Hookworms, Trichura, & Strongyloides

Unholy Trinity

- The Soil-Transmitted Helminths
  - *Ascaris lumbricoides*
  - *Trichuris trichiura* (whipworm)
  - *Necator americanus*, *Ancylostoma* duodenale (hookworms)

Hookworm Disease

'There is…Scarce blood enough in all their sticky veins

To give each naked curtleaxe a stain.'

William Shakespeare *(1564-1616),*
Henry V, IV, ii

'Her anaemia made her rather short of breath, and she held her mouth

Slightly open…

Her thin lips were pale and her skin was delicate, of a faint green colour,

Without a touch of red even in her cheeks.'

William Somerset Maugham *(1874-1965),*

Historical Perspective

- Hookworm infection in humans dating back to pre-Colombian times in the New World and Pharaonic times in the Old World.
- 1909 the Rockefeller hookworm eradication campaign was started in the southern USA, extended globally in 1913, and finally terminated in the 1950s.
- In 1941, Stoll's landmark paper, *This Wormy World*, provided the first global estimates of the numbers of people infected with hookworms and showed that hookworm infections accounted for an

Hookworm Disease

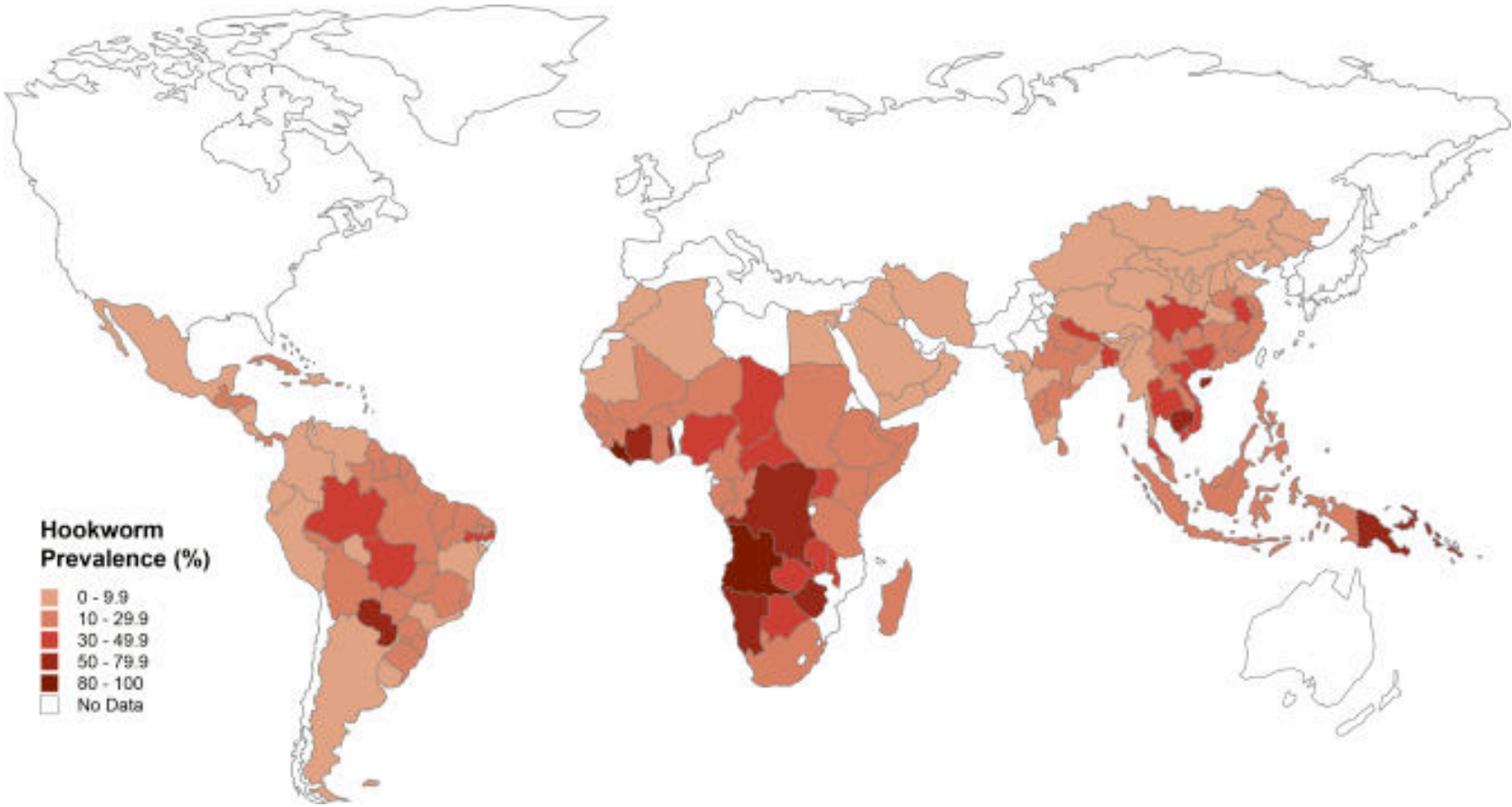
- Caused by infection by either one of the two (or both) human hookworm parasites.
  - *Ancylostoma duodenale*
  - *Necator americanus*
  - Also infection by the dog hookworm *A. caninum* has been documented to elicit eosinophilic enteritis.
- Infection causes a wide-range of signs and symptoms, most of which are related to blood loss caused by these intestinal parasites.
- Hookworm infection also known as


NO DOGS
ALLOWED
ON BEACH
MAY 1 - SEPT 30

Geographic Distribution

- *A. duodenale* and *N. americanus* are misleadingly called Old and New World hookworms, respectively.
- Both are widely distributed throughout the moist tropical and moist temperate regions of the world.
- Before control programs essentially eradicated them in several now highly developed areas, human hookworm infections occurred in the southeastern U.S. and much of Central and South America.

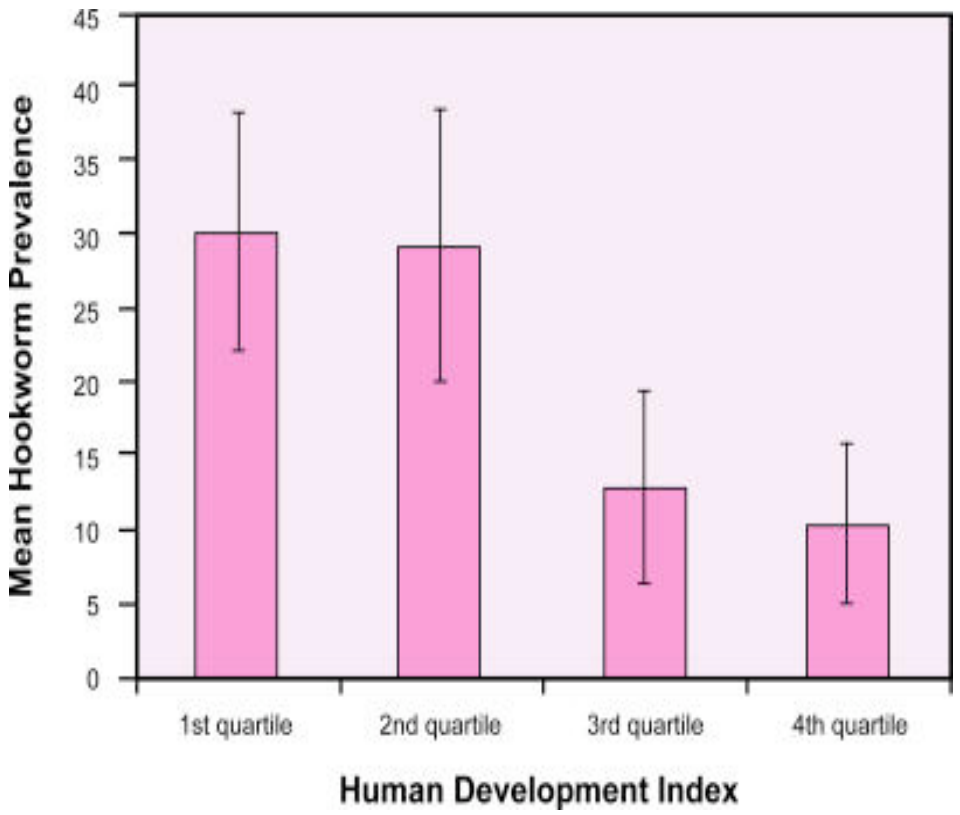
Hookworm Disease



Eradicated in the U.S., Europe, Korea, Japan, Taiwan, Australia and some of the Caribbean and Pacific Islands.

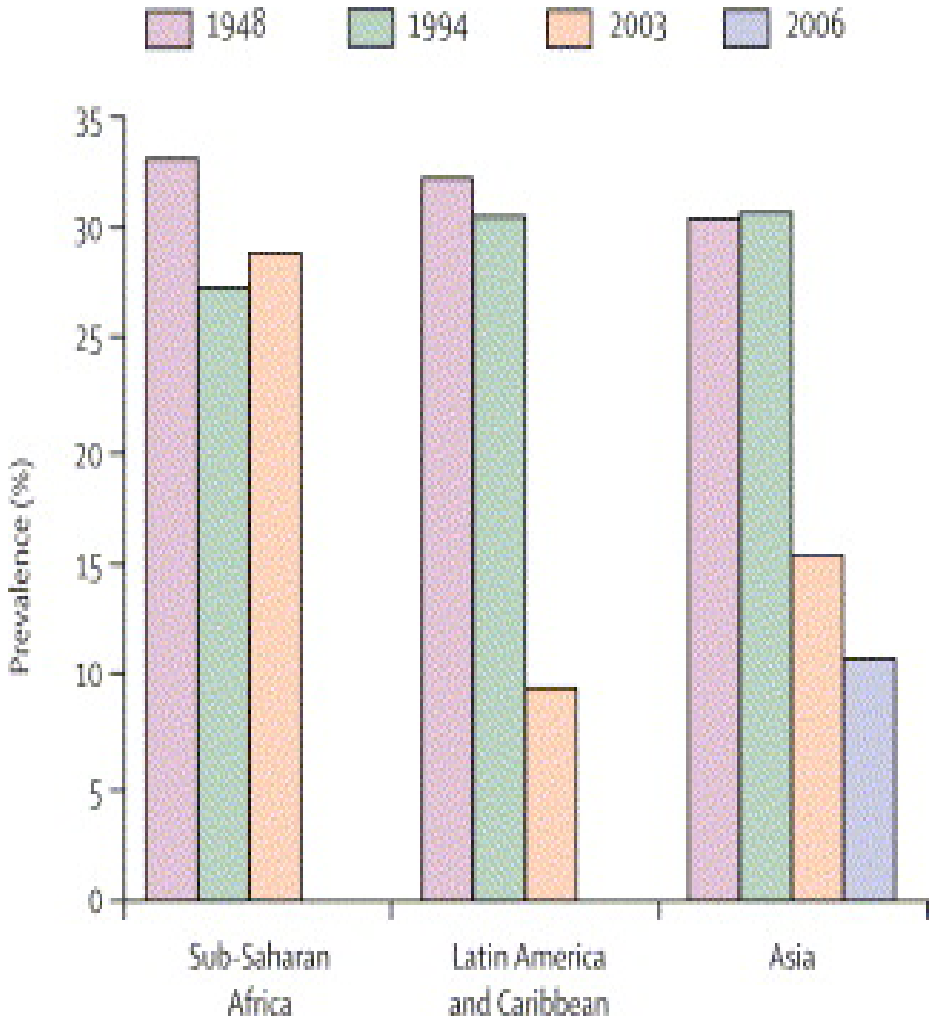
Geographic Distribution

- Striking relationship between hookworm prevalence and low socioeconomic status.
- Hookworm infections are concentrated among the world's poorest 2.7 billion people who live on less

