FILARIASIS
1. Lymphatic filariasis
   - *Wuchereria bancrofti*
   - *Brugia malayi*
   - *Brugia timori*

2. Subcutaneous filariasis
   - *Loa loa* (African eye worm)
   - *Onchocerca volvulus* (convoluted filaria or blinding filaria)
   - *Mansonella streptocerca*

3. Serous cavity filariasis
   - *Mansonella ozzardi*
   - *Mansonella perstans*

4. Zoonotic filariasis
   - *Dirofilaria*
   - *B. beaveri, B. leporis, B. pahangi*
History

1863 - Demarquay - microfilaria of *W. bancrofti* in hydrocele fluid from a patient in Cuba

1868- Wucherer - microfilaria in urine (Brazil)

1872- Lewis - microfilaria in human blood (Calcutta)

1899- Manson - Periodicity of microfilaria & role of Culex mosquito as vector (China)

1927- Brug, described it as a new species (Sumatra)
Morphology

Adult worm

• Creamy white, slender, 2-10cm (female Oncocerca 35-50 cm)

• Male smaller in size than female, cork screw like tail & have 2 spicules at posterior end

• Viviparous

Larva

Four larval stages

• 1st stage larvae - Microfilaria
• 3rd stage larvae - Filariform larva - Infective form
Adult worm

L 1 in mosquito vector

L 2 in mosquito vector

L 3 in mosquito vector
Microbial Periodicity

Nocturnal Periodicity
• Night time (10 pm to 4 am)
• Wucherechia & Brugia

Diurnal Periodicity
• Day time (12noon-2pm)
• Loa loa

Sub-periodic
• Present throughout, with slight increase in the afternoon

Non-periodic
• Any time
• Mansonella & Onchocerca
• Biological & evolutionary co-adaptation of the microfilariae to the feeding habit of the mosquito

• Culex bites at night, Aedes bites in daytime

• Sleeping pattern of the individual, temperature & other climatic conditions also contribute

• Microfilariae are present in pulmonary blood vessels, when not in peripheral blood
<table>
<thead>
<tr>
<th>Parasite</th>
<th>Location of Adults</th>
<th>Location of Microfilaria</th>
<th>Microfilaria Periodicity</th>
<th>Vector</th>
<th>Epidemiology</th>
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<tr>
<td><em>W. bancrofti</em></td>
<td>Lymphatic tissue</td>
<td>Blood</td>
<td>Nocturnal (mostly)</td>
<td>Culex – worldwide Anopheles in rural Africa</td>
<td>Cosmopolitan (South America, Africa, South Asia)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Subperiodic (rare)</td>
<td>Aedes</td>
<td>Pacific islands</td>
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<td><em>B. malayi</em></td>
<td>Lymphatic tissue</td>
<td>Blood</td>
<td>Nocturnal (Mostly)</td>
<td>Mansonia Anopheles</td>
<td>South-east Asia, Indonesia &amp; India</td>
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<tr>
<td></td>
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<td></td>
<td>Subperiodic (rare)</td>
<td>Coquillettidia Mansonia</td>
<td>South-East Asia</td>
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<tr>
<td><em>B. timori</em></td>
<td>Lymphatic tissue</td>
<td>Blood</td>
<td>Nocturnal</td>
<td>Anopheles</td>
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<td><em>Loa loa</em></td>
<td>Subcutaneous tissue &amp; Conjunctiva</td>
<td>Blood</td>
<td>Diurnal</td>
<td><em>Chrysops</em> (Deerflies)</td>
<td>West &amp; Central Africa</td>
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<tr>
<td><em>Onchocerca volvulus</em></td>
<td>Subcutaneous tissue</td>
<td>Skin &amp; Eye</td>
<td>None</td>
<td><em>Simulium</em> (Blackflies)</td>
<td>South &amp; Central Africa</td>
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<td><em>Mansonella streptocerca</em></td>
<td>Subcutaneous tissue</td>
<td>Skin</td>
<td>None</td>
<td>Culicoides (Midges)</td>
<td>West &amp; Central Africa</td>
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<td><em>Mansonella perstans</em></td>
<td>Body cavities &amp; Mesentery</td>
<td>Blood</td>
<td>None</td>
<td>Culicoides (Midges)</td>
<td>South &amp; Central America &amp; Africa</td>
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<tr>
<td><em>Mansonella ozzardi</em></td>
<td>Body Cavities</td>
<td>Blood</td>
<td>None</td>
<td>Culicoides (Midges)</td>
<td>South &amp; Central America</td>
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<td>Caribbean island</td>
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Life Cycle

