2. LITERATURE REVIEW

2.1 Lymphatic filariasis

Lymphatic filariasis is an inflammatory parasitic infection of lymphatic vessels caused by the filarial roundworms *Wuchereria bancrofti*, *Brugia malayi*, and *Brugia timori*, which results in massive lymphoedema (elephantiasis) of the affected tissues. The adult worms inhabit the lymphatics where they elicit an inflammatory response that causes acute lymphangitis and eventualy lymphatic obstruction leading to severe lymphoedema.

2.1.1 Epidemiology

WHO (1995) reported that lymphatic filariasis is widespread throughout the tropical and subtropical areas of Asia, Africa, the Western Pacific and some parts of the America. More than 1.1 thousand million people (20% of the world's population) now live in areas where they are at risk of infection with lymphatic filarial parasites and a minimum of 120 million people is currently infected (about 107 million with *W. bancrofti* and 13 million with *B. malayi* or *B. timori*). A total of 44 million persons currently suffer from one or more of the overt manifestations of the infection: lymphoedema and elephantiasis of the limbs or genitals, hydrocele, chyluria, pneumonitis, or recurrent infections associated with damaged lymphatic vessels. The remainder of the 120 million infected has "preclinical" hidden damage of their lymphatic and renal systems.

2.1.1.1. Geographical distribution

Lymphatic filariasis is known to occur in 73 countries (Figure 1); 38 in the Africa region, 7 in the region of the America, 4 in the Eastern Mediterranean region, 8 in the South-East Asia region and 16 in the Western Pacific region. The condition has been previously reported, and might still

occur in another 40 countries. The highest numbers of infected persons live in the South-East Asia region with India alone accounting for 45.5 million. In Sub-Sahara Africa, the estimate of 41 million cases is less precise, and there is a particular need to determine more accurately the distribution of infection and disease in affected countries. Several countries in Asia have large numbers of infectious cases that are very prevalent in many of the pacific islands as well. Brugian filariasis is most highly endemic in India and China (32% and 20% respectively, of the global burdens). It is also prevalent in Indonesia, Thailand, Malaysia, the Philippines, Vietnam, and Republic of Korea (Ottesen *et al*., 1997).

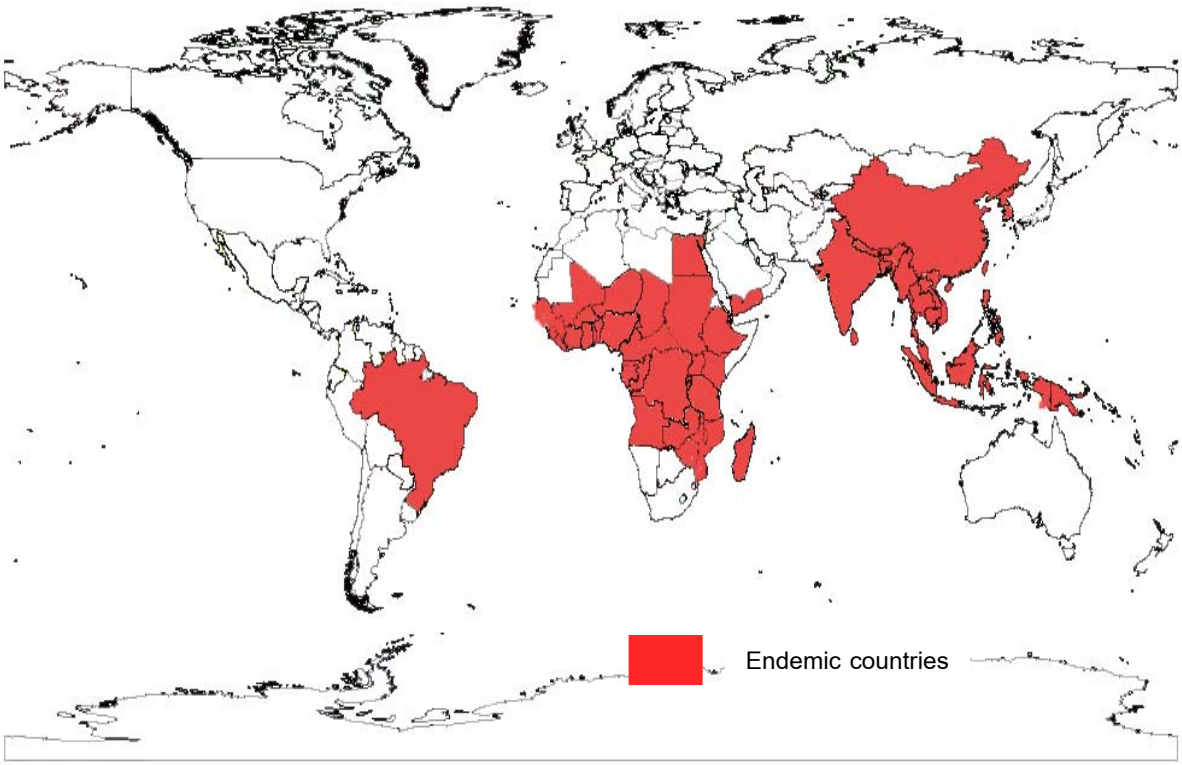


Figure 1 Distribution of lymphatic filariasis

(From WHO, 1992)

2.1.1.2 Situation of lymphatic filariasis in Thailand

Lymphatic filariasis is an important health problem in many Asian countries. The largest numbers of people both at risk and infected live in India, but disease is a severe problem in Indonesia, Malaysia, Sri Lanka, the Philippines and Thailand (WHO, 1992).

In Thailand, lymphatic filariasis is found in the rural areas. Its spreading and abundance are different depending on character of areas. Among 76 provinces, only 10 of them have some foci of lymphatic filariasis transmission, namely Mae Hong Son, Lamphun, Tak, Kanchanaburi, Ratchaburi, Ranong, Surat Thani, Krabi, Nakhon Si Thammarat and Narathiwat (Figure 2).

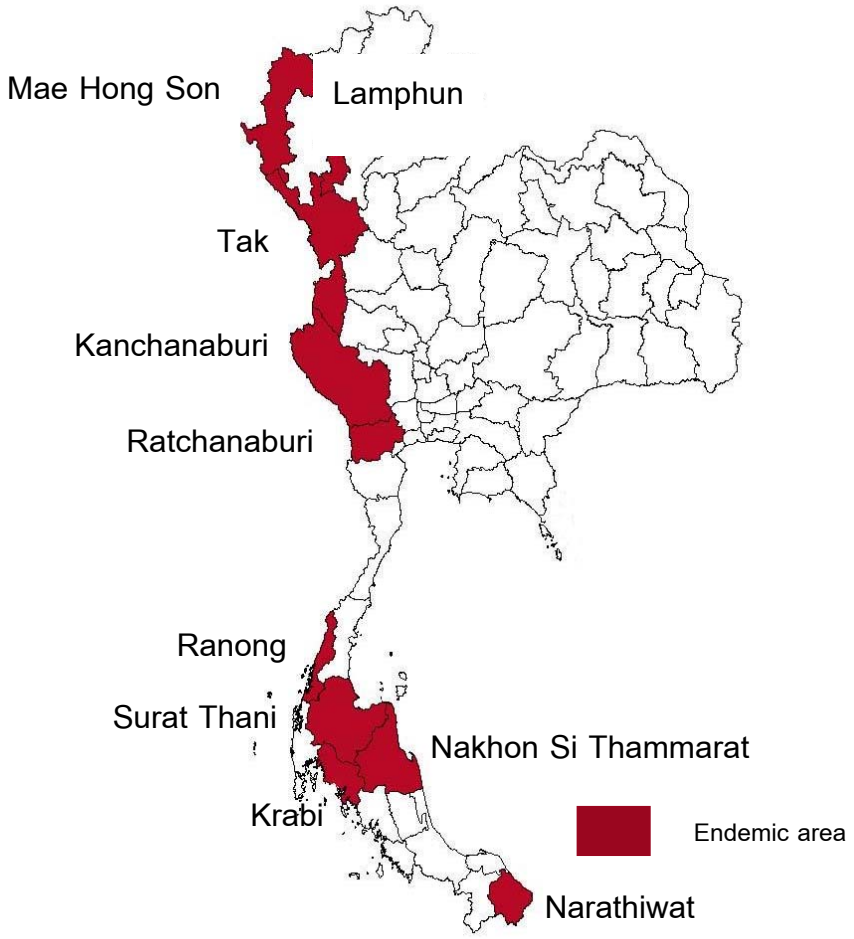


Figure 2 Endemic areas of lymphatic filariasis by provinces in Thailand (Modified from Annual report of Filariasis Division, 2004)

It has been reported that there were 326 patients with lymphatic filariasis registered between October 2001 and September 2002 (Annual report of Filariasis Division, 2002). Most of them (194 cases; 59.51%) were asymptomatic, 110 cases (33.74%) were patients having antigen of microfilariae in blood. The rest were lymphadenitis stage (11 cases; 3.37%) and in elephantiasis stage (11 cases; 3.37%). The prevalence rate of lymphatic filariasis was highest in Narathiwat (21.79 per a hundred thousand) and Mae Hong Son (20.92 per a hundred thousand). The highest rate of patients, who were in elephantiasis stage, was found in Nakhon Si Thammarat province (0.46 per a hundred thousand) and Surat Thani province (0.22 per a hundred thousand). Overall, the prevalence rates of lymphatic filariasis during previous 10 financial years (from 1992 to 2002) have decreased from 11.16 per a hundred thousand in 1992 financial year to 0.53 per a hundred thousand in 2002 financial year (Figure 3) (Annual report of Filariasis Division, 2002).

