

MEDICAL HELMINTHOLOGY

LEARNING OBJECTIVES

At the end of this session, students should be able to:

- Understand medically important helminthes including their life cycles, modes of transmissions, clinical features, diagnosis, treatment and prevention.
- Describe blood, intestinal, liver and lung flukes.
- Understand common round worms.
- Understand different species of Cestodes.

INTRODUCTION

Medical helminthology is concerned with the study of helminthes or parasitic worms. Helminthes are trophoblastic metazoa (multi-cellular organisms).

Helminthes are among the common parasitic causes of human suffering. They are the cause of high morbidity and mortality of people worldwide. They cause different diseases in humans, but few helminthic infections cause life-threatening diseases.

They cause anemia and malnutrition. In children they cause a reduction in academic performance. Helminthes also cause economic loss as a result of infections of domestic animals. There is age dependent distribution of infections from geohelminthes and schistosomes. As a result of predisposing behavioral and immunological status, children disproportionately carry the burden of schistosomes and geo-helminthes.

The sources of the parasites are different. Exposure of humans to the parasites may occur in one of the following ways:

1. Contaminated soil (Geo-helminthes), water (cercariae of blood flukes) and food (Taenia in raw meat).
2. Blood sucking insects or arthropods (as in filarial worms).
3. Domestic or wild animals harboring the parasite (as in echinococcus in dogs).
4. Person to person (as in *Enterobius vermicularis*, *Hymenolopis nana*).
5. Oneself (auto-infection) as in *Enterobius vermicularis*.

They enter the body through different routes including: mouth, skin and the

respiratory tract by means of inhalation of airborne eggs.

The helminthes are classified into three major groups. These are:

1. Trematodes (Flukes)
2. Nematodes (Round worms)
3. Cestodes (Tape worms)

INTRODUCTION

Platyhelminthes (*platy* means flat; *helminth* means worm) are divided into two classes: Cestoda (tapeworms) and Trematoda (flukes).

Tapeworms consist of two main parts: a rounded head called a **scolex** and a flat body consisting of multiple segments. Each segment is called a **proglottid**. The scolex has specialized means of attaching to the intestinal wall, namely, suckers, hooks, or sucking grooves. The worm grows by adding new proglottids from its germinal center next to the scolex. The oldest proglottids at the distal end are gravid and produce many eggs, which are excreted in the feces and transmitted to various intermediate hosts such as cattle, pigs, and fish.

Humans usually acquire the infection when under-cooked meat or fish containing the larvae is ingested. However, in two important human diseases, cysticercosis and hydatid disease, it is the eggs that are ingested and the resulting larvae cause the disease.

There are four medically important cestodes: *Taenia solium*, *Taenia saginata*, *Diphyllobothrium latum*, and *Echinococcus granulosus*. , .

TAENIA

There are two important human pathogens in the genus *Taenia*: *T. solium* (the pork tapeworm) and *T. saginata* (the beef tapeworm).

1- *Taenia solium*

Disease

The adult form of *T. solium* causes taeniasis. *T. solium* larvae cause cysticercosis.

Important Properties

T. solium can be identified by its scolex, which has **four suckers and circle of hooks**, and by its gravid proglottids, which have 5 to 10 primary uterine branches. The eggs appear the same microscopically as those of *T. saginata* and *Echinococcus* species

In taeniasis, the adult tapeworm is located in the human intestine. This occurs when humans are infected by eating raw or undercooked **pork** containing the larvae, called **cysticerci**. (A cysticercus consists of a pea-sized fluid-filled bladder with an invaginated scolex.) In the small intestine, the larvae attach to the gut wall and take about 3 months to grow into adult worms measuring up to 5 m. The gravid terminal proglottids containing many eggs detach daily, are passed in the feces, and are accidentally eaten by pigs. Note that pigs are infected by the worm eggs; therefore, it is the larvae (cysticerci) that are found in the pig. A six-hooked embryo (oncosphere) emerges from each egg in the pig's intestine. The embryos burrow into a blood vessel and are carried to skeletal muscle. They develop into cysticerci in the muscle, where they remain until eaten by a human. Humans are the definitive hosts, and pigs are the intermediate hosts.

In cysticercosis, a more dangerous sequence occurs when a person **ingests the worm eggs** in food or water that has been contaminated with human feces. Note that in cysticercosis, humans are infected by eggs

TABLE –1 Features of Medically Important Cestodes (Tapeworms)

Cestode	Mode of Transmission	Intermediate Host(s)	Main Sites Affected in Human Body	Diagnosis	Treatment
<i>Taenia solium</i>	(A) Ingest larvae in undercooked pork	Pigs	Intestine	Proglottids in stool	Praziquantel
	(B) Ingest eggs in food or water contaminated with human feces		Brain and eyes (cysticerci)	Biopsy, computed tomography (CT) scan	Praziquantel, albendazole, or surgical removal of cysticerci
<i>Taenia saginata</i>	Ingest larvae in undercooked beef	Cattle	Intestine	Proglottids in stool	Praziquantel
<i>Diphyllobothrium latum</i>	Ingest larvae in undercooked fish	Copepods and fish	Intestine	Operculated eggs in stool	Praziquantel
<i>Echinococcus granulosus</i>	Ingest eggs in food contaminated with dog feces	Sheep	Liver, lungs, and brain (hydatid cysts)	Biopsy, CT scan, serology	Albendazole or surgical removal of cyst

excreted in human feces, *not* by ingesting undercooked pork. Also, pigs do not have the adult worm in their intestine, so they are not the source of the eggs that cause human cysticercosis. The eggs hatch in the small intestine, and the oncospheres burrow through the wall into a blood vessel. They can disseminate to many organs, especially the eyes and brain, where they encyst to form cysticerci . Each cysticercus contains a larva.

Pathogenesis & Epidemiology

The adult tapeworm attached to the intestinal wall causes little damage. The cysticerci, on the other hand, can become very large, especially in the **brain**, where they manifest as a **space-occupying lesion** . Living cysticerci do not cause inflammation, but when they die, they can release substances that provoke an inflammatory response. Eventually, the cysticerci calcify.

The epidemiology of taeniasis and cysticercosis is related to the access of pigs to human feces and to consumption of raw or undercooked pork. The disease occurs worldwide but is endemic in areas of Asia, South America, and Eastern Europe. Most cases in the United States are imported.

Clinical Findings

Most patients with adult tapeworms are asymptomatic, but anorexia and diarrhea can occur. Some may notice proglottids in the stools. Cysticercosis in the brain causes headache, vomiting, and seizures. Cysticercosis in the eyes can appear as uveitis or retinitis, or the larvae can be visualized floating in the vitreous. Subcutaneous nodules containing cysticerci commonly occur. Cysts also are commonly found in skeletal muscle.

Laboratory Diagnosis

Identification of *T. solium* consists of finding gravid proglottids with 5 to 10 primary uterine branches in the stools. In contrast, *T. saginata* proglottids have 15 to 20 primary uterine branches. Eggs are found in the stools less often than are proglottids. Diagnosis of cysticercosis depends on demonstrating the presence of the cyst in tissue, usually by surgical removal or computed tomography (CT) scan. Serologic tests (e.g., enzyme-linked immunosorbent assay [ELISA]) that detect antibodies to *T. solium* antigens are available, but they may be negative in neurocysticercosis.

TABLE –2 Medically Important Stages in Life Cycle of Cestodes (Tapeworms)

Organism	Insect Vector	Stage That Infects Humans	Stage(s) in Humans Most Associated with Disease	Important Stage(s) Outside of Humans
<i>Taenia solium</i>	None	1. Larvae in undercooked pork 2. Eggs in food or water contaminated with human feces	Adult tapeworm in intestine Cysticercus, especially in brain	Larvae in muscle of pig None
<i>Taenia saginata</i>	None	Larvae in undercooked beef	Adult tapeworm in intestine	Larvae in muscle of pig
<i>Diphyllobothrium latum</i>	None	Larvae in undercooked fish	Adult tapeworm in intestine can cause vitamin B ₁₂ deficiency	Larvae in muscle of freshwater fish
<i>Echinococcus granulosus</i>	None	Eggs in food or water contaminated with dog feces	Hydatid cysts, especially in liver and lung	Adult tapeworm in dog intestine produces eggs

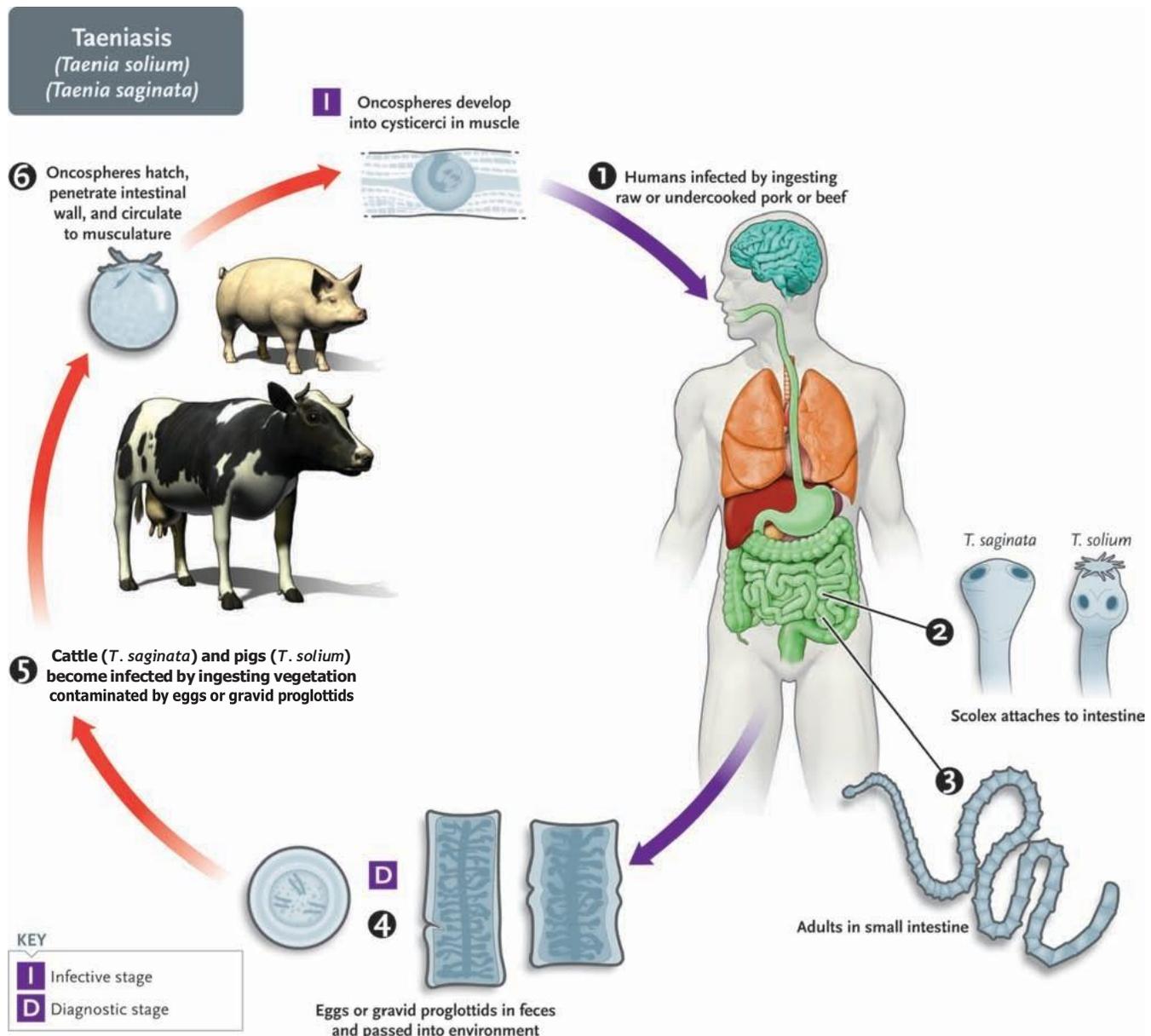


FIGURE –1 *Taenia solium* and *Taenia saginata*. Life cycle.

Treatment

The treatment of choice for the intestinal worms is praziquantel. The treatment for cysticercosis is either praziquantel or albendazole, but surgical excision may be necessary.