Definitive host: Man

Intermediate host: Mosquito

Infective Form: 3rd stage filariform larvae (L₃)

Mode of transmission:

L₃ filariform larvae get deposited in skin by mosquito bite
Human cycle

- Development into adults
- Adults lay L₁ larvae or microfilariae

Mosquito cycle

- Transmission of microfilaria
- Exsheathing
- Migration to thoracic muscle
- Develop into L₃ filariform larvae (Infective form to man)
Pathologic change

Inflammatory damage to lymphatics

1. Lymphatic dilatation & thickening of the vessel walls due to migration of live adult worms

2. Tissue alterations related to antigen & toxic metabolites released from dead adult worm

3. Secondary bacterial & fungal infections
4. Host’s inflammatory response to both live & dead parasite

Infiltration of plasma cells, eosinophils & macrophages in the infected vessels along with endothelial & connective tissue proliferation

Leads to tortuosity of the lymphatics & damage to lymph valves

Lymph edema of limbs & brawny edema on the overlying skin
Damaged Lymphatic vessels remain patent till the worm remain viable

Death of worm leads to

- Enhanced granulomatus reaction
- Thrombi formation
- Fibrosis of lymph vessels with extensive perilymphangitis

Severe lymphatic obstruction
Host immune response

- Both cellular & humoral immune response

- Antigen presenting cells (macrophages) process the antigens of both adult worms & microfilaria & present them to T helper cells

- T helper cells are differentiated into T helper 1 cells & or T helper 2 cells
| Early Infection (Amicrofilaremic individuals) | Activation of parasite specific Th cells  
•Th1 cytokines IL -2 & IFN γ  
•Th2 cytokines IL -4 & IL -5 are elevated | Profound Eosinophilia  
High IgE titers |
|-----------------------------------------------|---------------------------------------------------------------------------------|
| **Microfilaraemic individuals** (Asymptomatic & Acute stage) | Diminished parasite specific T cell proliferation  
Predominant **T helper 2 cells response**  
- Elevated IL-4, IL-5, IL -13 ,IL 10  
- Low production of IFN γ ,IL -2 | Profound Eosinophilia  
Increased parasite specific IgG-4 antibodies  
Hyper IgE levels |
| **Chronic Filariasis** | Increased production of **T helper 2 cells induced cytokines**, IL-4,IL-5,IL-13 | Increased levels of parasite specific IgG-1,IgG -2 & IgG -3 |
Clinical manifestations

1. Asymptomatic microfilaremia
2. Acute adenolymphangitis
   - Fever
   - Lymphangitis
   - Lymphadenitis
   - Lymphedema
   - Lymphoangiovarix
3. Chronic lymphatic disease
   - Hydrocoele
   - Lymphorrhagia
   - Elephantiasis
Occult Filariasis or Tropical Pulmonary Eosinophilia

- Hypersensitivity to microbial antigen

- Microfilariae are rapidly cleared from the blood stream & filtered, lodged & destroyed in lungs initiating an allergic response

- Microfilariae are not detected in blood
Laboratory Diagnosis

Detection of Microfilaria

- Examination of unstained wet mount of blood
- Giemsa stained thick & thin blood smear
- QBC

Detection of Adult worm

- Lymphnode biopsy
- Imaging methods

Other tests

- Immunodiagnosis
- Molecular methods
Microscopy

Specimen

- Blood
- Hydrocele fluid
- Urine (10-20 ml of 1\textsuperscript{st} early morning urine)
- Other body fluids (Lymph)
- Biopsy from Lymphnodes
DEC Provocation Test

• Done to collect blood in day time

• 2mg/kg DEC orally

• Microfilariae comes to the blood after 30 mins
Direct Wet Mount

- Demonstrates serpentine movement of microfilariae

Stained Film

- Thick & thin smear - Stained with Leishman’s, Giemsa or Hematoxylin & Eosin stain
- Thick smear dehaemoglobinized by distilled water
- Low power – observe microfilarae in thick smear
- Thin smear -- observe the morphology (sheath & nuclei of microfilaria)
Tail up pointed

Microfilariae brancofti

Tail tip rounded with two nuclei at tail-tip

Microfilariae malayi
Concentration Method

When microfilaria density is low

**Membrane filtration technique**

5ml blood filtered through milipore or nucleopore membranes

(3µm diameter *Mansonella perstans microfilariae*)

