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ESSENTIALS OF APPLIED MICROBIOLOGY FOR NURSES

Including Infection Control and Safety

Semester III

2nd Edition





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As per the Revised INC Syllabus for BSc Nursing

Apurba S Sastry • Sandhya Bhat

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CHAPTER 49

Intestinal Nematodes

CHAPTER PREVIEW

- *Trichuris trichiura*
- *Enterobius vermicularis*
- *Ascaris lumbricoides*
- Hookworm
- *Strongyloides stercoralis*

NEMATODES

Nematodes are developmentally higher helminths, probably the most widespread helminths occurring in the world.

Classification Based on Habitat

Based on their habitat, nematodes infecting humans are classified into two groups.

Intestinal nematodes: Include five common parasites.

1. Small intestinal nematodes—*Ascaris,* hookworm, and *Strongyloides.*
2. Large intestinal nematodes—*Trichuris* and *Enterobius*.

Somatic or tissue nematodes: They reside in various tissues or organs.

3. Filarial nematodes: They comprise of several vector-borne parasites.
   - *Wuchereria bancrofti* and *Brugia malayi* cause lymphatic filariasis.
   - *Loa loa, Onchocerca volvulus* and *Mansonella* cause cutaneous filariasis.
4. *Dracunculus medinensis:* Causes Guinea-worm disease; presents as painful cutaneous blisters.
5. *Trichinella spiralis*: Produces profuse watery diarrhea and myalgia.

Apart from the above list, there are several nematodes of lower animals that rarely cause intestinal and somatic disease in man (described later in this chapter).

Classification Based on They Lay Egg or Larva

Nematodes can be classified into three groups based on whether they lay eggs or larvae after fertilization of male and female worms.

- **Oviparous:** Here following fertilization, eggs are produced that are released in feces. Most of the intestinal nematodes are oviparous except for *Strongyloides.* Here, eggs are the diagnostic form in the stool.
- **Viviparous:** Female worms directly give birth to larvae; there is no egg stage. All somatic nematodes are viviparous. Here, the diagnosis is made by the detection of larvae in tissues or blood.
- **Ovoviviparous:** Here following fertilization, eggs are produced, from which the larvae immediately hatch out—e.g. *Strongyloides* species. The larvae are excreted in stool and are the diagnostic form.

Morphology

Nematodes have three morphological forms—adult worm (male and female), egg, and four larval stages (L_1–L_4). The adult form of nematodes is elongated, cylindrical in shape, and vary in size from a few mm (hookworm, *Trichinella*, and *Strongyloides*) to as long as one meter (*Dracunculus*).

TRICHURIS TRICHIURA

Trichuris trichiura is a soil-transmitted helminth (STH), that commonly affects children. It is also called **whipworm** (as the adult worm resembles a handle of a whip).

- **Life cycle (Fig. 49.1):** Humans are the only host, acquire infection by ingestion of contaminated food and water containing embryonated egg (infective form).
  - **In the large intestine**: Eggs hatch out to form L_2 larva→L_3 larva→L_4 larva→ adult worms→ fertilize to produce eggs → are excreted in feces.
  - **Environment:** The eggs become embryonated (L_2 larvae) in the environment and the cycle continues.

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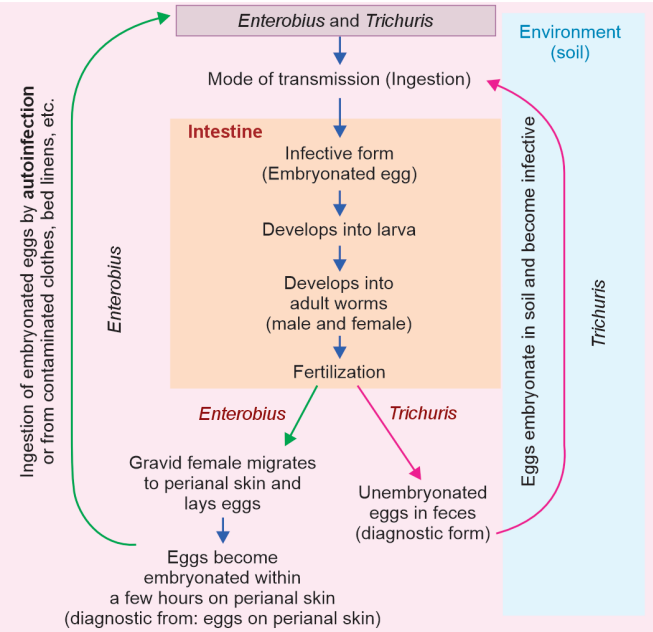


Fig. 49.1: Life cycles of *Trichuris* and *Enterobius*.

