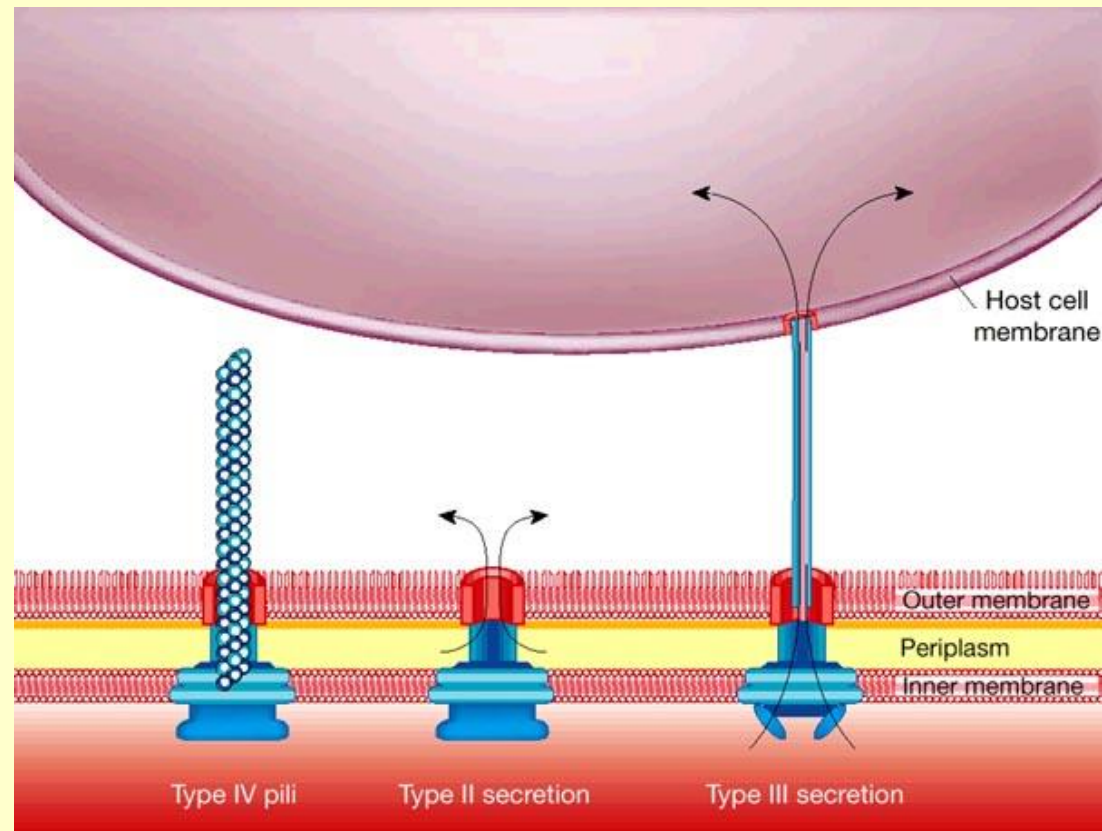


Type III Secretion System

- Complex protein secretion system employed by many Gram-negative pathogenic bacteria
- Transport bacterial effector proteins across three membrane barriers into eukaryotic host cytoplasm
- The effector proteins delivered by TTSS are capable of modulating and interfering with the host cellular processes,
 - plague,
 - typhoid fever,
 - bacterial dysentery
- Composed of more than 20 structural proteins, effector proteins, and chaperones.



Recombinant DNA

- NIH established the RAC (recombinant DNA Advisory Panel) in 1974 in response to concerns over safety of manipulating genetic material using recombinant DNA techniques.
- The RAC issues recommendations to NIH Director that are then conveyed through NIH OBA (Office of Biotechnology Activities) in the form of NIH Guidelines for Research Involving Recombinant DNA Molecules
- http://oba.od.nih.gov/rdna/nih_guidelines_oba.html
- Mandatory compliance for all institutions receiving NIH funds for Research involving recombinant DNA

Recombinant DNA

- Recombinant DNA molecules are either: 1) molecules which are constructed outside living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell; or 2) DNA molecules that result from the replication of those described in 1).
- Synthetic DNA segments which are likely to yield a potentially harmful polynucleotide or polypeptide (e.g., a toxin or a pharmacologically active agent) are considered as equivalent to their natural DNA counterpart.
 - However, if the synthetic DNA segment is not expressed *in vivo* as a biologically active polynucleotide or polypeptide product, it is exempt from the *NIH Guidelines*.
- Genomic DNA of plants and bacteria that have acquired a transposable element, even if the latter was donated from a recombinant vector no longer present, are not subject to the *NIH Guidelines* unless the transposon itself contains recombinant DNA.

IBC Approval

- Principal Investigator must submit a registration document to the Institutional Biosafety Committee which contains the following information:
 - (i) the source(s) of DNA;
 - (ii) the nature of the inserted DNA sequences;
 - (iii) the host(s) and vector(s) to be used;
 - (iv) if an attempt will be made to obtain expression of a foreign gene, and if so, indicate the protein that will be produced; and
 - (v) the containment conditions that will be implemented as specified in the *NIH Guidelines*

Required Approval

§ III-A. Experiments that Require Institutional Biosafety Committee Approval, RAC Review, and NIH Director Approval Before Initiation

- The deliberate transfer of a drug resistance trait to microorganisms that are not known to acquire the trait naturally

§ III-B. Experiments That Require NIH/OBA and Institutional Biosafety Committee Approval Before Initiation

- Deliberate formation of recombinant DNA containing genes for the biosynthesis of toxin molecules lethal for vertebrates at an LD50 of less than 100 nanograms per kilogram body weight (e.g., microbial toxins such as the botulinum toxins, tetanus toxin, diphtheria toxin, and *Shigella dysenteriae* neurotoxin); exemption for *E. coli* K-12 (100ng -100µg)

§ III-C. Experiments that Require Institutional Biosafety Committee and Institutional Review Board Approvals and RAC Review Before Research Participant Enrollment

- The deliberate transfer of recombinant DNA, or DNA or RNA derived from recombinant DNA, into human research participants (human gene transfer)

§III-D Experiments that Require IBC Approval Prior to Initiation

- §III-D-1. Experiments Using Risk Group 2, Risk Group 3, Risk Group 4, or Restricted Agents as Host-Vector Systems
- §III-D-2. Experiments in Which DNA From Risk Group 2, Risk Group 3, Risk Group 4, or Restricted Agents is Cloned into Nonpathogenic Prokaryotic or Lower Eukaryotic Host-Vector Systems
- §III-D-3. Experiments Involving the Use of Infectious DNA or RNA Viruses or Defective DNA or RNA Viruses in the Presence of Helper Virus in Tissue Culture Systems
- §III-D-4. Experiments Involving Whole Animals
- §III-D-5. Experiments Involving Whole Plants (BL 2P+ and above)
- §III-D-6. Experiments Involving More than 10 Liters of Culture
- §III-D-7. Experiments Involving Influenza Viruses

§III-E. Experiments that Require IBC Approval Simultaneous with Initiation

- §III-E-1. Experiments Involving the Formation of Recombinant DNA Molecules Containing No More than Two-Thirds of the Genome of any Eukaryotic Virus
- §III-E-2. Experiments Involving Whole Plants (BL1 and 2 P)
- §III-E-3. Experiments Involving Transgenic Rodents

§III-F. Exempt Experiments

- §III-F-1. Those not in organisms or viruses
- §III-F-2. Those consisting entirely of DNA from a single chromosomal or viral DNA source, though one or more of the segments may be a synthetic equivalent
- §III-F-3. Those that consist entirely of DNA from a prokaryotic host including its indigenous plasmids or viruses when propagated only in that host (or a closely related strain of the same species), or when transferred to another host by well established physiological means.
- §III-F-4. Those that consist entirely of DNA from an eukaryotic host including its chloroplasts, mitochondria, or plasmids (but excluding viruses) when propagated only in that host (or a closely related strain of the same species).
- §III-F-5. Those that consist entirely of DNA segments from different species that exchange DNA by known physiological processes, though one or more of the segments may be a synthetic equivalent.
- §III-F-6. Those that do not present a significant risk to health or the environment, as determined by the NIH Director, with the advice of the RAC, and following appropriate notice and opportunity for public comment.