Prevention

Prevention of taeniasis involves cooking pork adequately and disposing waste properly so that pigs cannot ingest human feces. Prevention of cysticercosis consists of treatment of patients to prevent autoinfection plus observation of proper hygiene, including handwashing, to prevent contamination of food with the eggs.

2-Taenia saginata

Disease

T. saginata causes taeniasis. T. saginata larvae do not cause cysticercosis.

Important Properties

T. saginata has a scolex with four suckers but, in contrast to T. solium, no hooklets. Its gravid proglottids have 15 to 25 primary uterine branches, in contrast to T. solium proglottids, which have 5 to 10. The eggs are morphologically indistinguishable from those of T. solium.

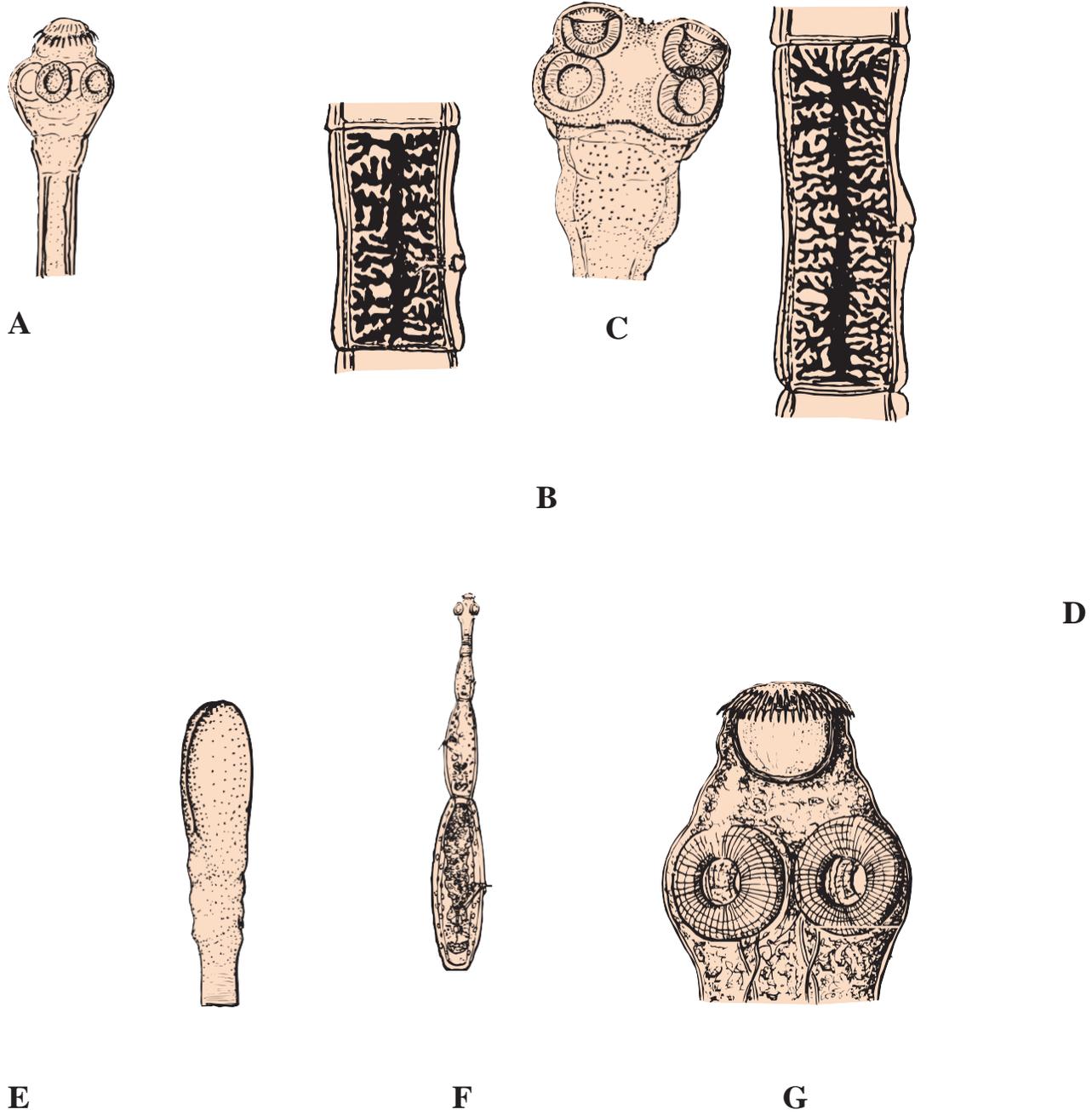


FIGURE –2 **A:** *Taenia solium* scolex with suckers and hooks (10×). **B:** *Taenia solium* gravid proglottid. This has fewer uterine branches than does the proglottid of *Taenia saginata* (see panel D) (2×). **C:** *T. saginata* scolex with suckers (10×). **D:** *T. saginata* gravid proglottid (2×). **E:** *Diphyllobothrium latum* scolex with sucking grooves (7×). **F:** Entire adult worm of *Echinococcus granulosus* (7×). **G:** *E. granulosus* adult scolex (70×).



FIGURE –3 *Taenia solium*—scolex and several proglottids.

Long arrow points to one of the four suckers on the scolex of *T. solium*. Short arrow points to the circle of hooklets. Proglottids can be seen extending from the scolex toward the left side of the image.



FIGURE –4 **A:** *Taenia solium* egg containing oncosphere embryo. Four hooklets are visible. *Taenia saginata* and *Echinococcus granulosus* eggs are very similar to the *T. solium* egg but do not have hooklets. **B:** *Diphylobothrium latum* egg with an operculum on the top (300×).

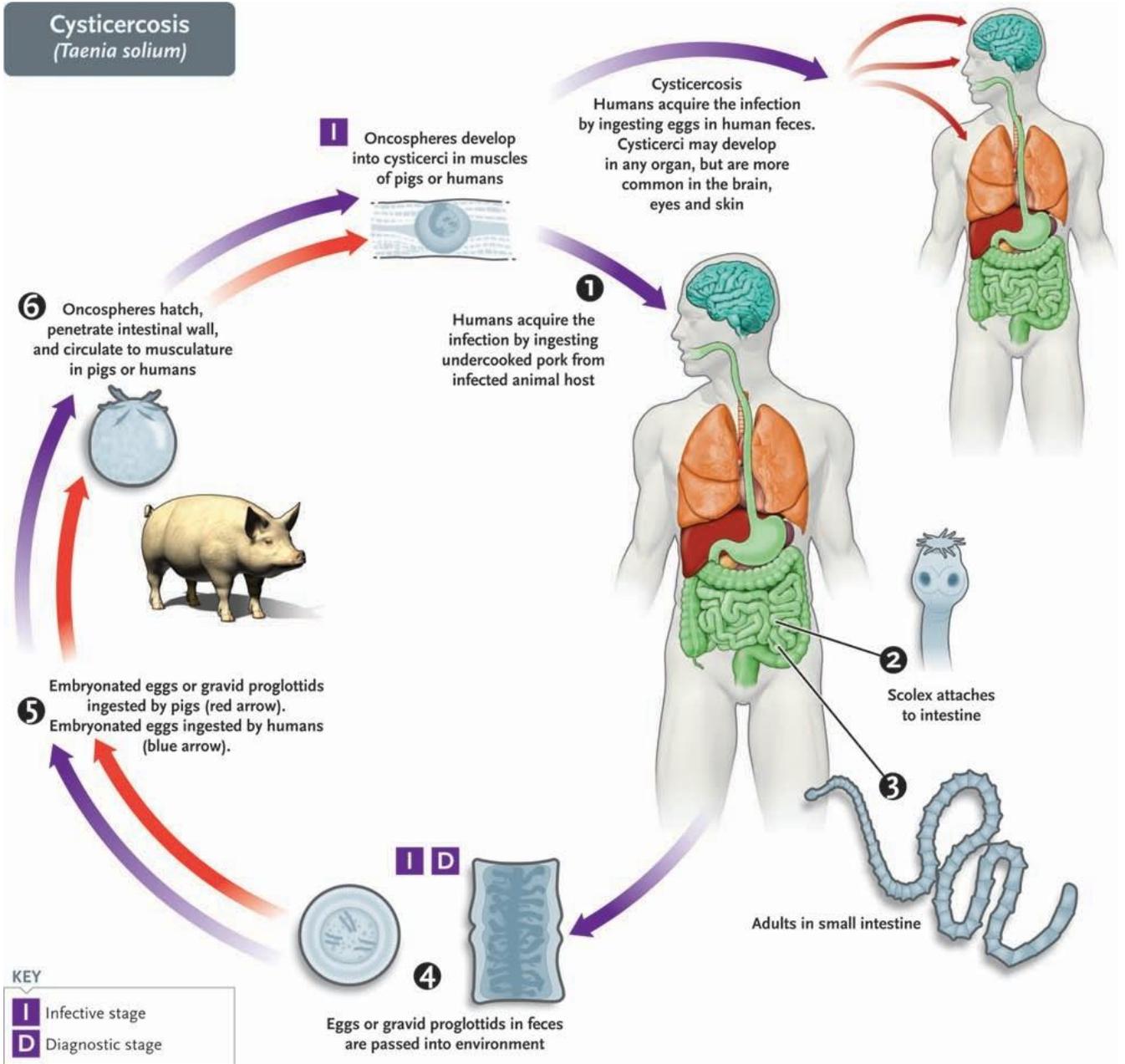


FIGURE 54-5 *Taenia solium*. Life cycle including cysticercosis stage.

The life cycle of *Taenia saginata* is shown in. Humans are infected by eating raw or undercooked **beef** containing larvae (cysticerci). In the small intestine, the larvae attach to the gut wall and take about 3 months to grow into adult worms measuring up to 10 m . The gravid proglottids detach, are passed in the feces, and are eaten by cattle. The embryos (**oncospheres**) emerge from the eggs in the cow's intestine and burrow into a blood vessel, where they are carried to skeletal muscle. In the muscle, they develop into cysticerci. The cycle is

completed when the cysticerci are ingested. Humans are the definitive hosts and cattle the intermediate hosts. Unlike *T. solium*, *T. saginata* **does not cause cysticercosis** in humans.

Pathogenesis & Epidemiology

Little damage results from the presence of the adult worm in the small intestine. The epidemiology of taeniasis caused by *T. saginata* is related to the access of cattle to human feces and to the consumption of raw or undercooked beef.

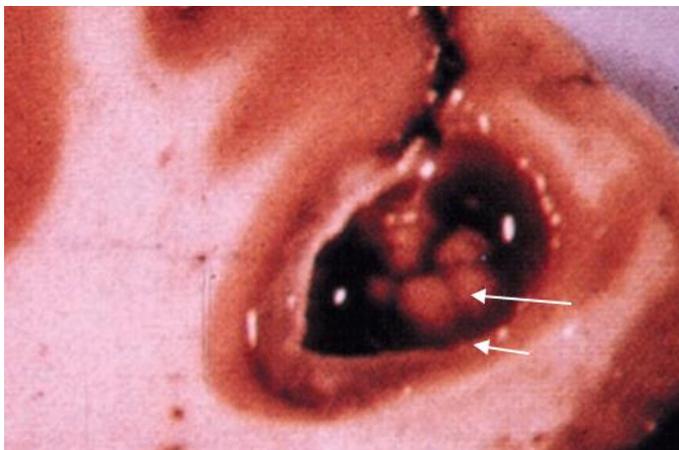


FIGURE –6 Cysticercus of *Taenia solium* in brain—long arrow points to a larva of *T. solium*. Short arrow points to the wall of the cysticercus (sac) that surrounds the larva. (Figure courtesy of Rhodes B. Holliman, PhD, Professor Emeritus, Virginia Tech.)

Treatment

The treatment of choice is praziquantel.

Prevention

Prevention involves cooking beef adequately and disposing waste properly so that cattle cannot consume human feces.

The disease occurs worldwide but is endemic in Africa, South America, and Eastern Europe. In the United States, most cases are imported.

Clinical Findings

Most patients with adult tapeworms are asymptomatic, but malaise and mild cramps can occur. In some, proglottids appear in the stools and may even protrude from the anus. The proglottids are motile and may cause pruritus ani as they move on the skin adjacent to the anus.