- **Clinical manifestations:** Adult female worms get buried in the large intestinal mucosa, which leads to abdominal pain, dysentery, iron deficiency anemia, recurrent rectal prolapse, malnutrition leading to growth retardation.
- **Laboratory diagnosis:** Stool microscopy reveals characteristic eggs—barrel-shaped with mucus plugs at the ends. Eggs measure 50 × 22 µm in size, are bile-stained, and float in saturated salt solution **(Fig. 49.2A)**.
- **Treatment:** Mebendazole or albendazole is the drug of choice.

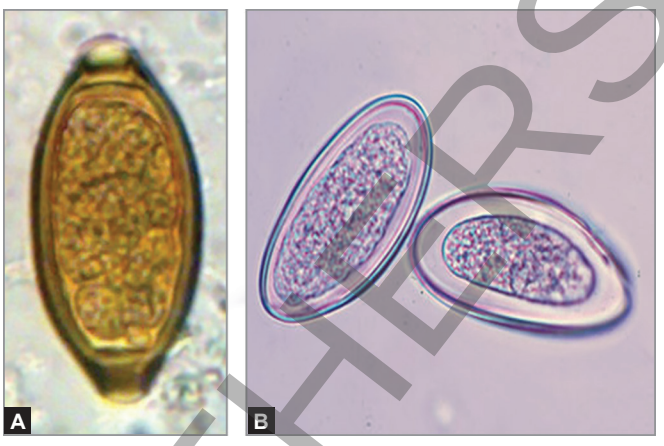
ENTEROBIUS VERMICULARIS

E. vermicularis is a common large intestinal nematode affecting children. It is also called **pinworm** or **threadworm** (as the adult worm of *Enterobius* is small, white, and thread-like).

Life Cycle (Fig. 49.1)

Humans are the only host. Embryonated eggs are infective to man.

- **Transmission:** Infection occurs by:
  - Ingestion of embryonated eggs from contaminated hands, food, surfaces, clothes, or bed linen.
  - **Autoinfection:** Usually occurs by transfer of eggs from the perianal region to the mouth via contaminated fingers, especially with nail biting.
  - **Retroinfection:** Larvae may hatch on the perianal skin and migrate back through the anus into the intestine.



Figs. 49.2A and B: Saline mount of stool showing: (A) Egg of *Trichuris trichiura* (bile stained); (B) Egg of *Enterobius vermicularis* (nonbile stained).

Source: DPDx Image Library, Centers for Disease Control and Prevention (CDC), Atlanta (*with permission*).

- Larvae hatch out from eggs in the cecum and then develop into adult worms.
- After fertilization, the gravid female worms migrate to the large intestine (rectum, colon) and start laying eggs on the perianal skin.
- The eggs are embryonated (carrying L_2 larvae) and are the infective stage to man.

Pathogenicity and Clinical Features

Children are commonly affected. The most cardinal symptom is **perianal pruritus,** which often gets worse at night as a result of the nocturnal migration of the female worm.

- The worms may be found in undergarments and lying in the buttock area of infected children.
- Repeated scratching is the main reason of contaminated fingers; which causes autoinfection.

Laboratory Diagnosis

Microscopy of the perianal skin samples is the test of choice which detects characteristic eggs.

- **Methods:** Specimen is collected by two methods—cellophane tape or by NIH swab (developed by National Institute of Health, USA).
  1. Cellophane tape is applied onto the perianal region and then the tape is mounted with a drop of saline on a clear glass slide **(Fig. 49.3A)**.
  2. **NIH swab:** It consists of a glass rod attached to a cellophane tape by a rubber band. The cellophane part of the glass rod is rolled over the perineal skin area to collect the sample **(Fig. 49.3C)**.