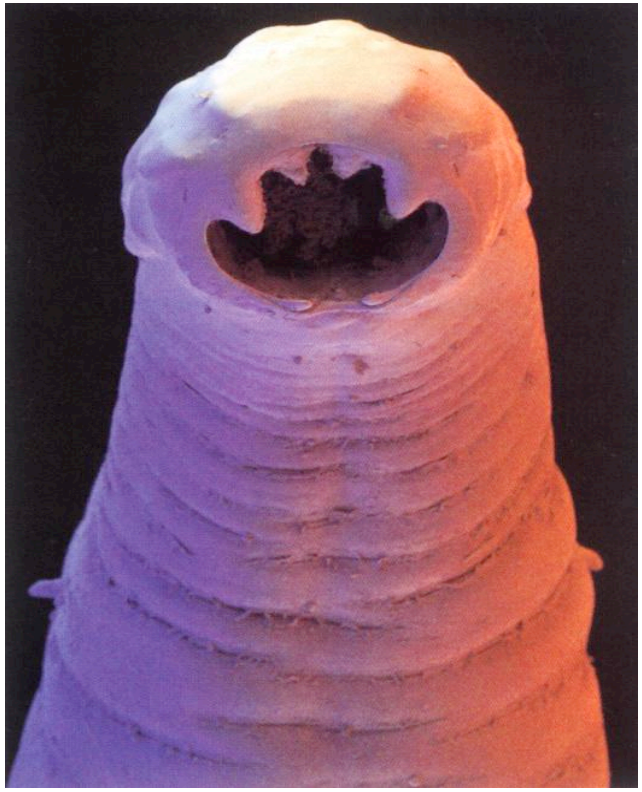


Hookworm Prevalence by Region over Time

- Although there have been precipitous decrease in prevalence over time, estimated prevalence rates for sub-Saharan Africa are equivalent to those first estimated by Stoll more that 60



The Hookworms-Intestinal Parasites

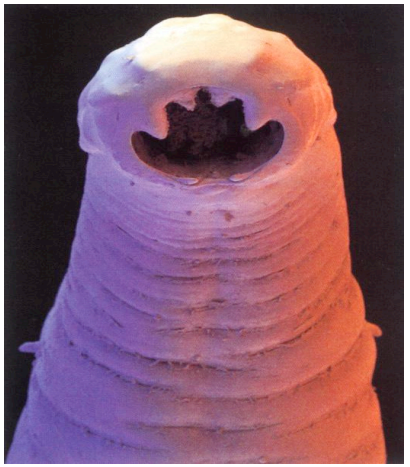


A. duodenale

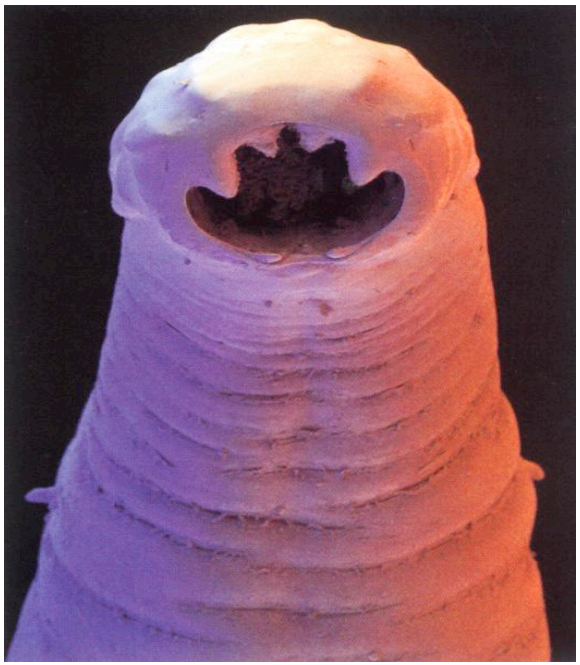
N. americanus

The Hookworms

- Both species affecting humans are relatively short, bursate nematodes, *i.e.*, male and females have terminal copulatory bursa.
- In the opening of the mouth there are structures designed to lacerate tissue.
- *A. duodenale*-two pairs of teeth.
- *N. americanus*-cutting

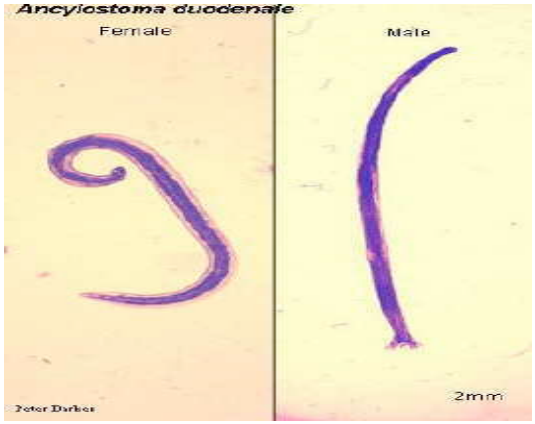


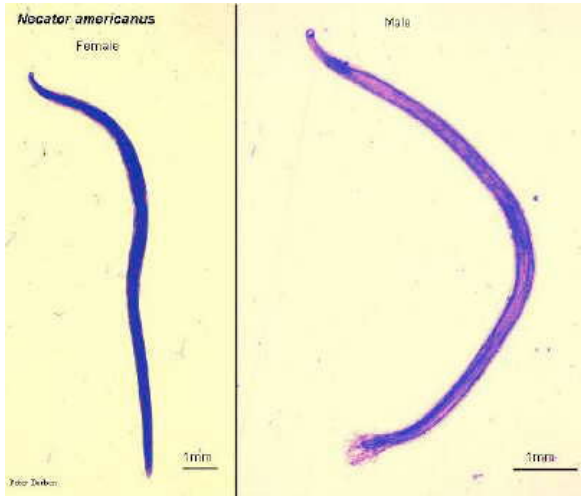
The Hookworms



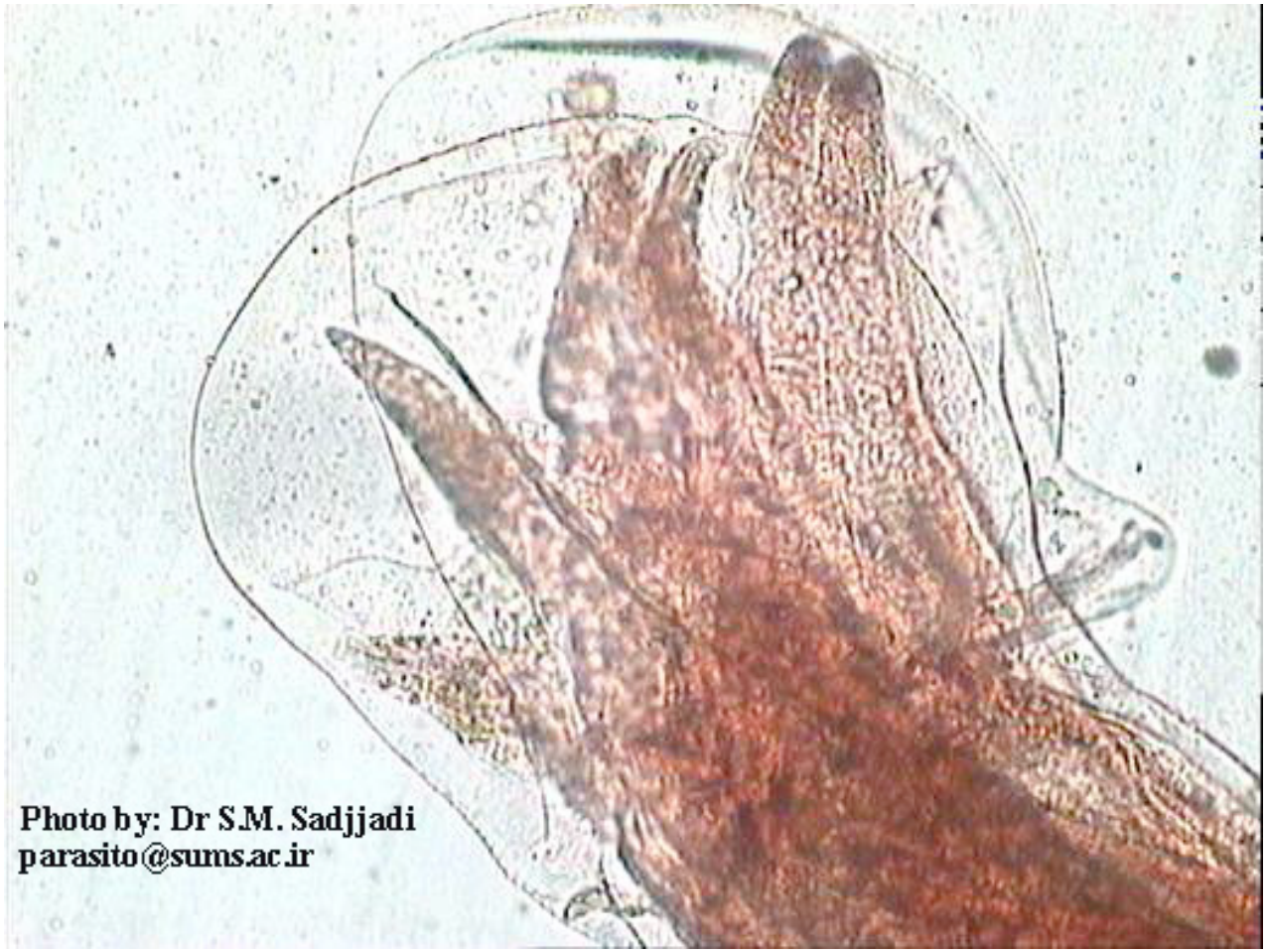
The Hookworms

- *N. americanus* is more slender, longer.
- The tail of the female is conical; that of the male ends in a complex, bell-shaped copulatory bursa, which serves to hold the female during copulation.
- Males also have a pair of extrusible chitinoid copulatory spicules.



