period of about twelve months in the human host during which period they either die or enter the vector mosquito while taking a blood-meal. Development takes place in the new host for a period of two weeks and the infective larvae thus produced enter the human host during a subsequent bite and cause infection.

Symptoms seen in filariasis vary from person to person eg. feverishness, temporary swellings, cough, red patches, enlarged lymph nodes, aches and pains, joint swelling, itching swelling of hands and feet, painful red streaks in hands and legs passage of milky-white urine, swelling of breasts and testicles etc. Elephantoid swelling develop when the disease progresses and the damage becomes irreversible.

Figure 2: Elephantoid swelling of a leg caused due to filariasis

Figure 3: A Swollen hand developed due to long standing filariasis.

To detect microfilariae blood must be taken at night. Even though it is not practicable best time to take blood is between 10 p.m. and 2 a.m. Florescent antibody test, though very popular is not a reliable test for diagnosing filariasis.

People with microfilariasis are treated by giving the drug Diethyl Carbamazine Citrate (DEC) for two weeks and followed up for two years until they are free of infection. While on drug, some patients develop adverse reactions like fever, headache, and transient swellings in the body, itching, constipation or diarrhoea. These last for 1-2 days and therefore drugs must be continued without interruption. These reactions are more common in people with high microfilaraemia.

Today, filariasis is prevalent in densely populated urban areas due to rapid, unplanned urbanisation that has taken place in these areas producing several manmade mosquito breeding places. The vector breeds in highly polluted stagnant water containing decaying organic matter, which is found rampantly in these areas. Therefore, getting rid of temporary breeding sites, and treating the permanent breeding places with larvicides, using mosquito nets and repellents to minimise man vector contact are some of the methods used today to control disease by vector control.

In the control of filariasis more attention is paid to parasite control and is done by screening the entire population in the endemic area by subjecting them to an annual night blood examination during home visits. The positives detected are treated with DEC and are followed up for 2 years. Within limited resources this coverage is not very satisfactory. Vector control using larvicides is limited to few urban council areas. High cost of larvicides, its temporary effect and the long term hazards caused by environmental pollution has to be considered in using them for vector control.

Today more than 120 million people in the world are affected by lymphatic filariasis in at least 80 countries of whom about 79 million are asymptomatic carriers. A large percentage of the affected people live in South-East Asia. Globally, the infection has been recognised as the second leading cause of permanent and long-term disability with deforming, mutilating disease of the limbs and genitals resulting not only in physical crippling but also in serious psycho-social crippling in economic less. Recent dramatic advances in treatment methods, both for controlling transmission and for disease management, along with improved techniques in diagnosis of the disease, has led an independent International Task Force for Disease Eradication to identify lymphatic filariasis as one of only six infectious diseases that are considered to be eradicable. Therefore, in 1997 World Health Assembly adopted a resolution to eliminate lymphatic filariasis as a global public health problem.

Based on this resolution on the recommendation of the WHO, instead of giving selective treatment after screening, single dose mass drug administration will be carried out in the entire endemic area annually for a period of 5-6 years. Here a combination of two drugs (DEC and Albendazole) will be used to achieve better targets instead of using DEC alone. Albendazole has an antihelmenthic effect in addition to being macrofilaricidal and therefore benefits achieved in administering multi-drug therapy has been considered for the future.

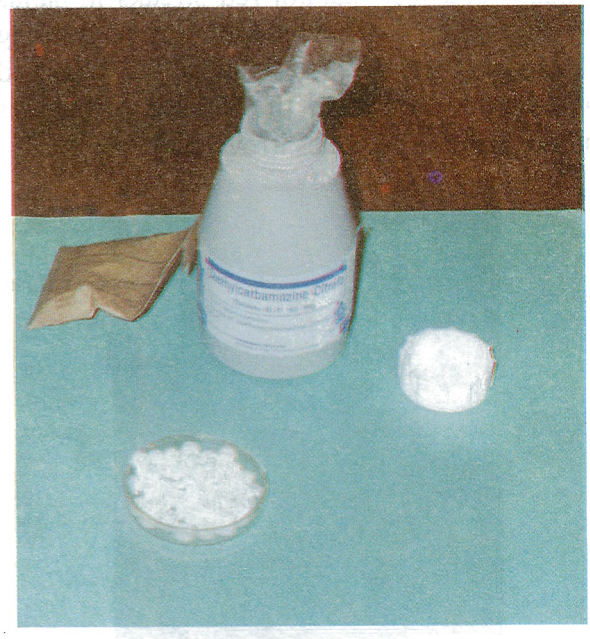

Figure 4: Medicine used in controlling filariasis

Single dose mass treatment using DEC only, commenced in our country in 1997. A population of 0.7 million were thus treated in 1998. This programme was extended to more endemic areas and in October 1999 and in April 2000, this was implemented as a National Programme. Action is being taken to commence implementing the programme using the multi drug regimen in March 2001. Here drugs will be administered to the entire population at risk (above 2 years of age, leaving pregnant and lactating mothers). To get the desired effect coverage of more than 80% will have to be achieved.

Lymphatic filariasis is an eradicable disease. This could be achieved by eliminating the worm-load in the community and by controlling the vector.

This single dose treatment will not be adequate to people harbouring microfilariae and for those with clinical manifestations. These patients must receive the full course of treatment and those with progressive clinical symptoms must be relieved by preventing further progress of the disease, by advising them to attend to proper foot hygiene.

Thus to reach our goal of eliminating lymphatic filariasis as a public health problem, community support and participation is a grave necessity and if these are received fully this dreadful disease could be eradicated from Sri Lanka during the next decade.

MALARIA

Dr Kapila Piyasena
Eye Hospital

Malaria is a disease affecting 100 million people annually with a mortality rate of 1%. It is caused by a parasite *Plasmodium* which lives in blood and other tissues.

Malaria means "bad air", was so named because of association of the disease with the odorous air of swamps. Parasite was discovered in the blood by Laveran in 1880. In 1898 Ross experimentally proved the mosquito transmission of the disease. Afterwards Grassia and his pupils worked independently and described the cycle of human malaria in *Anopheles*.

Endemic and epidemic malaria are found in all countries between the 30° South and 40° North lines of latitude. Malaria is primarily a disease of hot humid countries at altitudes less than 2200m above mean sea level, where conditions are ideal for prolific breeding of mosquito vector *Anopheles*. It is endemic in South Asian countries, in parts of Africa and in parts of South and Central America. Other than the mosquito, malaria also can be transmitted by blood transfusion.

At present malaria is the most important communicable disease in Sri Lanka, which has devastating results both economically and socially. During the year 1998, 1.3 million blood samples of fever patients were screened and 15.8%, were positive, compared to 16.4% positive in 1997. Of which 80%, were due to *Plasmodium vivax*. The rest was due to *Plasmodium faliciparum*. Sixty two percent (62%) of total malaria cases were reported from the North-Eastern Province.

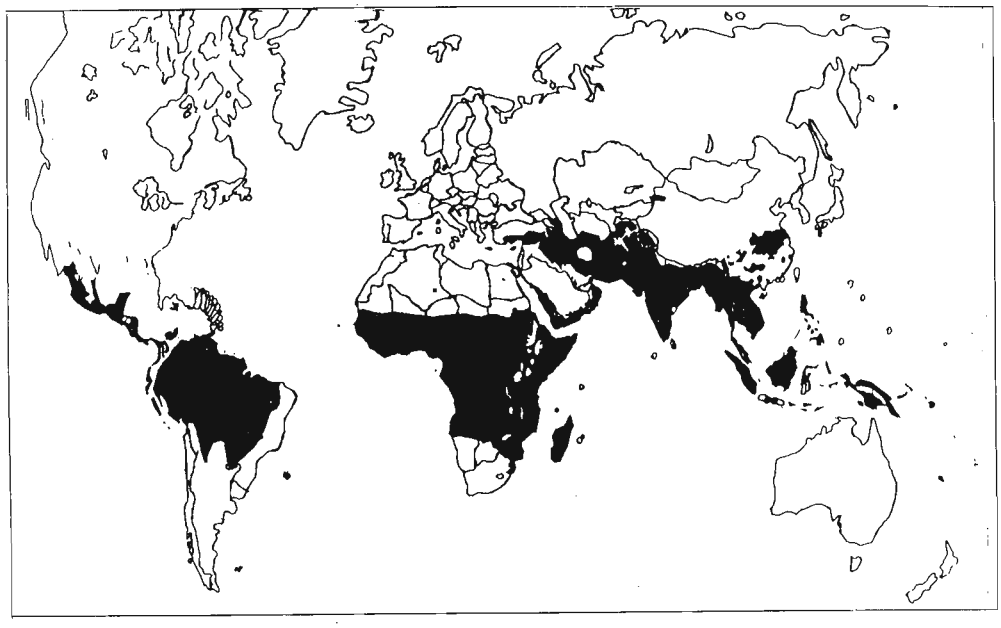

Figure 1: Distribution of Malaria in the world

During 1998, 115 deaths were due to malaria. Of which 106 (92%) were from North Eastern Province.

The main reason for the increase in mortality and morbidity due to malaria in these areas was the practical difficulties encountered in case detection and treatment of malaria patients. Still the predominant parasite species continues to be *Plasmodium vivax*. The principal vector continues to be *Anopheles culicifacies*. Secondary vectors are *Anopheles subpictus* and *Anopheles annularis*.

Four species of *Plasmodium* are capable of causing malaria in man.

Plasmodium vivax
Plasmodium falciparum
Plasmodium malariae
Plasmodium ovalae

P.vivax is the commonest and most widely distributed species being prevalent in both tropical and temperate zones. *P.vivax* causes "tertian" or "benign tertian" malaria which is likely to cause relapses. *P.falciparum* is prevalent in the tropics but does not thrive as *P.vivax* in temperate countries. It causes "malignant tertian" malaria which is dangerous than other types. There is no animal reservoir for any of these human parasite diseases except possibly Chimpanzees for *P.malariae*. In Sri Lanka only *P.vivax* and *P.falciparum* malarial parasites are found.