Laboratory Diagnosis

Identification of *T. saginata* consists of finding gravid proglottids with 15 to 20 uterine branches in the stools. Eggs are found in the stools less often than are the proglottids.

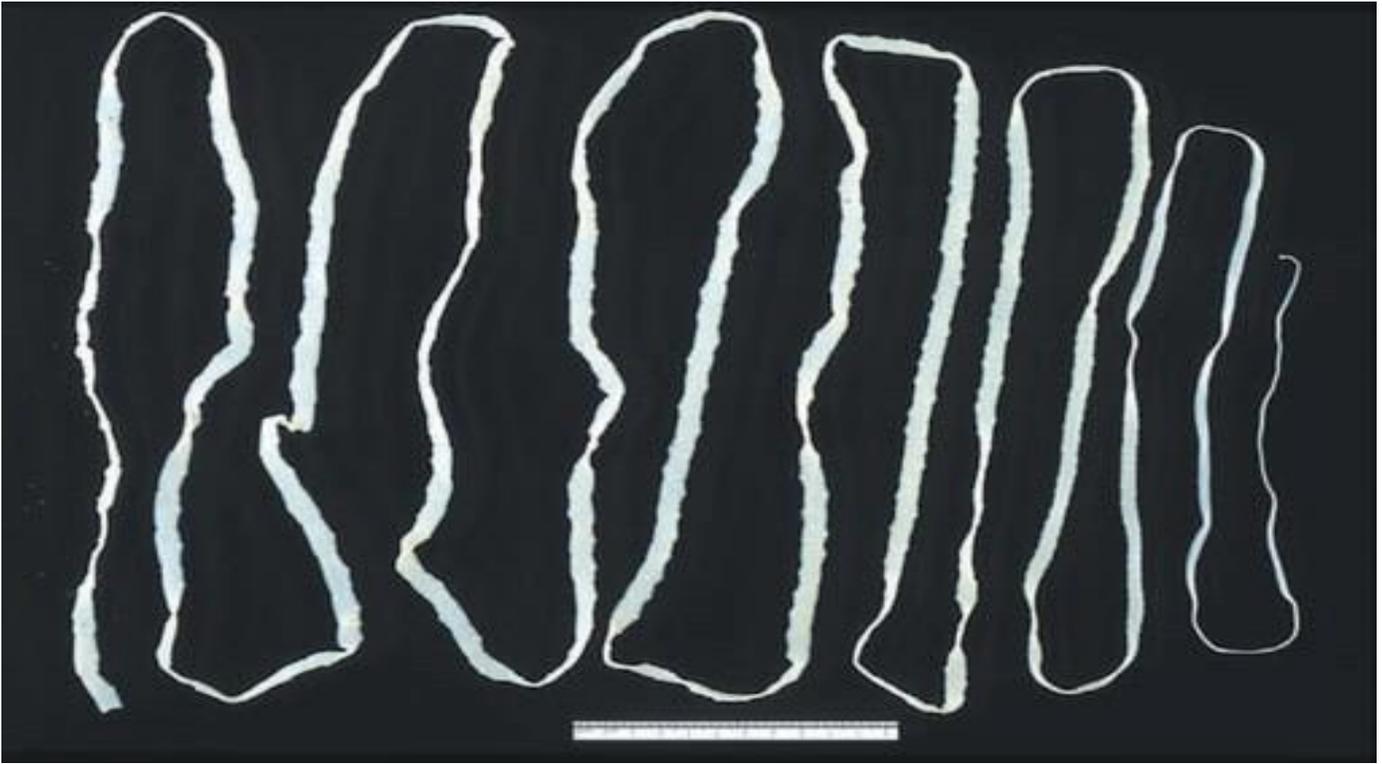


FIGURE 54-7 *Taenia saginata*—adult tapeworm. Note the tiny scolex on the right side of the image and the gravid proglottids on the left side of the image. White arrow points to the scolex. Ruler is 12 inches long.

ECHINOCOCCUS

Disease

Echinococcus granulosus (dog tapeworm) causes echinococcosis. The larva of *E. granulosus* causes unilocular hydatid cyst disease. Multilocular hydatid disease is caused by *Echinococcus multilocularis*, which is a minor pathogen and is discussed later.

Important Properties

E. granulosus is composed of a scolex and only three proglottids, making it **one of the smallest tapeworms**. The scolex has a circle of hooks and four suckers similar to *T. solium*. **Dogs** are the most important definitive hosts. The intermediate hosts are usually **sheep**. Humans are almost always dead-end intermediate hosts.

The life cycle of *E. granulosus* is shown in . In the typical life cycle, worms in the dog's intestine liberate thousands of eggs, which are ingested by sheep (or humans). The oncosphere embryos emerge in the small intestine and migrate primarily to the liver but also to the lungs, bones, and brain. The embryos develop into large fluid-filled **hydatid cysts**, the inner germinal layer of which generates many protoscoleces within "brood capsules." The life cycle is completed when the entrails (e.g., liver containing hydatid cysts) of slaughtered sheep are eaten by dogs.

Pathogenesis & Epidemiology

E. granulosus usually forms one large fluid-filled cyst (unilocular) that contains thousands of individual scoleces as well as many daughter cysts within the large cyst.

Individual

scolecetes lying at the bottom of the large cyst are called “hydatid sand.” The cyst acts as a space-occupying lesion, putting pressure on adjacent tissue. The outer layer of the cyst is thick, fibrous tissue produced by the host. The cyst fluid contains parasite antigens, which can sensitize the host. Later, if the cyst ruptures spontaneously or during trauma or surgical removal, life-threatening **anaphylactic shock** can occur. Rupture of a cyst can also spread proto- scolecetes widely.

The disease is found primarily in shepherds living in the Mediterranean region, the Middle East, and Australia. In the United States, the western states report the largest number of cases.

Clinical Findings

Many individuals with hydatid cysts are asymptomatic, but **liver cysts** may cause hepatic dysfunction. Cysts in the lungs can erode into a bronchus, causing bloody sputum, and cerebral cysts can cause headache and focal neurologic signs. Rupture of the cyst can cause fatal anaphylactic shock.

Laboratory Diagnosis

Diagnosis is based either on microscopic examination demonstrating the presence of brood capsules containing multiple protoscolecetes or on serologic tests (e.g., the indirect hemagglutination test).

Prevention

Prevention of human disease involves not feeding the entrails of slaughtered sheep to dogs.

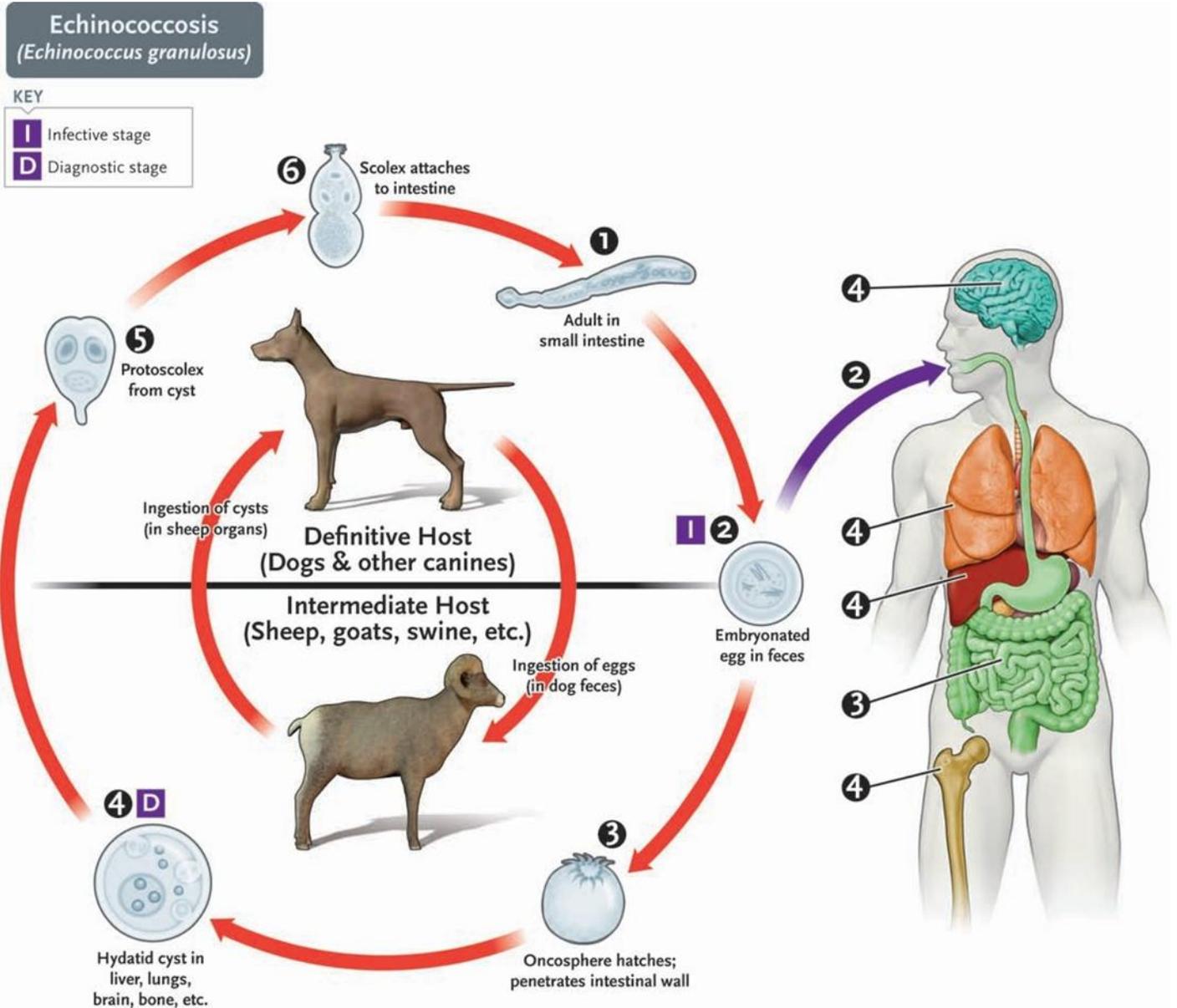


FIGURE-8 *Echinococcus granulosus*. Life cycle.

Hymenolepis nana

Hymenolepis Nana

Common name: Dwarf tape worm

Habitat

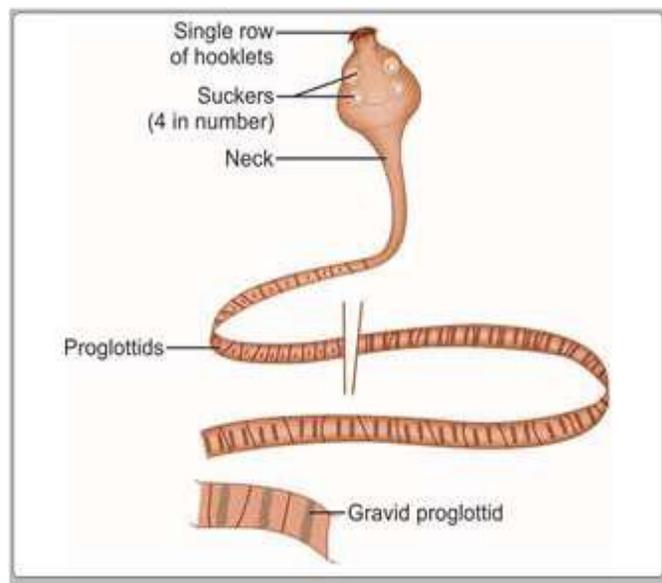
The adult worm lives in the proximal ileum of man. *H. nana* var. *fraterna* is found in rodents like mice and rats, where they are found in the posterior part of the ileum.