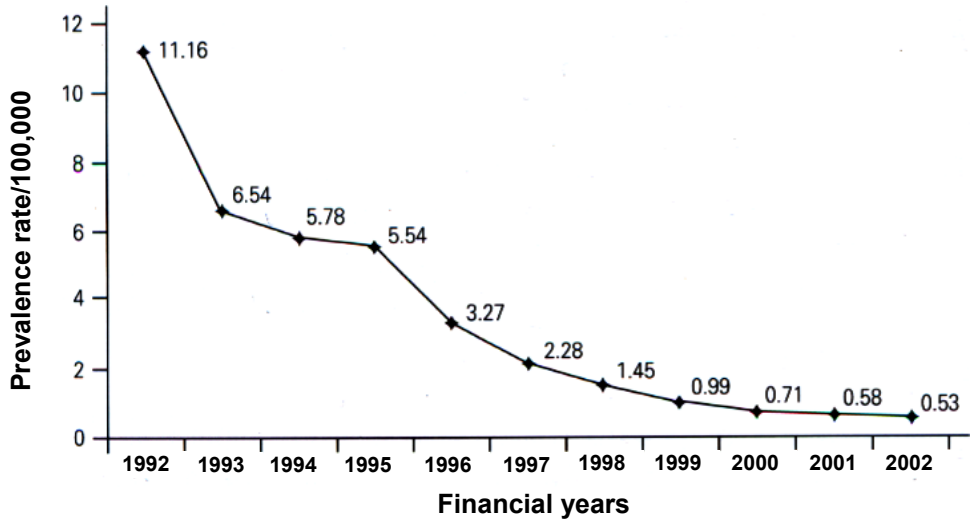


Figure 3 Prevalence rate of lymphatic filariasis in Thailand
(Modified from Annual report of Filariasis Division, 2004)

Iyengar (1953) examined lymphatic filariasis in the South of Thailand for the first time in four southern provinces, namely Nakhon Si Thammarat, Surat Thani, Patthalung and Pattani, and found that *B. malayi* was the cause of the

disease and spread equivalently among these provinces. When the territories of examinations were expanded, the disease also existed in the areas of Eastern South, namely Chumphon, Pattani, Nakhon Si Thammarat, Surat Thani, Patthalung,and Narathiwat (Harinasuta *et al*., 1970).

At present, lymphatic filariasis in the South of Thailand is under control and is limited in only 4 provinces. Number of patients with lymphatic filariasis who are registered for treating is as follow; Ranong, 2 patients, 1.25 per a hundred thousand; Surat Thani, 18 patients, 2.01 per a hundred thousand; Nakhon Si Thammarat, 8 patients, 0.52 per a hundred thousand; and Narathiwat, 148 patients, 21.79 per a hundred thousand (Annual report of Filariasis Division, 2002). In addition, blood examination of population in these areas for identifying the microfilaraemia subjects was preceded. Number of microfilaraemia subjects only caused by *B. malayi* was 10 subjects (0.10% of total population) in Surat Thani and 88 subjects (0.66% of total population) in Narathiwat (Annual report of Filariasis Division, 2002). However, spreading of the disease was found only in rural villages. Migration of population and non-cooperation of communities and patients are at risk.

2.1.2 Pathogenesis

Mosquito bites transmit infective larvae that migrate to lymphatic and lymph nodes. After maturing into adult forms over several months, the worms mate and the females release microfilariae into lymph nodes and the bloodstream. The manifestations of lymphatic filariasis result from the inflammatory response to degenerating adult worms in the lymph nodes.

The initial inflammatory response is an acute lymphangitis, which resolves in 1 to 2 week. Repeated filarial infections are common in endemic regions and produce repeated bouts of lymphangitis (filarial fevers), which eventually (over years) may cause extensive scarring and obstruction of

lymphatic vessels. The lymphatic vessels obstruction causes localized dependent edema, most commonly affecting legs, arms, genitalia and breasts. In its most severe form, which occurs in less than 5% of the infected population, this edematous distortion of body parts is known as elephantiasis (Genta & Conner, 1998).

2.1.2.1. Life cycle of the parasites

In Thailand, lymphatic filariasis is caused by *B. malayi* and *W. bancrofti*. Life cycles of both parasites are similar and divided into 2 stages as follows (Figure 4).

A. Mosquito stages

(1) A mosquito takes human blood containing microfilariae.

(2) Microfilariae shed theirs sheaths, penetrate mosquito's stomach wall and migrate to muscle of thorax.

(3) Microfilariae shapes become short, thick and are called sausage-stage or L_1 larvae. The L_1 larvae molt and transform to the L_2 larvae or pre-infective larvae, which has one or two papillae at the caudal end.

(4) L_2 larvae molt and transform to the L_3 larvae or infective larvae. Their shapes are longer than that of L_2 larvae.

(5) The L_3 larvae migrate to proboscis of the mosquito.

The time interval between microfilariae ingestion of mosquito and transformation to the L_3 larvae is approximately 7-14 days, which depend on the type of parasites and microfilarial body temperature. Generally, the microfilariae are rapidly developed at the optimal temperature.

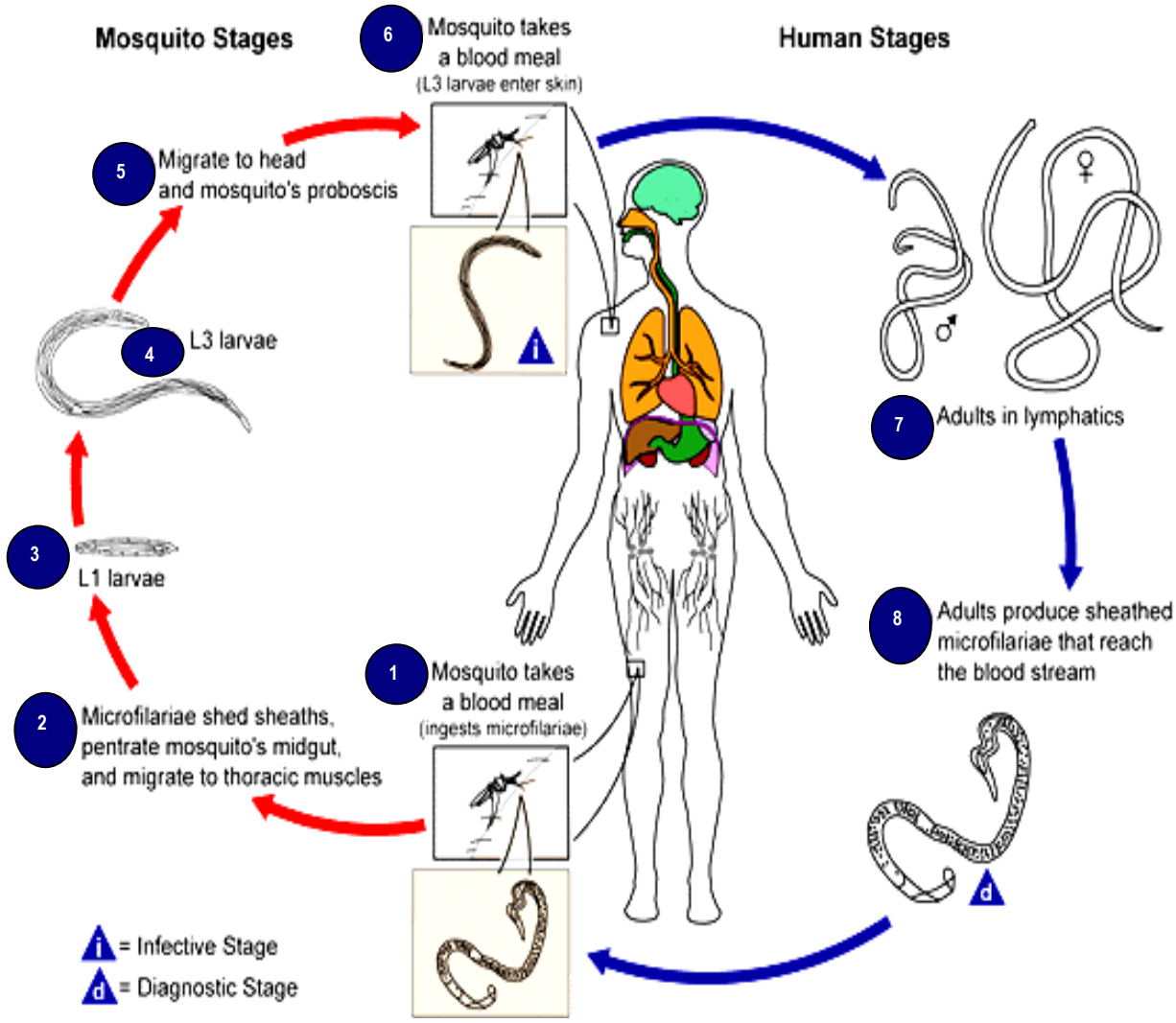


Figure 4 Life cycles of filarial parasites (*B. malayi*)

(Modified from Garcia, 2001)

B. Human stages

(๔) During a blood meal, the L_3 larvae from the proboscis of the mosquito penetrate human skin and enter in the lymphatic system.