Life Cycle

General nature of the life cycle of all species of *Plasmodium* is similar.

In human
- Exo-erythrocytic stage
- Erythrocytic stage

Mosquito cycle

Exo-erythrocytic stage

When a mosquito bites a human it introduces sporozoites (stage which is infective to human). The parasite does not enter red blood cells at once. Parasites leave the circulation and enter into the liver. Those sporozoites that are not removed by the body's defence mechanism undergo development within the liver cells. They go through development (at least two schizogonic cycles) within the liver cells before invading blood. Then parasites rupture the liver cells and invade the blood stream and enter into red blood cells. At this stage parasites are called micro merozoites.

Erythrocytic stage

About a week or ten days after introducing sporozoites the parasites (micro merozoites) invade red blood cells and pass through several stages of development. The earliest form is the "ring stage". Later it is developed and become trophozoites, shizants and finally merozoites. These parasitic forms are found in peripheral blood about 12 days after inoculation of the sporozoites in *P.vivax* infections and 9 days in *P.falciparum* infections.

Gametogeny (the process of development of sexual stages)

Within red blood cells the process may continue for a considerable period of time and stage of gametogeny occurs. At this stage few merozoites develop into sexual form of the parasite known as gametocytes. Mature forms are found in peripheral blood and now the patient is infective. When an *Anopheles* mosquito bites the patient this stage of parasite enter the mosquito stomach and the life cycle continues.

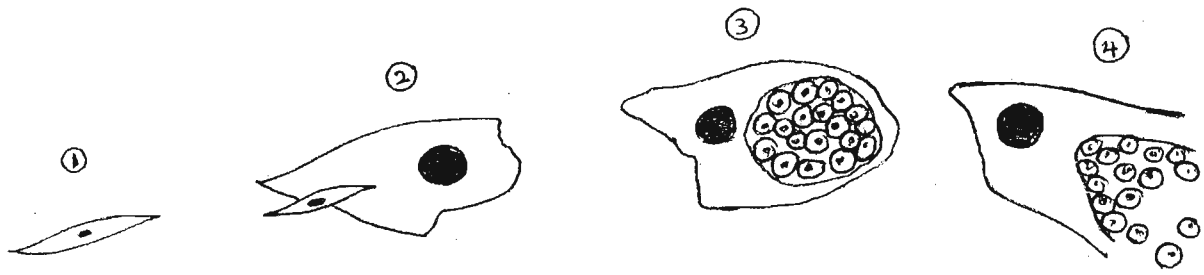

Figure 2: Exo-Erythrocytic stage

1. **Sporozoite**
2. **Sporozoite enters into liver cells**
3. **Development and multiplication within the liver cells**
4. **The liver cells rupture and micromerozoites liberate into blood**

Mosquito cycle

The gametocytes are of two types. Micro-gametocyte (male form) and Macro-gametocyte (female form). Both forms are found in mosquito stomach. Male gametocytes form flagella like structures and swim actively and unite with the inactive female gametocytes. Later an oocyst results and finally sporozoites break loose from the oocyst. Such an oocyst may contain more than 10,000 sporozoites and there may be about 50 oocysts on one mosquito stomach. Sporozoites make their way to salivary glands and assemble in the cell lining of the salivary glands. There may be up to 200,000 sporozoites in one mosquito. They enter the lumen of the ducts and discharge with saliva when the mosquito bites. It may take 20 or more bites to discharge them all.

Clinical features

Malaria clinical features usually precede by various premonitory symptoms and comprise of three stages.

Cold stage: Starts with shivering and a feeling of intense cold. Teeth chatter and patient covers himself with any available clothing and blankets. Skin becomes dry and pale with high pulse rate. Vomiting occurs and children often have convulsive seizures. This stage lasts for15 minutes to one hour.

Hot stage: Feeling of intense cold gives way to one of the distressing heat. The face is flush and skin becomes dry and burning, headache becomes intense, nausea and vomiting are common. The pulse rate is high. There is often intense thirst while the temperature may rise to 41°C (106°F) or more. This stage lasts for two to six hours.

Sweating stage: The patient breaks out in profuse sweat. So that his bedding become drenched. The temperature falls rapidly, often below the normal level. He usually falls into a deep sleep and on waking feels weak but other wise normal. This stage lasts for two to four hours.

In all types of infections the periodic febrile response is related to the time of rupture of a sufficient number of mature schizonts and consequent discharge of merozoites into the blood stream.

The incubation period in malaria covers the time between the infection and the first appearance of clinical signs of which fever is the most common. In *Plasmodium vivax* infections it is 13-17 days, (may go up to 9 months) whereas in *Plasmodium falciparum* infections it varies between 9-14 days.

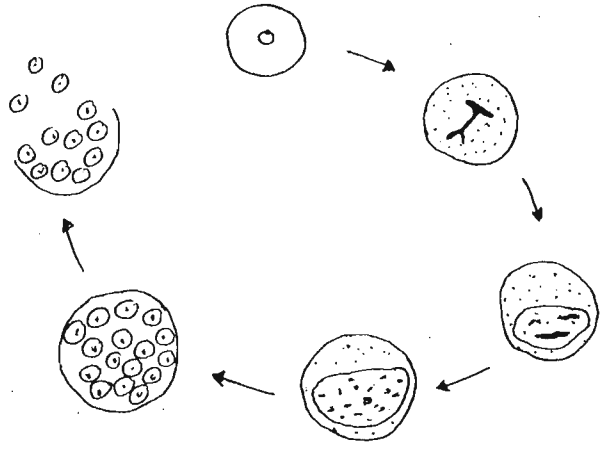

Figure 3: Erythrocytic stage
From ring stage to merozoites
Ring stage, development of the parasite within the RBC, release of merozoites in the blood

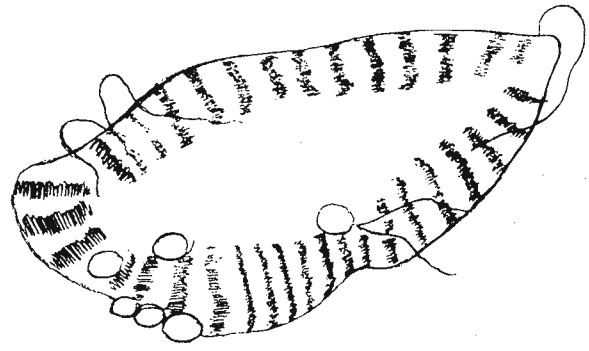

Figure 4: An oocyst of the parasite developing in mosquito stomach

Plasmodium falciparum is potentially the most severe form of malaria, with high levels of parasites in the blood. It affects kidneys, liver, brain and gastro intestinal tract. The fever has no particular pattern. If the amount of parasites in the blood exceeds more than 5% of red blood cells severe form of malaria may develop.

Cerebral malaria with elevation in body temperature and rapid deterioration in consciousness, convulsion coma and death may result.

Gastro intestinal symptoms with stools containing blood, mucus and pus together with abdominal pain.

Renal complications with blood in the urine and renal failure.

Treatment

General treatment: to control fever antipyretic as Asprin and Paracetamol can be given. Intravenous fluid may be required.

Drug treatment - Chloroquine is the drug of choice, some species especially *P.falciparum* has shown resistant to Chloroquine. They are treated with a combination of sulphurdioxine and pyrimethamine.

Control of Malaria

Control of Malaria started in Sri Lanka way back in 1946 by introducing DDT spraying. The spraying programme covers all houses in Malaria endemic areas. It was so effective; subsequently the malaria eradication programme was launched in 1958. Resulted near eradication in 1963 when only 17 cases were recorded within the country. But due to multitude of factors resurgence of the disease occurred often for a few years, which led to an epidemic situation in 1968-1969. The significant technical problem was the emergence of DDT resistance in the vector mosquito, which later resulted in the change of insecticide spraying to Malathion in August 1977. At first there was a significant drop in incidence, but in 1982 there was again a sharp rise in incidence.

So now the control of malaria would be a multi disciplinary approach. The major constraint in malaria control is that even when carried out efficiently and cost effectively, it is relatively expensive.

Control through treatment

The provision of easy access to treatment is the main approach in malaria control. In areas of malaria endemicity, radical treatment of cases of clinical malaria with appropriate drugs is often used to reduce transmission.

Chemoprophylaxis as a control tool

People living in the endemic areas primarily young children and pregnant women are given medicine even without the disease to protect from the disease.

Malaria Vaccines

Malaria vaccines are being developed against three stages of the parasite's life cycle with particular focus on *P.falciparum* malaria, due to its severity and high prevalence. Vaccines that destroy the initial stages of the parasite after entering the body. Such vaccines would prevent sporozoites from developing after being inoculated into human host, blocking infection and preventing clinical disease and transmission.

Another vaccine is targeting the blood stages blocking invasion, development and spread of merozoites in red blood cells.

Also, vaccines are being developed to prevent transmission by destroying sexual forms.

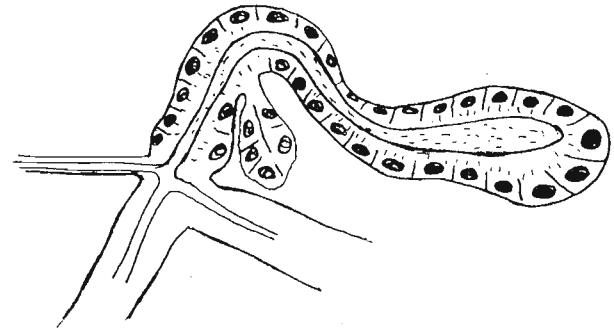

Figure 5: Salivary gland of the mosquito with merozoites within the cells and lumen

Prevention of Human-vector contact

Several self protective techniques exist which if used consistently can greatly reduce human vector contact and in some cases destroy the vector. *Anopheles* mosquitoes are usually found from dusk to dawn. Bed nets to protect against mosquito bites, while sleeping is very helpful. In recent years it has been shown that using bed nets in malaria endemic areas particularly nets impregnated with pyrethroid (insecticide) such as permethrin can be safe and an effective barrier between human and mosquito.