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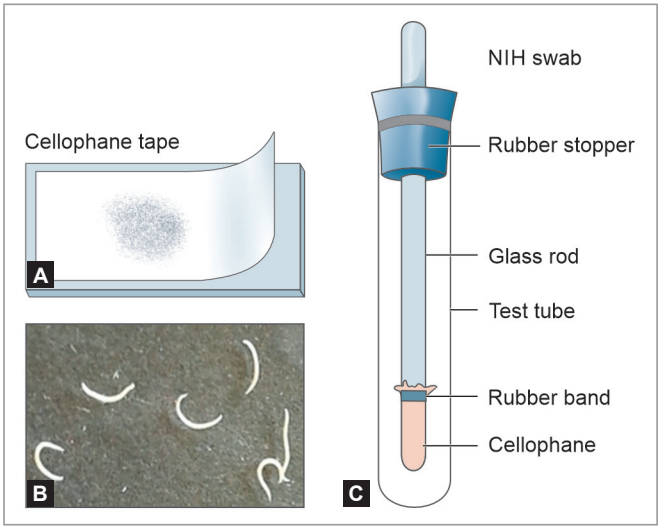

Figs. 49.3A to C: *Enterobius vermicularis*: (A) Cellophane tape; (B) Adult worms (actual size); (C) NIH swab (schematic diagram).

Source: (B) Head, Department of Microbiology, Meenakshi Medical College, Chennai (*with permission*).

- **Number of specimens:** A series of 4–6 consecutive tapes may be necessary as the female worms migrate intermittently **(Fig. 49.3B)**.
- **Timing:** Samples should be collected when the chance of egg deposition is more such as late evening, or early morning.

Eggs of *Enterobius*

Eggs are planoconvex (one side is convex and the other side is flat), and measure 50–60 µm long (**Fig. 49.2B**).

- Embryonated egg when freshly passed contains a tadpole-shaped larva inside.
- Nonbile-stained, colorless in saline mount.
- Floats in a saturated salt solution.

TREATMENT Enterobiasis

The recommended drugs are mebendazole or albendazole or pyrantel pamoate.

- The same treatment should be repeated after 2 weeks.
- Treatment of household members is advocated to eliminate asymptomatic reservoirs.

Prevention

Improving personal hygiene such as proper washing of bedclothes, keeping nails short and clean, and frequent hand washing are the key measures to contain the transmission.

ASCARIS LUMBRICOIDES

Ascaris lumbricoides is the largest nematode parasitizing the human intestine. It is commonly called as **roundworm**. It is a soil-transmitted helminth.

Morphology

Similar to other nematodes, *Ascaris* exists in three forms: adult, larvae (four stages), and egg. The adult worm is cylindrical and measures 15–31 cm. The female worms liberate two types of eggs—(1) fertilized eggs and (2) unfertilized eggs.

Life Cycle (Fig. 49.4)

Ascaris involves only one host (man). Embryonated eggs containing the L_2 larvae are the infective form.

- **Mode of transmission:** Ingestion of embryonated eggs from the contaminated soil, food, and water.
- **Migratory phase:** Following ingestion, the eggs hatch out to liberate the L_2 larvae → molt once to form L_3 larvae→ penetrate the intestine, reach the right side of the heart via portal circulation→ then enter the lungs → molt once to form L_4 larvae in the lungs → migrate up to reach pharynx → finally are swallowed to re-enter the intestine.
- **Intestinal phase:** The L_4 larvae undergo final molt to become adult worms in the small intestine. After fertilization, female worms lay fertilized eggs that are passed in feces. Unmated female worms may lay unfertilized eggs.
- **Development in soil:** The fertilized eggs molt twice and become embryonated (carrying L_2 larvae), which is infective to man, and the life cycle continues.

Pathogenesis and Clinical Feature

Clinical features occur in two stages.

1. **Pulmonary phase:** It occurs due to migrating larvae in the lungs, which provoke an immune-mediated

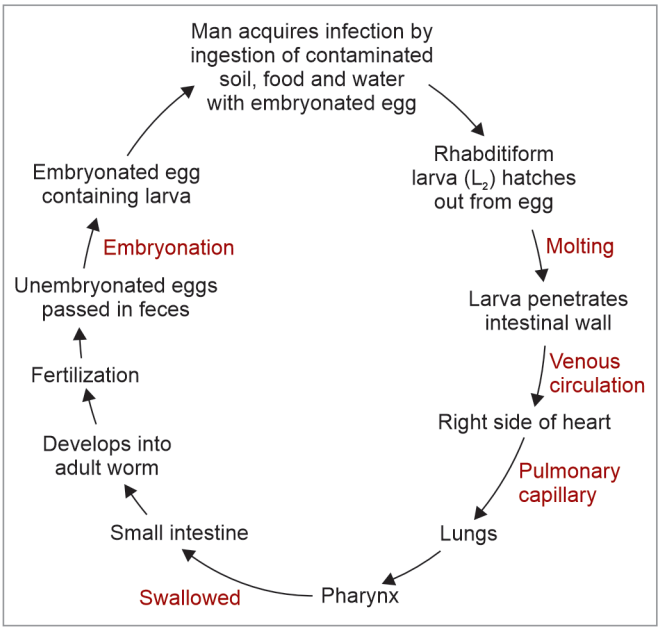

Fig. 49.4: Life cycle of *Ascaris lumbricoides*.