Morphology

Adult Worm

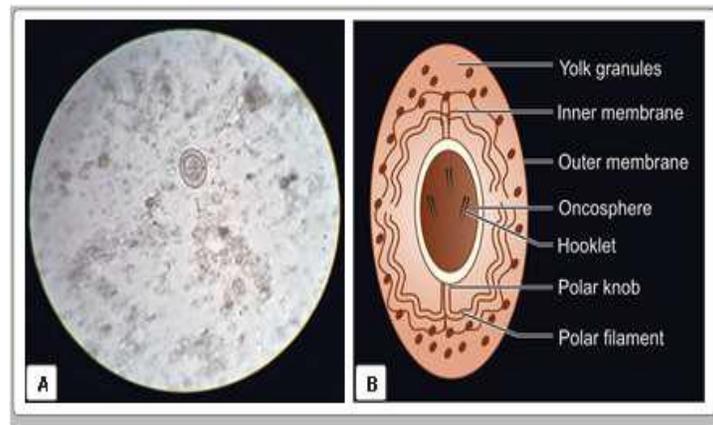
H. nana is the smallest intestinal cestode that infects man.

□ □ It is 5–45 mm in length and less than 1 mm thick. The *scolex* has 4 suckers and a retractile rostellum with a single row of hooklets



□

Adult worm of *Hymenolepis nana*



Egg of *Hymenolepis nana*. **A.** As seen under microscope; **B.** Schematic diagram

Life Cycle

Host: Man.

- There is no intermediate host.
- **Mode of transmission:** Infection occurs by ingestion of the food and water contaminated with eggs.
- **Internal autoinfection** may also occur when the eggs released in the intestine hatch there itself (Fig. 12.25).
- **External autoinfection** occurs when a person ingest own eggs by fecal oral route.
- *H. nana* is unusual in that it undergoes multiplication in the body of the definitive host.
- When the eggs are swallowed, or in internal autoinfection, they hatch in the small intestine.
- The **hexacanth embryo** penetrates the intestinal villus and develops into the cysticercoid larva.
- This is a solid pyriform structure, with the vesicular anterior end containing the invaginated scolex and a short conical posterior end.
- After about 4 days, the mature larva emerging out of the villus evaginates its scolex and attaches to the mucosae.
- It starts strobilization, to become the mature worm, which begins producing eggs in about 25 days.

A different strain of *H. nana* infects rats and mice. The eggs passed in rodent feces are ingested by rat fleas (*Xenopsylla cheopis* and others), which acts as the intermediate host. The eggs develop into cysticercoid larvae in the hemocele of these insects. Rodents get infected when they eat these insects. The murine strain does not appear to infect man. However, the human strain may infect rodents, which may, therefore, constitute a subsidiary reservoir of infection for the human parasite.

Clinical Features

Hymenolopiasis occurs more commonly in children.

- There are usually no symptoms but in heavy infections, there is nausea, anorexia, abdominal pain, diarrhea, and irritability.
- Sometimes pruritus may occur due to an allergic response.

Laboratory Diagnosis

The diagnosis is made by demonstration of characteristic eggs in feces by direct microscopy. Concentration methods like salt flotation and formalin ether may be readily used. ELISA test has been developed with 80% sensitivity.

Treatment

Praziquantel (single dose of 25 mg/kg) is the drug of choice, since it acts both against the adult worms and the cysticercoids in the intestinal villi.

- Nitazoxanide 500 mg BD for 3 days may be used as alternative.

Hymenolepis Diminuta

differs from *Hymenolepis nana* in that:

- The adult worm measures about 10-60 cm
- The rosetellum on the head has no hooks
- In the mature segment, there are two testes at one side and another testis on the other side.

Life cycle

The adult worms are present in the small intestine of man and rats. Eggs passed in stool are similar to the eggs of *H. nana* but are brown in color with no polar filaments arising from the polar thickening. The eggs are ingested by the rat flea where they develop to cysticeroid stage. Infection to man takes place accidentally by food or contaminated hands by cysticeroid stage.

Pathogenecity

Most infections are asymptomatic, but occasionally, patients may present with nausea, anorexia and diarrhea.

Treatment

same as *Hymenolepis nana*.

Dipylidium Caninum

This common tapeworm of dogs and cats, it may accidentally cause human infection, mainly in children.

Morphology

- □ The adult worm in the intestine is about 10–70 cm long
- □ The scolex has 4 prominent suckers and a retractile rostellum with upto 7 rows of spines (Fig. 12.26).
- □ The mature proglottid has 2 *genital pores*, 1 on either side, hence the name *Dipylidium* (*dipylos*—2 *entrances*).
- □ Gravid proglottids are passed out of the anus of the host singly or in groups.

Life Cycle

Definitive host: Dogs, cats, and rarely man.

Intermediate host: Fleas.

- □ Man acquires infection by ingestion of flea harboring cysticeroid larva.
- □ The eggs or proglottids passed in feces of dogs and cats are eaten by larval stages of dog and cat fleas, *Ctenocephalus canis* and *C. felis*.

The embryo develops into a tailed cysticeroid larva

Clinical Features

Human infection is generally asymptomatic, but the actively motile proglottids passed

in stools may raise an alarm.

Diagnosis

The diagnosis is made by detection of proglottids or eggs in stool.

Trematodes

INTRODUCTION

Trematoda (flukes) and Cestoda (tapeworms) are the two large classes of parasites in the phylum Platyhelminthes. The most important trematodes are *Schistosoma* species (blood flukes), *Clonorchis sinensis* (liver fluke), and *Paragonimus westermani* (lung fluke). Schistosomes have by far the greatest impact in terms of the number of people infected, morbidity, and mortality. Features of the medically important trematodes are summarized in Table 55–1, and the medically important stages in the life cycle of these organisms are described in Table 55–2. Three trematodes of lesser importance, *Fasciola hepatica*, *Fasciolopsis buski*, and *Heterophyes heterophyes*, are described at the end of this chapter.

The life cycle of the medically important trematodes involves a sexual cycle in humans (definitive host) and asexual reproduction in **freshwater snails** (intermediate hosts). Transmission to humans takes place either via penetration of the skin by the free-swimming **cercariae** of the schistosomes or via ingestion of cysts in undercooked (raw) fish or crabs in *Clonorchis* and *Paragonimus* infection, respectively.

Trematodes that cause human disease are not endemic in the United States. However, immigrants from tropical areas, especially Southeast Asia, are frequently infected.

SCHISTOSOMA

Disease

Schistosoma causes schistosomiasis. *Schistosoma mansoni* and *Schistosoma japonicum* affect the **gastrointestinal tract**,¹ whereas *Schistosoma haematobium* affects the **urinary tract**.

Important Properties

The life cycle of *Schistosoma* species is shown in. In contrast to the other trematodes, which are hermaphro- dites, adult schistosomes exist as **separate sexes** but live attached to each other. The female resides in a groove in the male, the gynecophoric canal (“schist”), where he continu- ously fertilizes her eggs. The three species can be distinguished by the appearance of their eggs in the microscope: *S. mansoni* eggs have a **prominent lateral spine**, whereas *S. japonicum* eggs have a very small lateral spine and *S. haematobium* eggs have a terminal spine. *S. mansoni* and *S. japonicum* adults live in the **mesenteric veins**, whereas *S. haematobium* lives in the veins draining the urinary bladder. Schistosomes are therefore known as **blood flukes**.

TABLE –1 Features of Medically Important Trematodes (Flukes)

Trematode	Mode of Transmission	Main Sites Affected	Intermediate Host(s)	Diagnostic Features of Eggs	Endemic Area(s)	Treatment
<i>Schistosoma mansoni</i>	Penetrate skin	Veins of colon	Snail	Large lateral spine	Africa, Latin America (Caribbean)	Praziquantel
<i>Schistosoma japonicum</i>	Penetrate skin	Veins of small intestine, liver	Snail	Small lateral spine	Asia	Praziquantel
<i>Schistosoma haematobium</i>	Penetrate skin	Veins of urinary bladder	Snail	Large terminal spine	Africa, Middle East	Praziquantel
<i>Clonorchis sinensis</i>	Ingested with raw fish	Liver	Snail and fish	Operculated	Asia	Praziquantel
<i>Paragonimus westermani</i>	Ingested with raw crab	Lung	Snail and crab	Operculated	Asia, India	Praziquantel

Humans are infected when the free-swimming, fork-tailed **cercariae** penetrate the skin. They differentiate to larvae (schistosomula), enter the blood, and are carried via the veins into the arterial circulation. Those that enter the superior mesenteric artery pass into the portal circulation and reach the liver, where they mature into adult flukes. *S. mansoni* and *S. japonicum* adults migrate against the portal flow to reside in the mesenteric venules. *S. haematobium* adults reach the bladder veins through the venous plexus between the rectum and the bladder.

In their definitive venous site, the female lays fertilized eggs, which penetrate the vascular endothelium and enter the gut or bladder lumen, respectively. The eggs are excreted in the stools or urine and must enter fresh water to hatch. Once hatched, the ciliated larvae (miracidia) penetrate **snails** and undergo further development and multiplication to produce many cercariae. (The three schistosomes use different species of snails as intermediate hosts.) Cercariae leave the snails, enter fresh water, and complete the cycle by penetrating human skin.

Pathogenesis & Epidemiology

Most of the pathologic findings are caused by the presence of eggs in the liver, spleen, or wall of the gut or bladder. Eggs in the liver induce granulomas, which lead to fibrosis, hepatomegaly, and portal hypertension. The granulomas are

formed in response to antigens secreted by the eggs. Hepatocytes are usually undamaged, and liver function tests remain normal. Portal hypertension leads to **splenomegaly**.

S. mansoni eggs damage the wall of the distal colon (inferior mesenteric venules), whereas *S. japonicum* eggs damage the walls of both the small and large intestines (superior and inferior mesenteric venules). The damage is due both to digestion of tissue by proteolytic enzymes produced by the egg and to the host inflammatory response that forms granulomas in the venules. The eggs of *S. haematobium* in the wall of the bladder induce granulomas and fibrosis, which can lead to **carcinoma of the bladder**.

Schistosomes have evolved a remarkable process for **evading the host defenses**. There is evidence that their surface becomes coated with host antigens, thereby limiting the ability of the immune system to recognize them as foreign.

The epidemiology of schistosomiasis depends on the presence of the specific freshwater snails that serve as intermediate hosts. *S. mansoni* is found in Africa and Latin America (including Puerto Rico), whereas *S. haematobium* is found in Africa and the Middle East. *S. japonicum* is found only in Asia and is the only one for which domestic animals (e.g., water buffalo and pigs) act as important reservoirs. More than 150 million people in the tropical areas of Africa, Asia, and Latin America are affected.

TABLE –2 Medically Important Stages in Life Cycle of Trematodes (Flukes)

Organism	Insect Vector	Stage That Infects Humans	Stage(s) in Humans Most Associated with Disease	Important Stage(s) Outside of Humans
<i>Schistosoma mansoni</i> , <i>Schistosoma haematobium</i> , <i>Schistosoma japonicum</i>	None	Cercariae penetrate skin	Adult flukes living in mesenteric or bladder veins lay eggs that cause granulomas	Miracidium (ciliated larvae) infect snails → cercariae infect humans
<i>Clonorchis</i>	None	Larvae in undercooked fish	Adult flukes live in biliary ducts	Eggs ingested by snails → cercariae infect fish
<i>Paragonimus</i>	None	Larvae in undercooked crab	Adult flukes live in lung	Eggs ingested by snails → cercariae infect crab