In addition, individual measures to prevent human vector contact and reduce vector population include impregnating curtains and small fumigation mats with pyrethroid, burning coils, applying repellents and spraying living places.

Installing windows and door screening and wearing protective clothing particularly after sunset also reduces contact with the mosquito. When selecting a site for new housing, temporary or permanent, should avoid places adjacent to mosquito breeding sites. Improving housing construction may protect humans against contact with mosquitoes.

Reducing Breeding sites

Draining or filling places around human dwellings where water collects, such as potholes, small ponds and old cans and covering wells or water tanks will reduce mosquito breeding sites.

Also careful planning of rural and urban development projects such as construction, road building, irrigation system and well digging is criticle.

Destruction of Mosquito Vector

There are several techniques used to destroy the mosquito in both its larval and adult stages. eg: biological control methods involving introducing mosquito pathogens or predators such as bacteria and larvivorous (larvae-eating) fish.

DENGUE

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National Science Foundation

Introduction

The word dengue (pronounced 'Dhen gey') is an African word and the word defines "breakbones" since the time when Benjamin Rush from Philadelphia first described it as 'breakbone fever' in 1780. There is no breaking of bones in a patient suffering from Dengue but the meaning indicates the severe pain that is being felt to the body. Dengue fever is a mosquito borne viral infection common throughout the tropical and subtropical regions of the world, predominantly in urban and peri-urban areas. It has now become a major international public health concern. It is a flu-like illness ranging from symptom free (non-specific viral syndrome) to classical fever with hemorrhagic manifestations. It affects infants, young children and adults but rarely causes death.

History of dengue in the World and Asia

Dengue virus was first isolated in 1943, is morphologically undistinguishable from the agent causing yellow fever. Dengue hemorrhagic fever (DHF) a potentially lethal complication was first recognized during the 1950s and today it is a leading cause of childhood mortality in several Asian countries. Its hemorrhagic nature as a new symptom was identified in 1953 in Philippines, after 3 years of this discovery; it was able to increase the death toll in Manila by 6%. The first reported epidemics of dengue fever occurred in 1779-1780 in Asia, Africa and North America, the near simultaneous occurrence of outbreaks on three continents indicates that these viruses and their mosquito vector have had a worldwide distribution in the tropics for more than 200 years.

During most of this time dengue fever was considered benign and nonfatal disease of visitors to the tropics. A global pandemic dengue began in Southeast Asia after World War11 and has intensified during the last 15 years. In South-east Asia epidemic DHF first appeared in the 1950s, but by 1975 it had become a leading cause of hospitalization and death among children in many countries in that region. Afterwards the disease appeared as outbreaks occasionally in South-East Asia. In the 1980s DHF began a second expansion into Asia when Sri Lanka, India and Maldive Islands had their first major DHF epidemics.

The global prevalence of dengue has grown dramatically in recent decades. The disease is now endemic in more than 100 countries in Africa, America, Eastern Mediterranian, South-East Asia and Western Pacific. According to WHO, 2500 million people (2/5) of the world population are now at risk from dengue. Current estimates show that there may be 50 million cases of dengue infection worldwide every year. Not only the number of cases increasing but the disease is also spreading to new areas. For example, in Brazil 475000 suspected cases were reported between January and October 1998.

Prevalence of the disease in Sri Lanka

It has been reported that clinical dengue like disease prevailed in the beginning of 19th century but it was serologically confirmed as dengue in 1962. It occurred as a plague in 1967. In the same year 08 people died from the disease and 28 were seriously ill. After that a less number of patients recorded in the country and in 1970 no suspected dengue patients were reported except a few occasional cases. But during the period 1987- 98, it was observed that the disease had been spread fast. From 1969 to 1988 it was witnessed that a circulation of multiple dengue serotypes with dengue fever in urban areas.

According to the Figure 1, before 1989 there had been reported only 8 patients from Sri Lanka. But in 1989,dengue suspected cases had been increased up to 203 out of which 20 were reported to be dead. In 1990, the number of suspected DF/DHF patients had been increased up to 1350 and 54 of them died. During the first 6 months of 2000, the situation worsened.

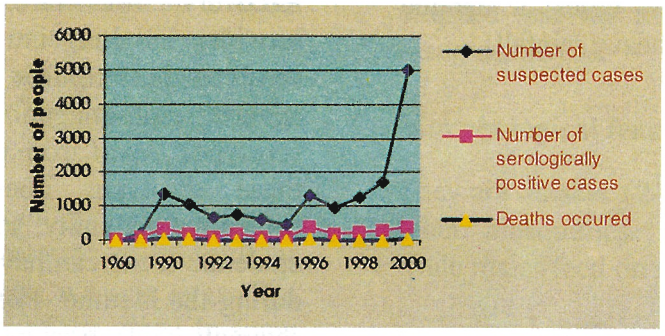

Fig. 1: Reported cases of DHF in Sri Lanka

It can be seen that during the period from 1991 to 1995 the number of suspected cases have been increased from 440 to 1048, out of which 54 died. In 1996, 1294 patients were reported and there were 54 deaths. Therefore dengue has reached the epidemic stage during the last 4 years in Sri Lanka. In 1996 and 1999, 1294 and 1699 cases were reported with an average case fatality rate of 1.8%.

According to Dr T.A. Kulathilake of Epidemiological Unit of Colombo General Hospital, there was a marked increase in the disease to the level of a plague in 2000 with about 5000 patients suspected of having dengue had been admitted to various hospitals around the country for treatments. Out of 5000, 400 were confirmed as Dengue patients and 30 had died from the diseases. In comparison, in 1999, 1699 patients were hospitalised and 300 were confirmed as having dengue and 14 died from the disease. These indicate how dengue has attained epidemic level during the year 2000 in Sri Lanka and how it has caused much effect on the normal life in Sri Lanka.

Prominent areas where Dengue outbreaks occurred

When we look into the areas where patients were reported, in 1990 the cases reported from Colombo and sub-urban areas are high compared with the number of cases reported from out-stations. The respective Colombo suburban areas were Nugegoda, Maharagama, Dehiwala, Mount-Lavinia, Kotte, Piliyandala, Ragama, Negombo, Kalutara, Beruwala, Moratuwa, Galle and Tangalle. Patients were reported from Anuradhapura too.

In 2000, Dengue cases were first reported from Matara area. The first death was also reported from Matara. However the patients were reported from other areas such as Colombo, Gampaha, Kegalle, Galle and Kurunegala where dengue is being reported frequently. In 1999 too, 1981 cases suspected of having dengue had also been reported from Colombo area.

The living agent responsible for Dengue

The dengue fever and dengue hemorrhagic fever are caused by a Flavi (Genus: *Flavivirus*, Family: Flaviridea) virus, which belongs to Arbo virus group. Arbo is defined as "Arthopodborne" (Arthropod origin). This virus has four flavors called serotypes, which are creatively named DEN-1, DEN-2, DEN-3, DEN- 4. They are closely related but antigenically distinct, RNA flavivirus. Symptoms may vary according to the serotypes. A serotype refers to how many antigens in different viruses have in common. In these types there can be virus types with minor changes. Epidemiologists believe that recently the dengue has spread in Sri Lanka as a plague causing a panic situation due to the infection by virulent type of viruses. It was observed that the suspected cases of dengue have been increased after a heavy rainfall.

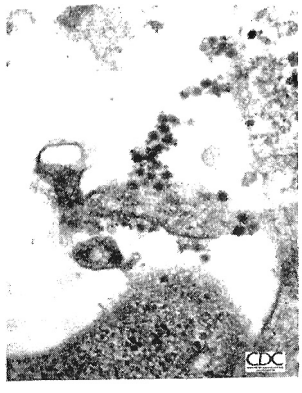

Fig 2 : Electro micrograph of Flavi virus (Source : Internet)

Difference of dengue from the traditional contagious diseases like measles and chicken pox

DHF is more likely to be developed in a person who had previously infected from Dengue than in a person who had not been infected from dengue. The reason for this situation could be that there are four distinct but closely related viruses (serotypes), which cause dengue. When one was ill from one serotype of virus at the first time, he or she could be ill at the second time by another serotype of the virus. But this situation is somewhat different from measles and chicken pox where it is very rare for a person to be infected from the virus of measles and chicken pox if the person had been suffered from the same at an earlier stage.

Recovery from infection by one serotype provides life long immunity against only that serotype but confers only partial protection against subsequent infections by other serotypes. Apart from that infection with one of these serotypes does not provide cross-protective immunity, so people living in dengue epidemic areas can have four dengue infections during the lifetime. There is good evidence that sequential infection increases the risk of more serious disease resulting in DHF. This is the fact that a person can have dengue for the second time although he or she had been contacted dengue earlier.

It has also been noticed that, dengue hemorrhagic fever (DHF) causes the condition critical for persons who are having the disease for the second time than for persons who have it for the first time. DHF is more likely to be developed if an individual previously infected with one serotype is later infected with a different viral strain. It has been observed that the antibodies formed in the body during first infection may cause some serious situation in

the second time. The antibodies that are produced from one serotype (virus type) in the body will act to facilitate the incoming and growth of a new virus type in the body rather than reacting against incoming of the new virus types into the body. Then the person will get the illness easily at the second time. This opinion is believed at present since 90% of cases that had the disease had been suffered from dengue more than once. Since these ideas are not yet proved, research must be performed to determine exactly what synergistic properties these serotypes exhibit.