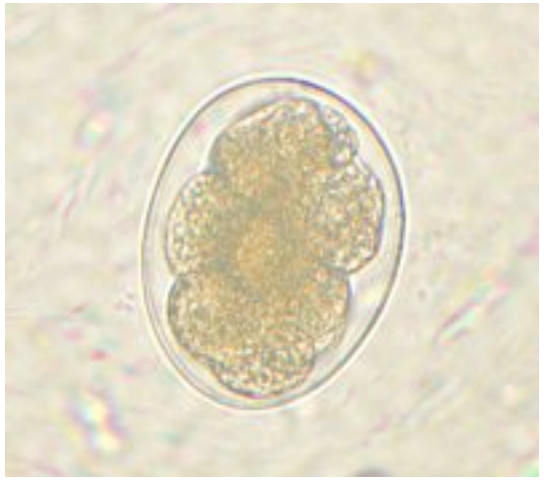


Bursa: *N. americanus*



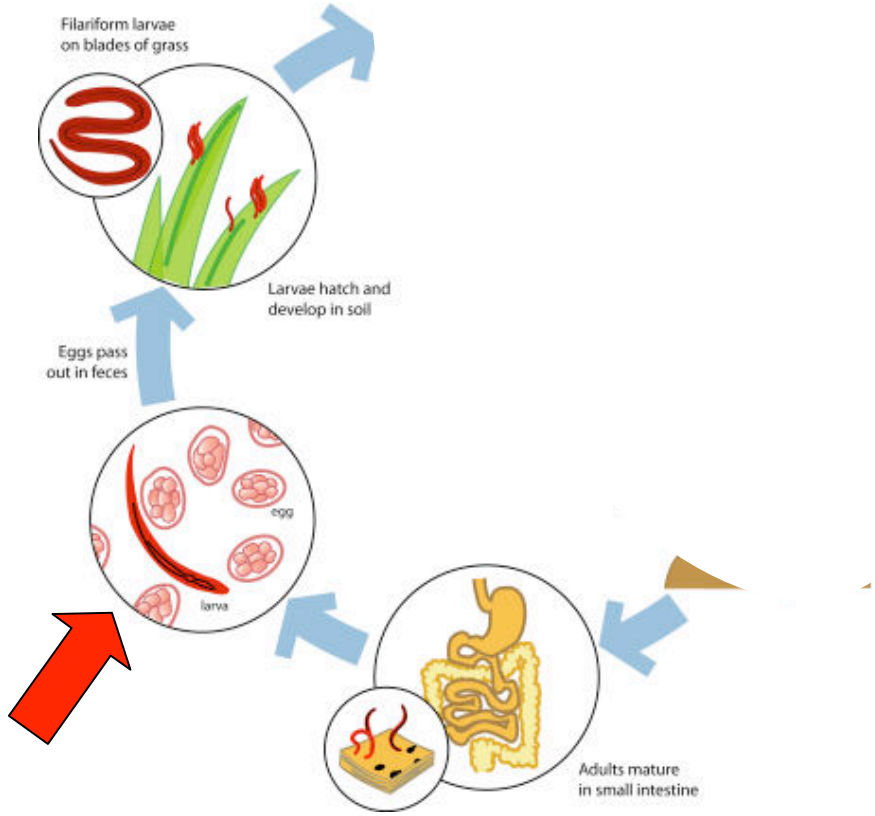
N. americanus

- The eggs, which measure 60 x 37 µm and 70 x 39 µm in *A. duodenale* and *N. americanus*, respectively, have a thin, small, colorless shell.
- The ovum passes in the feces has usually undergone two cleavages and is therefore in the four-celled stage.



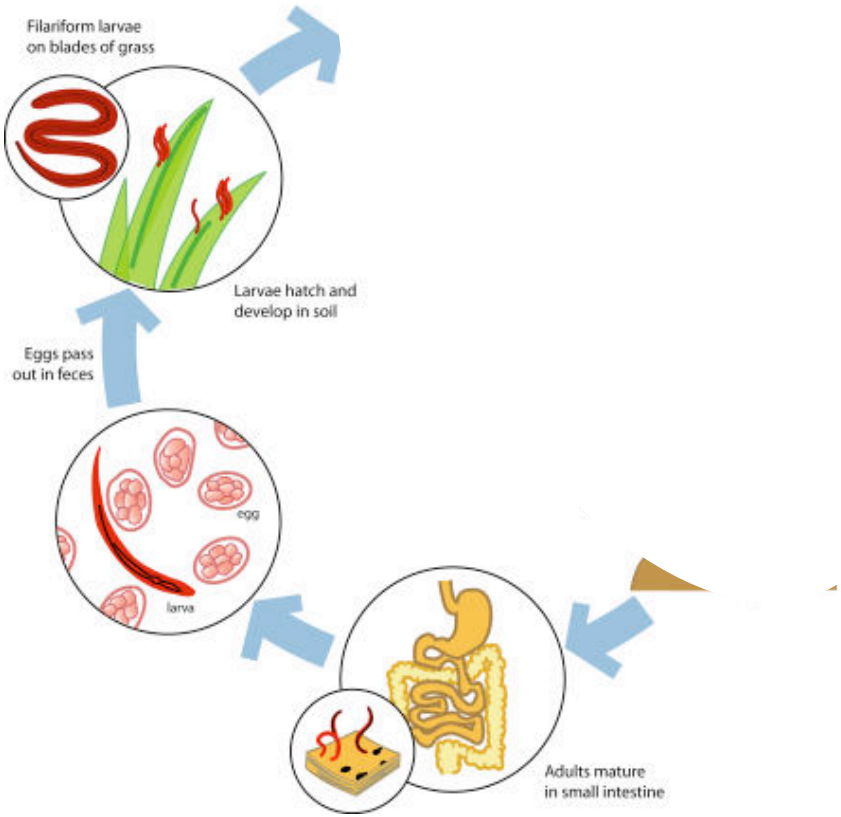
Life Cycle

- Eggs hatch in the soil.
- Vermiform larvae hatch.
- 2 growth stages.
- The infective 3rd stage: filariform larvae or L3.



Life Cycle

- Filariform larvae are ensheathed in the incompletely molted cuticle of the preceding stage.
- Highly resistant to environment.
- Susceptible to desiccation.
- Larva need moisture for survival and movement.
- If they dry; die in minutes.



Filariform larvae need moisture

- Endemic hookworm infection is only in those parts of the world where soil moisture is adequate.
- Need 100-125 mm rain/month (4-5 rainy days/week).

Adaptation for Infection

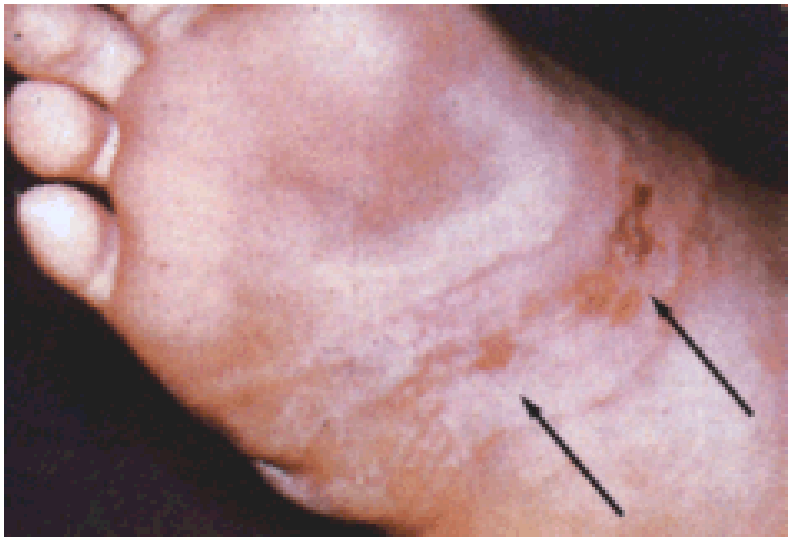
- Infective larvae migrate to the surface (from soil).
- They 'rest' positioned for host contact.
- Several larvae may reach the same micropromineence, creating a *Pilobolus*-like

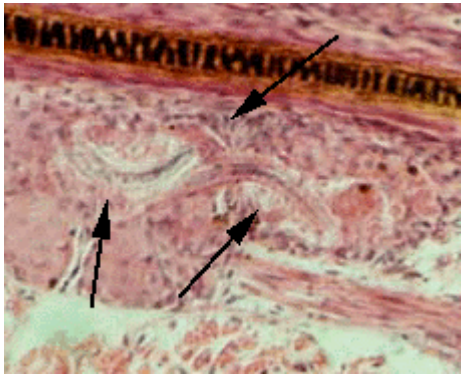
Adaptation for Infection

- Filariform larvae respond to vibrations, CO_2, warmth, and touch.
- Transfer to host in response to a chain of these signals.
- Skin, lipids, and serum constituents elicit penetration and feeding response.

Skin Penetration

- After skin penetration the larvae produce proteinases and a hyaluronidase.
- Results in severe itching and erythema (with in hours).
- Moderate lymphocytic perivascular infiltrates.
- Affected by degree of sensitization (means?).
- 'Ground itch' or 'dew



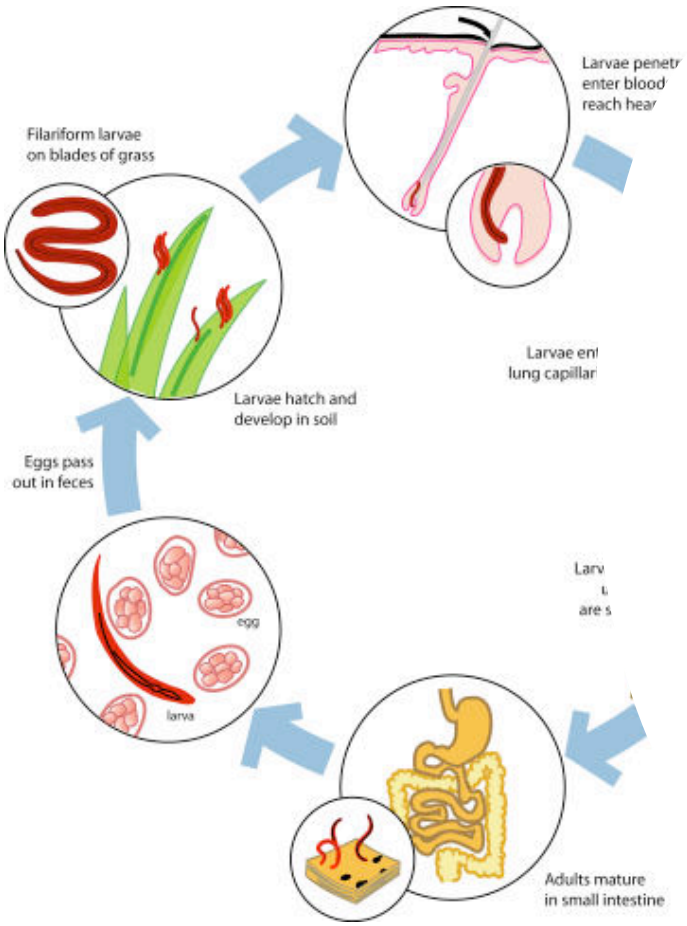


Skin penetration

- Experimental hookworm dermatitis in a volunteer exposed to larvae of *N. americanus*.