(๗) The L_3 larvae develop into L_4 larvae and enter adult stage containing male and female worms.

(๘) After fertilization, the female worms produce sheathed microfilariae reaching the blood circulation.

The period from the entering of L_3 larvae into human body to appearing

to sheathed microfilariae is approximately 3 months. Generally, microfilariae age 6-12 months and adult worms age 5-10 years (WHO, 1987).

2.1.2.2. Periodicity of lymphatic filariasis

In human, the microfilariae show circadian periodicity. They live mostly in pulmonary capillaries and enter blood circulation at certain intervals of time. Periodicity of microfilariae in the blood circulation relates to the time of blood meal and divides into 2 main types as follows.

1) Periodic types

a) Nocturnal periodic type

The microfilariae are found in the blood circulation during night-time.

b) Diurnal periodic type

The microfilariae are found in the blood circulation during day-time.

2) Subperiodic types

a) Nocturnal subperiodic type

The microfilariae are found in the blood circulation during night-time and day-time with greater in density during night-time.

b) Diurnal subperiodic type

The microfilariae are found in the blood circulation during night-time and day-time with greater in density during day-time.

The periodicity of parasites in human host is similar to that in animals. For example, the periodicity of *B. malayi* in an infected cat is nocturnal subperiodic, so the periodicity of parasites in infected human is nocturnal subperiodic too (Edeson, 1959). In the South of Thailand, lymphatic filariasis is generally caused by *B. malayi*, which is mainly nocturnal subperiodic type (Kanjanopas *et al*., 2001). The *B. malayi* microfilariae have unique circadian rhythms manifested as a variability of their number or concentration in the blood circulation (Hawking *et al*., 1966; Hawking & Gammage, 1968). The

nocturnal subperiodic microfilariae exhibit their highest levels of parasites in blood circulation at night, but 40 to 60% of peak levels persist during the day (Dondero *et al*., 1971). The mosquitoes, the vectors of *B. malayi*, also have a circadian rhythm, which describes their feeding time. The highest concentration of microfilariae in the blood circulation occurs at a time concurrent with the period of the most active feeding by the local vector. This coincidence suggests that the parasites have adapted their periodicity to the vector feeding behavior, possibly to facilitate their transmission (Wang & Saz, 1974). The periodicity of *B. malayi* is dependent primarily on the daily activities of the host and not on alternations of day and night. Thus, if the human host reverses its routine sleep-and-wake cycle, the periodicity of microfilaremia is also reversed. Studies of *B. malayi* suggest that their periodicity is due to differences in the oxygen tension between the arterial and venous blood in the lungs (Hawking & Gammage, 1968; Burren, 1972). When microfilariae are absent from blood circulation, they accumulate primarily in the arterioles of the lungs If the difference in the arterial-venous oxygen tension is less than 50 mmHg, microfilaria will accumulate in the lungs (Hawking *et al*., 1966; Hawking, 1967). When the pulmonary arterial-venous oxygen tension difference exceeds 50 mmHg during sleep, microfilariae migrate from the pulmonary vasculature and appear in the blood circulation (Hawking & Gammage, 1968).

2.1.3 Pathology

The adult nematode is a white, threadlike worm that is much convoluted within the lymph nodes where they reside. The female is twice the size of the male and measures 80 to 100 mm in length and 0.20 to 0.30 mm in width. In blood films stained with Giemsa, the microfilariae appear as gracefully curved worms, measuring about 300 μm in length.

The lymphatic vessels harboring the adult worms are dilated, and the endothelial lining is thickened. In the adjacent tissue, a chronic inflammatory infiltrate, consisting of lymphocytes, macrophages, plasma cells and eosinophils, surrounds the worms. A granulomatous reaction may develop, and degenerating worms can provoke acute inflammation. Microfilariae also provoke a chronic inflammatory reaction. After repeated bouts of lymphangitis, the lymph nodes and lymphatic vessels become densely fibrotic, often containing calcified remnants of the worms (Genta & Conner, 1998).

2.1.4 Clinical features

WHO (1987) reported that lymphatic filariasis is characterized by a wide spectrum of clinical manifestation with signs and symptoms often differing from one endemic area to another. The manifestations of disease can be divided into two distinct clinical types.

- Not causing from microfilariae or adult worms in the lymphatic system normally referred to lymphatic filariasis.

- Causing from an immune hyperresponsiveness of the human host against microfilariae resulted in occult filariasis, which includes tropical pulmonary eosinophilia (TPE).

The clinical courses of lymphatic filariasis can divide into asymptomatic, acute and chronic stages, generally progressing in that order. It is often initially asymptomatic with subsequent episodes of acute adenolymphangitis and finally the development of chronic lymphatic obstruction. In previously unexposed persons who move from non-endemic to endemic areas, this progress is often accelerated with early acute manifestations being followed much more rapidly by the chronic signs.

2.1.4.1. Exposure versus infection

In endemic area of lymphatic filariasis, there is always a certain

proportion of the population who, despite having been exposed to infective larvae. They do not show any clinical manifestations of lymphatic filariasis or any microfilaraemia with the presently available diagnostic procedures. It is impossible, in the absence of a reliable immunodiagnostic test, to determine whether people in this group have sub-threshold microfilaraemia or sub-clinical infection, or whether they are free from infection.

2.1.4.2. The asymptomatic stage

This is characterized by the presence of microfilariae in the peripheral blood, although there is no clinical manifestation of lymphatic filariasis. Some individuals remain asymptomatic for years, while others progress more rapidly to the acute and chronic stages.

2.1.4.3. The acute stage

The acute clinical manifestations of lymphatic filariasis are characterized by episodic attacks of lymphadenitis and lymphangitis associated with fever and malaise. There is no evidence to suggest that these are caused or accentuated by bacterial infection. Sometimes the fever precedes the adenolymphangitis for a few days. After several days or up to 4-6 weeks, the lymph nodes become enlarge and tender, edematous infiltration of the surrounding subcutaneous tissues or even formation of abscesses, which may turn to ulcerate and lead to scarring.

In most cases of Brugian filariasis, the lymphadenitis occurs in the inguinal region on one side, and there is lymphangitis on the medial side of the limb and foot on the same side. Occasionally the axillaries lymph nodes are involved and the lymphangitis may spread through the medial aspect of the arm to the hand. Infrequently lymphadenitis occurs at atypical sites such as the breasts, the popliteal lymph nodes, or elsewhere. Lymphadenitis rarely occurs at more than one site simultaneously.

2.1.4.4. The chronic stage

The chronic stage of lymphatic filariasis usually develops 10-15 years from the onset of the first acute attack. The incidence and severity of chronic clinical manifestations tend to increase with age.

As the inflammatory reaction continues, the area becomes firmer still, and pitting disappears. There is substantial encroachment on the subcutaneous tissue and consequent loss of elasticity of the overlying skin and develop to elephantiasis.

2.1.4.5. Tropical pulmonary eosinophilia of chronic filariasis

Tropical pulmonary eosinophilia (TPE) may result from human or non-human filarial parasites. It is characterized by immunological hyperresponsiveness of the human host to the parasite, especially the microfilariae. There is a marked increase in the production of IgE and IgG anti-parasite antibodies and a pronounced hypereosinophilia. In some areas, it is associated with paroxysmal nocturnal cough, breathlessness and wheezing, occasionally accompanied by a radiological picture of diffuse patchy infiltration of the lungs. In other areas, it is associated with lymphadenopathy, and sometimes hepatosplenomegaly. Microfilariae are almost never present in the blood, but remnants of microfilariae surrounded by aggregates of eosinophils are sometimes found in the spleen, liver, lymph nodes or lungs. The syndrome responds quite well to treatment with diethylcarbamazine citrate (DEC) (WHO, 1987).

In Brugian filariasis, the characteristic sites for elephantiasis are the legs below the knees or, less frequently, the arms below the elbows, although the lymph nodes affected during the acute stage are usually located in the inguinal or axillaries regions. In most cases, only the foot and the distal part of the lower leg are affected and, as the swelling usually occurs only below the

knee, the contour of the affected knee is more or less normal. The affected leg is usually less than twice its original size.

2.1.5 Diagnosis

Lymphatic filariasis diagnosis can be diagnosed following by WHO (1987). The diagnosis of disease can be divided into five types.

2.1.5.1 Detection of microfilariae

Microfilariae can be detected in blood, urine (in chyluria), hydrocele fluid, or tissue.