Transmission:

The dengue viruses are transmitted to humans through the bites of infective female mosquitoes. Mosquitoes generally obtain the virus while feeding on the blood of an infected person. Once infected, a mosquito is capable of transmitting the virus to susceptible individuals for the rest of the life during probing and blood feeding.

Infected female mosquitoes may also transmit the virus to the next generation of mosquitoes by transovarial transmission e.g. via eggs. But humans are the main host of the virus. In some regions of the world like Africa, monkeys may become infected and perhaps serve as a vector for uninfected mosquitoes. The virus circulates in the blood of infected humans for 2-7 days, at the same time they have fever, mosquitoes may get the virus when they feed on a person at this time.

Vector

The mosquitoes responsible for transmission of the flavi virus are of two species, which belongs to the genus *Aedes*. They are called *Aedes aegpti* and *Aedes albopictus*. These mosquitoes have been identified as living in clean water in vessels. Common mosquito breeding sites are

- discarded canned fish tin, clay pots
- used empty milk pots and bottles
- discarded automobile tyres
- coconut shell and discarded king-coconut
- water storage plastic vessels and polythene bags
- indoor and out door flower pots
- insect traps kept for table legs
- puddles of water left by rains

The main reason for the rapid growth of mosquito population is that even under some minor wet conditions the eggs of these mosquitoes could survive (when the rain stopped and the puddles evaporated) without being destroyed.

Morphological features of Mosquitoes

They are smaller (2-4 mm in size) compared to other mosquitoes. Only females suck blood while males feed on nectar.

Identification:

Aegypti	Albopictus
Distinctly visible black and white rings on the legs Silver markings on the surface of thorax	Horizontal single white line on the surface of the thorax

Normally they are active and bite people during daytime. It has been reported that they are very active during 6 to 9 in the morning and 3 to 5 in the evening of the day.

Eggs : Float as a collection, like a raft in water
Larvae: Swim at an angle to the water surface
Adults : Rest parallel to the surface

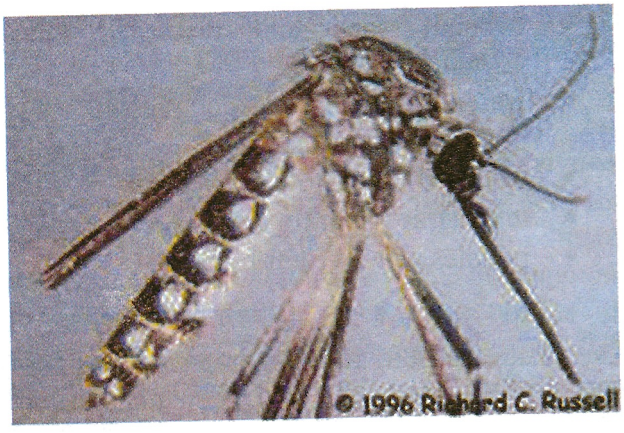

Fig 3: Vector mosquito (Genus : Aedes)

Symptoms

Symptoms of dengue appear within 5-8 days after entry of the virus into the body of an individual. Sometimes it can take 3 to 15 days. This is called the incubation period. Symptoms do not appear just after infection by the virus. The immune system of the body fight back with the virus and only if the body fails to overcome the virus, the symptoms would appear.

***Basic clinical features*:**

Dengue virus infections cause a spectrum of illnesses ranging from symptom free, mild fever to classical dengue fever (DF) and dengue fever with hemorrhagic conditions (DHF) and dengue shock syndrome (DSS).

a) *Classical dengue fever*:

Dengue fever is a severe, flu-like illness that affects children and adults but rarely causes death. Mild and nonspecific in most children associated with pharngitis, rhinitis and mild fever for 2-5 days. The basic symptoms of classical dengue fever most likely to occur in older children and adults can be mentioned as follows,

- fever
- severe headache
- pain behind the eyes
- muscle and joint pains ("breakbone" pain)
- back pain and skin rash, mild cough

At this stage it is advisable to rest and take drugs like Parasitamol to abate fever. If there is a doubt about dengue, patients should be prevented in taking drugs like Aspirin that will react in opposite of gathering blood platelets causing internal bleeding of the body.

b) *Dengue hemorrhagic fever*:

This stage is a potentially deadly complication characterized by the following features:

- High fever- continues for 2-7 days, can be as high as 40-41^{0}C
- Severe headache
- Hemorrhagic phenomina often with the enlargement of the liver, in severe cases circulatory failure, facial flush, spontaneous bleeding from eyes, nose, ears, mouth, skin and gums, bleeding with stools and urine, vomiting of blood

The reason for this hemorrhagic condition is the changes in blood clotting mechanisms. In addition fluid plasma in blood may secrete out from blood vessels. This internal bleeding could lead to the shock syndrome.

Fever can be abated by giving more fluids. Normally the fever is abated within 3-5 days and patient is also cured within several days.

c) *Dengue Shock Syndrome (DSS)*

The patient's condition may suddenly deteriorate after a few days of fever and the temperature drops and the patient may go rapidly into a critical state of fatal shock. The following features could be seen during the period,

- shock development
- drop in body temperature, sweating
- circulatory failure causing changes in blood pressure
- drop in platelet count and packed cell volume
- continuous internal bleeding (leakage phase)
- the body turns pale

WHO classification of dengue fever

Grade 1	Fever, constitutional symptoms
Grade 11	Grade1 + spontaneous bleeding (skin, gums, GI tract)
Grade 111*	Grade 11 + circulatory failure and agitation
Grade IV*	Grade 111+ profound shock, unrecordable blood pressure

* dengue shock syndrome

Treatment:

There is no specific treatment for dengue fever. Treatment is supportive. However careful clinical management will save the lives of DHF patients. Plenty of fluids are given to patients and it is important to take rest as much as possible. Currently no dengue vaccine exists.

Vaccine development is difficult because any of four different viruses may cause disease and protection against only one or two viruses could actually increase the risk of more serious disease. Research is underway in the development of vaccines and vaccines may not be immediately forthcoming since the cause of the disease itself requires more studies.

Prevention and Control:

- The only method of controlling and preventing the disease is to combat the breeding of vector mosquitoes
- Prevent from mosquito bites using mosquito nets, coils and fumes and wearing clothes
- Curing the patients from hospital treatments
- Identification of patients from blood tests, platelet counts etc.
- Launch programmes to create awareness about the disease especially at school level

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2. Hana Ibrahim, Dengue the scourge of the new millennium, Observer, published on 2000-11-20, 6-8 pp

http://www.cdc.gov.ncidod/dvbid/dengue/index.htm (CDC dengue fever home page)

CHARACTERISTICS OF DISEASES CARRYING MOSQUITOES

There are four major Mosquito borne diseases in Sri Lanka. They are namely Malaria, Filariasis, Dengue and Japanese Encephalitis. Not one single mosquito species is responsible for spreading all four diseases but different species. Though there are 16 types (Genera) comprising more than 140 species of Mosquitoes in Sri Lanka, disease carriers are limited to a few. That is also to their females. It such happens that these female mosquitoes need human blood meals to mature their eggs.

Male mosquitoes do not need blood meals but feed on plant juice, nectar and fruit juice. The number of disease carrying mosquitoes in Sri

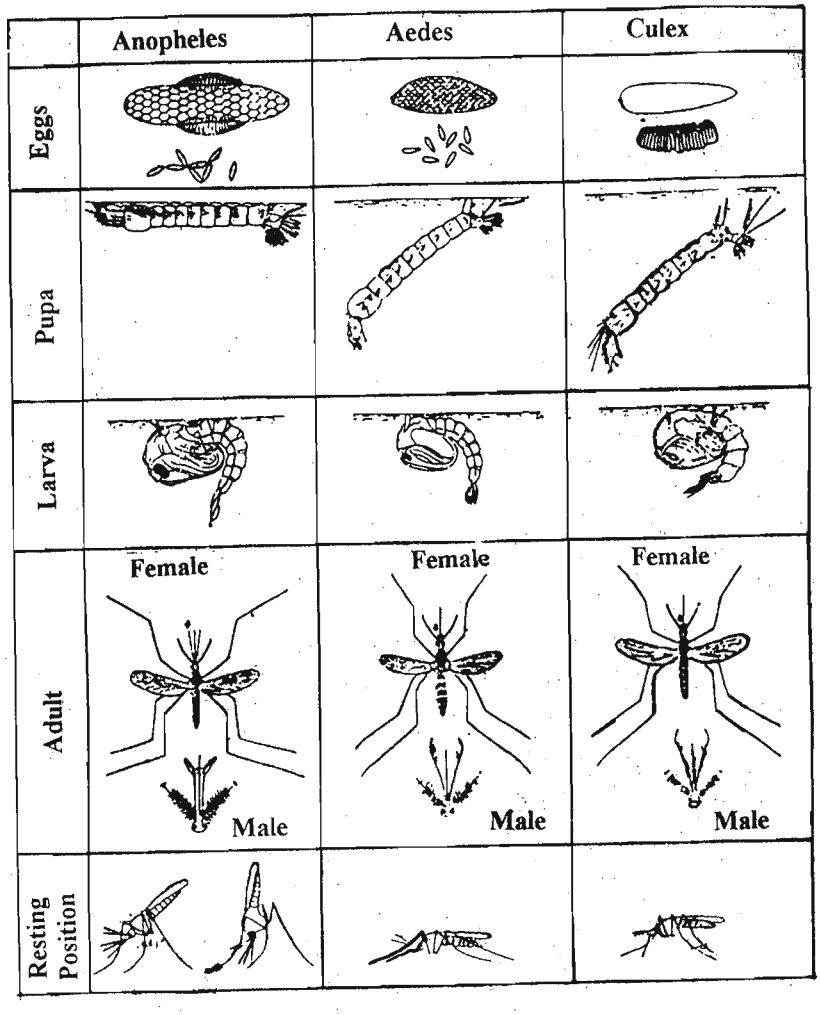

Figure 1: Phenotypic variation among life stages of mosquito genera

Lanka are only about 16. To prevent the spread of mosquito borne diseases one has to study the life cycle of Mosquitoes. It is important to understand the characteristic features of the four stages i.e. the egg, larva, pupa and the adult stage of different mosquito species. Figure-1 gives the differences, in the life cycles of the main mosquito species.