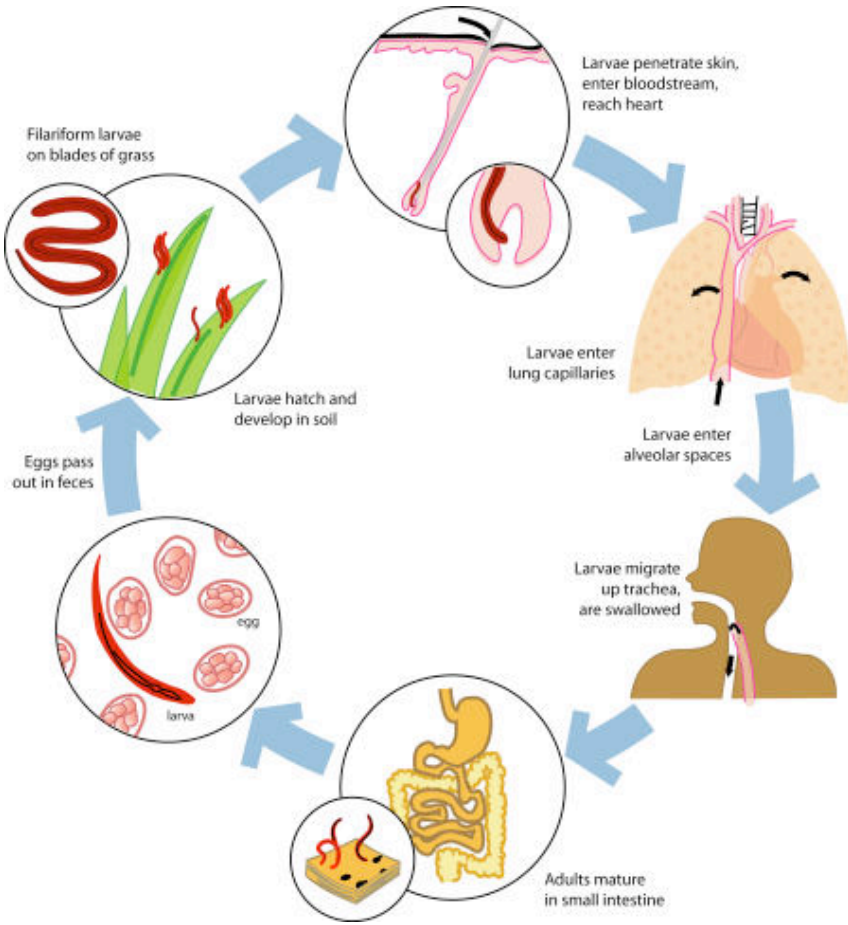
Skin, Blood…

- Circulation takes larvae to the…



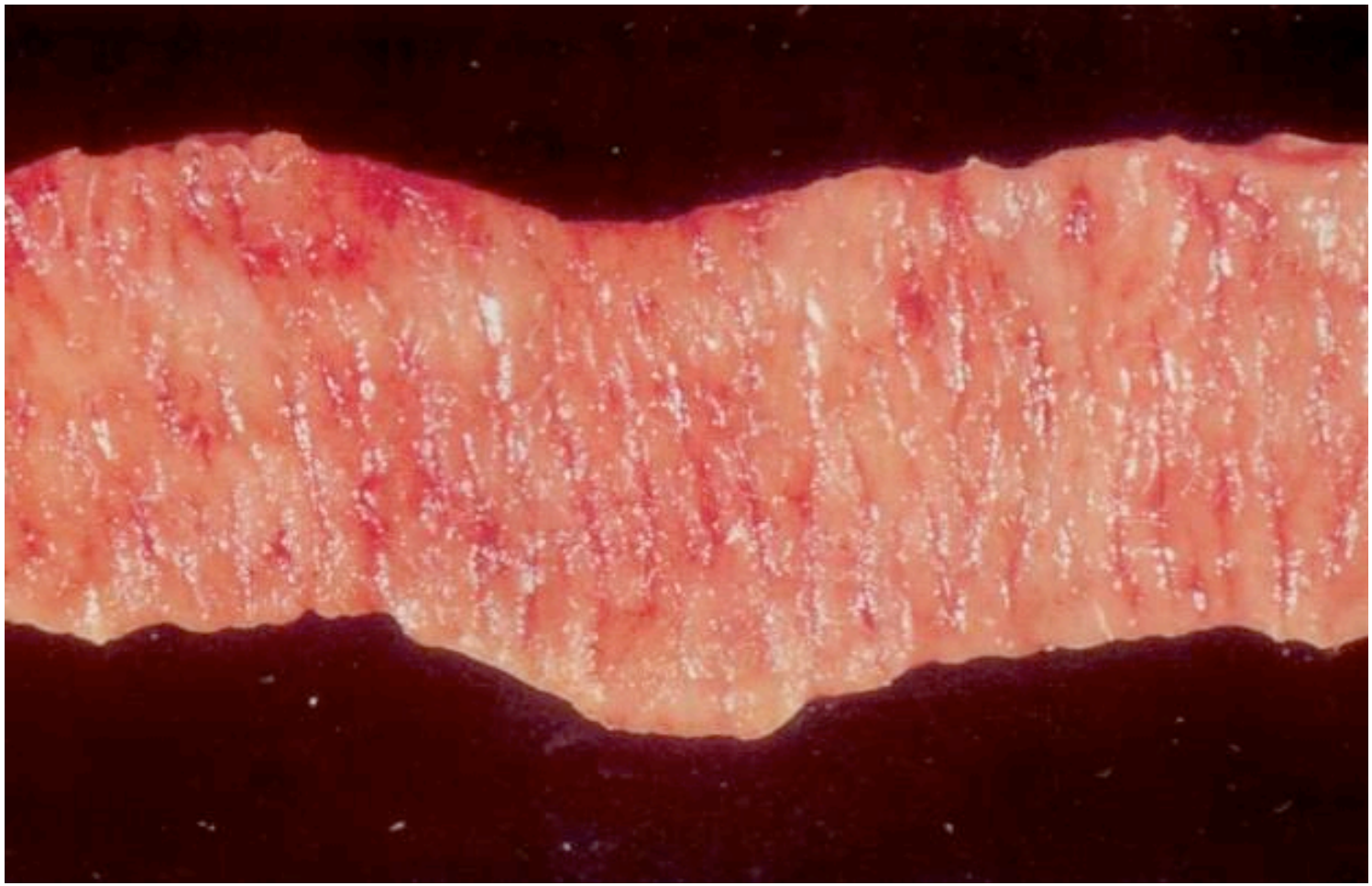
Skin, Blood, Lungs…

- Circulation takes larvae to the lungs.
- Forceful exit from the capillary bed causes microhemorarhages.
- Enter alveoli.
- The migration continues via the "mucociliary (tracheal) escalator" to the larynx.
- Successful larvae are swallowed.
- Both 4th stage larvae and adults have buccal capsules.
- Capsules allow parasites to suck in villi and to feed on host tissue and blood.
- 10,000-25,000 eggs/day.



Feeding Hookworm

Damage to the Intestine



Life Cycle Differences

- Similar for both *N. americanus* and *A. duodenale*, however, has a few unique features:
  - If swallowed, it will establish an infection (stomach will not act as a barrier).
  - Alternative developmental pathways are possible…

A. duodenale…'just have to be me'

- Larvae entering orally can either establish infection or remain as L3 larva.
  - These larva serve 2 functions:
    - Survival stage ("dauer larva"), lying dormant in the host during seasons when external ecologic conditions are unfavorable (*e.g.*, dry season).
    - Act as a source of infection for a new generation of susceptible hosts (*e.g.*, breast-feeding infants of infected mothers)

A. duodenale…

- Considered to be more virulent of the two species because:
  - Larger.
  - Causes more blood loss.
  - Produces more eggs (~2500/day).
  - Several modes of transmission.

Clinical Features-Lung

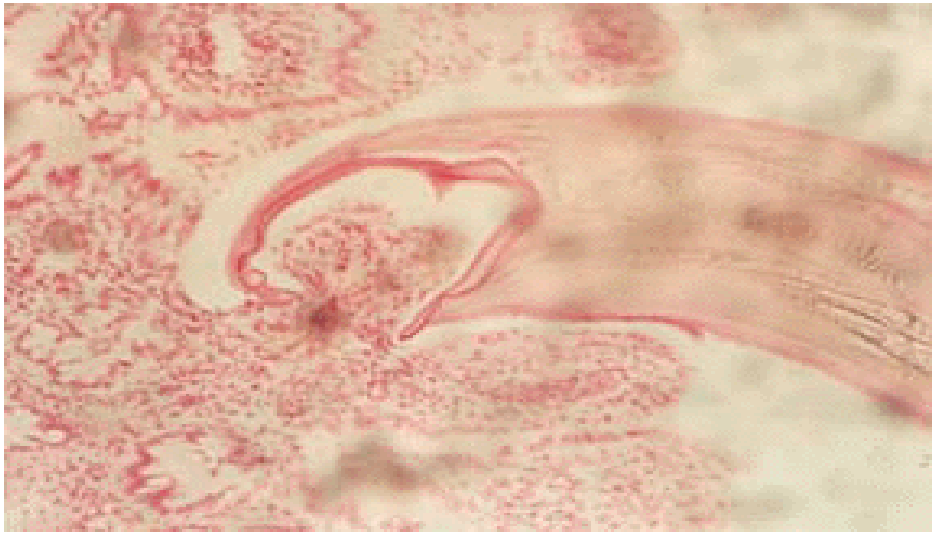
- Ground itch equivalent; short-lived punctuate hemorrhages at the site of penetrations.
- Hyper-pigmented macules lasting months.
- When dermatitis starts to wane, patients may develop paroxysmal shortness of breath with profuse mucus production resembling asthma.
  - Cough, bronchitis, and pneumonia (weeks-months)
- Examination of sputum will often reveal occult blood and eosinophils and occasionally migrating larvae

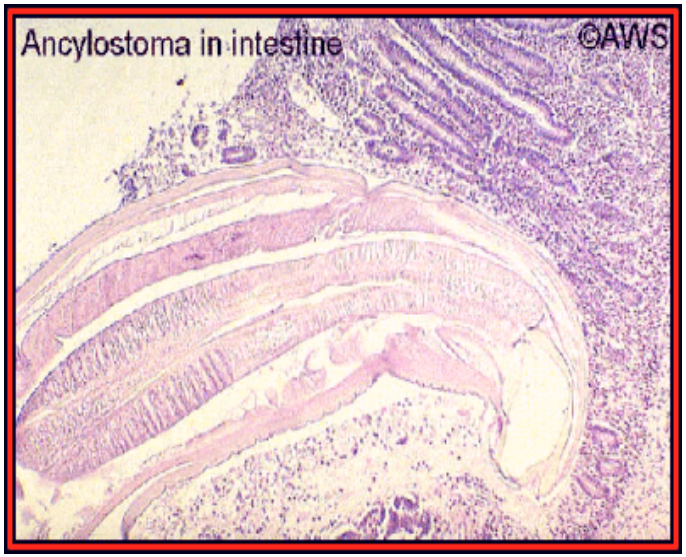
Clinical Features-Intestine

- As the pulmonary symptoms wane, there may be gastrointestinal manifestations related to adult worms in the small intestine.
  - Bloating, nausea, constipation, abdominal pain and rarely diarrhea.
- Gastrointestinal symptoms difficult to diagnose in the tropics because these ailments could be caused by so many other pathogens.

Clinical Features-Intestine

- Long standing infections=anemia.
  - Weakness, lassitude
  - Dizziness
  - Shortness of breath
- Subjects may be very pale and suffer tachycardia. Why?
- Children: symptoms more sever. Can present with plasma protein loss, abdominal distention, facial





Intestinal Pathology

- *N. americanus* prefers duodenum.
- *A. duodenale* prefers the proximal jejunum.
- Worms feeding continuously.
  - After several hours, detach and seek another villus.
  - Blood loss is from sucking and leaking of damaged villi (150 µl/worm/day).