A. Microfilariae in blood

The time of blood collection should be as close as possible to the peak of the microfilarial periodicity for the species, and strain concerned. Measured fingerprick blood samples of 20-120 µl are used, depending upon the purpose of the diagnostic test. For mass survey and treatment, a blood sample of 60 µl is recommended. To identify microfilaria-positive cases in a selective treatment program, a larger volume of blood (120 µl blood films or 1 ml for membrane filtration) should be taken for diagnosis.

- The blood slides

The conventional thick blood film is still the best method for fieldwork. It is particularly important for correct species identification in areas where mixed infections occur. It can be used for malaria detection at the same time. The slide should be perfectly clean, and the blood sample evenly spread, dried, carefully dehaemoglobinized, fixed, stained, correctly labeled and stored for examination.

- The counting chambers

The counting chambers can be used in areas where the parasite has been previously identified, but preferably not in an area where mixed infections are found.

- The DEC provocative test.

In this test, an adult is given 100 mg of DEC by oral administration in the daytime, and the peripheral blood is examined for microfilariae 30-45 minutes later. This test "provokes" microfilariae in the lung capillaries to invade the peripheral blood. It should only be used in areas where the microfilariae have a nocturnal periodicity, and where night-blood collection is impossible. It has a lower sensitivity than the night-blood sample. Furthermore, DEC should not be used in areas where *W. bancrofti* occurs together with *Onchocerca volvulus* or *Loa loa*, since it may provoke a severe reaction (the "Mazzotti reaction").

- Concentration techniques

Membrane filtration techniques using Millipore or Nuclepore membrane filters of 3-5 μm porosity are the most sensitive tools currently available for the detection of microfilariae. Venous blood samples of 1-10 ml or even more have been used by various workers. These methods are expensive, and are normally reserved for special purposes, such as for the diagnosis of individual cases, evaluation of treatment, as a research tool or in pilot control studies.

Knott's concentration technique is an alternative concentration method when membrane filters are not available. It has a lower sensitivity than membrane filtration, since microfilariae are more likely to be missed in the viscous sediment.

B. Microfilariae in hydrocele fluid or urine

Microfilariae are sometimes absent from the blood but present in hydrocele fluid or urine. The specimens should be processed and examined using one of the concentration techniques described above.

2.1.5.2 Clinical diagnosis

A. Acute adenolymphangitis in communities

A clinical history of recurrent fever associated with adenolymphangitis is strongly indicative of lymphatic filariasis and constitutes a syndrome that is usually well known to the inhabitants of endemic areas. The presence of scars at typical locations (over the inguinal and epitrochlea lymph nodes) supports the diagnosis of lymphatic filariasis. The presence of clinical cases with acute and chronic manifestations of lymphatic filariasis in the community provides more conclusive evidence.

B. Diagnosis of individual cases with clinical manifestations of lymphatic filariasis

A history of having lived in an endemic area of lymphatic filariasis should focus attention on a possible filarial etiology of adenolymphangitis. The detection of filarial parasites or anti-filarial antibodies provides supporting evidence for the diagnosis, as does the presence of a microfilaria-positive individual living in the same house as the patient.

C. Diagnosis of individual cases with bronchial asthma or lymphadenopathy

An eosinophil count of more than 3,000 cells per µl of blood gives rise to a suspicion of occult filariasis. Supportive evidence is a chest X-ray showing suggestive shadowing or the presence of a high titre of anti-filarial antibodies of the IgE class. A favorable response to DEC provides additional evidence.

2.1.5.3 Detection of adult or developing adult worms

Adults or developing adult worms are sometimes found in biopsy specimens of lymph nodes. Knowledge of the microanatomy of adult filarial worms in cross-section is essential for differentiation of human and animal

filarial parasites. The presence of microfilariae around the adult worm may help in establishing the species.

2.1.5.4 Detection of filarial larvae in mosquito vectors

After parasite controlling with DEC, mass dissection of mosquito vectors collected from the treated locality may aid in tracing microfilaria-positive individuals. The presence of human filarial L_3 larvae in these mosquitoes indicates a need for examination of blood samples from that locality for case detection.

2.1.5.5 Immunodiagnostic tests

None of the immunodiagnostic tests currently available is able to define accurately the presence of infection, either because of lack of specificity or inability to discriminate between present and past infection. However, the absence of anti-filarial antibodies in a patient residing in a no filarial endemic area excludes the possibility of a filarial etiology.

Recent advances in immunodiagnosis offer encouraging leads, and it is to be hoped that in the future these problems will be solved. Reagents (antibody and antigen) with high degrees of stage and species specificity are being sought and developed to detect the presence of active infection and to determine the intensity of infection (worm burden), to discriminate between present and past infection, to assess the effects of anti-parasite control measures, to detect previous exposure to infective larvae, and to solve other currently insoluble diagnostic problems.

2.1.6 Treatment

Generally, important criterion of lymphatic filariasis controlling is the blood examinations in the provinces where have been found the microfilaraemia patients together with the entomology. These patients are treated with DEC. Treatments of lymphatic filariasis are performed by following

WHO (1987), which can be divided into two types as follows.

2.1.6.1 Treatment of individual patients

The objective of treatment is to eliminate the parasite in order to reduce or prevent morbidity. DEC is currently the only drug available for treating of lymphatic filariasis that is effective, safe and relatively cheap. It kills almost all the microfilariae and a good proportion of adult worms. It is probably also effective against the L_3 and L_4 larval stages. DEC is generally very safe, but it is advisable not to give the drug to pregnant women; care should also be taken when treating people with chronic kidney or cardiac disorders.

The recommended dose of DEC is 6 mg/kg of body weight daily for 12 consecutive days (total dose, 72 mg/kg) for Bancroftian filariasis and 3 to 6 mg/kg of body weight (total dose from 18 to 72 mg/kg) for Brugian filariasis.

Repeated courses of treatment may be necessary to achieve radical cure. The drug is rapidly excreted from the body, mainly through the kidneys, and the possibility of a cumulative effect is minimal. A repeated course may be initiated approximately 2 weeks after the last dose of the previous course. The excretion of DEC in urine is highly pH-dependent. Under acid conditions (pH 5), some 70% of the given dose of DEC is excreted unchanged in the urine, while under alkaline conditions (pH 7.5 and above) only 5% of DEC is eliminated.

2.1.6.2 Treatment of communities

The target population and the objective of treatment should be clearly defined. The objective may be any of the following, namely 1) to reduce morbidity by treating clinical cases of lymphatic filariasis, 2) to reduce

transmission and consequently to reduce morbidity by treating people with microfilaraemia, 3) a combination of 1) and 2), and 4) to interrupt transmission and thus, unfavorable circumstances, to eliminate the parasite from the human.

A. Approach to community treatment

There is a basic difference in approach between individual and community treatment. In the first case, it is usually the patient comes to the doctor or to the community health worker. The patient realizes that he or she is ill, and needs help and is therefore more likely to comply with the treatment. This is particularly true in patients suffering from an acute attack of adenolymphangitis, who are likely to accept treatment with DEC, and in whom the treatment may be expected to prevent or delay the development of chronic disfiguring or disabling lesions and to reduce the chances of recurrent acute attacks in the future. In the community as a whole, on the other hand, usually only a few people will ever have had clinical lymphatic filariasis. Furthermore, at the time of mass or large-scale treatment, not all of this group will actually be suffering from the disease. It follows that the desire for help may be less.

B. Choice of treatment

- Selective treatment

In areas that found the microfilaraemia rate was found lower than at 1% of the all population, DEC is subsequently given only to those who have the microfilaraemia (selective drug administration). Only individuals with clinical manifestations and microfilaraemia are treated. If the objective is to reduce morbidity by treating all those with acute or acute-on-chronic attacks of adenolymphangitis and filarial fever, this can be affected on a permanent horizontal basis via the primary health care system. Each community health worker is given a 2-day course of training in the recognition and treatment of

these symptoms, and is provided with a supply of DEC to treat all such cases as they present themselves. Experience in India has shown that a 6-day course of 500 mg of DEC per day is acceptable and effective in these circumstances. The advantage of selective treatment is that acceptance of the drugs is better because infected individuals can be told that they are infected and forewarned about the possible side effects of treatment.

- Mass treatment

In areas of high endemicity where the microfilaraemia rate more than at 1% of the all population, DEC is given to almost everyone in the community irrespective of whether or not they have microfilaraemia or disease manifestations (mass drug administration). After that, the treatment is followed for 2 years, and estimated for controlling every 2 years for 10 years. However, the drug should not be given to infants, pregnant women, the elderly, or those with obvious debilitating disorders, especially those with cardiac or kidney disease.

The advantages of mass treatment are as follows.

1) It is not necessary to examine every member of the community. If the target population is large, examination of a statistically appropriate sample will give a valid estimate of the filarial endemicity of the community.

2) It is not necessary to use a highly sensitive method to detect microfilaria-positive individuals in the community.

3) There is no need to worry about false negative results, which may be due to the insensitivity of the method, inappropriate blood collection or processing, or technical errors.