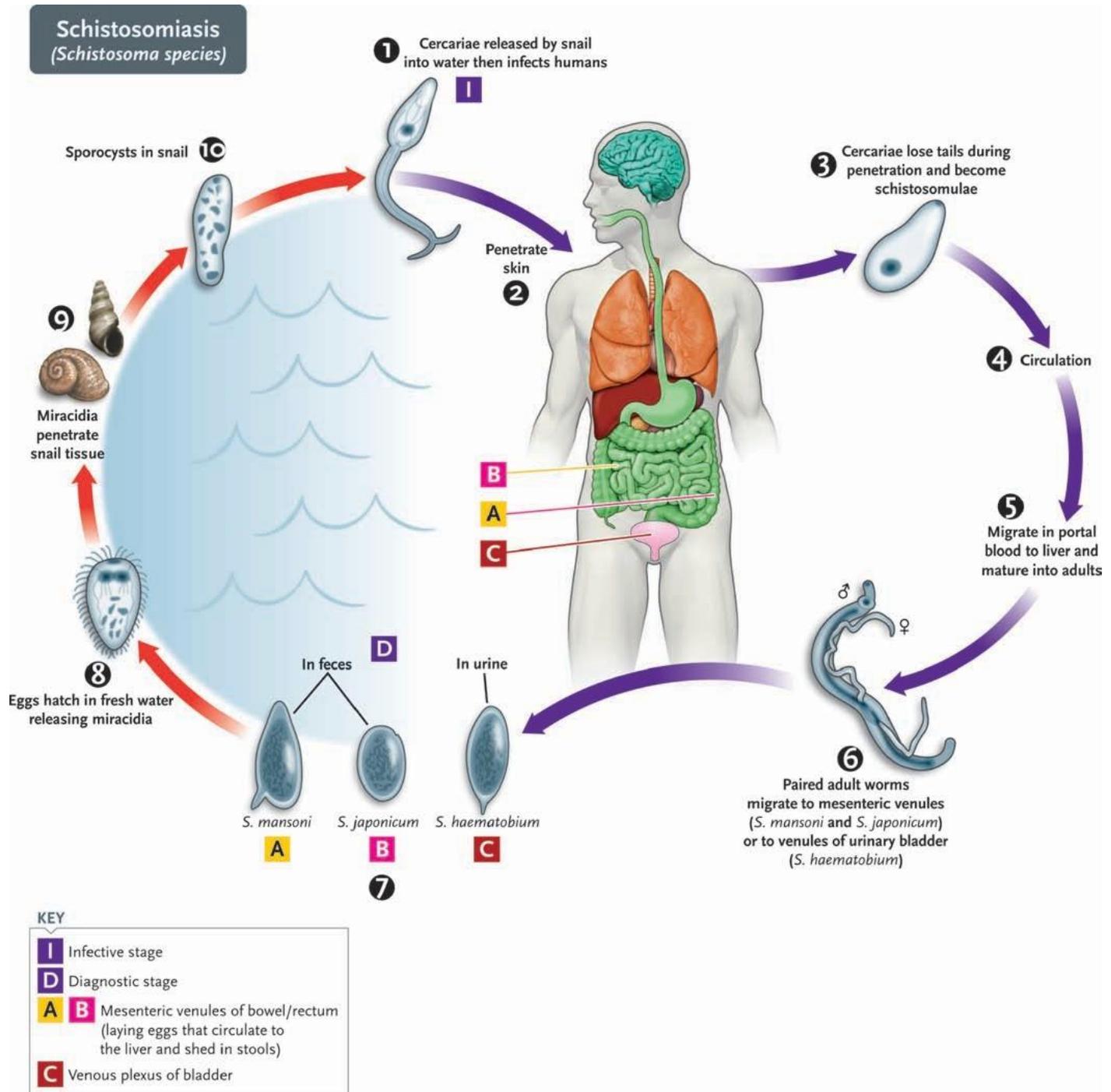
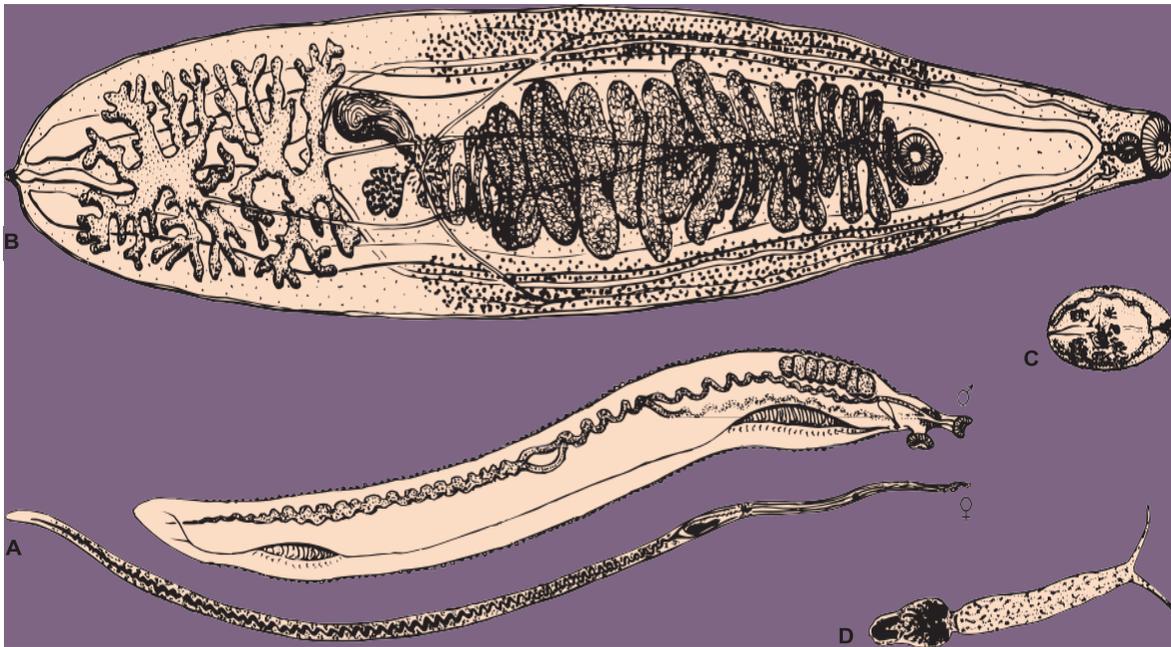


FIGURE –1 *Schistosoma* species.

Clinical Findings

Most patients are asymptomatic, but chronic infections may become symptomatic. The acute stage, which begins shortly after cercarial penetration, consists of itching and dermatitis followed 2 to 3 weeks later by fever, chills, diarrhea, lymph-adenopathy, and hepatosplenomegaly. Eosinophilia is seen in response to the migrating larvae. This stage usually resolves spontaneously.

The chronic stage can cause significant morbidity and mortality. In patients with *S. mansoni* or *S. japonicum* infection, gastrointestinal hemorrhage, hepatomegaly, and massive splenomegaly can develop.



FIGURE—2 A: Male and female *Schistosoma mansoni* adults. The female lives in the male's schist (shown as a ventral opening) (6×). B: *Clonorchis sinensis* adult (6×). C: *Paragonimus westermani* adult (0.6×). D: *S. mansoni* cercaria (300×).

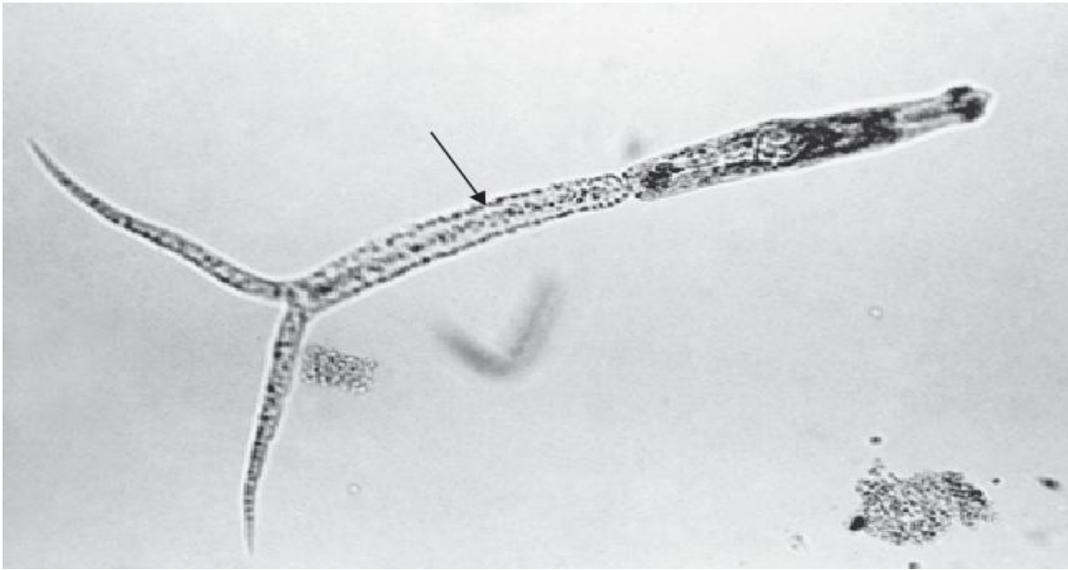


FIGURE 55–3 *Schistosoma*—cercaria. Arrow points to a cer- caria of *Schistosoma*. Note the typical forked tail on the left side of the image. (Figure courtesy of Minnesota Department of Health, R.N. Barr Library; Librarians M. Rethlefsen and M. Jones; Prof. W.Wiley, Public Health Image Library, Centers for Disease Control and Prevention.)

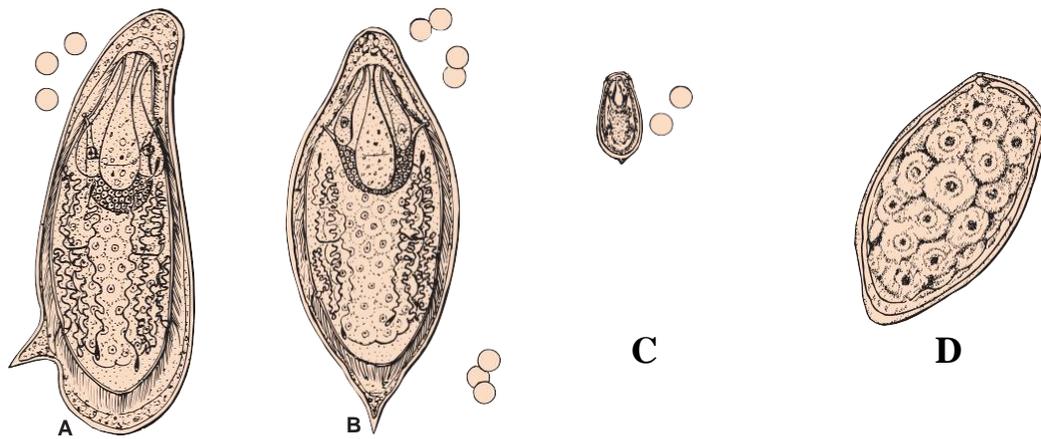


FIGURE 55–4 **A:** *Schistosoma mansoni* egg with lateral spine. **B:** *Schistosoma haematobium* egg with terminal spine. **C:** *Clonorchis sinensis* egg with operculum. **D:** *Paragonimus westermani* egg with operculum (300 \times). (Circles represent red blood cells.)



FIGURE 55–5 *Schistosoma mansoni*—egg. Long arrow points to an egg of *S. mansoni*. Short arrow points to its large lateral spine. (Figure courtesy of Public Health Image Library, Centers for Disease Control and Prevention.)



FIGURE 55–6 *Schistosoma haematobium*—egg. Long arrow points to an egg of *S. haematobium*. Short arrow points to its terminal spine. (Figure courtesy of Public Health Image Library, Centers for Disease Control and Prevention.)

cause of death is exsanguination from ruptured esophageal varices. Patients infected with *S. haematobium* have hematuria as their chief early complaint. Superimposed bacterial urinary tract infections occur frequently.

“Swimmer’s itch,” which consists of pruritic papules, is a frequent problem in many lakes in the United States. The papules are an immunologic reaction to the presence in the skin of the cercariae of nonhuman schistosomes. The pruritic papules appear within minutes to hours after exposure, indicating that this is an immediate (immunoglobulin [Ig] E–mediated) hypersensitivity. These nonhuman schistosomes are incapable of replicating in humans and do not cause disseminated disease.

Laboratory Diagnosis

Diagnosis depends on finding the characteristic ova in the feces or urine. The large lateral spine of *S. mansoni* and the rudimentary spine of *S. japonicum* are typical, as is the large terminal spine of *S. haematobium*. Serologic tests are not useful. Moderate eosinophilia occurs.

Treatment

Praziquantel is the treatment of choice for all three species.

Prevention

Prevention involves proper disposal of human waste and eradication of the snail host when possible. Swimming in areas of endemic infection should be avoided.

PARAGONIMUS

Disease

Paragonimus westermani, the lung fluke, causes paragoni- miasis.