Helminths

Helminths (Worms)

- Multicellular animals
- Some are human and/or animal parasites
- Eggs are small enough to pose environmental health problems from human and animal excreta in water, food, soil, etc.
- Several major groups:
 - Nematodes (roundworms): ex. *Ascaris*
 - Trematodes (flukes; flatworms): ex. Schistosomes
 - Cestodes (tapeworms): pork and beef tapeworms

What is Parasitology

- Lives on or in another organism its host
- Symbiosis - two types of organisms living together.
- Three types of symbiotic relationships
- Mutualism-
- Commensalism
- Parasitism

Human Parasitology

Medical
Protozoology

Medical
Helminthology

Medical
Arthropodology

- **Class Lobosea**
- **Class Zoomastigophorea**
- **Class Sporozoa**
- **Class Ciliophora**
- **Class Nematoda**
- **Class Trematoda**
- **Class Cestoda**
- **Class Metacanthocephala**
- **Class Insecta**
- **Class Arachnida**
- **Class Crustacea**
- **Class Chilopoda**

Parasites in the United States

- *Ascaris lumbricoides*
- *Necator americanus*
- *Trichinella spiralis*
- *Giardia lamblia*
- *Enterobius vermicularis*

Impacts of Helminthic Parasites

- >2.5 billion helminthes infections
- 1/3 of 3 billion people living below \$2 per day in developing regions are infected with **1 or more** heminth
- School age children harbor the greatest numbers
- Poor nutrition leads to reduced resistance
- High calorie demand (up to 5,000 calories/day)
- Estimated 60 million people die every year

The major human helminthiases and their global prevalence and distribution

Disease	Major etiologic agent	Global prevalence	Regions of highest prevalence
Soil-transmitted nematodes			
Ascariasis	<i>Ascariasis lumbricoides</i> (roundworm)	807 million	Developing regions of Asia, Africa, and Latin America
Trichuriasis	<i>Trichuris trichiura</i> (whipworm)	604 million	Developing regions of Asia, Africa, and Latin America
Hookworm	<i>Necator americanus</i> ; <i>Ancylostoma duodenale</i>	576 million	Developing regions of Asia, Africa, and Latin America (especially areas of rural poverty)
Strongyloidiasis	<i>Strongyloides stercoralis</i> (thread worm)	30–100 million	Developing regions of Asia, Africa, and Latin America (especially areas of rural poverty)
Filarial nematodes			
LF	<i>Wuchereria bancrofti</i> ; <i>Brugia malayi</i>	120 million	Developing regions of India, Southeast Asia, and sub-Saharan Africa
Onchocerciasis (river blindness)	<i>Onchocerca volvulus</i>	37 million	Sub-Saharan Africa
Loiasis	<i>Loa loa</i>	13 million	Sub-Saharan Africa
Dracunculiasis (guinea worm)	<i>Dracunculus medinensis</i>	0.01 million	Sub-Saharan Africa
Platyhelminth flukes			
Schistosomiasis	<i>Schistosoma haematobium</i> ; <i>Schistosoma mansoni</i> ; <i>Schistosoma japonicum</i> (blood flukes)	207 million	Sub-Saharan Africa Sub-Saharan Africa and Eastern Brazil China and Southeast Asia
Food-borne trematodiasis	<i>Clonorchis sinensis</i> (liver fluke); <i>Opisthorchis viverrini</i> (liver fluke); <i>Paragonimus spp.</i> (lung flukes); <i>Fasciolopsis buski</i> (intestinal fluke); <i>Fasciola hepatica</i> (intestinal fluke)	>40 million	Developing regions of East Asia
Platyhelminth tapeworms			
Cysticercosis	<i>Taenia solium</i> (pork tapeworm)	0.4 million (Latin America only)	Developing regions of Asia, Latin America, and sub-Saharan Africa

Zoonosis

- Wild or domestic animals serve as reservoir hosts in transmission to man.
- Sylvatic or enzootic – wild host
 - *Trichinella spiralis* - wild boar, bear, walrus
- Urban or domestic - domestic animals
 - *Trichinella spiralis* - domestic pigs

Terms Describing Parasites

- Ectoparasites (develop outside host) and Endoparasites (develop inside host)
- Obligatory (dependent on host for survival) and Facultative (can live independent of host; also used for ability to feed on live or dead material)
- Accidental or Incidental (other than normal host)
- Permanent (most of life cycle in host) and Temporary (part of life cycle in host, rest freeliving)
- Heteroecious (various hosts for different life stages) and Autoecious (one host)
- Parasitoid (single organism as host, kills organism)

Terms Describing Hosts

- Definitive (parasite passes its adult and sexual stage)
- Intermediate (parasite passes its larval or nonsexual stage)
- Paratenic or transport host (substitute intermediate hosts, generally due to ingestion of original host)
- Reservoir host (passive carrier)
- Vector (carries from one host to another)

Reproductive Potential of Parasites

- Extremely complex life cycles
- Reproductive system highly specialized
- Small chance of any one individual living so there is a large initial reproductive output
 - Female *Ascaris* produces 200,000 eggs /day
 - Many animals have both asexual and sexual cycle

Helminthes

	Cestodes	Trematodes	Nematodes
Shape	Segmented plane	Unsegmented plane	Cylindrical
Body cavity	No	No	Present
Digestive tube	No	Ends in cecum	Ends in anus
Sex	Hermaphroditic	Hermaphroditic, except Schistosoma which are dioecious	Dioecious
hook organ component	Oral sucker, botridias, and double Rostellar hooks	Oral sucker and ventral sucker	Lips, teeth, filariform extremities, and dentary plates

Helminths (Worms): Some Important Ones

Most acquired from ingestion of or contact with feces-contaminated soil or food

- Nematodes (Roundworms):

- *Ascaris lumbricoides*

GI illness; pneumonitis

- *Trichuris trichuria*

chronic GI

- Hookworms:

- *Ancylostoma duodenale*

chronic anemia

- *Necator americanus*

chronic anemia

- *Strongyloides stercoralis*

chronic anemia

- Cestodes (tapeworms):

- *Hymenolepis nana*

GI illness

Worm burdens tend to be low in the United States; little illness occurs

Still major cause of illness in developing countries

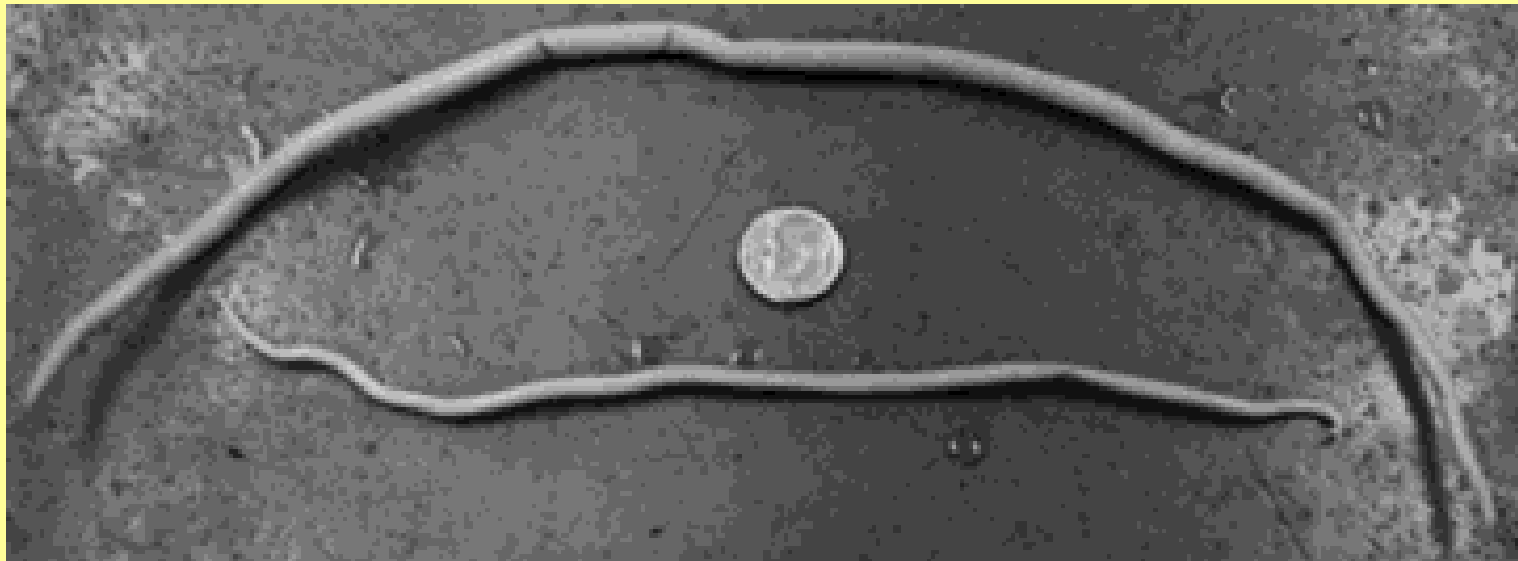
Ascaris lumbricoides

- Large intestinal roundworm of man
- In some areas in Asia, 100% of the population is infected.
- Most infections light but heavy infections may occur. Reported record (5,000)
- Glycogen consumption of Ascarids in China equal to glycogen of 143,000 tons of rice.