Blood Loss vs. Worm Burden

Worm Burden	Eggs	Mean Blood Loss/Day
• 0	0	1.24 ml
• Light	1-999	1.46 ml
• Moderate	1000-4999	2.96 ml
• Heavy	>5000	8.79 ml

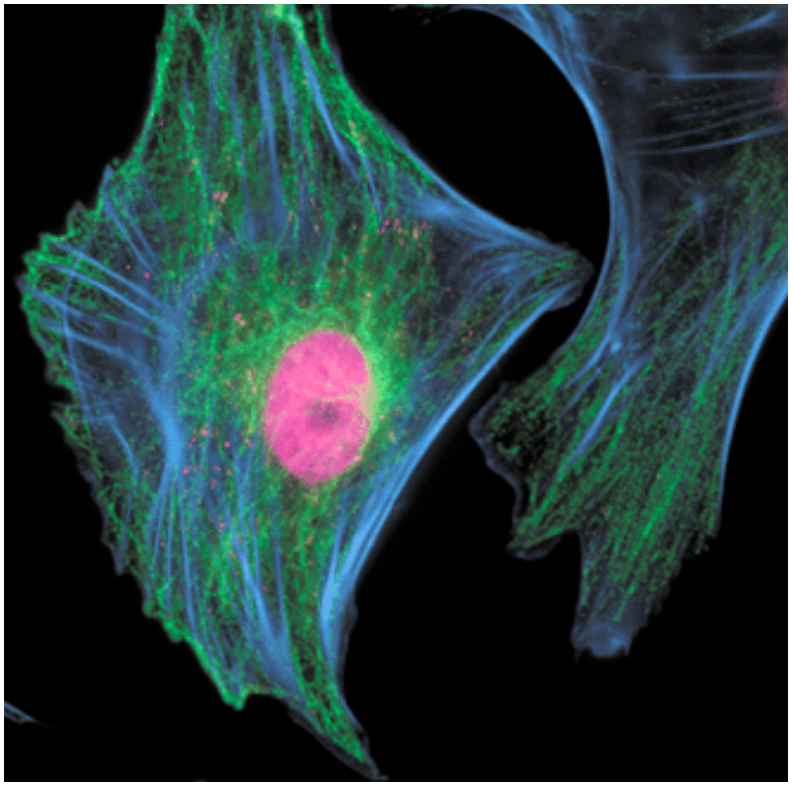
- Heavy=40-160 worms.
- N=50 for each group

Diagnosis/Treatment

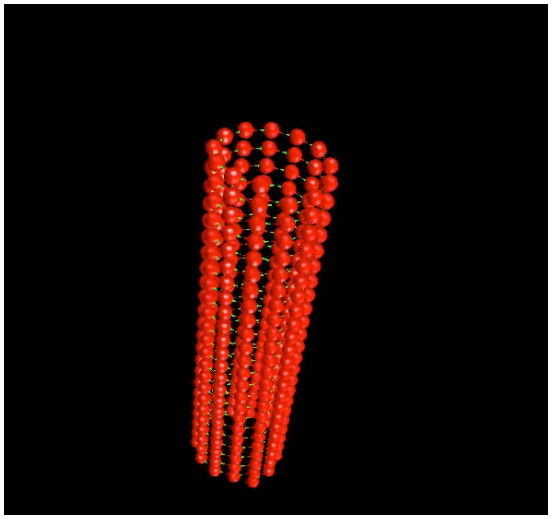
- You guessed it! Eggs in feces.
  - Quantification of eggs/gram allow for estimating worm burden.
- Eggs will hatch within 24 hours in stool if specimens kept at room temperature.
  - Can tell species apart only by comparing larvae.
- Mebendazole and albendazole.
- Drug-resistant hookworms reported.
  - Tubulin alleles.

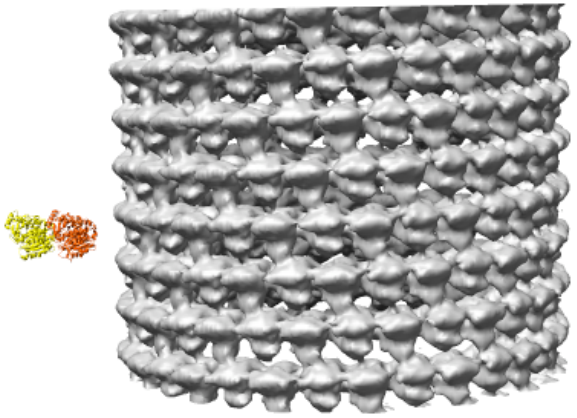
Alleles

Stain for Tubulin (Green)



Tubulin molecule





Prevention

- Sanitary disposal of feces.
- Health education.
- Encouraging the use of shoes.
  - Not working however because…
    - *A. duodenale* infect via ingestion too.
    - *N. americanus* can penetrate all aspects of the body.
    - High occupational exposure rate.
    - Use of human feces for fertilization.

Vaccine?

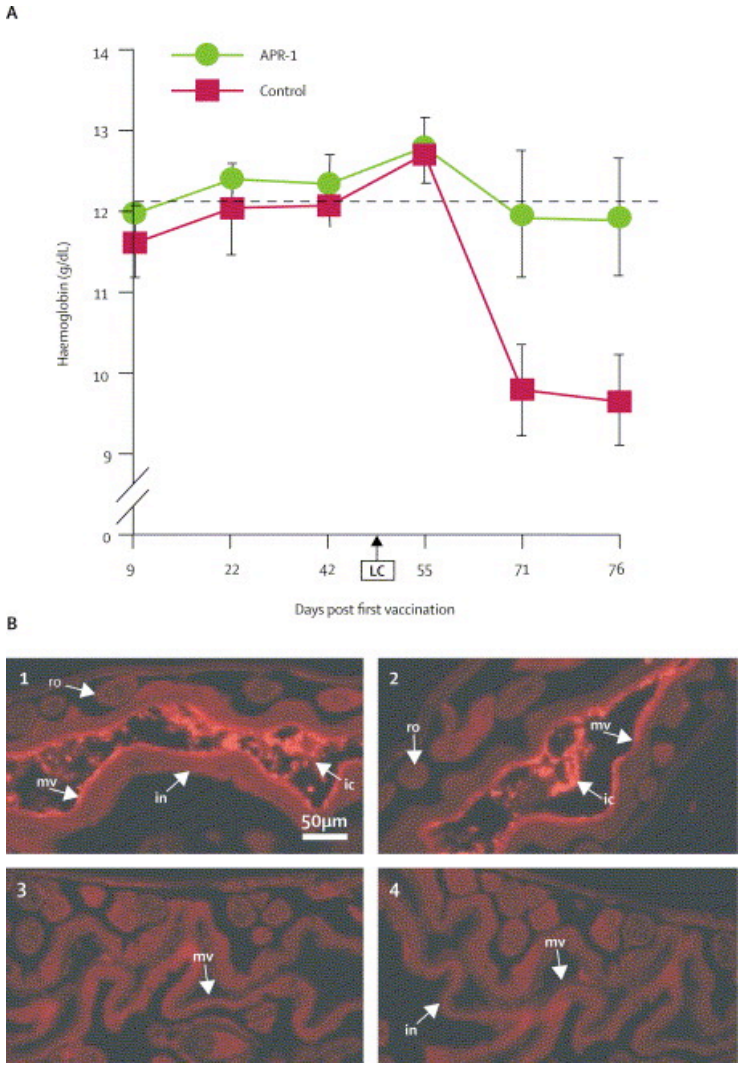
- Infection elicits poor immunity *i.e.*, reinfection very common.
  - Opposite to other STHs in that infections increase with age.
- Hematophagous lifestyle may prove their downfall.
- The hookworm is armed with an array of molecules essential for blood feeding and digestion (anticoagulants

Vaccine

- Recent study using one of these proteases (haemoglobinase) protected against blood loss in an animal model of hookworm infection.
- Antibodies to proteins that the worm needs to feed could neutralize their activity and effectively starve the worm.

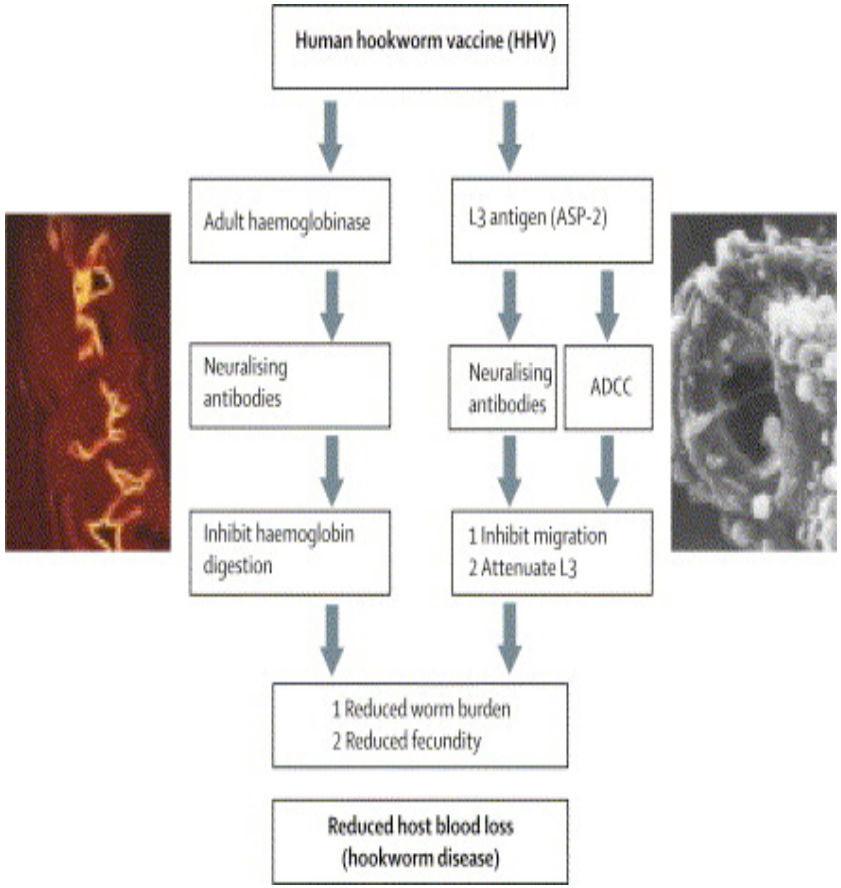
Haemoglobinase vaccines interrupt blood feeding

- **Anti Ac-APR1 (aspartic protease-1) reduces anaemia.**
- **Antibodies from vaccinated dogs bind to the *A. canium* gut (bottom 2 frames) but not antibodies from control dogs (top 2 frames).**
    - **Loukas *et al.*, The Lancet, 6:733. 2006.**



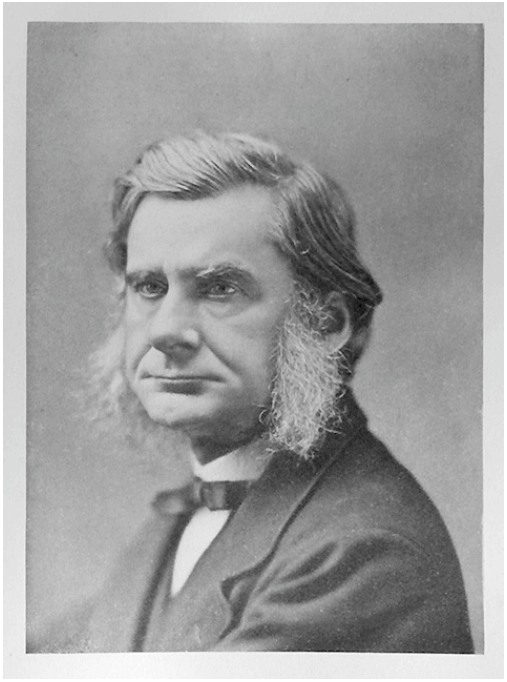
Roadmap for the development of a bivalent human hookworm vaccine.

- **Combination of recombinant haemoglobinase and L3 antigens.**
- **Overall goal would be to significantly reduce morbidity.**



Trichuris trichiura
(Whipworm)

'I cannot but think that he who finds a certain proportion of pain and evil inseparably woven up in the life of the very worms, will bear his own share with more courage and submission.'



Thomas Henry Huxley (1825-1895)

Trichuriasis: Definition & Distribution

- Trichuriasis is parasitization of the colon by *Trichuris trichiura*.
  - Related to *T. spiralis*.
- Common infection in the warm, wet parts of the tropics.
- 500 million people are infected.
- 90% prevalence in some tropical countries.