Important Properties

Humans are infected by eating raw or undercooked **crab meat** (or crayfish) containing the encysted larvae (metacer- cariae). After excystation in the small intestine, immature flukes penetrate the intestinal wall and migrate through the diaphragm into the **lung** parenchyma. They differentiate into hermaphroditic adults and produce eggs that enter the bronchioles and are coughed up or swal- lowed . Eggs in either sputum or feces that reach fresh water hatch into miracidia, which enter snails (first intermediate hosts). There, they differentiate first into larvae (rediae) and then into many free-swimming cercariae. The cercariae infect and encyst in freshwater crabs (second intermediate hosts). The cycle is completed when undercooked infected crabs are eaten by humans.

Pathogenesis & Epidemiology

Within the lung, the worms exist in a fibrous capsule that communicates with a bronchiole. Secondary bacterial infection frequently occurs, resulting in bloody sputum. Paragonimiasis is endemic in Asia and India. In the United States, it occurs in immigrants from these areas.

Clinical Findings

The main symptom is a chronic cough with bloody sputum. Dyspnea, pleuritic chest pain, and recurrent attacks of bacterial pneumonia occur. The disease can resemble tuberculosis.

Laboratory Diagnosis

Diagnosis is made by finding the typical operculated eggs in sputum or feces. Serologic tests are not useful.

Treatment

Praziquantel is the treatment of choice.

Prevention

Cooking crabs properly is the best method of prevention.

INTRODUCTION

Nematodes (also known as Nematelminthes) are roundworms with a cylindrical body and a complete digestive tract, including a mouth and an anus. The body is covered with a noncellular, highly resistant coating called a cuticle. Nematodes have separate sexes; the female is usually larger than the male. The male typically has a coiled tail.

The medically important nematodes can be divided into two categories according to their primary location in the body, namely, **intestinal** and **tissue** nematodes.

(1) The intestinal nematodes include *Enterobius* (pinworm), *Trichuris* (whipworm), *Ascaris* (giant roundworm), *Necator* and *Ancylostoma* (the two hookworms), *Strongyloides* (small roundworm), and *Trichinella*. *Enterobius*, *Trichuris*, and *Ascaris* are transmitted by ingestion of eggs; the others are transmitted as larvae. There are two larval forms: the first- and second-stage (**rhabditiform**) larvae are noninfectious, feeding forms; the third-stage (**filariform**) larvae are the infectious, nonfeeding forms. As adults, these nematodes live within the human body, except for *Strongyloides*, which can

also exist in the soil.

(2) The important tissue nematodes *Wuchereria*, *Onchocerca*, and *Loa* are called the “filarial worms,” because they produce motile embryos called **microfilariae** in blood and tissue fluids. These organisms are transmitted from person

to person by bloodsucking mosquitoes or flies. A fourth species is the guinea worm, *Dracunculus*, whose larvae inhabit tiny crustaceans (copepods) and are ingested in drinking water.

The nematodes described above cause disease as a result of the presence of adult worms within the body. In addition, several species cannot mature to adults in human tissue, but their larvae can cause disease. The most serious of these diseases is visceral larva migrans, caused primarily by the larvae of the dog ascarid, *T. canis*. Cutaneous larva migrans, caused mainly by the larvae of the dog and cat hookworm, *Ancylostoma caninum*, is less serious. A third disease, anisakiasis, is caused by the ingestion of *Anisakis* larvae in raw seafood.

2. In infections caused by certain nematodes that migrate through tissue (e.g., *Strongyloides*, *Trichinella*, *Ascaris*, and the two hookworms *Ancylostoma* and *Necator*), a striking increase in the number of eosinophils (**eosinophilia**) occurs. Eosinophils do not ingest the organisms; rather, they attach to the surface of the parasite via IgE and secrete cytotoxic enzymes contained within their eosinophilic granules. Host defenses against helminths are stimulated by interleukins synthesized by the Th-2 subset of helper T cells (e.g., the production of IgE is increased by interleukin-4, and the number of eosinophils is increased by interleukin-5 [IL-5]).

TABLE –1 Features of Medically Important Nematodes

Primary Location	Species	Common Name or Disease	Mode of Transmission	Endemic Areas	Diagnosis	Treatment
Intestines	<i>Enterobius</i>	Pinworm	Ingestion	Worldwide	Eggs on	Mebendazole or
	<i>Trichuris</i>	Whipworm	of eggs	Worldwide, especially tropics	skin Eggs	pyrantel pamoate
	<i>Ascaris</i>	Ascariasis	Ingestion	Worldwide, especially tropics	in stools	Mebendazole
	<i>Ancylostoma</i> and <i>Necator</i>	Hookworm	of eggs	Worldwide, especially tropics	Eggs in	Mebendazole or pyrantel pamoate
	<i>Strongyloides</i>	Strongyloidiasis	Ingestion of eggs	Worldwide, especially tropics (<i>Ancylostoma</i>), United States (<i>Necator</i>)	stools Eggs in	Mebendazole or pyrantel pamoate
	<i>Trichinella</i>	Trichinosis	Larval penetration of skin	Tropics primarily	stools	Ivermectin
	<i>Anisakis</i>	Anisakiasis	Larval penetration of skin, also autoinfection	Japan, United States, Netherlands	Larvae in stools	Thiabendazole against adult worm
			Larvae in undercooked meat		Larvae encysted in muscle; serology	No drug available
			Larvae in undercooked seafood		Clinical	
Tissue	<i>Wuchereria</i>	Filariasis	Mosquito bite	Tropics	Blood	Diethylcarbamazine
	<i>Onchocerca</i>	Onchocerciasis (river blindness)	Blackfly bite	primarily Africa, Central America	smear Skin biopsy	Ivermectin
	<i>Loa</i>	Loiasis	Ingestion of copepods in water	Tropical Africa	Blood	Diethylcarbamazine
	<i>Dracunculus</i>	Guinea worm	Ingestion of eggs	Tropical Africa	smear Clinical	Thiabendazole prior to extracting worm
		Visceral	Penetration of			

<i>Toxocara larvae</i>	larva migrans	skin	and Asia	Clinical and serologic	Albendazole or mebendazole
<i>Ancylostoma larvae</i>	larva migrans	Cutaneous	Worldwide	Clinical	Thiabendazole
			Worldwide		

TABLE –2 Medically Important Stages in Life Cycle of Intestinal Nematodes (Roundworms)

Organism	Insect Vector	Stage That Infects Humans	Stage(s) in Humans Most Associated with Disease	Important Stage(s) Outside of Humans
<i>Enterobius</i>	None	Eggs	Female worm migrates out anus and lays eggs on perianal skin, causing itching	None
<i>Trichuris</i>	None	Eggs	Worms in colon may cause rectal prolapse	Eggs survive in environment
<i>Ascaris</i>	None	Eggs	Larvae migrate to lung, causing pneumonia	Eggs survive in environment
<i>Ancylostoma</i> and <i>Necator</i>	None	Filariform larvae enter skin	Worms in colon cause blood loss (anemia)	Egg → rhabditiform larvae → filariform larvae
<i>Strongyloides</i>	None	Filariform larvae enter skin	Worms disseminate to various tissues in immunocompromised (autoinfection)	Egg → rhabditiform larvae → filariform larvae; also “free living” cycle in soil
<i>Trichinella</i>	None	Larvae in meat ingested	Larvae encyst in muscle causing myalgia	Larvae in muscle of pig, bear, and other animals
<i>Anisakis</i>	None	Larvae in fish ingested	Larvae in submucosa of GI tract	Larvae in muscle of fish

INTESTINAL NEMATODES

ENTEROBIUS

Disease

Enterobius vermicularis causes pinworm infection (entero- biasis).

Important Properties

The life cycle of *E. vermicularis* is shown in Figure 56–1. Infection occurs **only in humans**; there is no animal reservoir or vector. The infection is acquired by ingesting the worm eggs. The eggs hatch in the small intestine, where the larvae differentiate into adults and migrate to the colon. The adult male and female worms live in the colon, where mating occurs. At night, the female migrates from the anus and releases thousands of fertilized eggs on the perianal skin and into the environment. Within 6 hours, the eggs develop into embryonated eggs and become infectious. Reinfection can occur if they are carried to the mouth by fingers after scratching the itching skin.

Pathogenesis & Clinical Findings

Perianal pruritus is the most prominent symptom. Pruritus is thought to be an allergic reaction to the presence of either the adult female or the eggs. Scratching predisposes to secondary bacterial infection.

Epidemiology

Enterobius is found worldwide and is the **most common** helminth in the United States. Children younger than 12 years of age are the most commonly affected group.

Laboratory Diagnosis

The eggs are recovered from perianal skin by using the **Scotch tape** technique and can be observed microscopically .

Unlike those of other intestinal nematodes, these **eggs are not found in the stools**. The small, whitish adult worms can be found in the stools or near the anus of diapered children. No serologic tests are available.

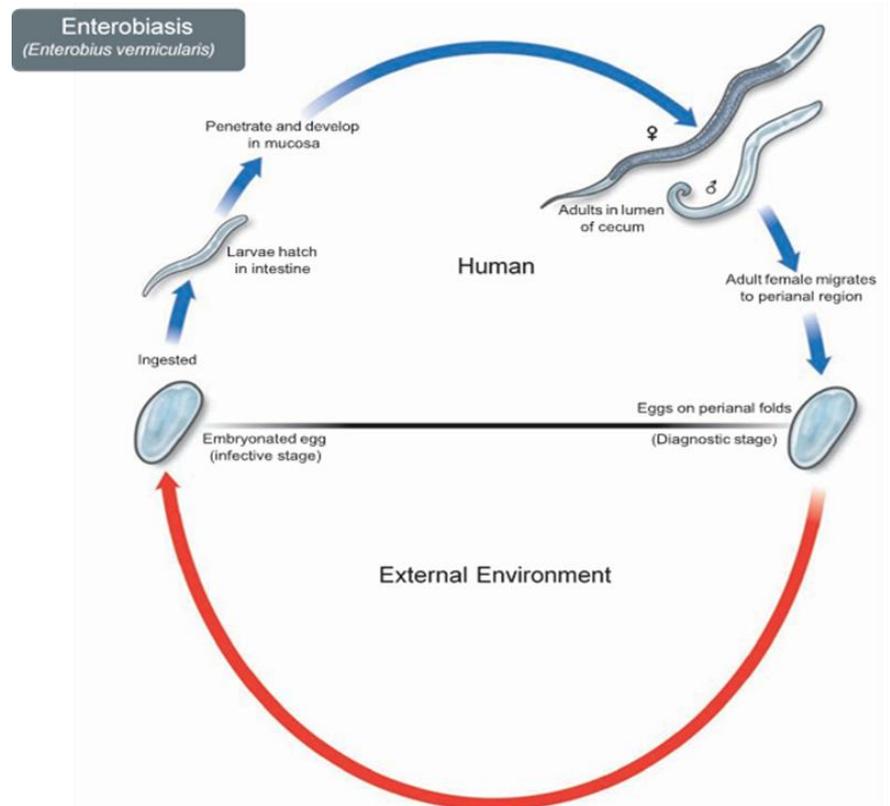
Treatment

Either mebendazole or pyrantel pamoate is effective. They kill the adult worms in the colon but not the eggs, so retreatment in 2 weeks is suggested. Reinfection is very common.

Prevention

There are no means of prevention.