There are two main groups of mosquitoes in Sri Lanka. They are called Anophylines and Culicines. The major difference that could be noted is the way they rest. The adult anophyline mosquito keeps its body at an angle to the resting surface, while its larva stays parallel to the water surface. The adult culicine mosquito rests with its body parallel to the resting surface while the larva hangs down from the water surface.

Table 1: Characteristic differences in Lifestyle of various Mosquito species.

STAGE OF THE LIFE CYCLE	GENUS		
	Anophelines	Culicines	
	Anopheles	**Aedes**	**Culex**
Eggs	Floats singularly in water	Floats as a collection of Eggs	Floats as a collection (about 100 eggs together)
Breeding Places	Pools formed in river and stream beds during droughts, margins of slow flowing irrigation channels, water collected in brick pits and abandoned gem pits rain water collections, non polluted water and in paddy fields, marshes etc.	Water collections in discarded tins, pots coconut shells, blocked rain gutters, flower vases, ant traps discarded tyres (container breeders)	Polluted waters in cess pits, arecanut pots, coconut shell pits, blocked drains, marshes etc.
Larvae	Swims parallel to water surface	Swims with an angle to water surface	Swims with an angle to water surface
Adults	Small in size. Forms an angle to the surface when resting. Black in colour. Black spots on the wings.	Normal in size. Rests parallel to the surface. Usually black in colour. Far end looks sharpened. No black spots on the wings. Characteristic white patterns seen on the thorax	Normal in size. Rests parallel to surface. Dark brown and light brown in colour.
Active Period	6.00 p.m. – 10.00 p.m. 4.00 a.m. - 6.00 a.m.	Day time 7.00 a.m. – 9.00 a.m. 3.00 p.m. - 5.00 p.m.	6.00 p.m. – 4.00 a.m. 4.00 p.m. - 10.00 p.m.
Preferred Meal	Cattle blood	Human blood	Pig, goat and cattle blood

(Reference: Dr Mervin B. Wickramasinghe, "Disease carrying mosquitoes" SAPATHA, 1990, Vol. 31(1-4), 9-11 pp.)

MOSQUITO CONTROL

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Introduction

Mosquitoes are considered as the most dangerous group of animals to human welfare. Apart from their biting nuisance, they transmit fatal human diseases. The total number of deaths due to malaria, one of the mosquito borne diseases, exceeds one million per year in Africa alone. Therefore, the control of mosquitoes has been an important issue since late 19th century, when mosquitoes were first identified as vectors of human diseases.

Taxonomically mosquitoes belong to the Class Insecta, Order Diptera and Family Culicidae. There are about 38 genera and 3450 species worldwide. In Sri Lanka about 140 species can be found. Male mosquitoes feed on sugary substances like fruit juices. Females feed on blood. A blood meal is essential for a female to develop its eggs. They can transmit various pathogens to the host while they feed. There are four major mosquito borne diseases in Sri Lanka (Table 1).

Biology

Females lay eggs (eggs mature 2-3 days after a blood meal) on the water surface or on the wall of a water container. Breeding sites differ from species to species. For example, *Anopheles culicifacies* prefers water pools in riverbeds, *Culex tritaeniorhynchus* prefers paddy fields, *Aedes aegypti* prefers tree holes and small water containers like water filled tyres, tins or buckets. Eggs are laid singly eg. *Anopheles* or in rafts eg. *Culex*. Out of all the types, eggs of

Table 1: Major mosquito borne diseases, and vectors in Sri Lanka

Disease	Major Vector	Pathogen
1. Malaria	*Anopheles culicifacies* *Anopheles subpictus*	Protozoan (*Plasmodium*)
2. Filariasis	*Culex quinquefasciatus*	Nematode worm (*Wuchereria*)
3. Dengue	*Aedes aegypti* *Aedes albopictus*	Virus
4. Japanese encephalitis	*Culex tritaeniorhynchus* *Culex gelidus*	Virus

It is important to understand the biology of a mosquito species before developing control strategies.

Aedes species are well known for their resistance to desiccation. Mosquito larvae live in water and often come to the surface for breathing. There are four larval stages (instars) and the whole larval life is about one week in the tropics. Then the larva becomes a pupa and after about 2-3 days the adult emerges. On average the life span of an adult is 1 - 2 weeks. Mosquitoes usually fly within a range of two kilometres.

However, wind currents may carry them considerable distances. Some mosquito species prefer to rest inside houses (indoor resting or endophilic) while others prefer to stay outside (outdoor resting or exophilic). Some prefer to feed on humans (anthropophilic) while others prefer to feed on other animals (zoophilic). Some species prefer to feed during daytime and others prefer nighttime feeding. It should be clear now that the habits and habitats of mosquitoes differ from species to species. Therefore, according to the species of interest, control methodologies should be developed. Mosquito control can be carried out at various levels. Larval control programmes and adult control programmes are launched to reduce mosquito abundance. Also, measures can be taken to protect you from mosquitoes. These strategies can be discussed under four topics; personal protection, house design and the habitats in and around houses, modification and manipulation of the environment and use of insecticides.

Personal Protection

Various chemicals such as deet (N,N- diethyl-3- toluamide) are available in the market as mosquito repellents. They are in the form of sprays, body lotions/oils *etc.* and not toxic as insecticides. In villages, various smokes (eg. cashew husks) and herbal extracts (*eg.* neem) and oils (eg. *Citronella* oil) are used to repel mosquitoes. Also wearing cloths to cover the whole body reduces the chances of getting bitten. As a preventive measure, visiting risky places during peak biting hours (for most of the mosquito species peak biting times are immediately before sunset and immediately after sunset) should be avoided. Another measure is the use of bed nets. However, bed nets will not be effective against daytime biting mosquitoes except to protect babies and young children sleeping during the daytime.

House design and the habitats in and around houses

Houses close to water bodies have a higher exposure to mosquitoes. If the direction of the wind is from the water body to the house, wind currents often bring newly emerged adults to the house. Therefore, these factors should be avoided if possible. A proper ceiling will not allow mosquitoes to enter houses through the gap between the wall and the roof. Screening other openings with mosquito nets or mesh is also important. If the screening of windows and doors is not possible they should be kept closed at least during the peak biting hours. Inside the houses, water containers, which can act as mosquito breeding sites should be managed properly. For example water of flower vases should be replaced once in two days, small water cups used as ant traps (*eg.* cups kept under table legs) should be filled with salt or soap mixed water so that larvae can not develop in them. In the garden, small containers such as cans, coconut husk shells should be removed or destroyed. Small depressions, tree holes, abundant ditches etc. which can act as breeding sites should be filled with soil, rubble, rubbish or cement. Gutters, blocked with leaves making small water pools, should be cleaned regularly. Overhead tanks, sewage pits etc. should be covered with

removable mosquito proof lids/cement slabs or expanded polystyrene beads, which are non-toxic. Polystyrene beads can be used even to cover larger bodies like abandoned gem pits.

Modification and manipulation of the environment

Drainage systems used in agriculture or for the transportation of sewage and rainwater are often important sources of breeding because of poor design and maintenance. Proper drainage systems should be established. It should follow the natural flow of water whenever possible. Sharp bends should be avoided. Margins or banks should be smooth and deep so that small water pools cannot be created. In larger canals regular fluctuation of the water level and flushing the breeding sites (stream sluicing) dislodge larvae. Marshy areas with high water tables often create good breeding sites for mosquitoes. Trees with high growth and evaporation rates *eg.* eucalyptus, can be grown to dry up these lands. Ponds, pools and lakes are excellent mosquito breeding sites. Certain species of fish, crustaceans and nematode worms feed on mosquito larvae and can be used in larval control. Rearing of larvivorous fish species is an easy and highly successful method used in larval control programmes. Fast growing water plants *eg. Azolla* that float on the surface can be used to cover the water surface.

Use of insecticides

Insecticides are the major tools used today in most of the mosquito control programmes. These are chemicals or biological materials that are used to control insect pests. Bio-insecticides have been produced using the bacteria species *Bacillus thuringiensis* and *Bacillus sphericus*. These bacteria produce toxic crystals, which can kill mosquito larvae once ingested. Crystals and spores of the bacteria are used as insecticides in the form of wettable powders. However, the use is restricted due to high production cost and low stability in storage.

The most successful synthetic insecticides are neuro-inhibitors. Despite the increasing cost and the risk of environmental contamination, these are the most widely used agents for insect pest control in the world today. Commonly used synthetic insecticides can be divided into four major groups; organochlorines, organo-phosphates (OPs), carbamates and pyrethroids. Today the use of organochlorines has been discontinued in many countries because of resistance and concerns for the environment but DDT still remains the most widely used insecticide for malaria control. All these insecticides attack the nervous system of the insect. For OPs and carbamates the target site is acetylcholinesterase (AChE), the enzyme which hydrolyses the neuro-transmitter acetylcholine. Cyclodienes, a sub-group of organochlorines, binds to the γ-aminobutyric acid (GABA) receptors in the Cl^- channels of the neurons and modulate the Cl^- conductance across the nerve membrane. The rest of the organochlorines (DDT + its analogues) and pyrethroids bind to Na^+ channel proteins of the nerve membrane inactivating their regulation. For organophosphates and carbamates the target site is acetylcholinesterase (AChE), the enzyme that hydrolyses the neurotransmitter acetylcholine.