Morphologic Features

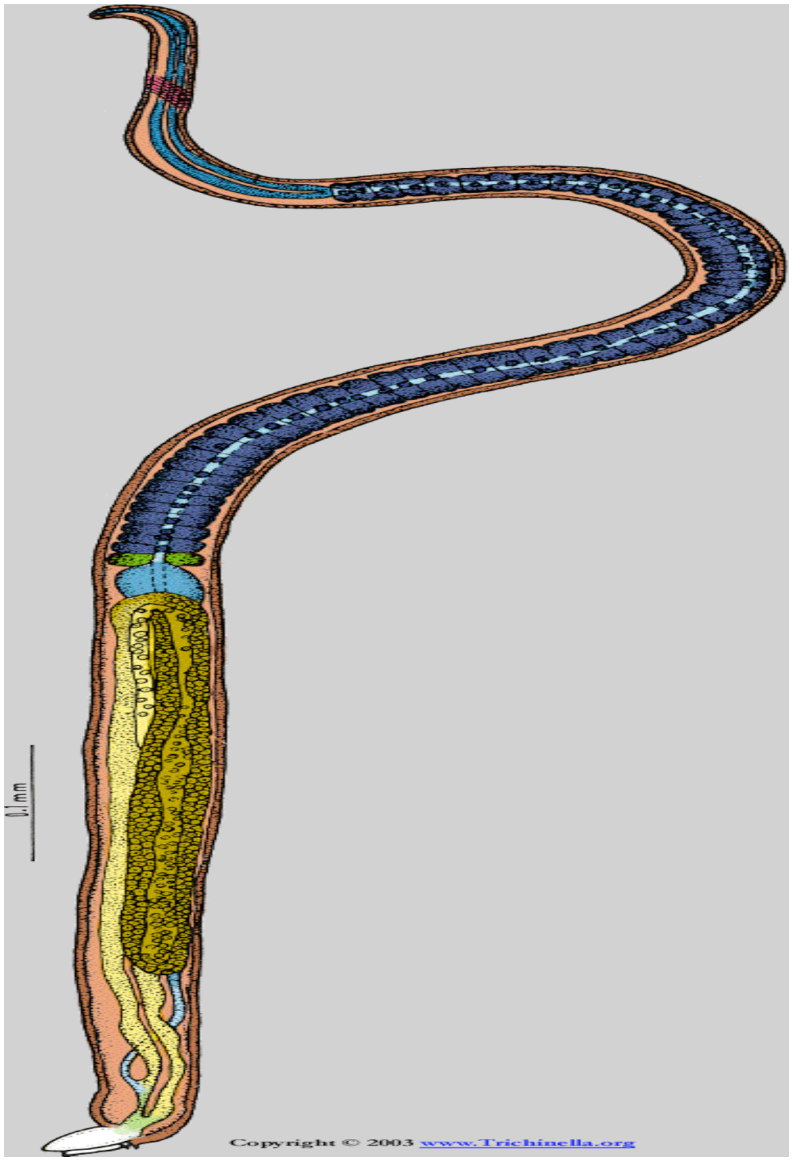
- Adult worms resemble a whip.
- Hairlike anterior part (the lash) makes up 3/5 of the length.
- 'Lash' comprised of large glandular cells called stichocytes.

30-45 mm long

35-50 mm long

Stichocytes

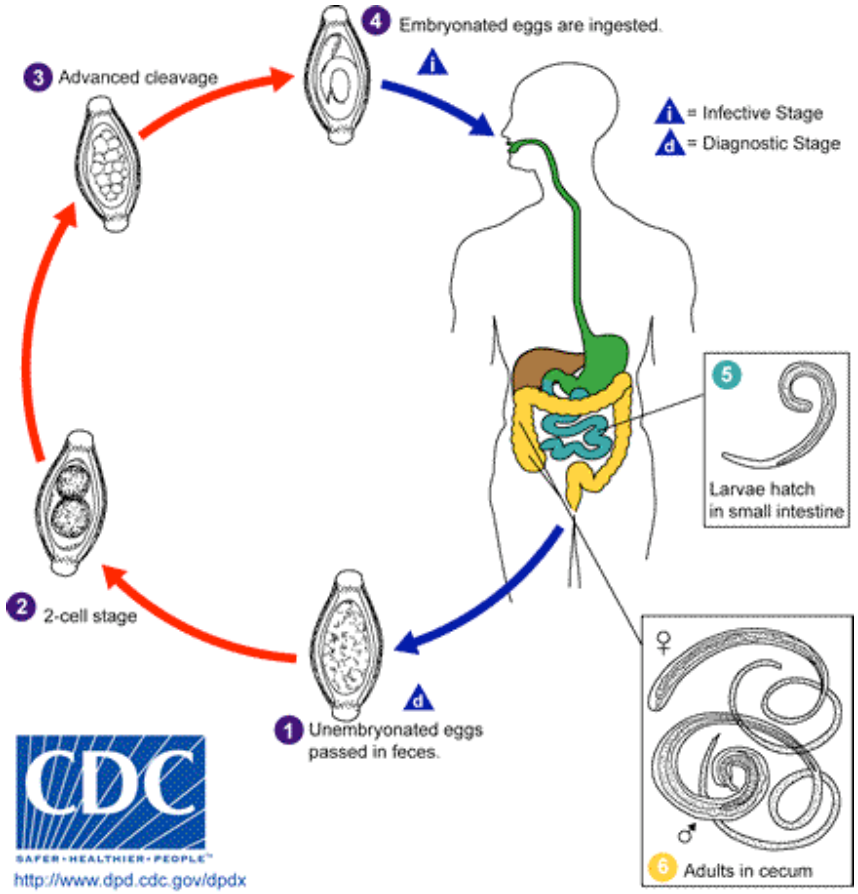
- Partially or completely enclose the esophagus.
- Mouth is simple (no lips).
- Stylet is present.



Stichocytes

Life Cycle

- Soil-transmitted.
- Females lay 2000-10,000 eggs/day.
- Adult worms live an average of 3 years (up to 8).
- Eggs take weeks to months to develop in soil.



Life Cycle

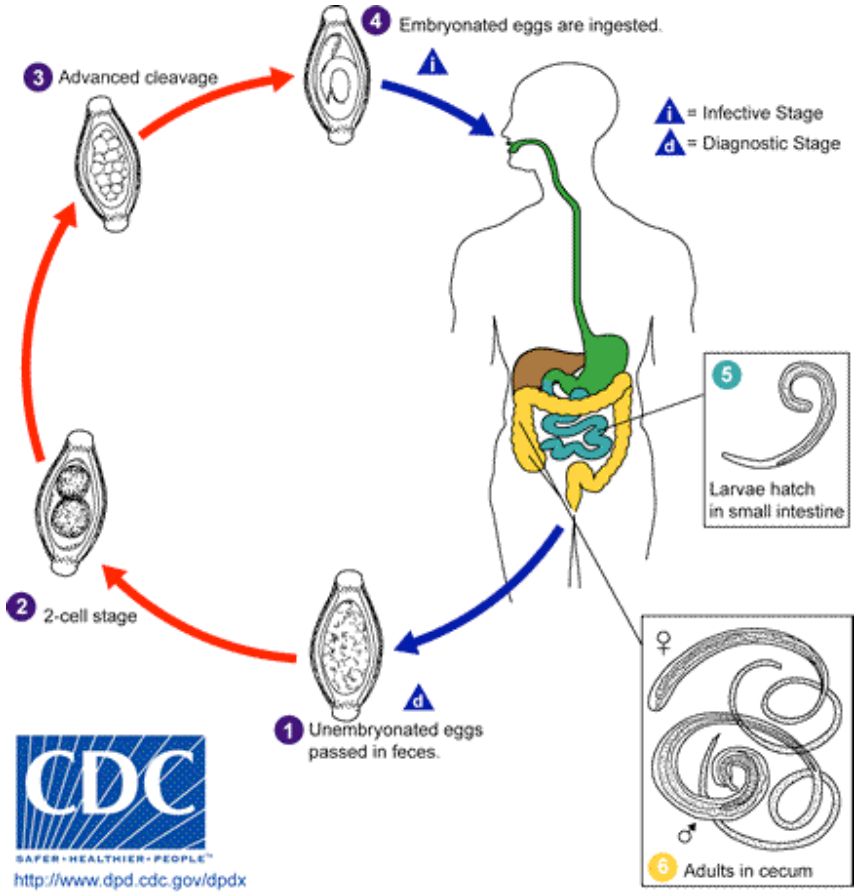
- Infective stage of egg contains the first-stage larva.
- Infection requires swallowing eggs in food or water contaminated by feces.

T. trichiura egg

- **Note the distinctive bipolar plugs.**

Life Cycle

- Larvae hatch in the small intestine.
- Penetrate the villi where the coil up and develop for about 1 week.
- Reenter the lumen of the cecum.
- 4 molts=Adult
- Egg laying begins 2 months after eggs



Terminal ileum: Infection

Clinical Manifestations

- Most patients asymptomatic.
- Clinical disease occurs mainly in children.
- Heavy infections characteristic of diarrhea, distention, flatulence, abdominal pain, headache, anorexia, tenesmus, weight loss, and sometimes rectal prolapse (toxic irritation of nerve

Clinical Manifestations

- Prolapse more common in undernourished children.
- A worm burden of 200-1000 can cause anemia from petechiae caused by probing stylets.
- 400 worms=4 ml blood/day.
- Dehydration and anemia important factors in fatal cases.
- Appendicitis if worms migrate to appendix.

Immunity & Pathogenesis

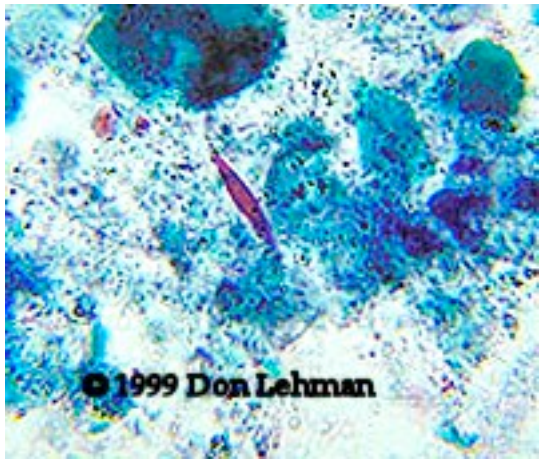
- Susceptibility to heavy Trichuris infections may depend on an inability to mount strong Th2 responses.
- Structural defects to the epithelium.
  - Release pore- and channel-forming protein.
  - Facilitate invasion and enable the parasite to maintain its syncytial environment.
- Low-grade inflammatory response to the presence of the adults occurs but cannot account for the observation that

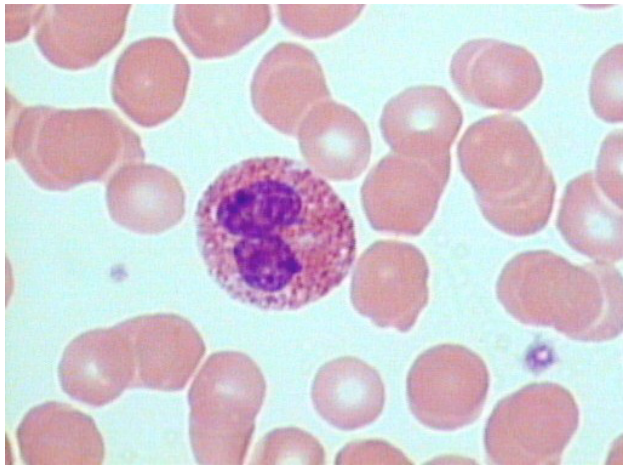
Prevention & Control

- As with Ascaris, direct sunlight for 12 hours or temperatures > 40°C will kill eggs.
- Eggs resistant to chemical disinfectants.
- Proper disposal of feces is the primary means of prevention.
- When used for fertilizer control impossible

Diagnosis/Treatment

- *T. trichiura* eggs in stool.
- Charcot-Leyden crystals in feces
- No single effective drug.
  - Mebendazole
  - Albendazole
    - Considerations during pregnancy.