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hypersensitivity response—called eosinophilic pneumonia (**Loeffler's syndrome**). Common symptoms are nonproductive cough, chest discomfort, fever, dyspnea, and transient patchy infiltrates seen in the chest X-ray along with transient peripheral eosinophilia.

2. **Intestinal phase:** It results due to the effect of adult worm in the intestine; characterized by:
   - *Malnutrition and growth retardation:* Due to robbing of the nutrition from the host by the adult worm. It is often associated with impairment of the educational performance, language learning, gross motor, and fine motor skills in children.
   - *Intestinal complications:* Intestinal obstruction, intestinal perforation, intussusception, or volvulus.

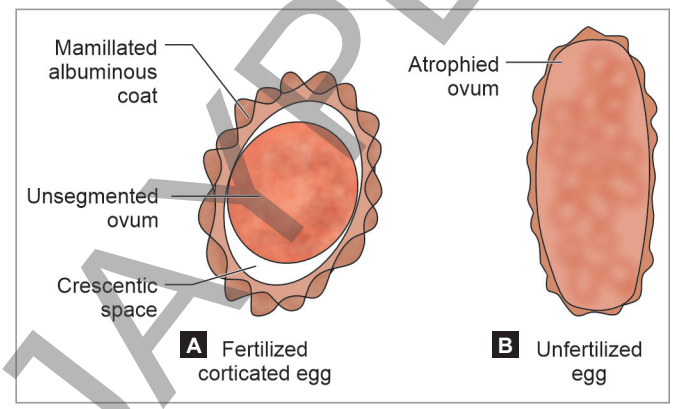
Laboratory Diagnosis

Stool examination: Fertilized and unfertilized eggs are detected by saline and iodine wet mount. Stool concentration by the sedimentation method is preferred to improve sensitivity.

Eggs of *Ascaris*

***Fertilized eggs* (Figs. 49.5A and 49.6A)**
Round to oval, measure 45–75 µm × 35–50 µm
- Surrounded by a thick mamillated, albuminous coat
- Contains a large, unsegmented ovum with clear crescentic space at both the poles
- Bile-stained, appear golden brown in saline mount
- Floats in saturated salt solution

***Unfertilized eggs* (Figs. 49.5B and 49.6B)**
Elongated, measure 85–95 µm × 43–47 µm
- Albuminous coat is thin, distorted and scanty
- Contains an unsegmented, small atrophied ovum
- Bile-stained, appear golden brown in saline mount
- Do not float in the saturated salt solution.



Figs. 49.5A to B : Eggs of *Ascaris lumbricoides*: (A) Fertilized eggs; (B) Unfertilized eggs (*schematic diagram*).

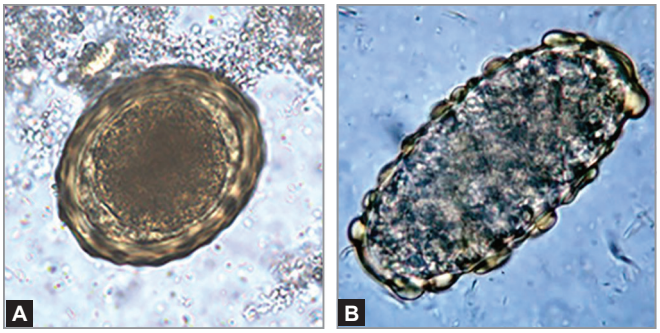
Other diagnostic modalities include:
- **Adult worms** may be detected occasionally in the stool or sputum of the patients.
- **Larvae** can be found in sputum or gastric aspirates during the early pulmonary migratory phase.
- **Antibody detection** by ELISA.
- **PCR** assay has been developed targeting *Ascaris*-specific genes in the stool.
- **Eosinophilia** is prominent during the early lung stage.
- Presence of **Charcot-Leyden crystals** in sputum and stool.

TREATMENT — **Ascariasis**

Albendazole or mebendazole is the drug of choice. It effectively kills the adult worm but has a limited effect on the larval migration phase.