Biology of the mosquito species should have been well understood to decide where to apply insecticides. *eg.* to control *Anopheles culicifacies* which breed in river beds, larvicides should be applied to rivers and other water streams; house spraying of adulticides is effective because they are indoor resting. But these applications may have very little effect on

other species. However, in situations where immediate results are needed eg. during outbreaks, vehicle or aircraft mounted aerosol generators are used to spray the whole area. Today mosquito coils are heavily used to kill /repel mosquitoes. Insecticides present in these coils are volatile pyrethroids. Recommended dosages of these insecticides have been proved to be harmless to humans. However, overnight exposure to the smoke of these coils which contains insecticides, other chemicals, carbon monoxide *etc*. may cause illnesses specially in the respiratory system.

Although it is not common in Sri Lanka, the use of insecticide impregnated bednets is a successful control measure in countries like Africa. Again the pyrethroids in harmless quantities are used. Villagers of rural areas are educated to prepare their own impregnated nets by the World Health Organization. Small quantities of insecticides can be incorporated into mosquito screening meshes, curtains, garments and even to wall paints.

Continuous exposure to insecticides (even to mosquito coils) can lead to the development of insecticide resistance in mosquitoes. Major mechanisms of insecticide resistance involve either an alteration in the rate of insecticide metabolism *ie.* detoxification, or an alteration within the target site of the insecticide. Increased metabolism is due to qualitative and/or quantitative changes of mosquito enzymes, which metabolise insecticides. Esterases, Glutathione-S-transferases and monoxygenases are the major groups involved. Target site alteration is often due to highly specific point mutations so that the altered target site does not respond to insecticides but performs its normal physiological functions within the mosquito body.

Sri Lanka, being a tropical agricultural country, heavily depends on synthetic insecticides, (mostly OPs and carbamates and recently pyrethroids) for control of agricultural insect pests and disease vectors such as mosquitoes. Mosquito control programmes have been mainly based on the usage of DDT prior to 1975-1977 and malathion (adulticide), temephos (larvicide) and fenthion (larvicide) until recently when other OPs and pyrethroids were introduced. It is always advisable to collect information about the underlying mechanisms of insecticide resistance in the field mosquito populations to decide the most effective insecticides.

As we all are aware insecticides pollute our environment. Therefore, it is desirable to go for other options *eg.* destroying the breeding sites, whenever possible. However, the last and the most effective option, which has to be taken in disease outbreaks, is the use of insecticides.

DNA PROBES FOR THE IDENTIFICATION OF MOSQUITO SPECIES

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Introduction

Mosquitoes are known to transmit a number of diseases to humans. The disease malaria for example, is transmitted to man through the bite of a mosquito infected with a protozoan parasite known as *Plasmodium.* The mosquito vector known as *Anopheles culicifacies* is mainly responsible for malaria in Sri Lanka. *An.culicifacies* is also a major vector of malaria in India and Pakistan.

Most mosquito species exist as a species complex comprising several sibling species within the same taxon. Sibling species arise due to reproductive isolation; they either do not cross-mate with other siblings or even if they cross-mate they do not produce viable offspring. However, their external features do not help to distinguish sibling species because they all look alike (isomorphic) and therefore the conventional taxonomic keys based on morphological features cannot be used to identify these siblings. In *An. culicifacies,* five sibling species have been identified up to now in India by the analysis of polytene chromosomes found in mosquito cells. Despite the similarity of these sibling species in their morphological features, differences between species have been found with respect to their seasonal prevalence, vectorial capacity and their response to insecticides. For example some sibling species are found to be more resistant to DDT insecticide, than the others. Each sibling species may therefore play a different role in transmission of malaria, due to Such behavioural differences amongst them. Hence it is very important to identify the sibling species of *An.culicifacies* present in areas where malaria is prevalent in Sri Lanka, in order to implement a successful vector control programme. For instance, if malaria vector species present in an area is known, the most suitable insecticide for that species could be sprayed. Proper use of insecticides will not only help to control the vector of malaria but also to reduce the wastage of resources.

Current Cyto-taxonomic Method of sibling species identification

The sibling species of the *An. culicifacies* are currently identified by staining and examination of the banding patterns of the polytene chromosomes present in the salivary glands or the ovaries of the mosquito. This is a technically difficult, time consuming method and certain stages of the life cycle can only be identified. A mosquito has several life cycle stages such as larvae, pupae and adult. Only fresh semi-gravid adult or mature larvae are identified by this procedure. Hence, many samples collected from the field cannot be identified due to this limitation.

DNA probes for mosquito sibling species identification

Recombinant DNA technology has contributed to the development of novel strategies

including DNA probes for the identification of mosquito vector species.

The use of DNA probes is an attractive alternative to the conventional techniques of vector identification due to the convenience, accuracy, sensitivity and reliability. In general, these techniques are not sex specific or life cycle stage specific and a large number of samples can be analysed at the same time. DNA probes are developed based on the differences found in the DNA of the members of a species complex.

What is DNA ?

DNA (deoxyribonucleic acid) is the genetic material found in an organism and contains the blueprint of life. It is found in the chromosomes present in the nucleus of cells. DNA is a giant molecule or a polymer made up of units called nucleotides (Fig.1). Each nucleotide is made up of three components; a phosphate group, a sugar and a nitrogenous base. There are four bases in the DNA; adenine (A), guanine (G), cytosine (C) and thymine (T) and hence a DNA molecule contains only four different nucleotides. One chromosome may contain as much as a billion nucleotides and the order of these nucleotide bases vary between regions giving rise to many different base sequences within the molecule. The genetic information in the DNA is encoded by this nucleotide base sequence of a DNA molecule. In other words, 'the language of DNA' has four different letters and by changing the order or the sequence of the four bases (letters) the information in the DNA can be varied giving rise to a large number of different DNA molecules carrying various messages.

DNA exists in the cell as double stranded molecules in helical form. The two strands are held together by specific H-bonding between adenine and thymine and cytosine and guanine base pairs (Fig.2). Hence, the two strands of the DNA are said to be 'complementary' to each other and if the nucleotide base sequence of

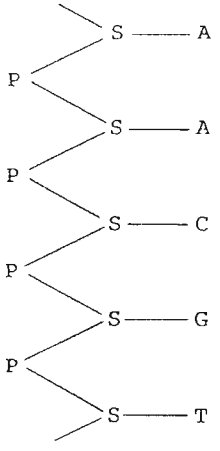

Fig. 1 : The DNA molecule is a polymer of nucleotides.
The nucleotide molecules are linked together producing an alternating sequence of phosphate (P) and sugar (S) residues, which is the backbone of the DNA molecule. Four types of bases (A,G,T and C) bind to the backbone via sugar residues.

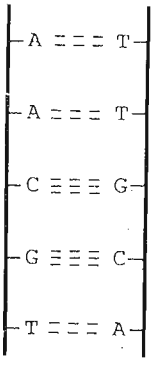

Fig: 2: Hydrogen bonding between the complementary strands of DNA.

Bases A and T bind to each other using 2 bonds, whereas G to C binding uses 3 bonds (Broken lines show hydrogen bonds).

one strand of the DNA is known, one can predict the sequence of the other strand. Since DNA is the primary source of variation among animal species, DNA sequences unique to an animal species, are found within the genome of the species. Such sequences can be isolated or cloned and developed as DNA probes for species identification.

What is a DNA probe?

A DNA probe is a short DNA fragment or a short sequence of nucleotides that is labelled with either a radioisotope or a non-radioactive chemical that can be detected by its signal and is used to detect a DNA sequence which is complementary to that of the probe. The probe binds to the target sequence only by complementary base pairing and this process of binding is referred to as 'nucleic acid hybridization' (Fig.3). During this process, the double stranded DNA molecules are denatured to separate the two strands and allowed to hybridize with the labelled single stranded DNA probe. Short DNA fragments, isolated or cloned from an animal genome, or synthesized in a laboratory, are usually developed as DNA probes.

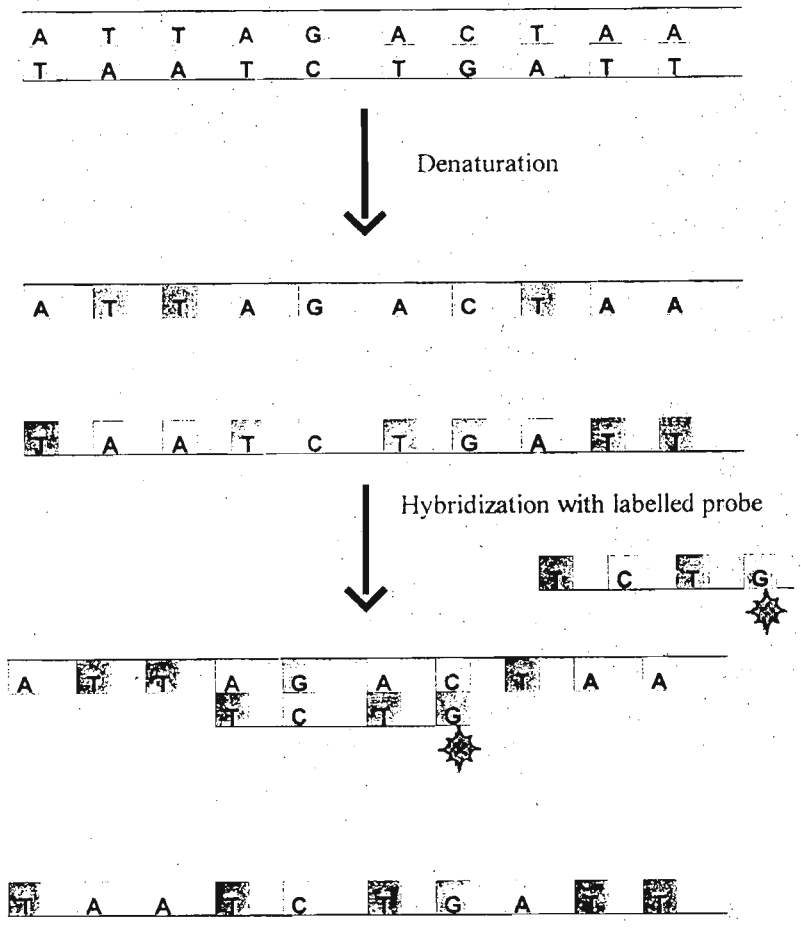

Fig. 3: Nucleic acid hybridization.
A single stranded labelled DNA base sequence containing usually 15 or more bases is used as the probe.