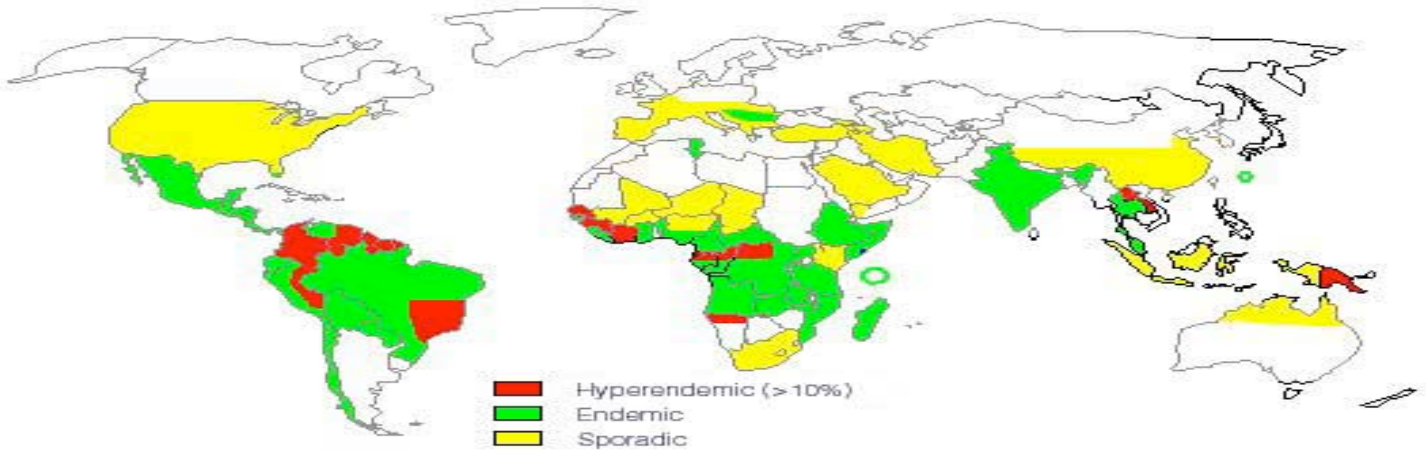
Strongyloidiasis

(*Strongyloides stercoralis*)

'Science and Peace will triumph over ignorance and war. Nations will unite, not to destroy, but to build, and the future will belong to those who have done most for suffering humanity.'

Louis Pasteur (1822-1895)

Geographic Distribution

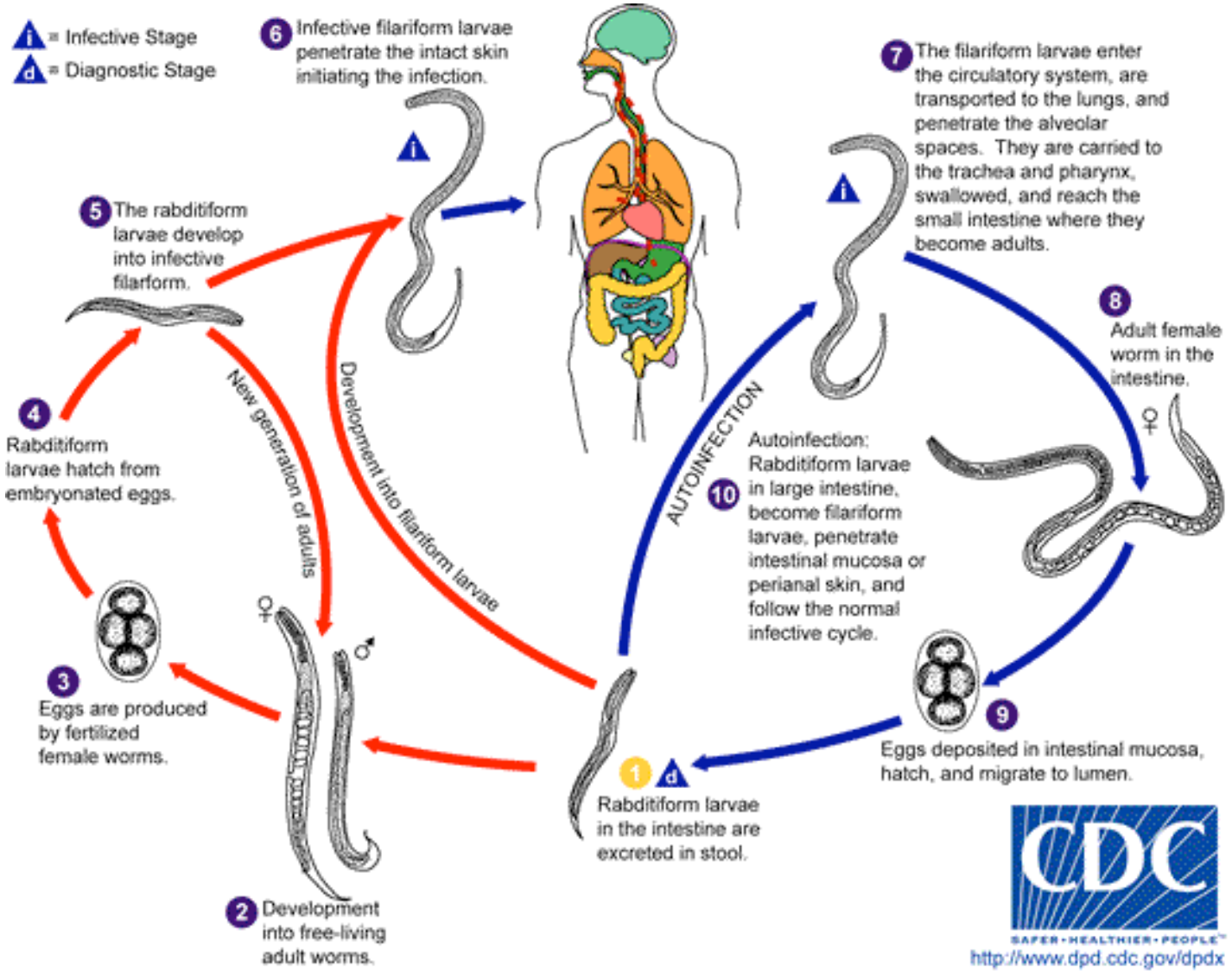


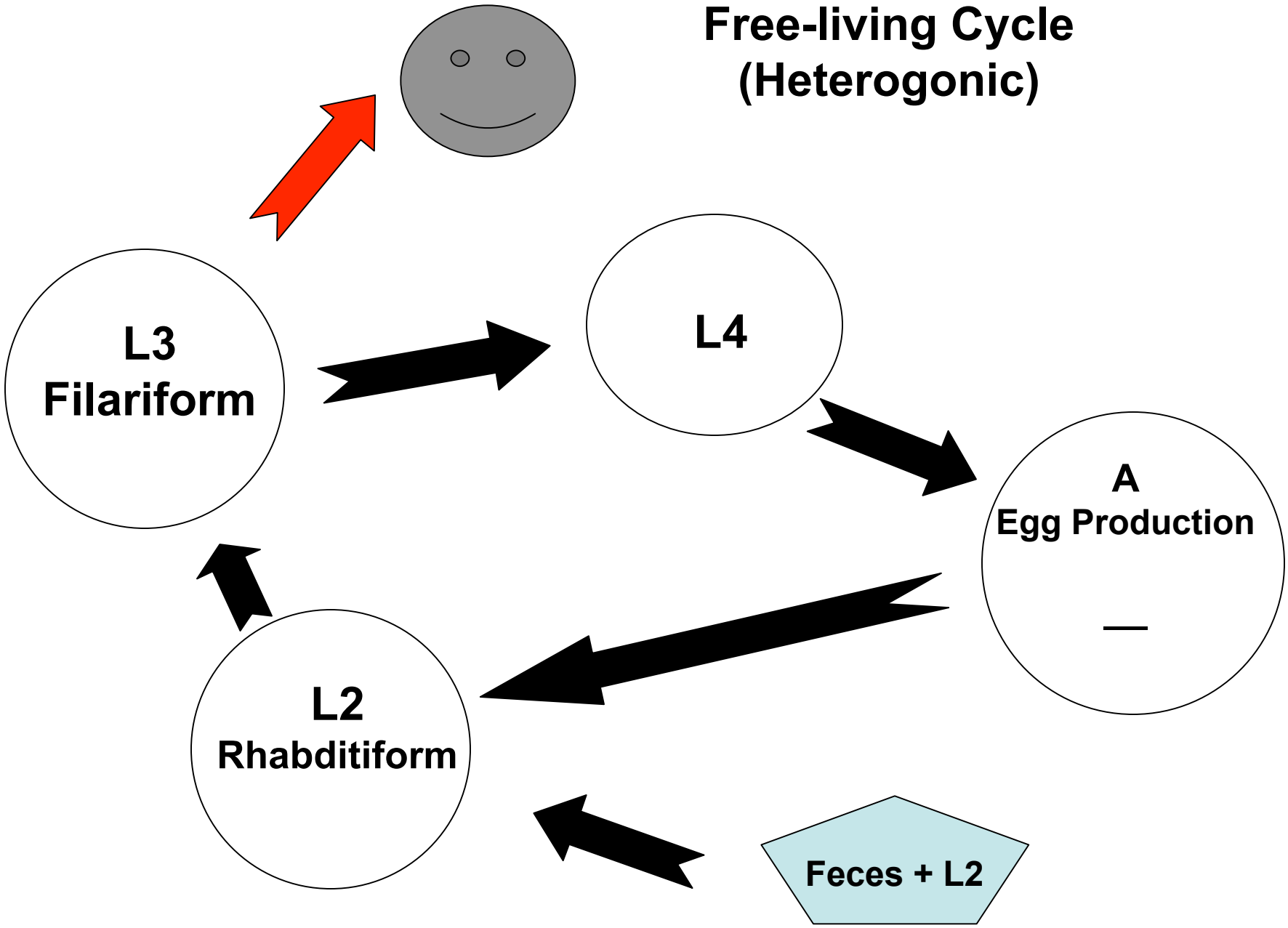
- World-wide distribution.
- Difficult to establish diagnosis because of long-lasting asymptomatic infections.
- True global prevalence unknown.
- Estimates suggest up to 100 million cases.

Targets for Infection

- Residents/immigrants of endemic area.
- Travelers to endemic area.
- Natives and residents of the Appalachian region of the US.
- Institutionalized persons.