HOOKWORM

Hookworm is a soil-transmitted helminth, one of the important causes of iron deficiency anemia.
- **Pathogens:** Two species are human pathogens: (i) *Ancylostoma duodenale* and (ii) *Necator americanus.*
- **Epidemiology:** Hookworm infection is widespread in the world and also in India.
- **Endemic index:** *Chandler's index* is used to estimate the morbidity and mortality in the community due to hookworm infection (which depends much upon the worm load).
- **Morphology:** Similar to other nematodes, hookworm exist as an adult worm, larvae (four stages), and egg.
  - The adult worm is small in size and has a bent in the anterior end (hence called as hookworm).
  - L_1 larva is called as rhabditiform larva whereas L_3 stage larva is called as filariform larva.



Figs. 49.6A to B: Eggs of *Ascaris lumbricoides*: (A) Fertilized eggs; (B) Unfertilized eggs.

Source: DPDx Image Library, Centers for Disease Control and Prevention (CDC), Atlanta (*with permission*).

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- *Ancylostoma* and *Necator* can be differentiated by the morphology of adult worm and third stage larva. Eggs and first-stage larvae of both are morphologically indistinguishable.

Life Cycle (Fig. 49.7)

Hookworm involves only one host (man). Third stage filariform (L_3) larva is the infective form.

- **Mode of transmission:** Through penetration of the skin by the L_3 larva; during walking barefoot in dampened soil.
- **Migratory phase**: Following penetration, the L_3 larvae are carried to the lungs through venous circulation. From the lungs, they migrate up to the pharynx, and finally, by swallowing sputum, they enter the GIT.
- **Intestinal phase:** The L_3 larvae molt twice in the small intestine to develop into adult worms, which attach to the intestinal mucosa by their teeth in the buccal capsule. Following fertilization, female worms lay eggs, which are excreted in the feces.
- **Development in soil:** Eggs released in feces become embryonated in soil and subsequently the L_1 (rhabditiform) larvae hatch out from eggs which molt twice to develop into L_3 larvae (infective form). Thus, the life cycle is continued.

Clinical Features

Affect Due to Migrating Larva

- Cutaneous lesions: (i) **ground itch:** pruritic maculopapular rashes at the site of skin penetration; (ii) **serpiginous tracks** may be formed due to subcutaneous migration of the larva.
- Mild transient pneumonitis due to the passage of migrating larvae through the lungs.

Affect Due to Adult Worm in Intestine

- **Early intestinal phase (less worm load):** Infected persons may develop epigastric pain, inflammatory diarrhea, etc. accompanied by eosinophilia.
- **Late intestinal phase (chronic infection with heavy worm load):** Patients develop iron deficiency anemia and protein-energy malnutrition resulting from