4) In mass treatment, the chance of not giving DEC to a person occurs only once such as during the distribution of the drug. In selective treatment, the chance of missing a person occurs twice: once during selection of

diseased and microfilaria-positive individuals and again during the distribution of the drug.

5) Mass treatment avoids the problems of discrimination whereby some microfilaria carriers, feeling themselves to be in perfect health, may wonder why they have been selected for treatment, while amicrofilaraemic people may wonder why they are excluded from receiving the medicine.

2.1.7. Prevention and control

WHO (1987) divided lymphatic filariasis controlling into four types of which the objective should be clearly defined. It may be any of the following, in increasing order of difficulty.

2.1.7.1 Reduction of morbidity

If the objective is merely to reduce, the existing filarial morbidity in the community there may be no need for blood examination or determination of microfilarial indices. What is needed is a team whose members are knowledgeable in the clinical manifestations and treatment of lymphatic filariasis. The problem with this approach is that many early infections that will eventually lead to morbidity may be missed. In order to determine which strategy should be employed to achieve the objective, as well as for subsequent evaluations of the campaign, it will usually be necessary to make observations on the prevalence of microfilariaemia as well as of clinical manifestations. However, if this is not feasible, a good practical solution is to make DEC available, through the primary health care system, to all those suffering from an acute or acute-on-chronic attack of filarial adenolymphangitis or fever.

2.1.7.2 Reduction of transmission

Transmission can be reduced by decreasing the microfilarial rate, the vector density, or the contact between the vector and the human host; or by

any combination of these methods. Reducing transmission will also bring about a decrease in the disease rate. The amount of transmission may be assessed by studying both the numbers of infective larvae of human lymphatic filarial parasites in the mosquito population, and by measuring the prevalence and intensity of microfilaraemia in the human population.

2.1.7.3 Reduction of morbidity and transmission

This is a general approach to lymphatic filariasis controlling in most endemic areas. There are no definite criteria as to the level to which the morbidity, the microfilarial rate and density, or the vector density and infectivity rate should decrease in order for lymphatic filariasis to cease to be a public health problem.

2.1.7.4 Interruption of transmission

Ideally, this is the ultimate objective. However, owing to the longevity of the adult worms and the limited resources of manpower and finance in most endemic countries, it is usually somewhat unrealistic to aim for elimination of lymphatic filariasis. So far only a few countries have achieved this goal. However, once it is accomplished and provided, there is no risk of reimportation or zoonotic transmission. There will be no need for continued costly maintenance or evaluation of the control program.

In addition, the offering of knowledge about individual protection from mosquitoes by using the mosquito net, the mosquito-repellent, and operation to cure the deformity in patients with lymphatic filariasis can be used to control of lymphatic filariasis also (Filariasis Division, 1995).

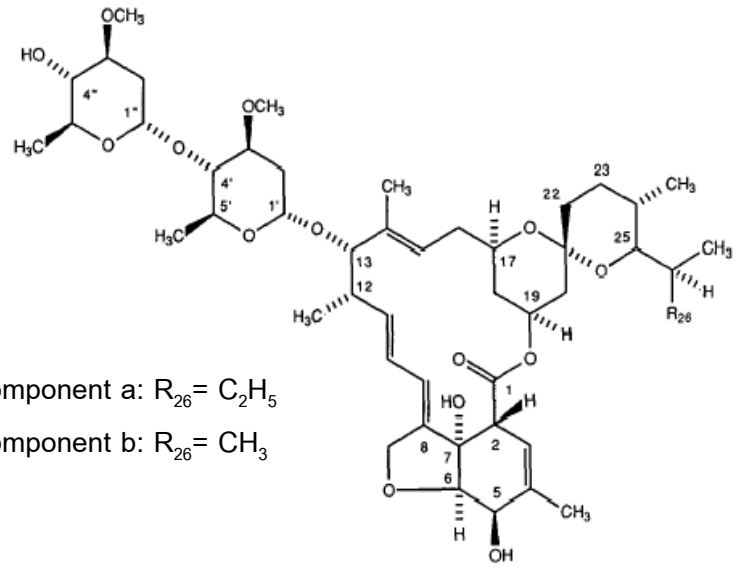
2.2 Ivermectin

Ivermectin has been known as an effective drug in treating lymphatic filariasis, and can be combined with DEC to reduce its side effects. Ivermectin has broader antiparasitic spectrum than DEC. It is highly effective for treating

both endo-parasites such as nematodes, which cause lymphatic filariasis and ecto-parasites such as ticks, insects, mites etc. In addition, it is effective against both immature and mature worms, and approved for using in dogs, cat, horses, cattle, sheep and swine in a variety of formulation (Campbell *et al*., 1983).

2.2.1 Chemistry

Ivermectin is a chemical modification of one of the avermectins, a series of naturally occurring macrocyclic lactones produced by the actinomycete *Streptomyces avermitilis*. Ivermectin (Figure 5) composes of more than 80% of 22, 23-dihydroavermectin B_{1a} (H_2B_{1a}; $C_{48}H_{74}O_{14}$ (R_{26}=C_2H_5); mw. 874) and less than 20% of 22, 23-dihydroavermectin B_{1b} (H_2B_{1b}; $C_{47}H_{72}O_{14}$ (R_{26}=CH_3); mw. 860).



Component a: R_{26}= C_2H_5

Component b: R_{26}= CH_3

Figure 5 Molecular structure of ivermectin

2.2.2 Antiparasitic activity

Ivermectin is active against two major phyla of animal parasites: the Nemathelminthes or nematodes (roundworms) and the Arthropods (insects, ticks and mites). However, it is unlikely to be active against species of the phylum Platyhelminths (Campbell *et al*., 1983).

2.2.2.1 Nematodes

Natural nematode infections of livestock usually involve several species and various development stages. Therefore, it is important that an anthelmintic drug for livestock should have a broad spectrum of action and should be active against both immature and mature worms (Armour *et al*., 1980). Ivermectin has an extremely broad spectrum of antinematodal activity in a variety of domestic animals, being active against genera of the superfamilies: Trichostrongyloidea, Strongyloidea, Oxyuroidea, Metastrongyloidea, Rhabditoidea, Ascandoidea, Spiruroidea, Filaroidea, and Trichuroidea (Benz & Ernst, 1981; Yazwinski *et al*., 1981; Klei & Torbert, 1980). Indeed, among the many nematode genera, which it has been tested, none has been found that is not affected by ivermectin during at least one stage of the life cycle (Lyons *et al*., 1981; Williams *et al*., 1981). In all but a few instances, the drug is highly active against both immature and mature worms. Ivermectin-susceptible nematodes include forms that live in the extra-intestinal tissues of the host as well as those with an intestinal habitat. Among the extra-intestinal worms are Filaroidea, some of which are important pathogens both in domestic animals and in man. With one or two exceptions, ivermectin has failed to show activity against adult filariae, but it has proved highly effective against other stages of several important filarial species. It prevents maturation of *Dirofilaria immitis* when given 1 day or 2 months after inoculation of larvae into dogs. These laboratory data suggest, and recent field trials confirm, that dogs can be fully protected against heartworm infection (and thus against heartworm disease) by monthly treatment with ivermectin (Blair & Campbell, 1980; Blair *et al*., 1982).

Efficacy of the drug against the skin-dwelling microfilariae of *Onchocerca* species in horses and cattle suggested that the drug might have

useful activity against *Onchocerca volvulus*, the causative agent of human onchocerciasis (including the condition known as river blindness). Preliminary studies in West Africa suggest that ivermectin does reduce the number of microfilariae in the skin of lightly infected patients (Aziz *et al*., 1982). Much further work will be required to determine the potential utility of the drug in the treatment or control of human onchocerciasis. Even if the drug does not prove beneficial in the therapy of patients with advanced forms of the disease, periodic treatment with ivermectin may well prove useful in preventing serious clinical manifestations (both dermal and ocular) in patients in whom such signs and symptoms have not yet developed.

Efficacy of ivermectin against *B. malayi* in animals has been established. Single oral dose (0.2-1.0 mg/kg) of the drug reduced microfilarial counts by 60-90% at 4-weeks post-treatment in *B. malayi* infected leaf monkeys (Mak *et al*., 1987). However, this drug 0.2-0.3 mg/kg monthly for 3 months can not prevent *B. malayi* infection in such animals subcutaneously inoculated with 100 infective larvae monthly for 3 months (Mak *et al*., 1987). In addition, ivermectin is found to inhibit the intrinsic exsheathing process of microfilariae in the mosquito, thereby block their development and further transmission of infection (Rao *et al*., 1992).

2.2.2.2 Arthropods

The effect of ivermectin on arthropod parasites has not been explored very extensively, but activity has been demonstrated against a wide variety of insect and acarine parasites. Parasitic fly larvae appear to be particularly susceptible to ivermectin. In the absence of suitable experimental models for cattle grub (ox warble) infection, efficacy has been evaluated by treating cattle under conditions of natural exposure. For this purpose the standard anthelmintic dosage of 0.2 mg/kg, injected subcutaneously, has been used.