FIGURE-
1 *Enterobius*
vermicularis. Life
cycle. **Top:** Blue
arrow at top left shows
eggs being ingested.
Adult



Ascaris lumbricoides

Disease

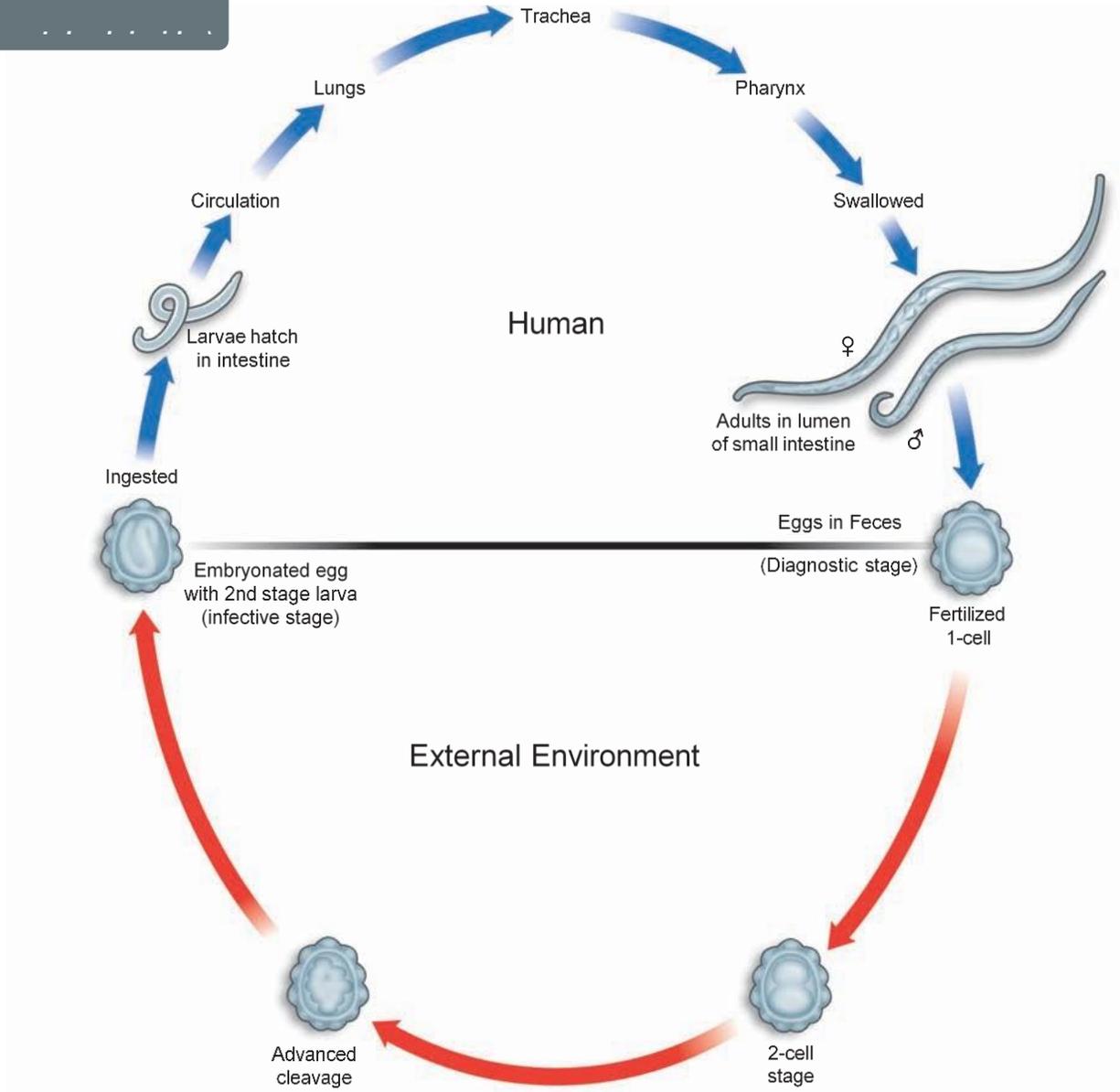
Ascaris lumbricoides causes ascariasis.

Important Properties

The life cycle of *A. lumbricoides* is shown in . Humans are infected by **ingesting worm eggs** in food or water contaminated with human feces. The eggs hatch in the small intestine, and the larvae migrate

through the gut wall into the bloodstream and then to the lungs. They enter the alveoli, pass up the bronchi and trachea, and are swallowed. Within the small intestine, they become adults. They live in the lumen, do not attach to the wall, and derive their sustenance from ingested food. The adults are the **largest intestinal nematodes**, often growing to 25 cm or more. *A. lumbricoides* is known as the “giant roundworm.” Thousands of eggs are laid daily, are passed in the feces, and differentiate into embryonated eggs in warm, moist soil . Ingestion of the embryonated eggs completes the cycle.

Ascariasis



Pathogenesis & Clinical Findings

The major damage occurs during larval migration rather than from the presence of the adult worm in the intestine. The principal sites of tissue reaction are the **lungs**, where inflammation with an **eosinophilic exudate** occurs in response to larval antigens. Because the adults derive their nourishment from ingested food, a heavy worm burden may contribute to malnutrition, especially in children in developing countries.

Most infections are asymptomatic. *Ascaris pneumonia* with fever, cough, and eosinophilia can occur with a heavy larval burden. Abdominal pain and even obstruction can result from the presence of adult worms in the intestine.

Epidemiology

Ascaris infection is very common, especially in the tropics; hundreds of millions of people are infected. In the United States, most cases occur in the southern states.

Laboratory Diagnosis

Diagnosis is usually made microscopically by detecting eggs in the stools. The egg is oval with an irregular surface. Occasionally, the patient sees adult worms in the stools.

Treatment

Both mebendazole and pyrantel pamoate are effective.

Prevention

Proper disposal of feces can prevent ascariasis.

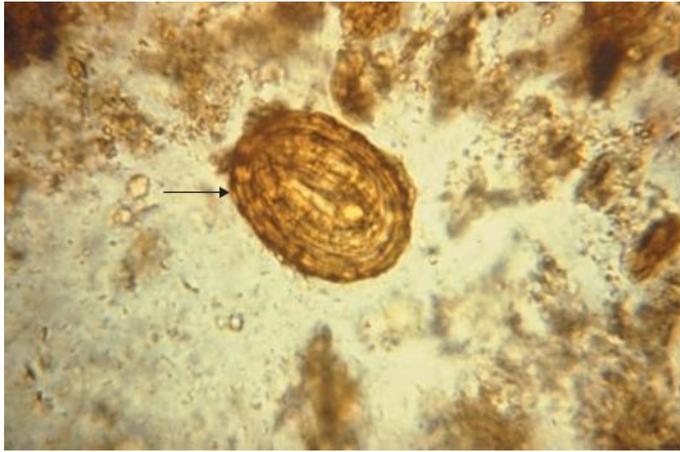


FIGURE –7 *Ascaris lumbricoides*—egg. Arrow points to an egg of *Ascaris*. Note the typical “scallop” edge of the *Ascaris* egg.



FIGURE –8 *Ascaris lumbricoides*—adult worms.,

STRONGYLOIDES

Disease

Strongyloides stercoralis causes strongyloidiasis.

Important Properties

The life cycle of *S. stercoralis* is shown in Figure 56–13.

S. stercoralis has **two distinct life cycles**, one within the human body and the other free-living in the soil. The life cycle in the human body begins with the **penetration of the skin**, usually of the feet, by **infectious (filariform) larvae** and their migration to the lungs. They enter the alveoli, pass up the bronchi and trachea, and then are swallowed. In the small intestine, the larvae molt into adults that enter the mucosa and produce eggs.

The eggs usually hatch within the mucosa, forming rhabditiform larvae (that are passed in the feces. Some larvae molt to form filariform larvae, which penetrate the intestinal wall directly without leaving the host and migrate to the lungs (**autoinfection**). Filariform larvae can also exit the anus and reinfect through the perianal skin. In immunocompetent patients, this is an infrequent, clinically unimportant event. However, in immunocompromised patients (e.g., those who have acquired immunodeficiency syndrome [AIDS] or are taking high-dose corticosteroids) or patients who are severely malnourished, autoinfection can lead to **massive reinfection**, with larvae passing to many organs and with severe, sometimes fatal consequences. Reinfection can also occur in those infected with human T-cell lympho- tropic virus (HTLV) because their ability to mount a protective T-cell response is diminished.

If larvae are passed in the feces and enter warm, moist soil, they molt through successive stages to form adult male and female worms. After mating, the entire life cycle of egg, larva, and adult can occur in the soil. After several free-living cycles, filariform larvae are formed. When they contact skin, they penetrate and again initiate the parasitic cycle within humans.

Pathogenesis & Clinical Findings

Most patients are asymptomatic, especially those with a low worm burden. Adult female worms in the wall of the small intestine can cause inflammation, resulting in watery diarrhea. In autoinfection, the penetrating larvae may cause sufficient damage to the

intestinal mucosa that **sepsis caused by enteric bacteria**, such as *Escherichia coli* and *Bacteroides fragilis*, can occur. Larvae in the lungs can produce a pneumonitis similar to that caused by *Ascaris*. Pruritus (ground itch) can occur at the site of larval penetration of the skin, as with hookworm.

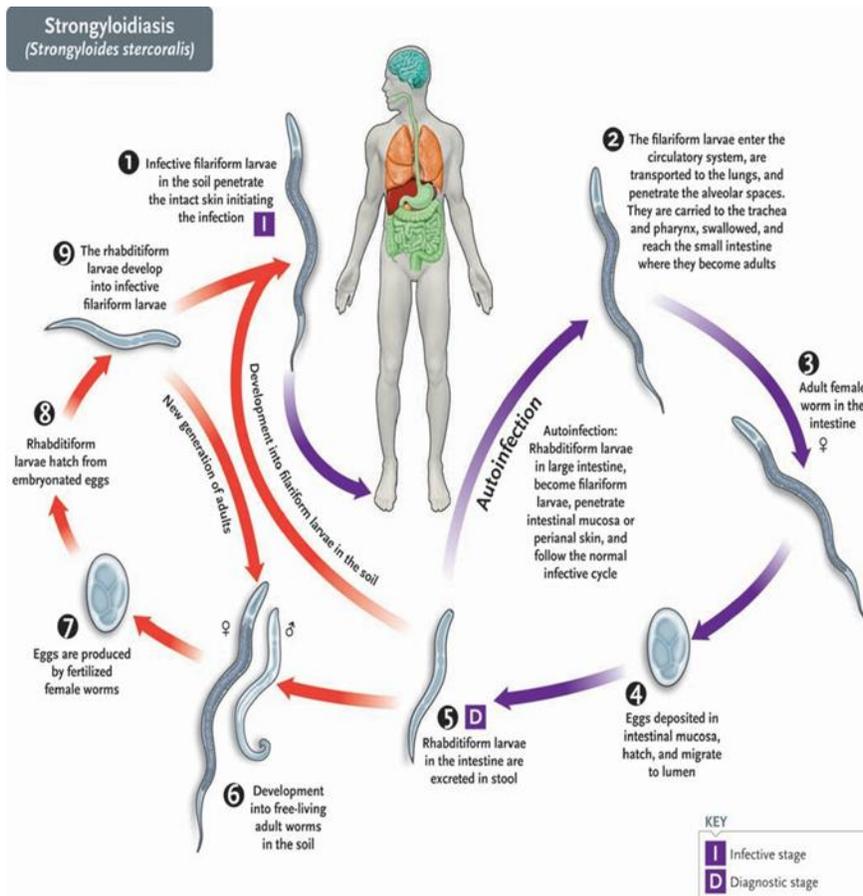
Epidemiology

Strongyloidiasis occurs primarily in the tropics, especially in Southeast Asia. Its geographic pattern is similar to that of hookworm because the same type of soil is required. In the United States, *Strongyloides* is endemic in the southeastern states.

Laboratory Diagnosis

Diagnosis depends on finding **larvae**, rather than eggs, in the stool . As with many nematode infections in which larvae migrate through tissue, **eosinophilia can be striking**. Serologic tests are useful when the larvae are not visualized. An enzyme immunoassay that detects antibody to larval antigens is available through the Centers for Disease Control and Prevention (CDC) in Atlanta.

Strongyloidiasis (*Strongyloides stercoralis*)



Treatment

Ivermectin is the drug of choice. Thiabendazole is an alternative drug.

Prevention

Prevention involves disposing of sewage properly and wearing shoes.

TRICHINELLA

Disease

Trichinella spiralis causes trichinosis.

Important Properties

. Any mammal can be infected, but **pigs** are the most important reservoirs of human disease in the United States (except in Alaska, where bears constitute the reservoir). Humans are infected by **eating raw** or **undercooked meat** containing larvae encysted in

the muscle The larvae excyst and mature into adults within the mucosa of the small intestine. Eggs hatch within the adult females, and larvae are released and distributed via the bloodstream to many organs; however, they develop only in **striated muscle cells**. Within these “**nurse cells**,” they encyst within a fibrous capsule and can remain viable for several years but eventually calcify

The parasite is maintained in nature by cycles within reservoir hosts, primarily swine and rats.

Humans are **end-stage hosts**, because the infected flesh is not consumed by other animals.