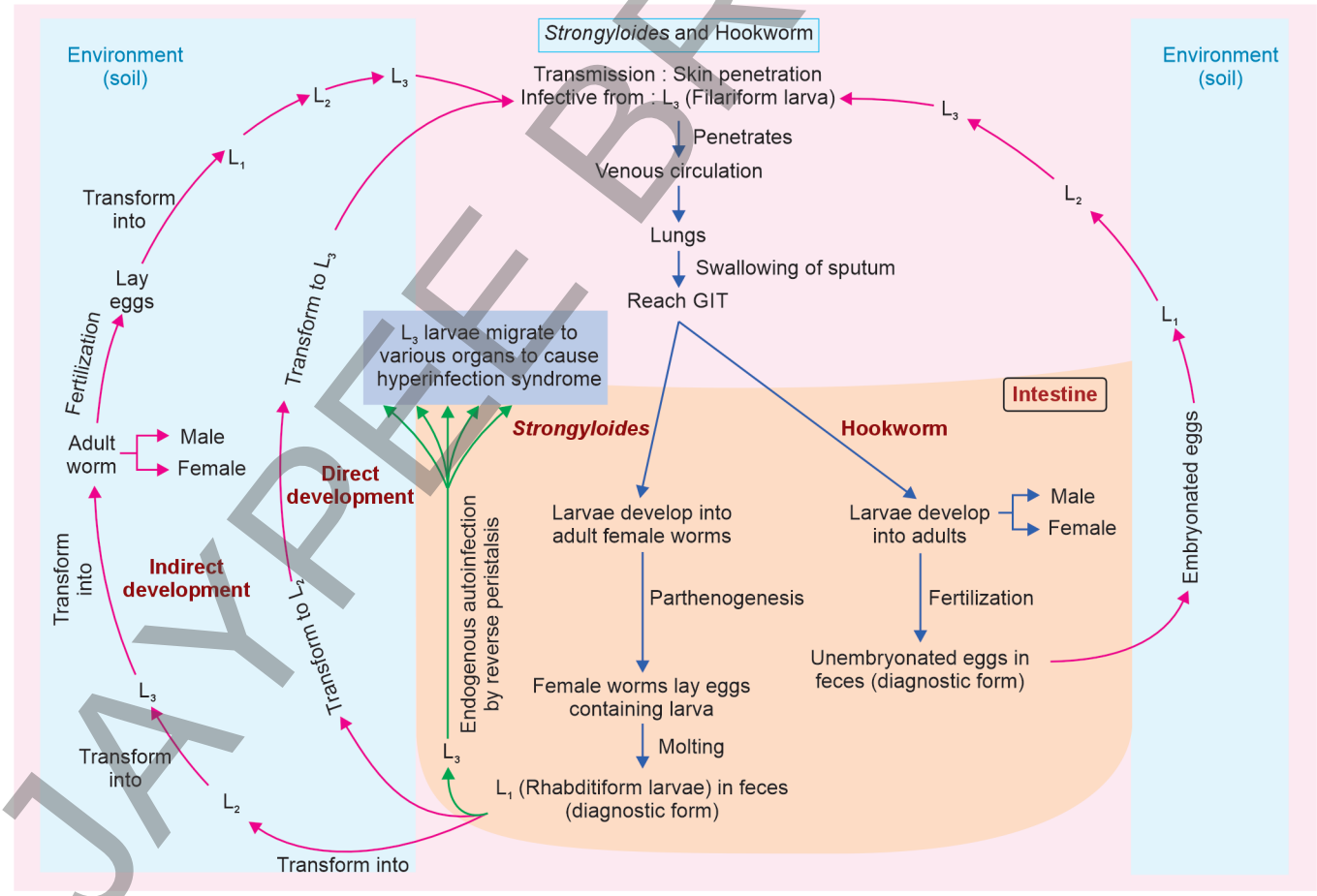

Fig. 49.7: Life cycles of hookworm and *Strongyloides*.

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blood loss. *Ancylostoma* is more pathogenic, and sucks more blood than *Necator.*

Laboratory Diagnosis

Stool Microscopy

The diagnosis is established by finding characteristic eggs in the feces.

- Stool concentration procedures may be required to detect eggs in case of lighter infections.
- **Egg counting:** The number of eggs per gram of stool can be counted (by the Kato Katz technique) to estimate the disease burden.

Eggs of Hookworm (Fig. 49.8)

Hookworm eggs are oval, measure 60 × 40 µm, surrounded by thin eggshell.

- Not bile stained, appear colorless in saline mount.
- Ovum is segmented; comprises of four blastomeres.
- Clear space between the eggshell and the embryo.
- Eggs float in saturated salt solution.
- Eggs of both *A. duodenale* and *N. americanus* are morphologically indistinguishable.

Stool Culture

The freshly passed stool samples can be cultured (so that the eggs hatch out to develop into L_3 stage filariform larvae) by Harada-Mori filter paper tube method or agar plate technique (more sensitive).

Molecular Diagnosis

PCR-based assays can differentiate between *Ancylostoma* and *Necator.*

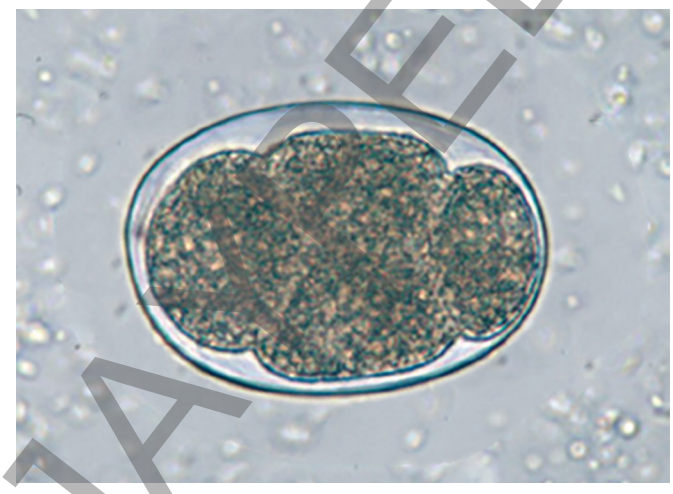

Fig. 49.8: Hookworm egg with four blastomeres.