Membranes examined as such or after staining

**Knott’s centrifugation technique**

1ml anticoagulated blood + 9ml 2% formalin

Centrifuge 500rpm for 1 minute

Smear from sediment stained & examined

**Disadvantage**

Microfilariae are killed by the formalin so not seen as motile organisms
QBC

- Centrifugation of blood
- Staining with acridine orange
- Examined under fluorescent microscope
- More sensitive than smear microscopy
Microfilariae may not be found in Blood

- Occult filariasis
- Chronic filariasis & endemic normal people
- Wrong time of blood collection
Lymphnode biopsy
Immunodiagnosis:

Important role in diagnosing amicrofilariaemic states

• Pre - patent period
• Chronic infection
• Tropical pulmonary eosinophilia
• Filarial granuloma
Antigen Detection

- Detects circulating antigens of *W. bancrofti Og4c3, WbSXP-1 & AD12*

  - **ELISA**
    - (Sensitive 100%, Specificity 99-100%)
  - **Immunochromatographic Filarial Card Test (ICT)**
    - (Sensitive 96-100%, Specific 95-100%)

- No antigen detection methods are available for Brugia infection
Advantages

• More sensitive than microscopy

• Can be detected in day time (Any time)

• Can differentiate the current & past infection as antigen disappears after clinical cure

• Can be detected in urine
Antibody detection

Older methods

- IHA
- IFA
- ELISA

Disadvantage

- Low specificity (cross reaction with intestinal round worms)
- Can’t differentiate between present & past infection
Newer approach

• Detection of IgG-4 antibodies against recombinant *W. bancrofti* antigen & BmR1 antigen of *B. malayi* by ELISA & ICT

• Less cross reactivity so more specific

• Correlate well with intensity, duration of filarial exposure & level of microfilaremia

• Anti-sheath antibodies are raised even before microfilariae appear in blood

• Can also be used to diagnose Brugia infection
Molecular methods

PCR
Detect DNA of *W. bancrofti* & *B. malayi* in blood
Can detect 1 microfilaria /ml of blood
Only if circulating microfilaria present not in chronic carrier state

PCR – RFLP
ITS 1 – rRNA gene as primer
Can differentiate all species of human & animal filarial parasite
Xenodiagnosis

• Mosquitoes are allowed to feed on the infected patients

• Are dissected 4-6 weeks later to demonstrate microfilariae

• Helpful in detecting low intensity microfilaremia
Skin test

- Intradermal injection of filarial antigen

(Extracts of microfilariae, adult worms & 3rd stage larvae of *B. malayi* or of *Dirofilaria immitis*)

- Not diagnostically important

- High false +ve & -ve reaction
Imaging methods

Ultrasound

– Anatomic abnormalities of lymphatics, dilated & tortuous vessels

– Filarial dance sign
  Serpentine movement of adult worms within the lymphatic vessels of scrotum
  Positive in 80% of cases
Lymphoscintigraphy

- Detects functional abnormalities of lymphatics in limbs (flow abnormalities)

- Useful in asymptomatic microfilaremic persons

X rays

- Dead & calcified worms

- Pulmonary infiltrates in patients with TPE
Other Methods

• Eosinophilia (Absolute Eosinophil count > 3000/µl)

• Elevated serum concentrations of IgE (> 1000 ng/ml)

• Cellular assays
  - Filarial skin test
  - Lymphocyte response to filarial antigen
Onchocerca volvulus (Onchocerca- curved tail)

- Convoluted filaria / blinding filaria / river blindness
- Intermediate host - female black flies (simulium)
- Adults- Subcutaneous nodules or Onchocercoma
- Microfilariae - lesions in skin & eyes

Ocular manifestations - Sclerosing keratitis , Secondary glaucoma, Optic atrophy, Chorioretinitis
Onchocerca volvulus

**Gold standard**

Detection of microfilariae in skin snip smear

**Most common sites**

- Both iliac crests or from calves & shoulders

- IgG 4 specific dip stick assay using antigen OV16

- PCR- Detect Onchocercal DNA in skin snips or skin scrapings
Onchocerca

Detection of Adult worm & Microfilaria

- Biopsy of nodules
  
  Less sensitive
Mazzotti Skin Test (DEC Patch Test)

- Topical application of DEC on skin leads to local reaction (erythema & itching) to dead worm
- Only done in light infection without eye involvement due to its severity in heavy infection
Loa loa

- Loiasis, calabar swellings, fugitive swellings- due to adult worm
- Intermediate host - chrysops (day biting fly)
- Microfilariae- in peripheral blood
- Isolation of *Loa loa* adult worm from eye
- Biopsy of subcutaneous swelling
- Nested PCR - Detection of DNA