This is probably well in excess of the minimum effective dosage, and the combined data from several dozen trials, involving approximately 2,000 cattle, indicated 100% efficacy against all parasitic stages of *Hypoderma bovis*. The susceptibility of lice to systemic treatment of the host with ivermectin is affected, not surprisingly, by the feeding habits of the parasite. Thus, the sucking lice (*Anoplura*) are more susceptible than the biting lice (*Mallophaga*). In cattle, for example, the field dosage of 0.2 mg/kg, subcutaneously, has been lightly effective against *Haematopinus eurysternus* and *Linognathus vituli*, but only moderately effective against *Damalinia bovis*. In swine, a high degree of efficacy against *Haematopinus suis* has been demonstrated at subcutaneous dosages as low as 0.02 mg/kg (Barth & Ernst, 1980).

Treatment of a host animal with ivermectin generally does not cause prompt death or detachment of ticks, but usually disrupts essential processes such as engorgement, molting, and reproduction (Drummond *et al*., 1981). In the case of *Boophilus microplus* (an important pathogen and pathogen-vector) effect of treatment was striking. When cattle received daily subcutaneous injections of ivermectin at 0.015 mg/kg, and were subjected to repeated infestation with an organophosphate-resistant strain of *Boophilus microplus*, no engorged adult ticks could be recovered so long as the treatment was continued (and for 2 weeks thereafter) (Nolan *et al*., 1981). Some kinds of ticks, for example *Otobius* species, have been reported to have little or no susceptibility to ivermectin at ordinary dosages (Craig & Kunde, 1981).

Some other acarine parasites are more susceptible than ticks to ivermectin treatment of their hosts. Mange mites (*Psoroptes* and *Sarcoptes* species) of cattle, for example, cease to be recoverable from skin scrapings about 2 weeks after treatment of the host with a single subcutaneous injection of ivermectin at 0.2 mg/kg, and a dramatic improvement in the clinical

condition of the host ensues (Melency, 1982). Efficacy against mites in cattle was substantially less when the drug was given orally (Melency, 1982). However, *Sarcoptes scabiei*, an important pathogen both of swine and of man, has been shown to be susceptible to orally administered ivermectin. Studies on experimental infestations in swine showed that a single oral dose at 0.18 mg/kg reduced the mite populations, and a dose of 0.3 mg/kg gave a 100% reduction in mote numbers and eliminated the clinical signs of injection (Lee *et al*., 1980).

2.2.3 Mechanism of action

Most studies on the mode of action of ivermectin have been carried out with avermectin B_1, but it is presumed that all avermectins share a common mechanism. Early studies on the free-living nematode *Caenorhabditis elegans* and on *Ascaris suum in vitro* (Kass *et al*., 1980) indicated that avermectin B_1 acted neither as a nicotinic agonist nor as a blocking agent of cholinergic nerve transmission. The same investigators dissected the anterior end of *Ascaris suum* to expose one pair of intact commissures of the dorsal excitatory neuron and the ventral inhibitory neuron for electrophysiological studies (Kass *et al*., 1980). The dorsal excitatory motorneuron could be stimulated indirectly by way of the ventral nerve cord, and a response recorded. Avermectin B_1, at a concentration of 5 μg/ml, abolished this response; washing the neurons with picrotoxin, an antagonist of γ-aminobutyric acid (GABA), restored it. These findings suggest that avermectin B_1 acts by blocking signal transmission from interneuron to excitatory motoneurons, and that GABA is the neurotransmitter that is blocked.

The effect of avermectin B_1 on nerve transmission to muscle was studied further by using the stretcher muscles in the walking legs of lobster (Fritz *et al*., 1979). The neuromuscular junction in this preparation is known to

be innervated by one excitatory axon regulated by GABA (Fritz *et al*., 1979). Avermectin B_1 inhibited both the excitatory and inhibitory postsynaptic potentials by reducing muscle membrane resistance. All responses were restored by picrotoxin. It is known that GABA receptors regulate the opening of chloride ion channels in crustacean muscle, and that those channels can be blocked with picrotoxin (Wang & Pong, 1982); hence, it is likely that avermectin B_1 stimulates GABA-mediated chloride ion conductance in this preparation. Thus, while avermectin B_1 appears to block interneuron-motorneuron transmission in nematodes and neuromuscular transmission in the lobster, the basic mechanisms are similar; both involve the GABA receptor (Wang & Pong, 1982).

If, as the data suggest, avermectin B_1 is anti-parasitic because it stimulates GABA-mediated chloride ion conductance, the overall effect could be due to avermectin B_1 acting as a GABA agonist, either at the GABA binding site or elsewhere on the protein, to stimulation of pre-synaptic GABA release, or to potentiation of GABA binding to its receptor (Campbell *et al*., 1983).

2.2.4 Method of determination

In the past, ivermectin has been detected by High Performance Liquid Chromatography (HPLC) with ultraviolet (UV) detection, UV absorption at 245 nm, but it was difficult due to matrix interference of ivermectin in plasma. Because of this drug with multihydroxy structure and high molecular weight are also quite unsuited for gas chromatography, much work has been done with fluorescent derivatization of ivermectin since the 1980s (Wei & Li, 2001).

Method of sample preparation used in this study was simple. It consisted of protein precipitation using acetonitrile and a sample clean-up using C_{18} solid-phase extraction (SPE). The whole clean-up procedure needed no additional organic solvent except for 2 ml of methanol and 2 ml of

water to precondition the SPE column. After extraction, analysts were evaporated to dryness before derivatization in the presence of trifluoroacetic anhydride (TFAA) and N-methylimidazole (NMIM) yielding a fluorescence product. The derivatives were determined. In a previous study (Tolan *et al.*, 1980), acetic anhydride and pyridine were used as a derivatizing reagent. The method required a reaction temperature of 100 ºC for 24 hours and derivative isolation. The derivatives were analyzed by liquid chromatography using fluorescence detection (excitation 364 nm and emission 480 nm). In the present study, derivatization of ivermectin was done at room temperature and the reaction was completed within 30 sec, using TFAA and NMIM, which were dissolved in acetonitrile, as reagents. The derivatives were assayed by liquid chromatography with fluorescence detection (excitation 365 nm and emission 475 nm). The derivative isolation was not required.

Almost all fluorescence derivatization involved with the same mechanism, i.e. acetylation, dehydration, and formation of an aromatic ring in conjugation with a butadiene (Wei & Li, 2001). Ivermectin contains a tertiary hydroxyl group at C7 and two secondary hydroxyl groups at C4 and C5. When ivermectin reacted with TFAA in the presence of a base catalyst (NMIM), all three hydroxyl groups were acetylated (Mrozik *et al.*, 1982). Subsequently, this acetylated derivative underwent dehydration at the C2-C7 and C5-C6 positions to form a fluorescent derivative having a six-member aromatic ring conjugated to a butadiene unit (Figure 6). White fog was suddenly formed and the solution turned light yellow.

Figure 6 Reaction of the formation of the fluorescent derivative of ivermectin following dehydration

The derivatization of ivermectin and abamectin (internal standard) in acetonitrile was complete in less than 30 sec at 25 ºC because the presence of the strong electron appeared to facilitate the dehydration of the acylated intermediate (De Montigny *et al*., 1990). The sensitivity and variety of derivatization were subject to the amount of solid residue and trace water in it after evaporation. The standards or samples must be dried completely before derivatizing reagents are added (Wei & Li, 2001). The use of abamectin as an internal standard ensured the reproducibility of derivatization and quantitative determination. Abamectin, which is very similar in chemical structure to ivermectin has the same fluorescence derivatizing mechanism and can be separated from analyzes well.

2.2.5 Pharmacokinetics

Pharmacokinetic data of ivermectin in normal volunteers have been established. Ette *et al*. (1990) have reported that peak levels of plasma ivermectin are achieved within 4 to 5 hours after single oral dose of 150 to 400 µg/kg. The long terminal half-life of about 57 hours in adults primarily reflects a low systemic clearance (about 1 to 2 L/hour) and a large apparent volume of distribution. The distribution of ivermectin to areas of filarial concentration, such as skin, eyes and nodules has not been characterized. Klotz *et al*., (1990) and Baraka *et al*., (1996) have confirmed that ivermectin, which is 93% bound to plasma proteins, has a relatively large apparent volume of distribution (46.9 L), indicating wide tissue distribution. The drug is extensively converted by hepatic CYP 3A4 to at least 10 metabolites, mostly hydroxylated and demethylated derivatives (Zeng *et al*., 1998). Virtually no ivermectin appears in human urine in either unchanged drug or conjugated form. In animals, ivermectin is recovered in feces, nearly all as unchanged drug (Perez

et al., 2001), and the highest tissue concentrations occur in liver and fat. Extremely low levels are found in brain, even though ivermectin would be expected to penetrate the blood brain barrier based on its lipid solubility.