Source: DPDx Image Library, Centers for Disease Control and Prevention (CDC), Atlanta (*with permission*).

Other Findings

Other findings include: (i) hypochromic microcytic anemia and (ii) eosinophilia.

TREATMENT Hookworm infections

Albendazole is the drug of choice.

Symptomatic treatment

- Iron-deficiency anemia with oral iron and folic acid.
- Malabsorption warrants nutritional support.

STRONGYLOIDES STERCORALIS

Strongyloides stercoralis is a small intestinal nematode that causes strongyloidiasis. The disease is particularly common in Southeast Asia (including India).

Morphology

S. stercoralis exists in three forms: adult, larvae (four stages), and egg.

- **Adult worm:** Only female worms are seen in the small intestine of man, and measure 2–3 mm long. Male worms are free-living in the environment, but not in the human intestine.
- **Eggs**: *Strongyloides* are ovoviviparous, i.e. eggs once laid, immediately hatch out to larvae.

Life Cycle (Fig. 49.7)

S. stercoralis involves only one host (man). L_3 larva (filariform) is the infective form.

- **Mode of transmission:** (1) Penetration of skin by the L_3 larva (by walking barefoot); (2) Autoinfection (internal autoinfection).
- **Migratory phase**: L_3 larvae are carried from the site of skin penetration to the lungs through venous circulation. From the lungs, they migrate up to the pharynx, and finally, by swallowing sputum, they enter the GIT.
- **Intestinal phase:** The L_3 larvae molt twice in the small intestine to develop into adult female worms, which are then buried in the intestinal mucosa. However, adult males are not found in the human intestine.
- **Laying eggs:** The female worms can directly lay eggs without fertilization, by a process called **parthenogenesis**.
- Being ovoviviparous, eggs immediately hatch out liberating the rhabditiform (L_1) larvae into the intestinal lumen, which are passed in the feces (diagnostic form) or transformed into (L_3) larvae to cause autoinfection.

Autoinfection: Sometimes, the L_1 larvae released in the human intestine do not pass in the feces but develop into filariform (L_3) larvae that eventually penetrate the intestinal wall or perianal skin, enter the venous circulation and reach the lungs. Autoinfection is responsible for disseminated infection.

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- **Development in the environment:** The L_1 larvae molt twice to form the L_3 larvae. Then two types of development take place:
  1. **Direct cycle:** The L_3 larvae act as the infective form to man. This cycle usually occurs in the temperate climate.
  2. **Indirect development:** The L_3 larvae develop into adult worms (male and female) → fertilize to produce eggs → L_1 larvae hatch out → molt twice to form the infective L_3 larvae. This cycle usually occurs in the tropical climate.

Pathogenesis and Clinical Feature

Effect Due to Migrating Larva

- **Cutaneous larva migrans:** Migrating larvae may produce the pathognomonic serpiginous urticarial rash (commonly on the thigh) called **larva currens**.
- **Pulmonary symptoms** are uncommon.

Effect Due to Adult Worm and Filariform Larva

- **Mild to moderate worm load:** May produce epigastric pain, nausea, diarrhea, constipation, and blood loss.
- **Heavy larva load:** Hyperinfection syndrome and disseminated strongyloidiasis are the important complications, observed in heavy larva load **(Table 49.1)**.

Laboratory Diagnosis

Stool Microscopy

Stool microscopy reveals the characteristic rhabditiform larvae (diagnostic form) **(Fig. 49.9)**. Repeated stool examination and concentration techniques can be performed to increase the yield.

Stool Culture

Various stool culture techniques used are: Harada-Mori filter paper tube method and agar plate technique. It is useful to differentiate rhabditiform larvae of *Strongyloides* from hookworm.

Other Diagnostic Tests

- **Serology** by ELISA using crude larval antigens has a greater sensitivity.
- **Coproantigen** detection in stool is useful.
- Molecular diagnosis by real-time PCR assays has high sensitivity.

Table 49.1: Complications of strongyloidiasis.

Hyperinfection syndrome
The underlying cause is the repeated autoinfection cycles; which lead to the generation of a large number of filariform larvae.
- **Risk factors:** Impaired host immunity, e.g. glucocorticoid therapy, transplant recipients, co-infection with HTLV-1 or rarely HIV.
- **Features:** Colitis, enteritis, or malabsorption, and in severe cases disseminated strongyloidiasis may develop.