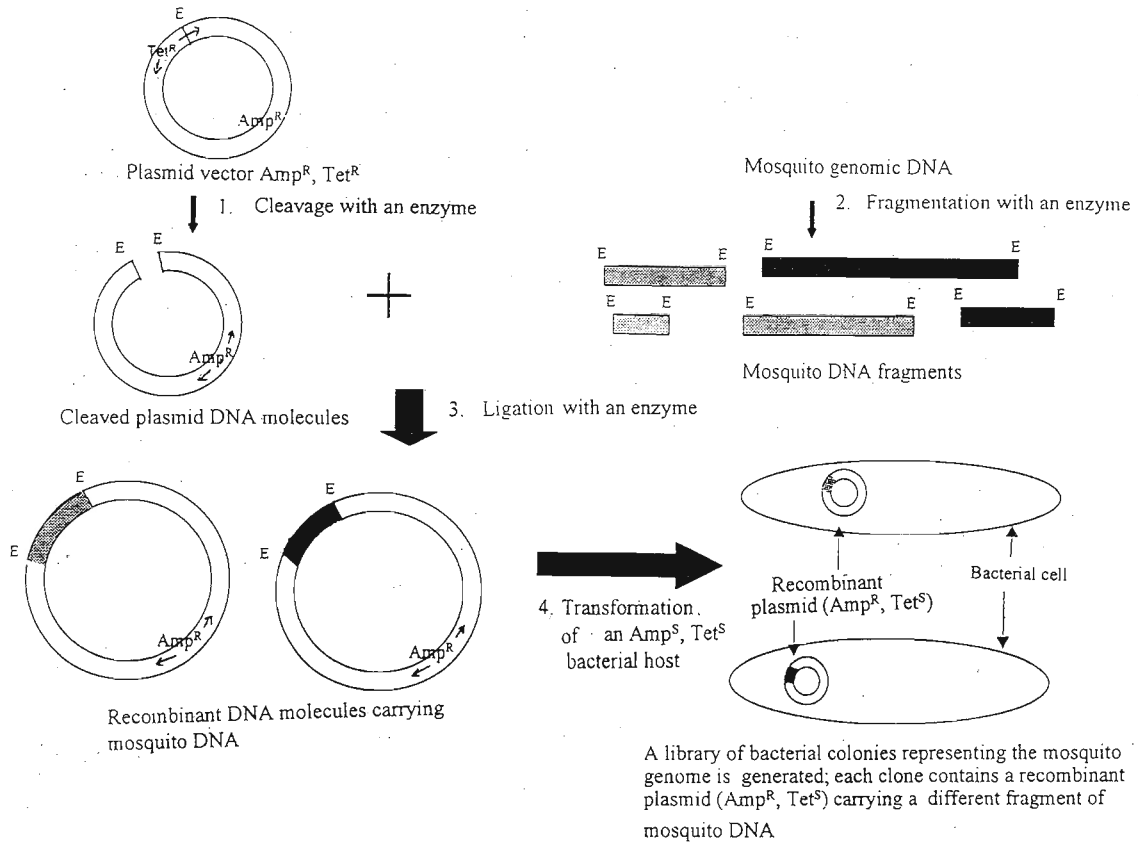

Fig. 4 - The steps in the construction of a mosquito genomic library

i. A plasmid vector containing genes for ampicillin and tetracycline resistance (Amp^R, Tet^R, respectively) is cleaved at a site (E) within the tetracycline resistance gene using an enzyme.

ii. The genomic DNA extracted from the mosquito species is also fragmented using an enzyme.

iii. The collection of cleaved plasmid DNA molecules generated and ligated to mosquito DNA fragments. A large number of recombinant DNA molecules containing mosquito DNA inserts are generated and each molecule contains a different DNA fragment of the mosquito genome. The tetracycline resistance gene is destroyed in each recombinant plasmid DNA molecule due to the insertion of a mosquito DNA fragment into this gene.

iv. This collection of recombinant plasmid DNA molecules is introduced into ampicillin and teracycline sensitive (Amp^S, Tet^S, respectively) bacterial host cells and grown in ampicillin medium. The bacterial clones containing recombinant plasmids are able to grow in ampicillin, however, they are unable to grow in tetracycline medium, and therefore can be identified.

Cloning of DNA fragments

Due to advances made in recombinant DNA technology it is now possible to separate a particular DNA fragment from all the other DNA extracted from the organism and make a large number of copies of that isolated DNA fragment. This process is known as 'DNA cloning'. Isolation of DNA fragments from the *An. culicifacies* genome is carried out by constructing a DNA library of the *An. culicifacies* mosquito genome. As the starting material for library construction, total genomic DNA is isolated from this mosquito species. Large chromosomal DNA molecules are then fragmented and ligated (using enzymes) into a cloning vector known as a 'plasmid'. A plasmid is a small, circular, double stranded DNA molecule, which is naturally present in many bacterial cells in addition to their chromosomal DNA. A plasmid is able to replicate independently inside a bacterial cell and in doing so, the genetic information present in the plasmid are expressed in the bacterial cell. For example, many plasmids carry genes for resistance to certain antibiotics, such as, ampicillin and tetracycline. The entry of such a plasmid into a bacterial cell would allow the bacterium to grow in a medium that contains ampicillin and tetracycline, as soon as the antibiotic resistant genes are expressed in the cell.

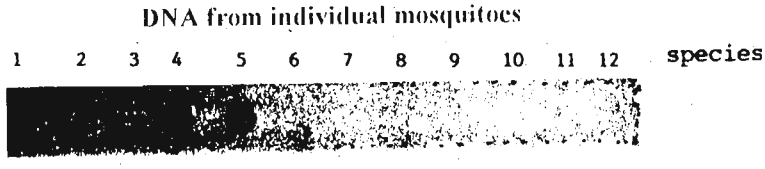

Fig. 5 : The DNA probe Rp 36 specifically identifies the *An. culicifacies* mosquito species.

The nucleic acid hybridization of genomic DNA extracts of various mosquito species (1-12) with radioisotope labelled DNA probe revealed hybridization signals only from *An. culificacies*, female (4) and *An. culicifacies*, male (5). (Experiments also revealed that the probe hybridizes with all three tested *An. culicifacies* sibling species; A, B and C).

1. *Aedes aegypti*
2. *Aedes togoi*
3. *Anopheles aconitus*
4. *Anopheles culicifacies (female)*
5. *Anopheles culicifacies (male)*
6. *Anopheles jamesi*
7. *Anopheles kaweri*
8. *Anopheles nigerrimus*
9. *Anopheles subpictus*
10. *Anopheles vagus*
11. *Culex quinquefaciatus*

The plasmid DNA molecules can easily be separated from the cell and taken into a test tube and purified from the bacterial chromosomal DNA. A Genetic Engineer can then make various changes in these plasmid DNA molecules. For example a plasmid can be cut and pasted to a piece of mosquito DNA using enzymes, and the recombinant DNA molecule so generated can be re-introduced to a bacterial cell. The characteristics of the bacterial cell will now be changed according to the genetic information present in the new DNA molecule. This process is referred to as 'transformation of bacteria'. Since a plasmid can be used to transfer one animal's DNA to another animal, it is known as a DNA 'vector'.

If we introduce the collection of recombinant DNA molecules generated by the ligation of mosquito genomic DNA fragments with cleaved plasmid DNA molecules into bacterial cells, millions of bacterial colonies harbouring recombinant plasmids are observed. Each such colony is referred to as a 'clone'. All the cells in a single clone contain copies of only one mosquito DNA fragment, since a colony starts from a single bacterial cell containing usually only one recombinant plasmid DNA molecule. Here, the plasmid replicates independently inside this bacterial cell and during the division of the bacterium, the daughter cells receive copies of the mosquito DNA fragment present in the plasmid. During bacterial transformation it is not only one recombinant DNA molecule that is introduced into bacteria but a whole collection of recombinants, each containing different regions of the mosquito genome. Each of these would enter a bacterial cell and give rise to a colony. Hence, this collection or the total pool of DNA clones is referred to as a 'genomic library' as it is assumed to be representing the complete genome of the mosquito. The main steps in the construction of such a library is illustrated in Fig.4. Next, we have to screen this genomic library to identify and isolate a DNA fragment that can be used as a DNA probe for the identification of the mosquito species.

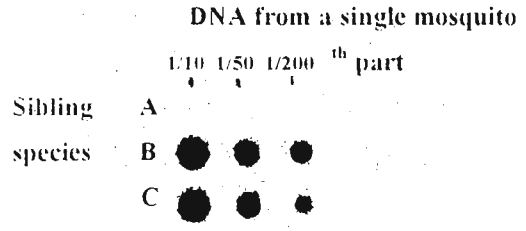

Fig. 6: The DNA probe Rp 217 distinguishes *An.culicifacies* sibling species A from species B and C.