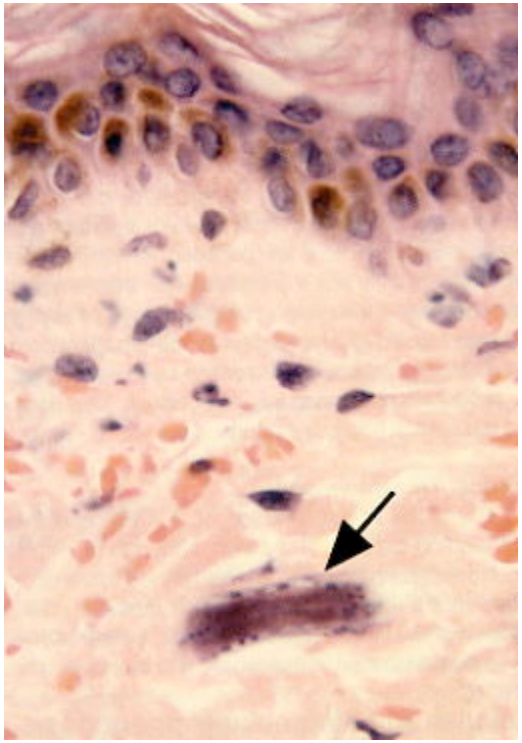
Life Cycle


i = Infective Stage
d = Diagnostic Stage
6 Infective filariform larvae penetrate the intact skin initiating the infection.
7 The filariform larvae enter the circulatory system, are transported to the lungs, and penetrate the alveolar spaces. They are carried to the trachea and pharynx, swallowed, and reach the small intestine where they become adults.
8 Adult female worm in the intestine.
5 The rabditiform larvae develop into infective filarform.
4 Rabditiform larvae hatch from embryonated eggs.
New generation of adults
Development into filariform larvae
AUTOINFECTION
Autoinfection: Rabditiform larvae in large intestine, become filariform larvae, penetrate intestinal mucosa or perianal skin, and follow the normal infective cycle.
10
3 Eggs are produced by fertilized female worms.
9 Eggs deposited in intestinal mucosa, hatch, and migrate to lumen.
1 d Rabditiform larvae in the intestine are excreted in stool.
2 Development into free-living adult worms.
CDC
SAFER·HEALTHIER·PEOPLE™
http://www.dpd.cdc.gov/dpdx


Free-living Cycle
(Heterogonic)
L3
Filariform
L4
A
Egg Production
L2
Rhabditiform
Feces + L2

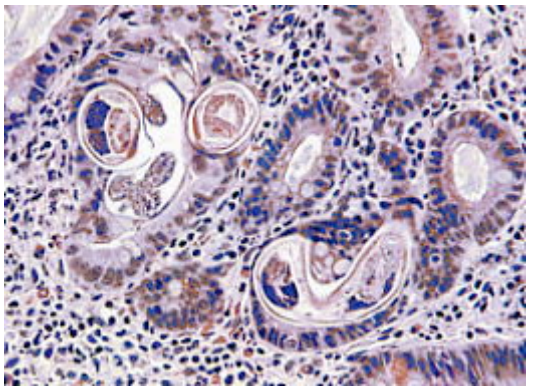
Parasitic Life Cycle

- Begins with filariform larvae penetrating the intact skin.
- Penetrate capillaries.
- Transported to lungs
- Break into alveoli.
- Migrate (grow) into the trachea.
- Swallowed.
- Lodge in the lamina propria of the duodenum.



Intestinal Phase

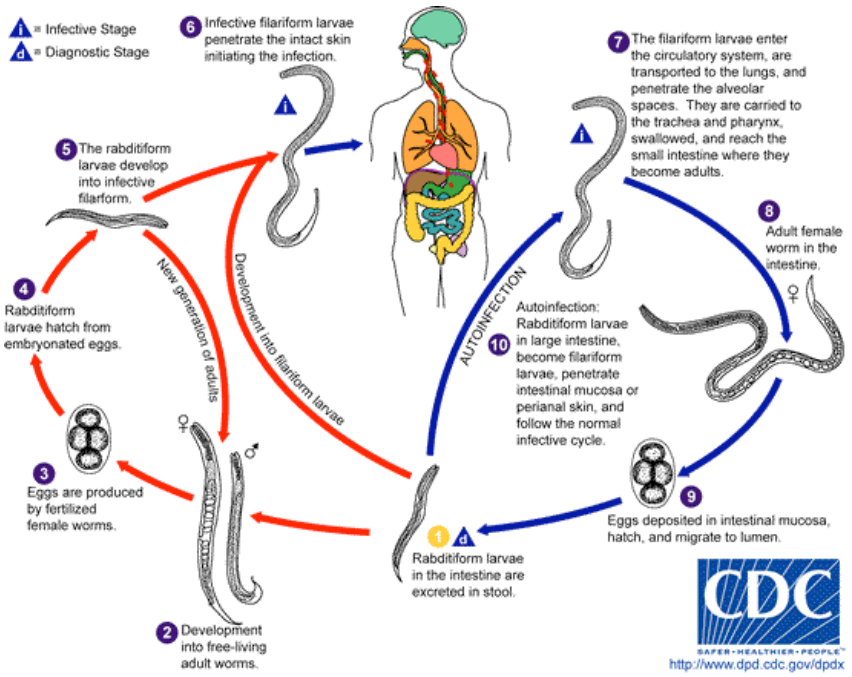
- Live in columnar cells.
- Similar to what other parasite?
- Reproduce by parthenogenesis.
- No parasitic male.
- Eggs hatch rapidly into L1.
- L1 to colon molt to L2 (rhabditiform), exit with feces or…



2 mm long 35 μm wide

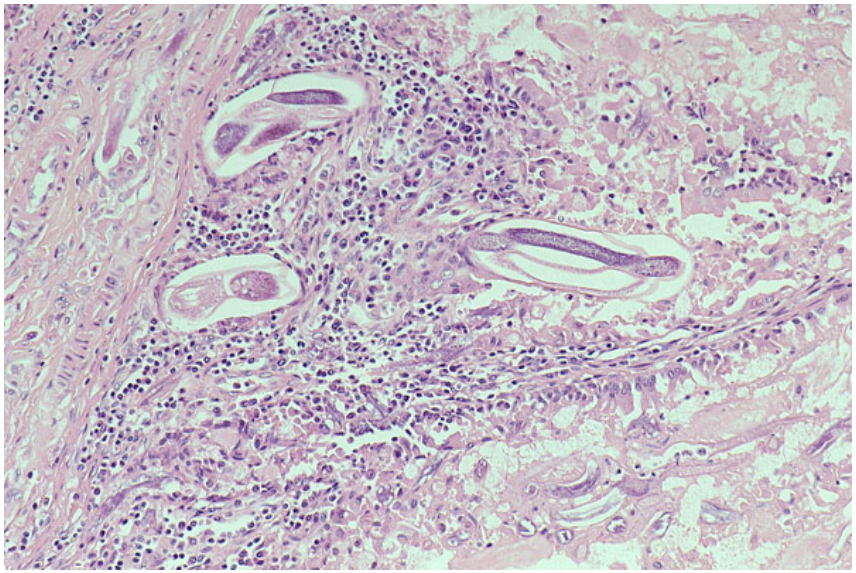
Autoinfection

- L2 rhabditiform larvae can molt into the infective filiariform larvae in the intestine.
- Penetrate the colonic wall.
- Repeat cycle.



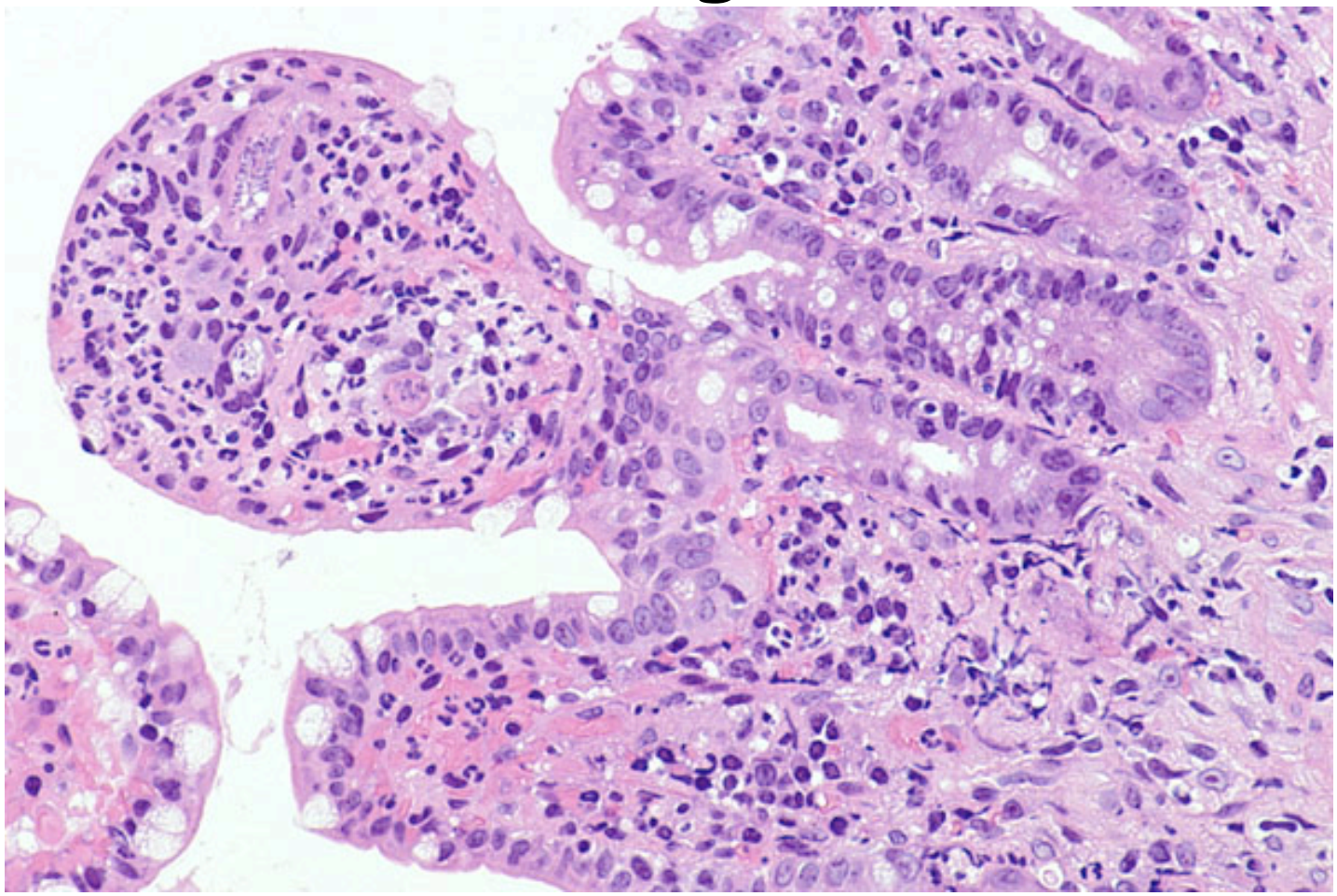
Pathogenesis and Clinical Features

- No human parasitic nematode associated with such a broad spectrum of manifestations.
- Typical symptoms:
  - Gastrointestinal ailments
  - Intestinal bleeding
  - Jejunal perforation, appendicitis, colitis, granulomatous hepatitis
  - Electrolyte imbalance
  - Sepsis
  - Pneumonia



Hyper-infected jejunum; autopsy.

Massive inflammation in T cell leukemia patient. Eosinophilia & shortening of villi.



Filaria-type larva in sputum of AIDS patient.

Systemic Disease

- In cases of hyper-infection or disseminated infection, penetrating larvae often carry enteric microorganisms.
- Can result in sepsis.

Diagnosis

- Stongyloides infection should be considered in patients with unexplained gastrointestinal symptoms and eosinophilia.
- Larvae in the stool is definitive method.
  - Difficult since as few as 50 larvae/day produced.
  - Larvae produced sporadically.

Diagnosis

- Serological tests now available but not easily done since no recombinant proteins available.
- Endoscopy of duodenal region very sensitive and excellent method.

Treatment

- Thiabendazole, 25 mg/kg orally twice a day for 2 days.
- For immunocompromised patients with hyperinfection or dissemination twice the daily dose for several weeks.
  - Simultaneous administration of corticosterioids essential.
  - Antibiotics.
  - 2/3 of patients die even with therapy.

Prevention & Control

- Reservoir hosts include dogs and primates (chimpanzees).
- Custodial institutions are the foci of infection.