Since ivermectin is widely used as an anti-parasitic drug in animals, its pharmacokinetics among different species has been established (Table 1). Scott & Mckellar (1992) studied the distribution and some pharmacokinetic parameters of ivermectin in pigs after being subcutaneously injected with 0.30 mg/kg in pigs. From the study, ivermectin was found to distribute well to all tissues and body fluids, which were sampled 24 hour post-injection. It was detected in the contents and mucus at all levels of the gastrointestinal tract. The drug was excreted in bile, with high concentration of the drug in the intestines and feces. High concentrations of ivermectin were measured in skin, ears and earwax, suggesting that the drug should be effective in the treatment of ecto-parasitic infestations, particularly ear mites. There was marked individual variation in the pharmacokinetics of ivermectin. In one pig, the $AUC_{0\rightarrow\infty}$ was particularly high. This may reflect individual variation in uptake and excretion of the drug. The $t_{1/2\ el}$ of the drug suggested that the drug is cleared slowly from pigs with drug detectable in plasma for 6-10 days.

Lifschitz *et al*. (1999a) studied bioequivalence of different formulations of ivermectin in pigs and cattle after being subcutaneously injected with either 0.30 mg/kg (pigs), or 0.20 mg/kg (cattle). None of the pharmacokinetic parameters calculated were statistically different between different drug formulations. In addition, bioequivalence studies on ivermectin disposition kinetics in both species were compared. When the drug was given by subcutaneous injection at recommended dose rates, the C_{max} and $AUC_{0\rightarrow\infty}$ values obtained were higher in cattle than in pigs. Those dose-dependent parameters were statistically different ($p<0.001$) between species, after

normalization by the administered dose rate. Differences in body composition may account for changes in the pattern of ivermectin tissue distribution. A more extensive distribution and deposit of ivermectin in adipose tissue in pigs compared to cattle, may account for the lower plasma availability obtained in this species.

Cerkvenik *et al*. (2002) studied pharmacokinetics of ivermectin in lactating sheep receiving a single subcutaneous dose of 0.2 mg/kg. Ivermectin concentrations in plasma and milk were determined. Results indicated that concentrations of ivermectin in plasma and milk did not differ. The mean time in which ivermectin concentrations fell below the limit of detection was 22 and 23 days for plasma and milk, respectively. Time course of ivermectin concentration in milk was following the time course of ivermectin in plasma, with an overall mean of milk and plasma ratio of 1.67 for the first 7 days of the experiment. A mean of 0.7% of the dose was excreted through milk. Disposition of this drug in the body is mostly influenced by its high lipophilicity (Lo *et al*., 1985), and manifested by large volumes of distribution and long elimination half-life. As such, expected that ivermectin transferred into milk is steadily increasing during the lactation period due to the affinity to milk fat content. Since the milk to plasma concentration ratio of ivermectin evidently differs from unity, $AUC_{0\rightarrow\infty}$, V_d/F and Cl/F values are different for the two matrices. Differences in elimination half-life ($t_{1/2\ el}$) between the two matrices can be ascribed to the relatively small size and considerable concentration variability of the analyses.

Echeverria *et al.* (2002) studied pharmacokinetics of ivermectin after subcutaneous administration (0.2 mg/kg) in healthy sheep and naturally infected sheep with mange (an ecto-parasitic disease produced by the mite *Psoroptes ovis*). Comparing the $AUC_{0\rightarrow\infty}$, there was not statistically

significant difference between sheep infected with mange and healthy ones (p>0.001), probably because the group sizes were too small to pick up a potential difference. However, the healthy animals showed a higher mean value. There were no statistically significant differences between elimination half life in normal and diseased animals (p>0.001). Absorption was faster in animals with mange. C_{max} was higher and T_{max} was shorter. These values are probably related to the change in the body condition of the animals infected with mange. These animals were in poor body condition and showed a reduction of body fat. It is well known that ivermectin is a lipophilic molecule, which distributes extensively into organic lipids. This is the cause of a high V_d/F. The animals with mange, with less lipids in the body, showed a lower V_d/F for ivermectin. A higher plasma affinity for the drug (because of less lipids in the body) gave rise to a higher C_{max} and a shorter T_{max}, indicating less transference to a smaller peripheral compartment. Although C_{max} was lower, the slow transference of the drug to and from tissues and a larger V_d/F generated a much higher $AUC_{0\rightarrow\infty}$ in healthy animals. The $MRT_{0\rightarrow\infty}$ was longer in healthy animals, which is a logical finding considering body condition.

Dupuy, *et al.* (2003) studied pharmacokinetics of ivermectin in yaks, (*Bos grunniens*) subcutaneously injected with a dosage of 0.20 mg/kg. The low value for $t_{1/2\ el}$ of 4.82 days compared with 17.20 days in cattle (Lanusse *et al.*, 1997) in association with a low value of $t_{1/2\ ab}$ (0.31 day), which represents the fifth of that obtained in cattle (1.15 day) (Lanusse *et al.*, 1997), leads a low $MRT_{0\rightarrow\infty}$. The low level of ivermectin concentrations in yaks represented by $AUC_{0\rightarrow\infty}$ should be explained by a rapid absorption process of drug associated with a low storage in fat. The results gave interesting informations on the ivermectin pharmacokinetics in yaks, which exhibit a peculiar

pharmacokinetic behavior in comparison with cows and other ruminants. This result is parallel with physiological differences (altitude adaptation, desert climate) and highlights the need of descriptive pharmacokinetics to avoid misuse of endectocides (extrapolation from different species) leading to emergence of parasites resistance. Furthermore, the ivermectin concentrations in edible tissues and milk should be different than those registered in other ruminants species, and would require different withdrawal times. Altogether these results demonstrate that further investigations are required to improve the use of ivermectin in yaks.

Table 1 Pharmacokinetic data of ivermectin in animals

Species	Dose (mg/kg) (route of admin.; No. of animals)	Pharmacokinetic parameters											Reference
		C_{max} (ng/ml)	T_{max} (days)	K_{ab} (day^{-1})	K_e (day^{-1})	$t_{1/2\ ab}$ (days)	$t_{1/2\ el}$ (days)	AUC $0\rightarrow\infty$ (ng.day/ml)	AUMC $0\rightarrow\infty$ ($ng.day^2/ml$)	MRT $0\rightarrow\infty$ (days)	Cl/F (L/day)	V_d/F (L/kg)	
Pigs	0.30 (SC.; 5)	28.4	1.1	-	-	-	1.5	71.4	-	-	-	-	Scott & Mckellar, 1992
Cattle[a]	0.20 (SC.; 20)	31.7±2.5	4.0±0.3	-	-	2.0±0.2	4.3±0.3	361.0±17.0	-	9.0±0.5	-	-	Toutain *et al*., 1997
Cattle[a]	0.20 (SC.; 7)	19.9±8.8	4.0±1.4	-	-	1.5±0.9	5.9±3.4	206.0±41.3	-	9.5±3.9	-	-	Lifschitz *et al*., 1999b
Pigs[b]	0.20 (SC.; 8)	33.3±4.1	2.8±0.4	-	-	-	3.5±0.5	165.0±21.6	995.0±108.0	6.3±0.6	-	-	Lifschitz *et al*., 1999a
Cattle[b]	0.20 (SC.; 8)	40.5±2.7	2.0±0.5	-	-	-	6.3±0.8	244.0±20.1	1530.0±244.0	6.1±0.5	-	-	Lifschitz *et al*., 1999a
Sheep[a]	0.20 (SC.; 6)	11.9±6.9	1.7±0.6	1.3±0.5	0.3±0.2	0.7±0.5	2.8±1.9	64.0±28.3	-	5.2±2.8	216.0±84.7	851.1±627.7[c]	Cerkvenik *et al*., 2002
Sheep[a]	0.20 (SC.; 6)	24.1±6.6	2.7±0.5	-	-	0.8±0.6	5.6±1.2	207.5±46.5	-	8.6±0.7	-	8.8±2.6	Echeverria *et al*., 2002
Sheep[a] (mange)	0.20 (SC.; 6)	41.2±16.2	0.9±0.2	-	-	0.2±0.2	5.5±1.4	179.9±90.6	-	6.7±1.8	-	6.5±1.7	Echeverria *et al*., 2002
Yaks[a]	0.20 (SC.; 4)	48.9±14.4	0.7±0.1	0.3±0.2	0.3±0.2	0.3±0.1	4.8±1.4	146.2±25.3	-	3.6±1.2	-	-	Dupuy *et al*., 2003

C_{max} = Peak plasma concentration

T_{max} = Time to maximum plasma concentration

K_{ab} = Absorption rate constant

K_e = Elimination rate constant

$t_{1/2\ ab}$ = Half-life of absorption

$t_{1/2\ el}$ = Half-life of elimination

AUC $0\rightarrow\infty$ = Area under the plasma concentration-time curve

AUMC $0\rightarrow\infty$ = Area under the first moment of the concentration-time curve

MRT $0\rightarrow\infty$ = Mean residence time

Cl/F = Clearance

V_d/F = Volume of distribution

S.C. = Subcutaneous administration

a = mean±SD.

b = mean±SE.

c = V_d/F was expressed by L unit

2.2.6 Therapeutic uses

Ivermectin has been used for treating several parasitic diseases as follows (Tracy & Webster, 2001).