The nucleic acid hybridization of DNA extracts of *An.culicifacies* sibling species A, B and C with the radioisotope labelled DNA probe did not reveal a hybridization signal with sibling species A. However, the sibling species B and C gave a hybridization signal with a minute quantity of DNA ($1/200^{th}$ part).

Screening of DNA libraries

Two important features that should be present in any species identification DNA probe are high sensitivity and specificity. In an animal genome there are sequences, which are present only once in the genome (single copy), as well as sequences that are repeated several times (multicopy; repetitive) in the genome. In order to develop a highly sensitive DNA probe for species identification it is very important to isolate a DNA fragment which is repeated in the genome, so that a minute amount of

mosquito sample (eg: a part of the head, a leg) is sufficient for its identification. Repetitive DNA sequences can be isolated from a DNA library by screening the library using a DNA probe made from a total mixture of genomic DNA fragments of the mosquito species. If a library DNA clone contains a mosquito DNA insert that is repeated in the genome, that clone would give a highly intense hybridization signal very quickly during the screening procedure. This is because a large number of copies of a repeat sequence is present in the total mixture of genomic DNA fragments labelled, and all these copies would quickly go and bind with the clone containing a complementary sequence as soon as it is met. Such positive clones are then isolated and the DNA inserts are then examined for their specificity i.e. a characteristic specific to a particular species so that one species can be distinguished from the other species. Here, we check to see whether the cloned DNA fragment hybridizes or not with the other mosquito species.

Using the procedures outlined above, our research group (B.G.N.K. de Silva, W. Abeywickreme and E.H. Karunanyake) was successful in developing the first DNA probe in the world that can distinguish all life cycle stages of *An. culicifacies* from other mosquito species (Fig.5). We also have developed a DNA probe technique that can distinguish *An. culicifacies* sibling species A from B and C and only a minute quantity of DNA is needed for this assay (Fig.6). The DNA probes developed in this study have also been tested and successfully applied in the field. The field investigations using these DNA probes revealed the absence of *An.culicifacies* sibling species A in Sri Lanka.

Next Vidurava issue, Vol. 21, Number 1

This issue will be published on "Science Communication". Contents will cover,

- Health Science Communication
- Journals in Science Communication
- Language in Science Communication
- Role of media in Science Communication
- Dramatisation of Science Communication

Using Fruit flies for Experiments to Study Diseases

It is very important to know how mosquitoes carry disease and transmit it from person to person. Scientists do not have much information about these mechanisms. The reason for this is that the insects are difficult to manipulate and not conducive to laboratory studies.

David Schneider, a fellow of White Head Institute says that fruit flies are more suitable to be used instead of mosquitoes for research studies on diseases that are transmitted from mosquitoes to man. He indicates that the characteristics of fruit flies have made them an excellent model to study human diseases. These characteristics have been highlighted as follows,

- flies have many genetic markers
- genetic screening can be conducted simply and in large numbers in flies
- parts of the immune system of humans are very similar to the immune system of flies

Schneider injected a form of *Plasmodium*, the parasite that causes malaria in chickens, into the body cavity of fruit flies. When he allowed carrier flies to infect chickens, the chickens developed malaria. He also observed that a component of the immune system of flies, knows as **macrophage,** was able to destroy *Plasmodium* in an attempt to fight the infection. In carrying malaria, mosquitoes act not only to pass infected blood from one person to another but they provide an environment for the parasite to grow and develop.

Another advantage of using fruit flies instead of mosquitoes is that genes can be knocked out to determine whether the parasite grows better or worse when a particular gene is missing. In addition the factors that are critical to a parasite's survival inside the mosquito can also be identified when flies are used. Because of these reasons the experiments have lead to develop drugs that prevent the growing of the parasite in mosquito. Sensitive stages in the parasite's life cycle could be potential targets for anti-malarial drugs and vaccines because parts of immune system of human are almost similar to the immune system of flies. This approach will definitely help scientists to understand how a fight is launched against infection and will hopefully lead to new ways to treat malaria.

Another possibility is that a better understanding of how mosquitoes carry malaria might lead to **transmission blocking vaccines**. Malaria blocking vaccines have been particularly elusive because Plasmodium hides inside human cells making it difficult for the immune system to locate and purge it from the body. Furthermore the parasite acts like a chameleon constantly changing its surface so that it is difficult for the immune system to recognize it.

David expresses the view that if transmission-blocking vaccines could be developed based on this new approach it will be a new beginning in disease control process for malaria and other diseases.

Reference:

"Can we win the malaria war" the article published in Sunday Observer dated 10 December 2000.

LOOKING FOR BLOOD MEALS

K.G. Janaka Karunasena
National Science Foundation

Dracula is famous for blood sucking from the human being, and most of the people are afraid of him. Although it is an imaginative thing, there are actual 'Draculas' that can be seen in our day-to-day life. Mosquito is such an animal familiar to us and they are frequent visitors to our homes.

Mosquitoes are more important in our lives and our economy because there are more than 3000 species of them in the world and they spread various different lethal diseases such as Malaria, Filariasis, Dengue, Japanese Encephalitis, Yellow fever, and West Nile Viral disease, etc. Therefore, mosquitoes are considered as the most dangerous animals in the world and they cause death to two to three million (2,000,000 to 3,000,000) people per year in the world by way of transmitting the above-mentioned diseases.

Male mosquitoes are innocent and they feed only on nectar from plants. Female mosquitoes are the ones who are looking for blood meals. They bite animals and human being many times in their 100-day long life period. Male mosquitoes are short lived, generally about 10 to 20 days. Female mosquitoes need blood protein of human and animal blood to develop their eggs. A female mosquito that consumes more than her own body weight in blood, can deposit around 250 eggs at once in water.

You may not known that there are certain people more at risk of mosquito bites, and hence to the mosquito borne diseases. Pregnant women, young children, adults over age of fifty, people with a weaken immune systems are at greater risk of mosquito bites than other people.

Mosquitoes are known to be attracted to Carbon Dioxide and various smells (volatile substances) released from our skin. It was reported that pregnant women are at a greater risk being bitten by a mosquito because they are twice as attractive to mosquitoes as non-pregnant women. It was found that women in an advanced stages of pregnancy enable 21 % more than non-pregnant women, abdomen of pregnant women were 33 degrees hotter and release more volatile substances from their skin. This results to be more attractive by mosquitoes.

Although mosquitoes create problems to people from generations, it is not found yet a 100 % successful method to eliminate this menace. One of the best methods to eliminate them to a considerable amount is use of a device invented in USA called 'Mosquito Magnet'. This is really a breakthrough of technology and this instrument emits a stream of Carbon Dioxide that mosquitoes mistake for exhaled breath of their pray. When mosquitoes get attracted in to the Mosquito Magnet, they are vacuumed into a net where they dehydrate and die within 24 hours. A 20 lb propane tank powers the Mosquito Magnet and it will last about 18 – 20 days. This technique is environmentally friendly and safe since uses no pesticides.

Countries like Sri Lanka cannot afford the cost of eliminating mosquitoes by using

high technology. Therefore, it is always advisable to follow up methods for prevention of spreading the mosquito borne diseases. The most effective way to do this to get rid of them before they appear. Some important tips to follow are given bellow.

- Not allowing of standing water to accumulate for more than two days. Better to check: old tires, un-used buckets, and plastic containers, base of flowerpots, plastic covers or any container that may collect water.
- Changing of water in birdbaths, and fountains at least once a week
- Cleaning of debris from rain gutters and removing of standing water under or around structures.
- A thin layer of oil will kill mosquitoes already present in the standing water.
- Stock minnows or goldfish in ornamental pools. They eat mosquito larvae on the water surface.
- Fill or drain large puddles, ditches and swampy areas.
- Remove, drain or fill tree holes stumps with mortar.
- Keep hedges and bushes trimmed to reduce shade. Mosquitoes can hide in the shade of tall grass.

Source: Internet

VIDURAVA

GUIDELINES TO CONTRIBUTORS (PROVISIONAL)

TYPES of PAPERS CONSIDERED FOR PUBLICATION

Articles written on the themes related to science and technology are accepted. Papers published or communicated for publication anywhere also can be submitted for publication in VIDURAVA. In such cases it is the author's responsibility to obtain written permission to reproduce the material. Articles submitted may be reviewed and edited by the members of the Editorial Committee. Submission of manuscripts in both languages (in Sinhala and English) will be much appreciated. Authors are encouraged to prepare articles in Tamil too, if possible.

GUIDE TO PREPARATION OF MANUSCRIPTS

Script

Manuscripts of papers should be typewritten in double space on one side white sheet. Adequate margins (4 cm) should be left with sufficient spacing at the top and bottom of each page. The typescript should be free of corrections and should be complete and in final form. **It is preferable if the article could be sent together with the diskette on MS Word 98.**

Length and Style

Articles should be written to a given theme; related to science and science activity. Articles should be well focused, preferably organized, structured and *avoid the "text book" style*. They should be written clearly and concisely. No maximum length of contributions is prescribed, but articles should *not normally exceed 2000 words*. Short articles are encouraged.

Manuscript layout

- *Title of the articles* should be brief and the a*uthor's names* and affiliation should also be mentioned.
- *Tables and figures* should be on separate sheets and should be fully labeled and captioned.
- *Graph and other line drawings* should be drawn in Indian ink on tracing paper or white drawing paper preferably art paper not bigger than A4 size.
- *Photographs* must have good contrast.
- Use names of chemical compounds and not their formulae in the text. Zoological names as well as Botanical names should be properly spelled and typed in italics or underlined. Mathematical applications, such as statistical analysis should be avoided as far as possible.
- Reference and footnotes should be numbered consecutively and given at the bottom of each page. References should be as much as possible be only to referred text books use for advanced level classes and abbreviations should be avoided in the references. Provide complete name of the textbook.

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