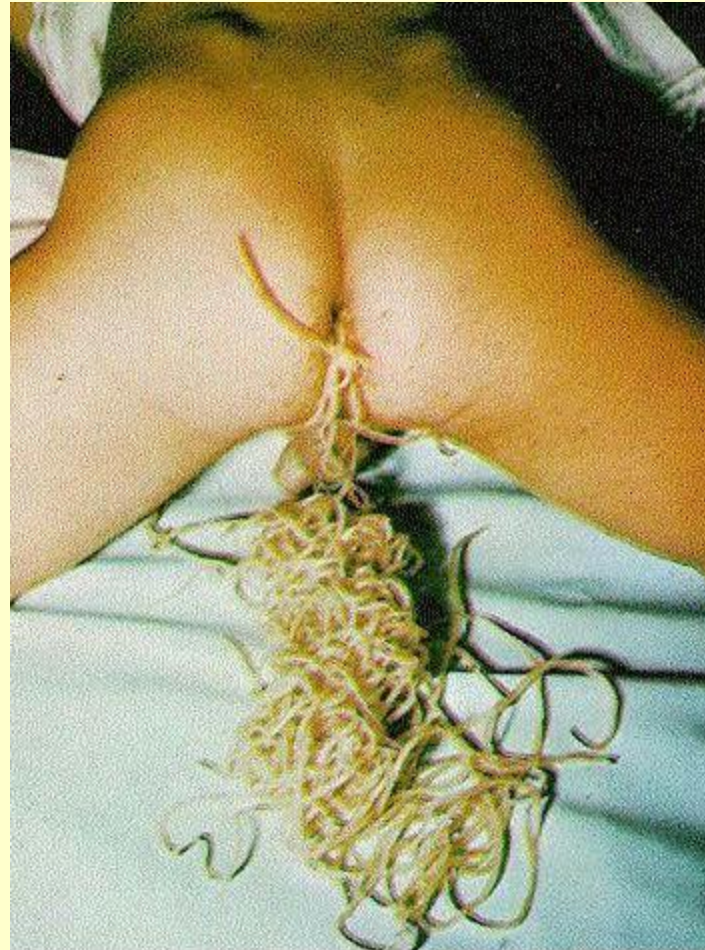
Roundworm: *Ascaris lumbricoides*

Ascaris female and male size comparison



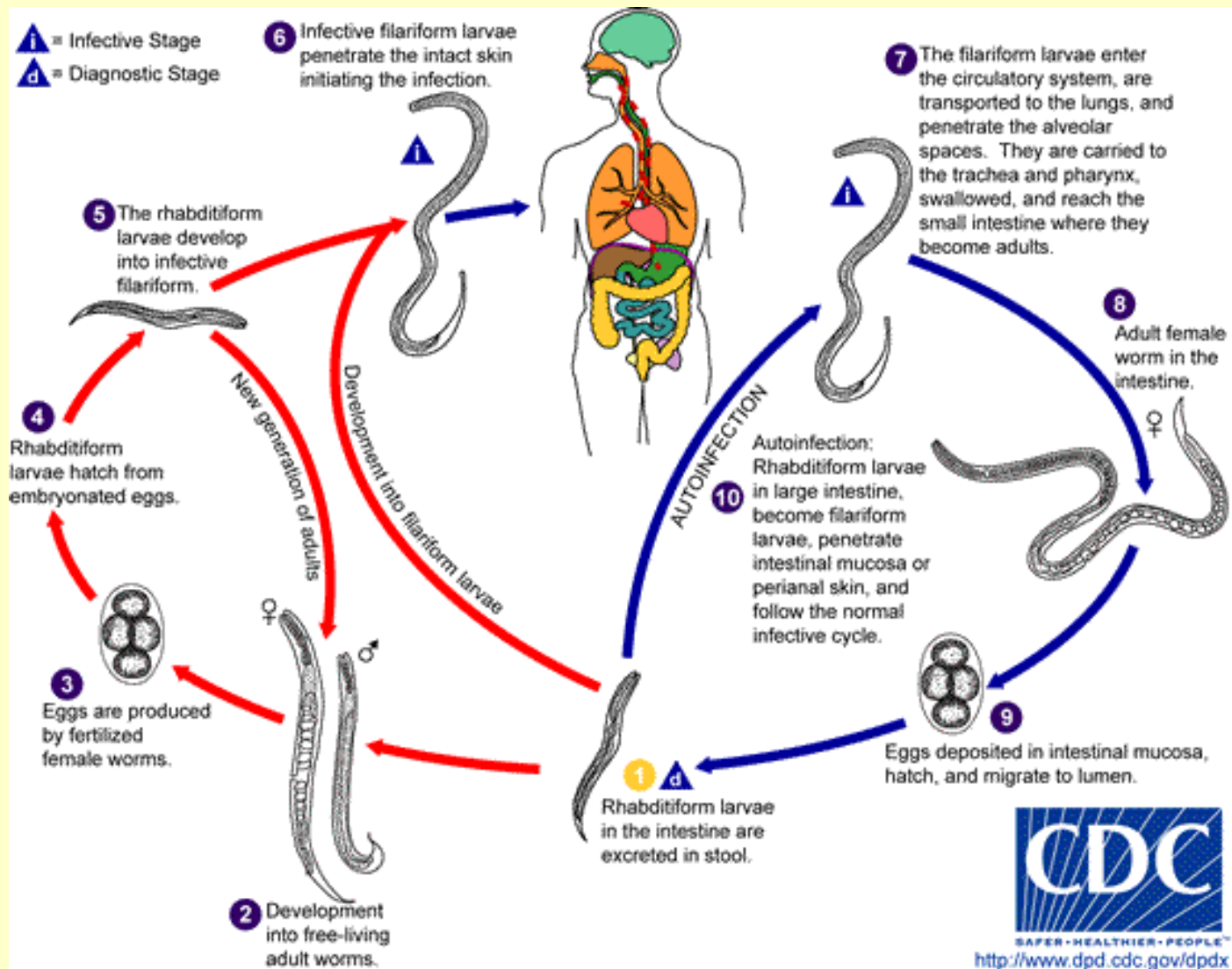
The female is the larger organism in the upper portion of the image. The male is smaller and typically possesses a hooked or curved tail.

Ascaris Shedding after Anthelmintic Drug Use



Massive Ascaris infection in child. A large bolus of roundworms expelled following anthelmintic treatment.

Strongyloides stercoralis (Threadworms)



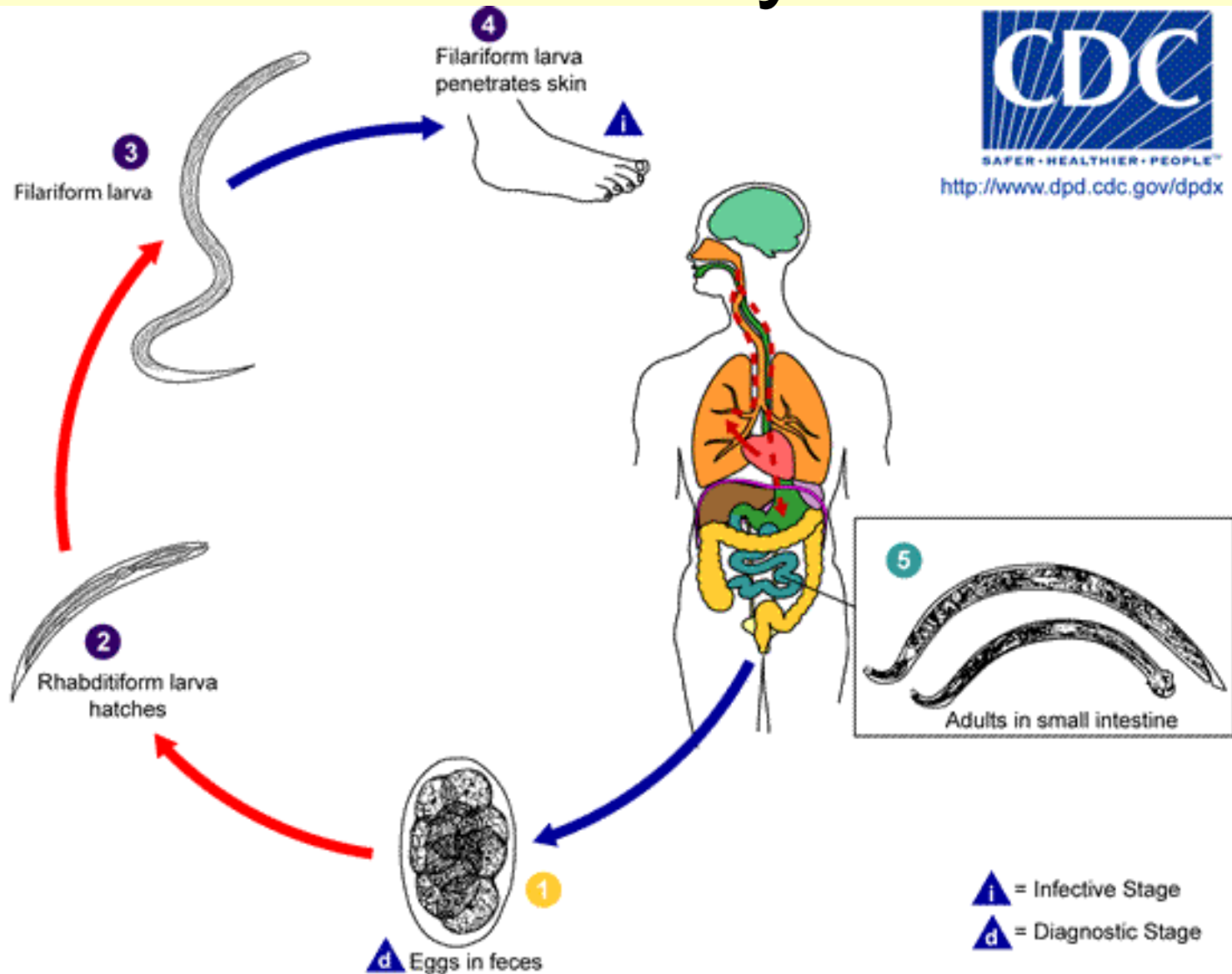


www.rotten.com

Family Ancylostomidae

- Commonly known as hookworms
- Live in intestines feed on blood and tissue fluids obtained from intestinal mucosa
- Morphology-especially important copulatory bursa of male.