Pathogenesis & Clinical Findings

A few days after eating undercooked meat, usually pork, the patient experiences diarrhea followed 1 to 2 weeks later by **fever, muscle pain, periorbital edema, and eosinophilia**.

Subconjunctival hemorrhages are an important diagnostic criterion. Signs of cardiac and central nervous system disease are frequent, because the larvae migrate to these tissues as well.

Death, which is rare, is usually due to congestive heart failure or respiratory paralysis.

Epidemiology

Trichinosis occurs worldwide, especially in Eastern Europe and west Africa. In the United States, it is related to eating home-prepared sausage, usually on farms where the pigs are fed uncooked garbage. Bear and seal meat also are sources. In many countries, the disease occurs primarily in hunters who eat undercooked wild game.

Laboratory Diagnosis

Muscle biopsy reveals **larvae within striated muscle**. Serologic tests, especially the bentonite flocculation test, become positive 3 weeks after infection.

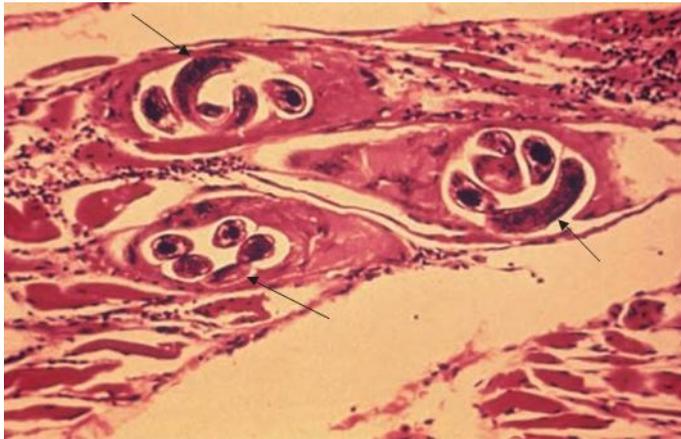
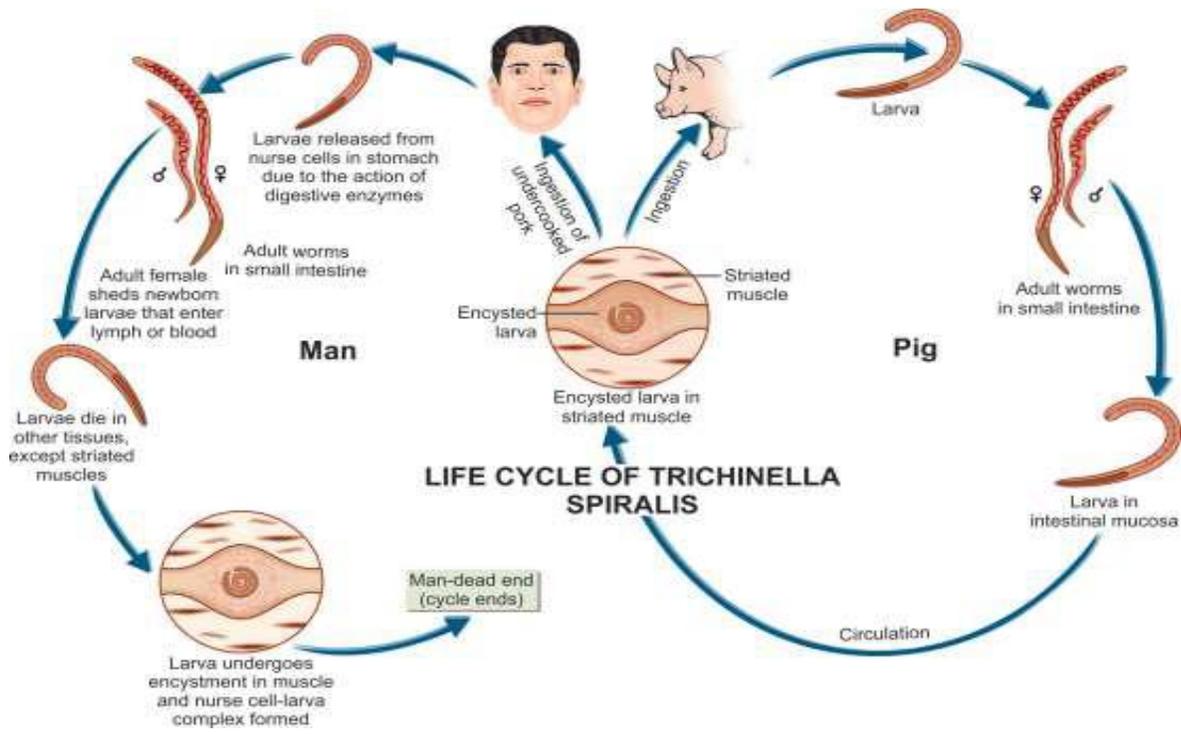


FIGURE-15 *Trichinella spiralis*—larvae in skeletal muscle. Three arrows point to *Trichinella* larvae within “nurse cells” in skeletal muscle.

Kato Katz technique

Kato Katz technique is used for qualitative and semi-quantitative diagnosis of intestinal helminthic infestations; caused by *Ascaris lumbricoides*, *Trichuris trichiura*, hookworm and especially *Schistosoma* spp.

WHO has recommended Kato Katz technique in areas with moderate to high transmission rates of soil transmitted helminths (i.e. where the proportion of infected individuals is >20– >50%) or intestinal schistosomiasis (>10–50%). Where the prevalence of soil transmitted helminths (STH) is <20%, the specificity of this technique makes it less appropriate and more sensitive tools should be used

Principle

People infected with STH or intestinal schistosomes pass the eggs of the worms through their faeces. In the Kato-Katz technique faeces are pressed through a mesh screen to remove large particles. A portion of sieved sample is then transferred to the hole of a template on a slide. After filling the hole, the template is removed and the remaining sample is covered with a piece of cellophane soaked in glycerol. The glycerol clears the faecal material from around the eggs. The eggs are then counted and the number calculated per gram of faeces

Materials

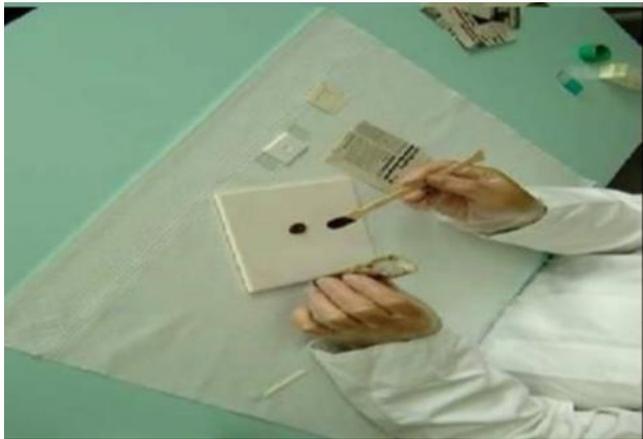
- 1_Kato-set (Template with hole, screen, nylon or plastic, plastic spatula)
- 2_Newspaper or glazed tile
- 3_Microscope slides
- 4_Cellophane as cover slip, soaked in Glycerol-malachite green or or glycerol-methylene blue solution.
- 5_Fresh stool
- 6_Gloves



Procedure of Katz-Kato Technique

Label a glass slide with the sample number and then place a plastic **template** on top of it. Place a small amount of the faecal sample on a newspaper and press a piece of nylon screen on top. Using a spatula, scrape the sieved faecal material through the screen so that only the debris remains. Scrape up some of the sieved faeces to fill the hole in the template, avoiding air bubbles and levelling the faeces off to remove any excess. Carefully lift off the template and place it in a bucket of water mixed with concentrated detergent so that it can be reused. Place one piece of the cellophane, which has been soaked overnight in methylene blue glycerol solution, over the faecal sample. Place a clean slide over the top and press it evenly downwards to spread the faeces in a circle. Carefully remove the slide by gently sliding it sideways to avoid separating the cellophane strip. If done well, it should be possible to read newspaper print through the stool smear. Place the slide with the cellophane upwards.

Note: If hookworm is present in the area the slide should be read within 30–60 minutes. After that time, the hookworm eggs disappear



Examination and Results ▪

Place the slide under a microscope and examine the whole area in a systematic zigzag pattern. ▪ Record the number and the type of each egg of each species on a recording form alongside the sample number. ▪ Finally, multiply the number of eggs by the appropriate number (see inlet-information of the kato-set) to give the number of eggs per gram (epg) – the standard measurement to assess the intensity of infection

Advantages

- Best method to identify eggs of Ascaris, hookworms and S. mansoni .

- Allows both identification and quantification of those eggs so it is easy to calculate the worm burden in a patient .
- It is easy to perform and to transfer madepreparations .
- Therefore, it is good for field studies as the preparation can be done in the field then transferred to the equipped reference laboratory

Disadvantages:

Not suitable for liquid stool (not for diarrheal samples) Suitable only for stool specimens that contain at least 20 egg/gram of feces .

Harada–Mori Technique for Cultivation of Hookworm

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Harada–Mori Technique for Cultivation of Hookworm

1. Introduction

Hookworms are medically important intestinal parasites that infect humans, mainly:

- *Ancylostoma duodenale*
- *Necator americanus*

They are a major cause of iron-deficiency anemia and malnutrition, particularly in tropical and subtropical regions.

2. Definition of Harada–Mori Technique

The Harada–Mori technique is a simple laboratory method used to cultivate hookworm larvae from stool samples for diagnostic and educational purposes.

3. Principle

The technique is based on:

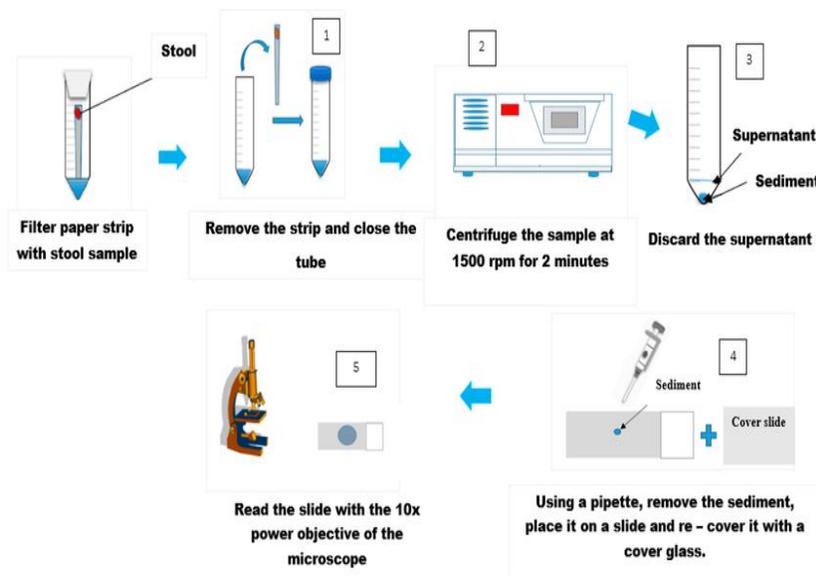
- Placing a stool sample on filter paper
- Providing a moist and warm environment
- Allowing eggs to hatch and larvae to migrate downward into water due to their motility

4. Materials Required

1. Fresh stool sample
2. Filter paper strip
3. Test tube
4. Distilled water
5. Light microscope

5. Procedure

1. A small amount of stool is placed on a strip of filter paper.
2. The filter paper is inserted into a test tube containing a small amount of water, without immersing the stool.
3. The tube is incubated at room temperature or 25–30°C for 5–7 days.
4. After incubation, the water at the bottom of the tube is examined microscopically.
5. Larvae are observed and identified.



6. Expected Results

- Rhabditiform larvae may be seen in early stages.
- Filariform larvae may appear after prolonged incubation.

7. Diagnostic Importance

- Useful when direct stool examination is negative.
- Helps differentiate hookworm larvae from *Strongyloides stercoralis*.
- Commonly used in teaching laboratories.

8. Advantages and Disadvantages

Advantages:

- Simple and inexpensive

- Requires minimal equipment
- Reliable for larval recovery

Disadvantages:

- Time-consuming
- Risk of contamination if not handled properly