2.2.6.1 Onchocerciasis

Single oral doses of ivermectin (150 µg/kg) given every 6 to 12 months are considered effective, safe and practical for the control of onchocerciasis in adults and children 5 years or older. Most importantly, such therapy results in reversal of lymphadenopathy and acute inflammatory changes in ocular tissues and arrests the development of further ocular pathology due to microfilariae. Marked reduction of microfilariae in the skin and ocular tissues is noted within a few days and lasts for 6 to 12 months; the dose then should be repeated. Cure is not attained because ivermectin has little effect on adult *Onchocerca volvulus*. Ivermectin has been used since 1987 as the mainstay for onchocerciasis control programs in all 34 countries in Africa and in the Middle East and Latin America where the disease is endemic. Nearly 20 million people have received at least one dose of the drug, and many have received 6 to 9 doses. Annual doses of the drug are quite safe, and can cause a substantial reduction in transmission of this infection. How long such therapy should continue is unknown.

2.2.6.2 Lymphatic filariasis

Initial studies indicate that single annual doses of ivermectin (400 µ g/kg) are both effective and safe for mass chemotherapy of infections with *W. bancrofti* and *B. malayi*. Ivermectin is as effective as DEC for lymphatic filariasis controlling, and can be used in regions where onchocerciasis, loiasis, or both infections are endemic. Recent evidence indicates that a single annual dose of ivermectin (200 to 400 µg/kg) and a single annual dose of albendazole (400 mg) are even more effective in lymphatic filariasis controlling

than either drug alone. The period of treatment is about 4 to 6 years based on the estimated fecundity of the adult worms. This dual drug regimen also reduces infections with intestinal nematodes. The drug combination now serves as the treatment standard for mass chemotherapy and control of lymphatic filariasis.

2.2.6.3 Infection with intestinal nematodes

A single dose of 150 to 200 μg/kg of ivermectin can cure human strongyloidiasis, this drug also effective against coexisting ascariasis, trichuriasis and enterobiasis. A single dose of 100 μg/kg of ivermectin is as effective as traditional treatment of intestinal strongyloidiasis with thiabendazole but less toxic. However, the efficacy of ivermectin against disseminated strongyloidiasis has yet to be established.

2.2.6.4 Other indications

Although ivermectin has activity against microfilaria, but not against adult worms of *L. Loa* and *M. Ozzardi*, it is not used clinically for treating infections with these parasitic worms. Taken as a single 150 to 200 μg/kg oral dose, ivermectin is a first-line drug for treatment of cutaneous larva migrants caused by dog or cat hookworms. Similar doses of this compound also are safe and highly effective against human head lice and scabies, the latter even in HIV-infected individuals.

2.2.7 Side effects, toxicity, and safety

2.2.7.1 Side effects

Ivermectin is generally well tolerated. Almost all of the side effects occurring during ivermectin therapy appear to be a consequence of an immunological reaction to dead microfilariae rather than being directly attributable to ivermectin. Side effects reported most commonly include myalgia, rash, lymph node tenderness and swelling, itching, fever and chills,

and joint or facial swelling. These are usually of mild to moderate severity, and respond to analgesics or antihistamines (Goa *et al*., 1991).

Chijioke & Okonkwo (1992) studied side events following mass ivermectin therapy for onchocerciasis. Mass ivermectin therapy single oral dose of 150 μg/kg were given the Achi community of southeastern Nigeria to control endemic onchocerciasis. Of 9,995 people eligible for dosing, 7556 (75.6%) received ivermectin. In 992 patients (13.1%) complained of side effects, mostly within one week of dosing. Side events were mainly of the Mazzotti type. Worsening of pruritus affected 71.2% of those complaining of side effects; 44.7% complained of fever (feeling hot, chills) but only 0.5 % had a temperature above 37.5 ^{o}C. Headache affected 46.4%, limb swelling 47.4%, and worsening of rash 24.4%. The incidence of side effects was greater in villages with a high load of microfilarial infection.

Coutinho *et al*. (1994) studied ivermectin treatment of Bancroftian filariasis in Recife, Brazil. Single oral dosages of ivermectin ranging between 20 and 200 μg/kg were studied to determine the effectiveness of ivermectin in 43 microfilaraemia patients with Bancroftian filariasis, caused by *W. bancrofti.* Side effects (predominantly fever, headache, weakness, and myalgia) occurred to some degree in almost all patients, but generally lasted only 24-48 hours and were easily managed symptomatically. Side effects were significantly milder in those receiving the lowest (20 μg/kg) of ivermectin dose and they were significantly correlated with individual's pretreatment microfilaremia levels in all groups.

2.2.7.2 Toxicity

Amounts approaching the therapeutic doses in animals (100 to 200 μ g/kg) are not hazardous to humans. Ingestions of large quantities (10 to 100 times the animal therapeutic dosage) may produce symptoms resembling

those observed in animal toxicology studies at high toxic levels. An adult female accidentally self injected a small quantity (approximately 200 μg/kg, subcutaneously). In 12 hours later, she experienced colicky pain with nausea but recovered within 12 hours (MSD, 1988). In children, a 16-month-old boy weighing 15 kg was approximately given 100 to 130 mg of ivermectin (an injectable solution). In 10 hours post-injection, he had mydriasis in one pupil with frequent vomiting, pallor, 35 ^{o}C temperature, tachycardia, somnolence and variable blood pressure. He developed urticaria the following day, and had recovered after three days (MSD, 1988).

In animals, the oral LD_{50} values of ivermectin that have been reported are 80 mg/kg in dogs, in mousses and rats ranges between 11.6-41.6 and 42.8-44.3 mg/kg, respectively (Merck & Co., Inc; 1979: 1981). At relatively high doses in animal toxicity studies, Central nervous system (CNS) effects and visual disturbances have been observed. High dose caused death due to respiratory depression. Ivermectin given to rat in intravenous administration at a dose of 4 mg/kg produced moderate incoordination of muscles. A dose of 6 mg/kg induced a state resembling anesthesia, which began one minute after injection and lasted for 4 to 5 hours. Higher doses caused death due to respiratory depression (Hayes & Laws, 1991). Oral toxicity study of dogs given at 0.5 mg/kg/day had no related effects. Dogs given 1 and 2 mg/kg/day developed mydriasis and lost a small amount of weight. Four of eight dogs given 2 mg/kg/day developed tremors, ataxia, anorexia and became dehydrated (MSD, 1988). Dogs given oral doses of ivermectin at 10 mg/kg produced ataxia with tremor and death occurred due to respiratory depression at 40 mg/kg (Campbell & Benz, 1984).

Lewis *et al*. (1994) studied ivermectin toxicosis in a kitten with ear mites, subcutaneously injected with 0.30 mg/kg. Signs of toxicosis usually

developed within 1 to 2 hours after administration of the drug, and include abnormal behavior, ataxia, lethargy, weakness, recumbency, apparent blindness, coma and death. Four kittens that received 1 mg/kg of ivermectin, subcutaneously of a formulation of ivermectin labeled for use in cattle, developed diarrhea and ataxia. Two of the 4 kittens deteriorated into a coma, and 1 kitten, the only female developed mild miosis and hinge limb ataxia, but all 4 kittens recovered completely in 5 to 7 days (Rischke & Hunt, 1991).

There is only one report of ivermectin toxicosis in an adult cat. The cat received a large dose (4 mg/kg of ivermectin) of paste preparation of ivermectin labeled for oral administration to horses. The cat developed ataxia, anorexia, mydriasis, blindness, and tremor, but recovered (Houston, 1985).

2.2.7.3 Safety

One major difference between invertebrates and mammals is that in mammals GABA-mediated nerves occur only in the CNS, whereas in many invertebrates (nematodes and arthropods) such nerves regulated peripheral muscles. Thus, a compound such as ivermectin, which acts on GABA-mediated nerves, should have a wide margin of safety in mammals, if it doses not readily cross the blood-brain barrier (Campbell *et al*., 1983). Studies in transgenic mice suggest that a P-glycoprotein efflux pump in the blood-brain barrier prevents ivermectin from entering the CNS. Thus, the limited affinity of ivermectin for CNS receptor may explain the paucity of CNS side effects and the relative safety of this drug in mammals (Schinkel *et al*., 1994). However, at higher concentrations ivermectin can also potentiate GABA gated chloride channels in mammals. This has led to suggestions that ivermectin and related drugs may be toxic in mammals having a deficiency in their blood brain barrier (Etter *et al*., 1999). Recent findings suggest that the severe CNS side effects seen in various mammals following ivermectin treatment may be due to an

absence of, or functional deficiency of P-glycoprotein (Kwei *et al*., 1999).

Generally, ivermectin is highly lipophilic substance but show unexpectedly poor penetration of the blood brain barrier. It is now believed that this phenomenon is a result of the actions of drug efflux transporters, which is P-glycoprotein that has been most widely studied. P-glycoprotein is an integral component of the mammal's blood brain barrier and plays a central role in limiting drug uptake into the brain. Altered expression or function of P-glycoprotein could conceivably allow elevation of brain concentration of ivermectin and produce severe neurotoxicity. Therefore, therapeutic dose ivermectin used in mammals has safety (Edward, 2003)