Necator and Ancylostoma



Necator americanus

- "The American Killer" New World Hookworm
 - Probably introduced by slave trading
 - Major impact on development of southern U.S.
 - In 1947 estimated 1.5 million cases in North America
 - Current estimates in S.E.U.S. 4-5

Necator americanus



(by P.W. Pappas and S.M. Wardrop)

Ancyclostoma duodenale

- - Old World Hookworm
 - Predominately found in southern Europe,
 - northern Africa, India, China, southeastern Asia
 - In mines of England and Belgium



Ancylostoma duodenale

Hookworm disease

- Sometimes asymptomatic-pathology depends on worm load and nutritional condition of the infected person
- Disease restricted to warmer regions of world also adequate amounts of moisture
- White people 10 (ten) times more susceptible to hookworm than black persons -"poor white trash"

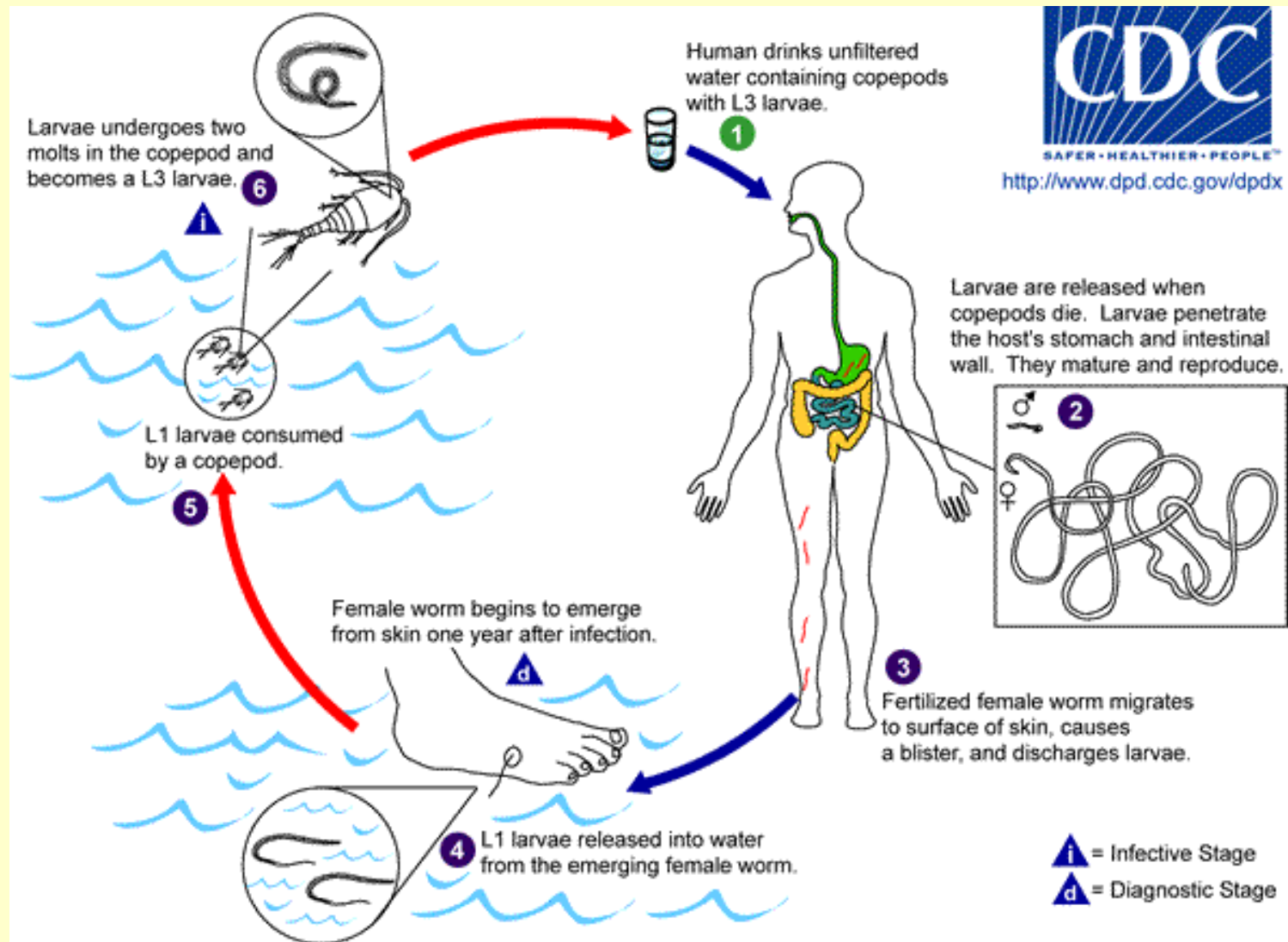
Family Toxocaridae

- *Toxocara canis* (dog) and *Toxocara cati* (cat) – common parasite of domestic dogs and cats and can be parasites of humans.
- Nearly 100 % of puppies and kittens infected. 98% puppies
- Adults able to repress worms. When females become pregnant, the worm is awoken and migrates to the offspring.

Family Toxocaridae

- If unnatural host (small child) becomes infected, worms have a tendency to migrate throughout organs. This is known as **visceral larval migrans**.
- Can be a very important parasite depending upon which organs it migrates into.

Guinea Worm Disease (Dracunculiasis)



Guinea Worm Disease





Dracunculiasis - Guinea worm disease
Dracunculus medinensis parasite

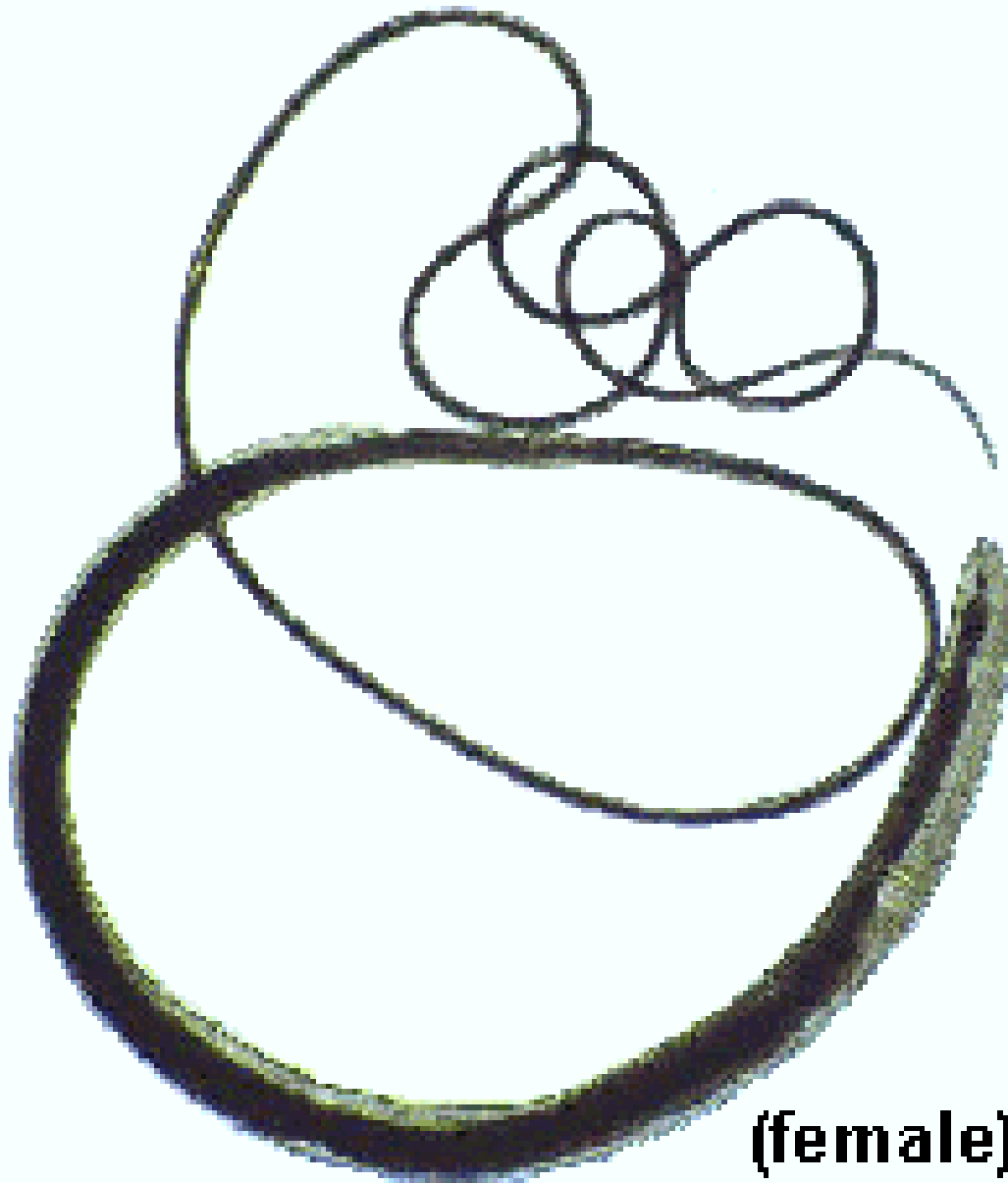
Family Trichuridae - Whipworms

- Morphology thick relatively short, posterior end with long thread - like anterior end, whip like in appearance (Trichocephalus) thread-head used in some texts.