Disseminated strongyloidiasis
Larvae may invade the GIT and migrate to various organs.
- **CNS invasion:** Leads to brain abscess and meningitis.
- **Passage of enteric flora** through disrupted mucosa leads to gram-negative bacterial sepsis/meningitis.
- **The mortality rate** is very high if left untreated.

(HTLV-1: human T cell lymphotropic virus)



Fig. 49.9: Rhabditiform larva of *Strongyloides stercoralis* (Iodine mount).
Source: Department of Microbiology, Meenakshi Medical College, Chennai (*with permission*).

TREATMENT **Strongyloidiasis**

Ivermectin is the drug of choice and is more effective than albendazole.

LARVA MIGRANS

Larva migrans refer to the lesions produced by nematodes of lower animals when they accidentally infect man.

Arrested Life Cycle and Pathogenesis

There are a number of nematodes of lower animals for which humans are an abnormal accidental host.
- Larvae of these lower animal nematodes when accidentally infect man, they are not able to complete their normal development (because humans are the unusual host for them) and their life cycle gets arrested. The larvae wander around aimlessly in the body. This is called larva migrans (LM).
- In some cases, transmission to man occurs through ingestion of embryonated eggs (infective form) contaminated in soil. Larvae hatch out and then wander aimlessly.

Larva migrans occur in—cutaneous and visceral forms.

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Cutaneous Larva Migrans

It is also called **creeping eruption**. Larva migration occurs in the skin and subcutaneous tissue, following which the life cycle gets arrested.

- It is mainly caused by nonhuman hookworm species such as *Ancylostoma brasiliensis, A. caninum,* and *A. ceylanicum.*
- Rarely, can be caused by human nematodes such as *Strongyloides stercoralis* (larva currens), *Ancylostoma duodenale,* and *Necator americanus* (ground itch).
- **Diagnosis** made mainly by clinical feature (presence of the linear tracks) and history of exposure. Larvae are usually not detected in skin biopsy. Elevated eosinophilia may be seen in peripheral blood or sputum.

A list of agents causing cutaneous larva migrans is given in **Table 49.2**.

Visceral Larva Migrans

Larva migrates to viscera, following which the life cycle gets arrested. It is caused by various nematodes of lower animals such as:

Table 49.2: Etiology of larva migrans (LM).

Causes of cutaneous larva migrans (CLM)	
Important causes (nonhuman *Ancylostoma* species): • *A. brasiliensis* • *A. caninum* • *A. ceylanicum*	**Rare causes:** Occasionally human nematodes may cause LM: • *Strongyloides stercoralis* • *Ancylostoma duodenale* • *Necator americanus*
Causes of visceral larva migrans (VLM)	
Important causes: *Toxocara* species (*T. canis* and *T. cati*)	
Other agents: • *Angiostrongylus* species • *Gnathostoma spinigerum*	• *Baylisascaris procyonis* • *Anisakis species*

- *Toxocara canis*: Infects the liver (hepatomegaly) and other organs. It can also infect eyes (ocular larva migrans) causing painless chorioretinal granuloma.
- *Angiostrongylus cantonensis:* Infects CNS and causes eosinophilic meningitis.

A list of agents causing visceral larva migrans is given in **Table 49.2**.

EXPECTED QUESTIONS

I. Write essays on:

1. Discuss the life cycle, clinical manifestations and laboratory diagnosis of *Ascaris lumbricoides* infection.
2. Discuss the life cycle, clinical manifestations, and laboratory diagnosis of hookworm infection.

II. Write short notes on:

1. Laboratory diagnosis of enterobiasis.
2. Laboratory diagnosis of *Trichuris trichiura* infection.
3. Laboratory diagnosis of strongyloidiasis.
4. Larva migrans.

III. Multiple choice questions (MCQs):

1. All of the following intestinal nematodes are oviparous, *except*:
   a. Roundworm
   b. *Strongyloides*
   c. Hookworm
   d. *Enterobius*

2. Barrel-shaped eggs are seen for:
   a. Pinworm
   b. Roundworm
   c. Hookworm
   d. Whipworm

3. Nocturnal anal pruritus is seen for:
   a. Pinworm
   b. Roundworm
   c. Hookworm
   d. Whipworm

4. Hyperinfection syndrome is seen for:
   a. Roundworm
   b. *Strongyloides*
   c. Hookworm
   d. *Enterobius*

Answers

1. b **2.** d **3.** a **4.** b

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