Trichuris trichiura

- 30-50mm long
- Produces 1000-7000 eggs per day - eggs embryonate in soil.
- When swallowed infective juvenile hatches in small intestine enters intestinal crypts.
- After development reenters lumen of intestine matures.
- Can live a long time (many years).

Trichuris trichiura

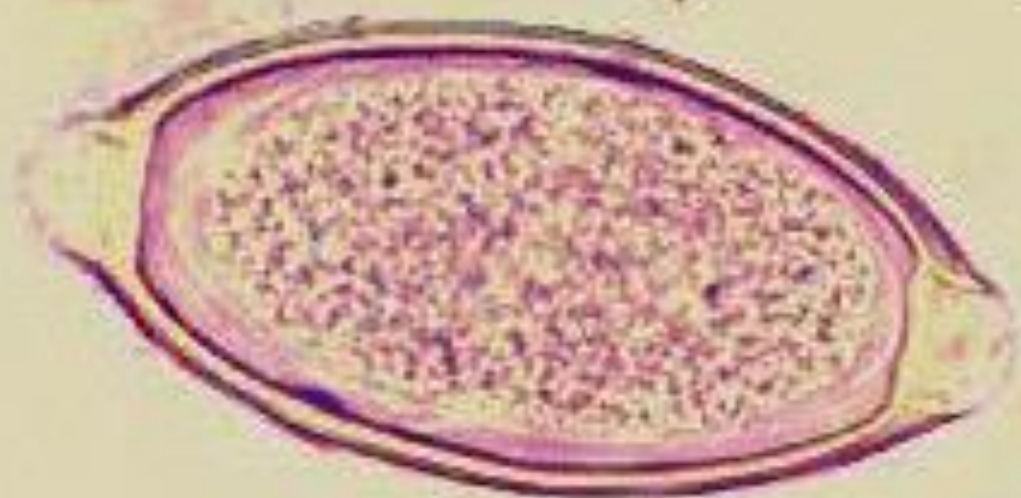


(female)



(male)

Trichuris trichiura

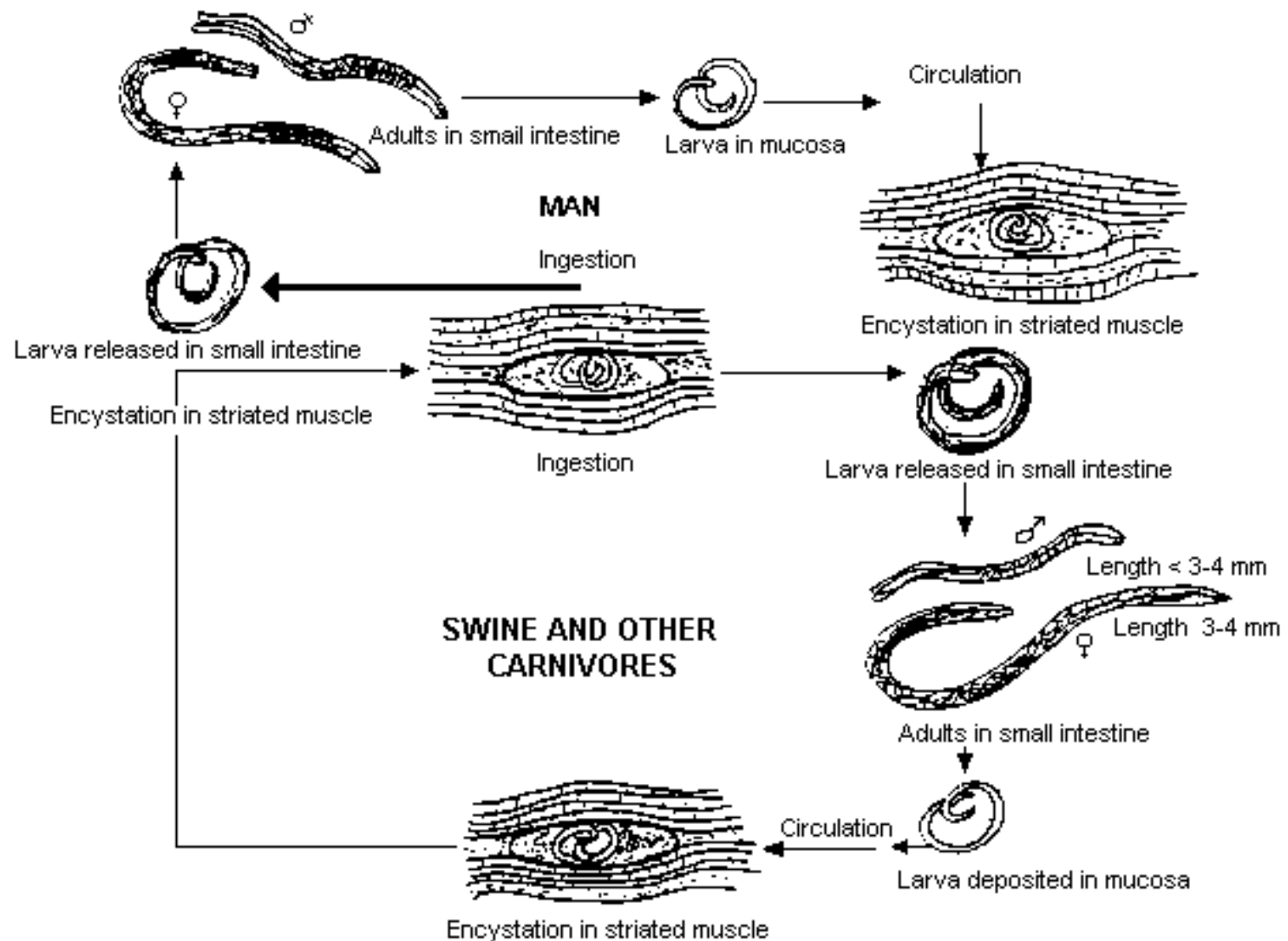


egg

(by Peter Darben)

Trichinella spiralis

- Causes disease trichinosis, trichiniasis, or trichinelliasis
 - Vague symptoms leads to misidentification
 - Morphology
 - Males 1.4-1.6mm long females 2.8-3.2mm long
 - Slender at anterior end
 - Biology
 - same animal can serve as definitive and intermediate host with juvenile and adults located in different organs.



LIFE CYCLE of *TRICHINELLA SPIRALIS*

Adapted and redrawn from NCDC

Family Oxyuridae – Pinworms

- *Enterobius vermicularis*
- *Small* worms of colon area.
- Females leave anus at night to lay eggs (contain embryonic juveniles. Severe rectal itching results.
- Children often reinfect themselves.
- If the anal folds are not cleaned, the worms may hatch and the larvae reenter the anus causing retroinfection.

Family Oxyuridae – Pinworms

- Epidemiology – bedding, clothing, stuffed animals, become seeded with ova. Very light can be carried in the air.
- Children often scratch where it itches, then insert fingers in mouth.
- Footed pajamas, mittens, wash with very hot water, treat the whole family.

Tapeworms

- All tapeworms are parasites
- Most parasitize wild animals few important parasites of men.
- Only orders Pseudophyllidea and other Cyclophyllidea contain tapeworms of importance to humans or parasites of man.
- Can cause cysticercosis (Subcutaneous tissue, brain or eyes)

Most Important Species

- *Taenia solium*
- *Taeniarrhynchus saginatus*
- *Echinococcus granulosus*
-
- *Echinococcus multilocularis*

Cestodes

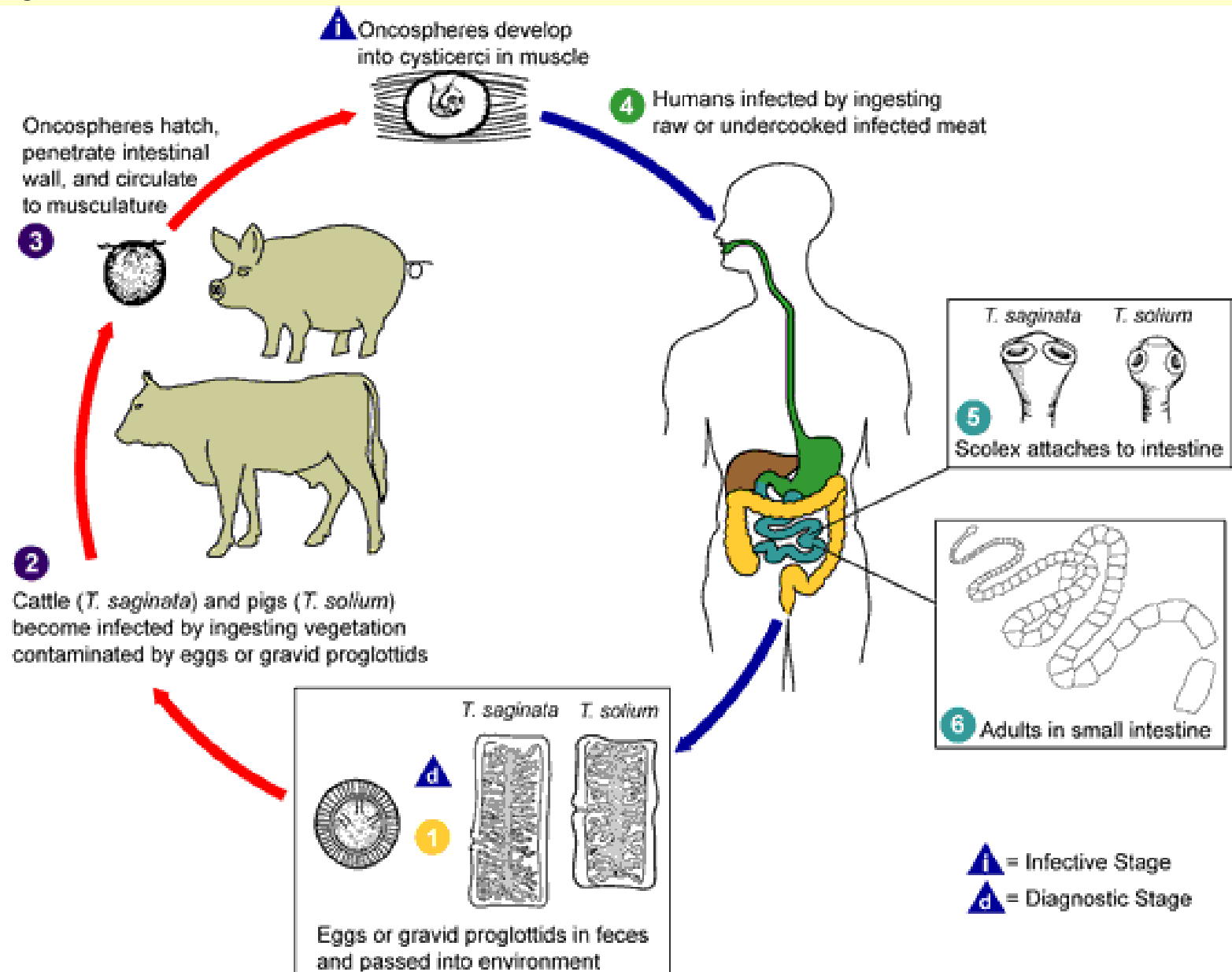
- ◆ Tapeworms
- ◆ scolex (head) →
- ◆ Proglottids (segments) ↙



Taenia sp. Scolex

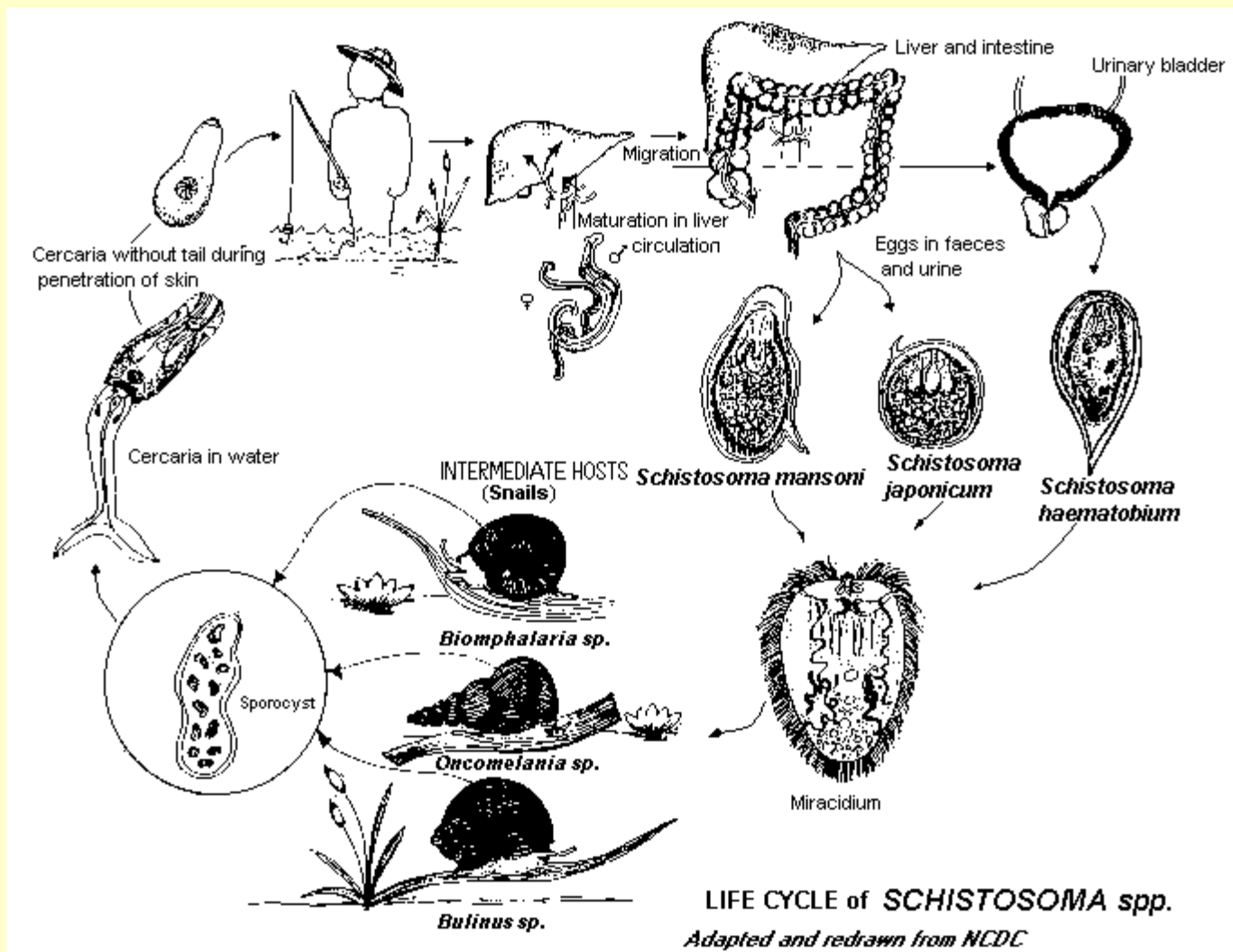


Taenia



Schistosoma sp.

- Important parasites of man and some domesticated animals
- Three species infect man
- *Schistosoma mansoni*
- *Schistosoma japonicum*
- *Schistosoma haematobium*



Trematodes

◆ Schistosomes (blood flukes)



Schistosoma japonicum.

- Common in parts of Japan, China, Taiwan, Philippines, Thailand, and other parts of Southeast Asia.
- Most pathogenic and most difficult to control
- Located in blood vessels of small intestine.
- Eggs may lodge in brain causing CNS damage, coma, and paralysis.
- Low host specificity

Schistosoma mansoni

- Common in Egypt, the Middle East, parts of Africa, and parts of South and Central America.
- Found in portal veins draining large intestine
- The sharp lateral spine is distinctive
- Primary pathological effects come from the damage done by eggs.

Schistosoma mansoni

- In heavy infections eggs become trapped in the mucous and submucosa of the gut and cause granuloma formation
- If extensive, they can cause colon blockage and significant blood loss.
- In liver can cause hepatomegaly.
- Destruction of lungs and heart tissue.
- Reservoir hosts are of limited or no importance.
-

Schistosoma haematobium

- often referred to as Bilharzia after Theodore Bilharz who discovered it.
- found in parts of Africa, and parts of the Middle East, southern Europe and some parts of Asia.
- Found primarily in the veins of the urinary bladder. Eggs released in urine.
- They are least pathogenic

Schistosome cercarial dermatitis or swimmers itch

- Schistosomes of animals other than man (usually rodents and birds) try to penetrate the skin of man, they can not establish themselves in the blood vascular system of man.
- Often cause a dermatitis which can be severe and in some cases life threatening.
- Allergic reaction

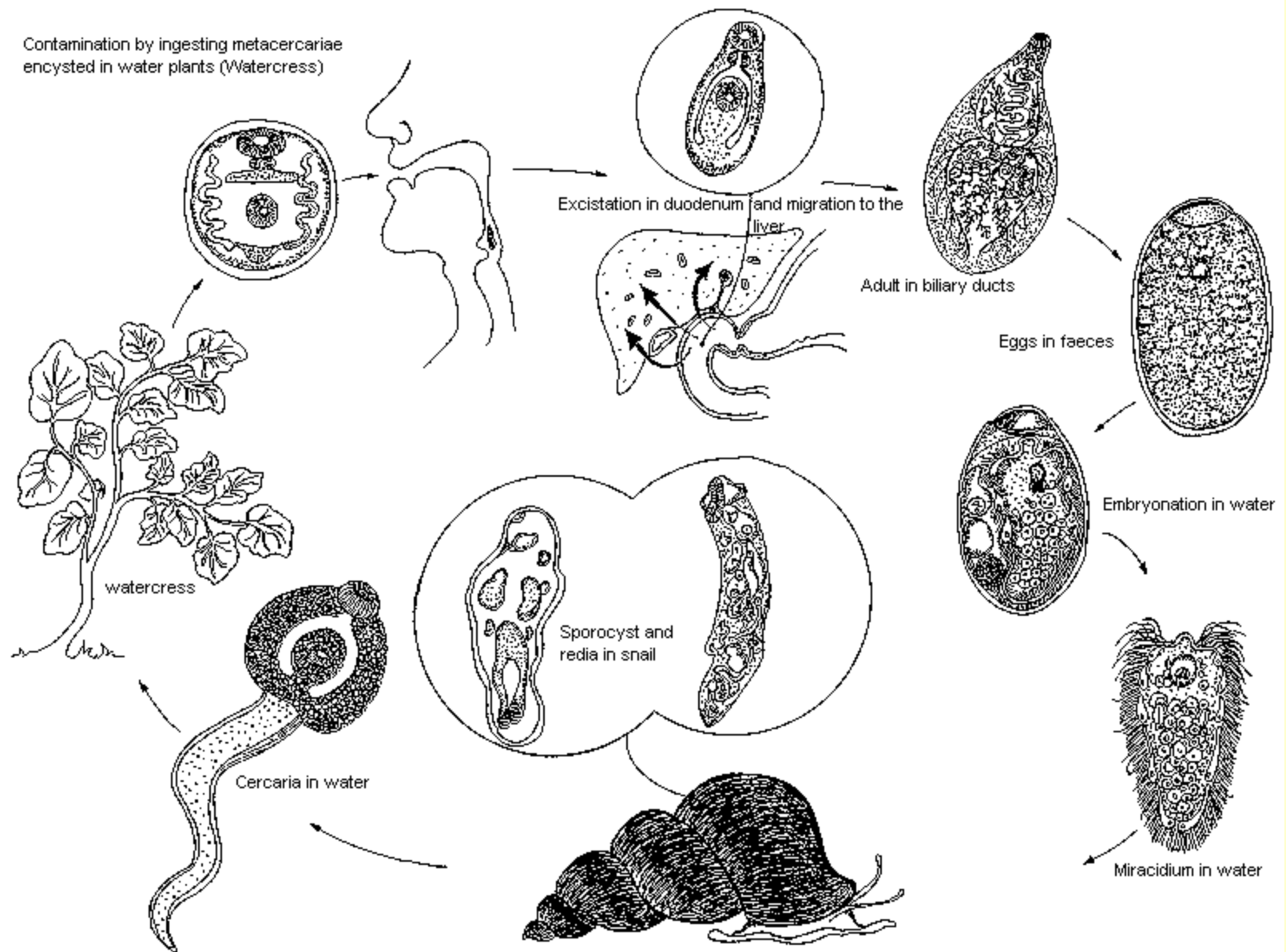
Swimmer's Itch



Fasciola hepatica

- Commonly known as the sheep liver fluke
- Important parasite of sheep and cattle (other grazers) can be found in humans.
- Morphology
 - Large size, frequently over 30 mm long
 - Characteristic cone-shaped projection at anterior end followed by wide shoulders

Contamination by ingesting metacercariae
encysted in water plants (Watercress)

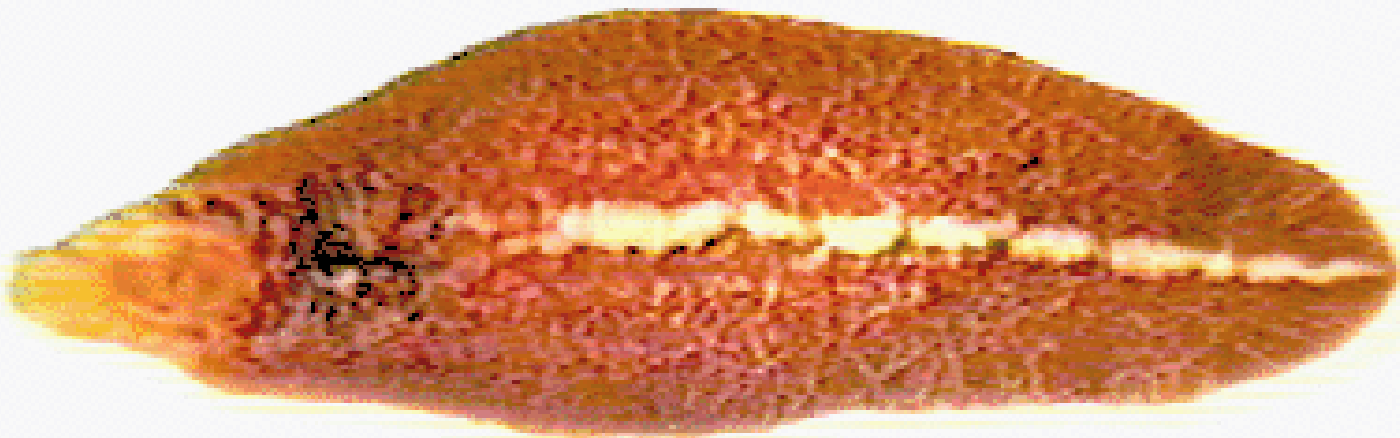


Adapted and redrawn from NCDC

LIFE CYCLE of **FASCIOLA HEPATICA**

Fasciola hepatica adult

Fasciola hepatica



(by P.W. Pappas and S.M. Wardrop)

Fasciola hepatica

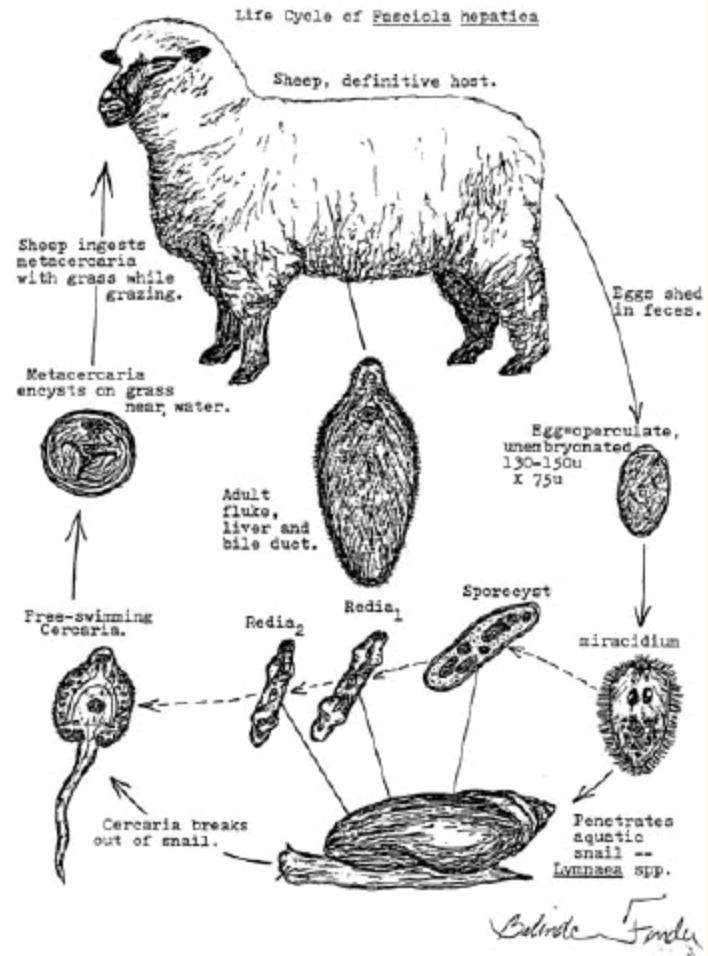
- Adult in bile duct of definitive host passes eggs in feces.
- If eggs land in water, they hatch into miracidium that actively swims until it finds an appropriate snail.
- Penetrates snail, develops into germinal sac (sporocyst), asexual stages of rediae and cercariae formed.

Fasciola hepatica

- Cercariae leave snail, encyst on vegetation, and form metacercaria.
- Herbivore infected when it ingests vegetation with metacercaria.
- Metacercaria develop into adult penetrates gut wall, moves to the liver.
- Humans infected by eating watercress that has metacercaria on it.

Fasciola hepatica (sheep liver fluke)

miracidium (free living ciliated larva, penetrates snail)
 sporocyst (bag-like creature)
 redia (develop inside sporocysts)
 daughter redia (develop inside redia)
 cercariae (develop inside daughter redia, exit snail encyst on vegetation)
 metacercaria develops from encysted cercaria



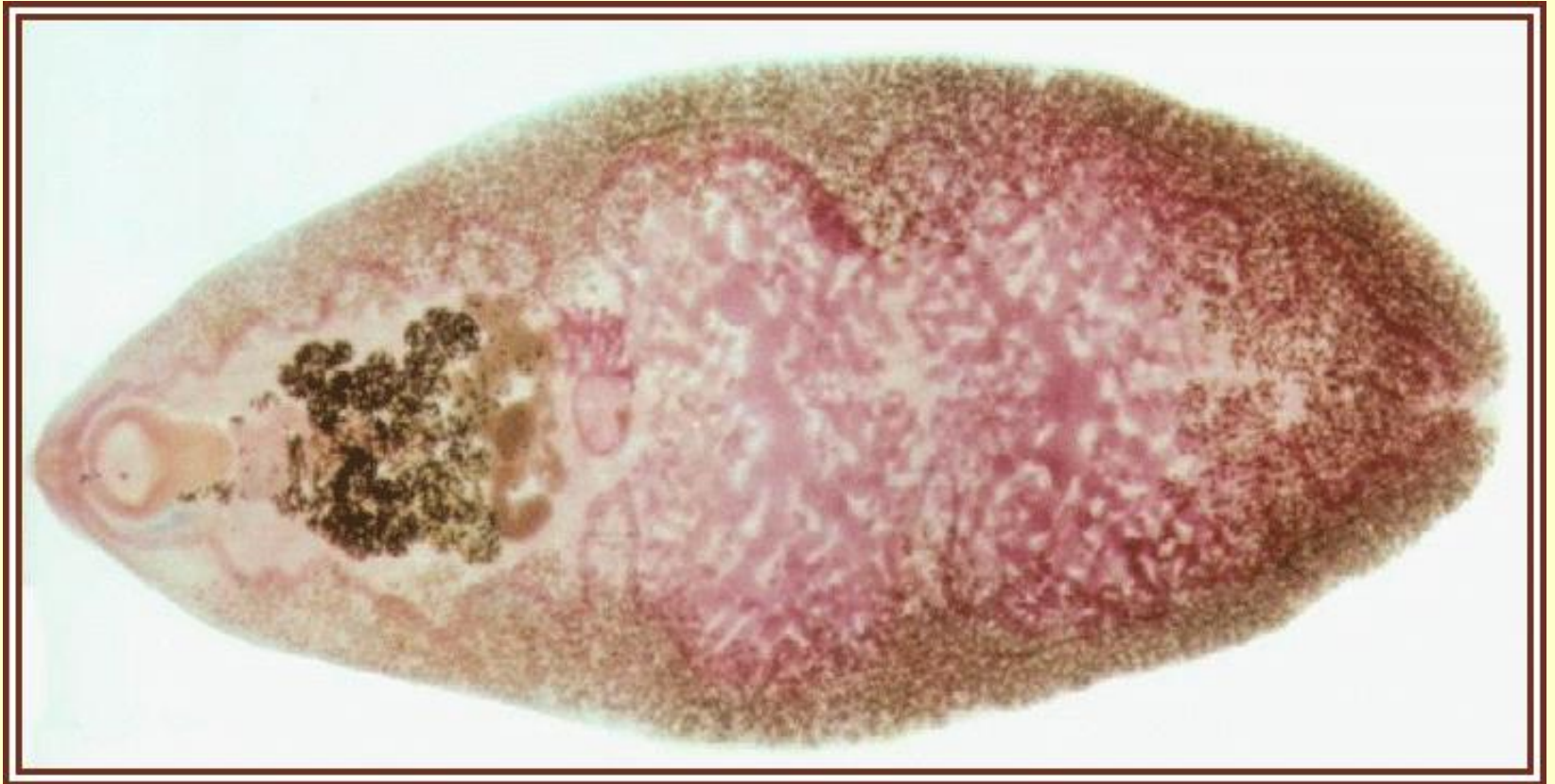
Fasciola hepatica Epidemiology

- liver blockage and watercress consumption
- Prevention - Eschewing (shunning or avoiding) watercress.
- Rabbits are probably important in spreading
- In some parts of southeastern United States, it is important parasite of domestic animals

Fasciolopsis buski - Intestinal fluke of man

- large fluke infects man when he ingests metacercaria found on vegetation including water chestnuts, bamboo, and water caltrop.
- eat these raw or peel or crack with teeth.
- elimination of feces (human and animal) into water and use of night soil for farming

Intestinal Fluke



Other Helmithic Parasites

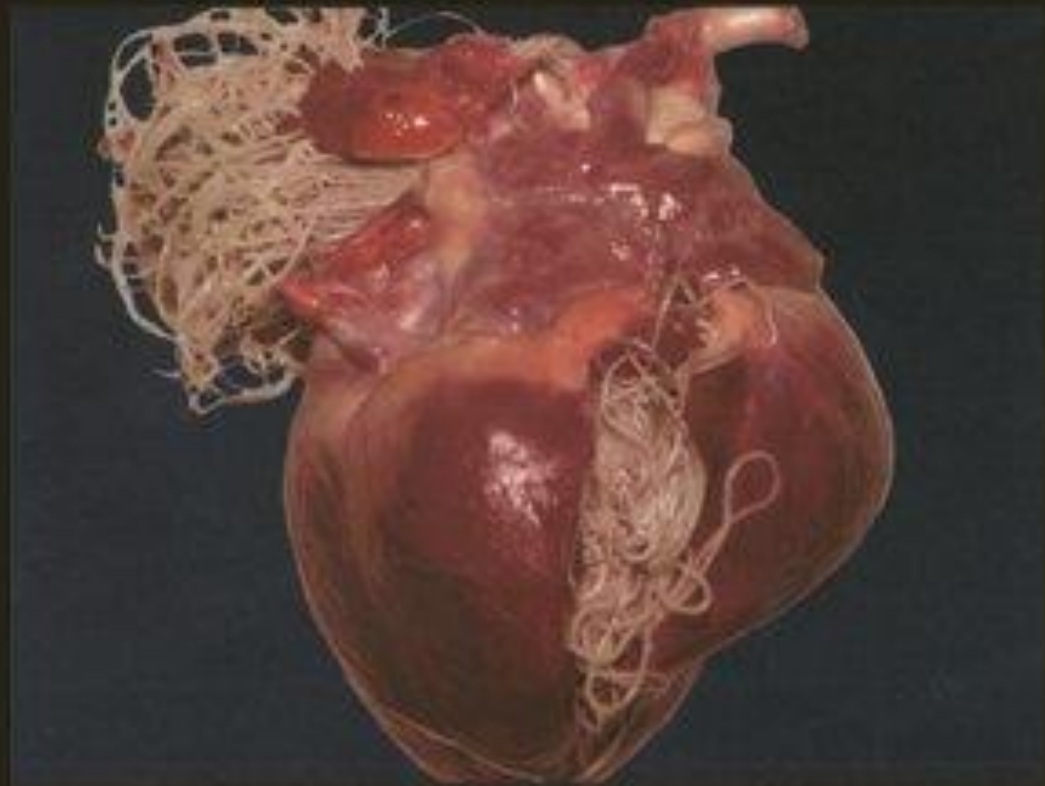
- *Baylisascaris procyonis* (Raccoon Round Worm)
- Human echinococcosis (hydatidosis, or hydatid disease)
 - caused by the larval stages of cestodes (tapeworms) of the genus *Echinococcus*
- Hymenolepiasis (*Hemnolepis nana* and *dimnuta*) (dwarf and rat tapeworms)
- *Dipylidium caninum* (dog tapeworm)

Filariasis

- Eight Species in Humans
- *Wuchereria bancrofti* and *Brugia malayi* cause lymphatic filariasis
- *Onchocerca volvulus* causes onchocerciasis (river blindness)
- *Loa loa*, *Mansonella perstans*, *M. streptocerca*, *M. ozzardi*, and *Brugia timori*. (The last species also causes lymphatic filariasis.)
- Vectored by insect vectors (e.g. Black Fly)



HEARTWORMS



Other Helminthic Infections

- *Angiostrongylus* spp. (rat lungworm) (nematode)
 - *A. cantonensis* (human eosinophilic meningitis)
 - *A. costaricensis* (intestinal angiostrongyliasis)
- *Anisakis simplex* and *Pseudoterranova decipiens* (Anisakiasis) (nematode)
- *Capillaria* spp. (nematode)
 - *C. philippinensis* (abdominal)
 - *C. hepatica* (liver)
 - *C. aerophila* (lung)
- *Clonorchis sinensis* (Chinese or oriental liver fluke)
- Gnathostomiasis (nematode)
 - *Gnathostoma spinigerum* and *Gnathostoma hispidum*

Other Helminthic Infections

- Opisthorchiasis (trematode)
 - *Opisthorchis viverrini* (Southeast Asian liver fluke) and *O. felineus* (cat liver fluke)
- Paragonimiasis (trematode)
 - *Paragonimus* spp.
- Diphyllobothriasis
 - *Diphyllobothrium latum* (Fish tapeworm; largest human tapeworm) (cestode)
- Heterophyasis
 - *Heterophyes heterophyes* (trematode)
- Metagonimiasis
 - *Metagonimus yokogawai* (smallest human fluke)