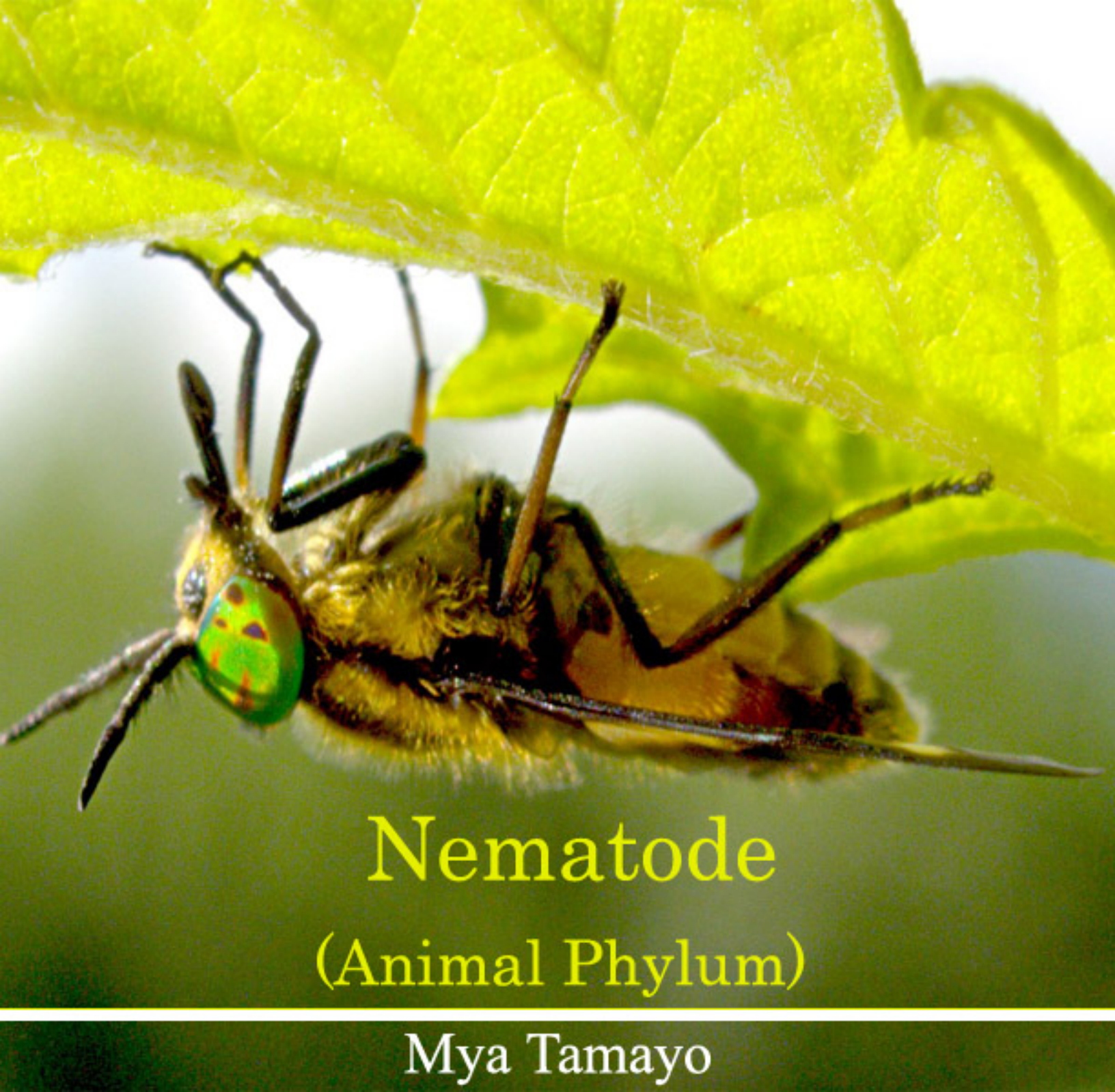


Nematode

(Animal Phylum)

Mya Tamayo

First Edition, 2012

ISBN 978-81-323-4194-9

Published by:
White Word Publications
4735/22 Prakashdeep Bldg,
Ansari Road, Darya Ganj,
Delhi - 110002
Email: info@wtbooks.com

Table of Contents

Chapter 1

Nematode

Nematodes

Unidentified roundworm from wet soil. The mouth is at the top left corner.

Scientific classification [e]	
Kingdom:	Animalia
clade:	Nematoida
Phylum:	**Nematoda** Diesing, 1861

Classes

Chromadorea (disputed)
Enoplea (disputed)
Secernentea

Synonyms

Adenophorea
Aphasmidia
Nematoidea Rudolphi, 1808
Nematodes Burmeister, 1837
Nemates Cobb, 1919
Nemata Cobb, 1919

The **nematodes** are the most diverse phylum of pseudocoelomates, and one of the most diverse of all animals. Nematode species are very difficult to distinguish; over 28,000 have been described, of which over 16,000 are parasitic. It has been estimated that the total number of nematode species might be approximately 1,000,000. Unlike cnidarians

or flatworms, roundworms have a digestive system that is like a tube with openings at both ends.

Habitats

Nematodes have successfully adapted to nearly every ecosystem from marine to fresh water, from the polar regions to the tropics, as well as the highest to the lowest of elevations. They are ubiquitous in freshwater, marine, and terrestrial environments, where they often outnumber other animals in both individual and species counts, and are found in locations as diverse as mountains, deserts and oceanic trenches. They represent, for example, 90% of all life on the seafloor of the Earth. Their many parasitic forms include pathogens in most plants and animals (including humans). Some nematodes can undergo cryptobiosis.

One group of carnivorous fungi, the nematophagous fungi, are predators of soil nematodes. They set enticements for the nematodes in the form of lassos or adhesive structures.

Taxonomy and systematics

Eophasma jurasicum, a fossilized nematode

The group was originally defined by Karl Rudolphi in 1808 under the name **Nematoidea**, from Ancient Greek νῆμα (*nêma, nêmatos*, 'thread') and -ειδής (*-eidēs*, 'species'). The vernacular word "nematode" is a corruption of this taxon, reclassified as family **Nematodes** by Burmeister in 1837 and order **Nematoda** by K. M. Diesing in 1861.

At the origin, the "Nematoidea" included both roundworms and horsehair worms. Along with Acanthocephala, Trematoda and Cestoidea, it formed the group Entozoa. The first differentiation of roundworms from horsehair worms, though erroneous, is due to von Siebold (1843) with orders Nematoidea and Gordiacei (Gordiacea). They were classed along with Acanthocephala in the new phylum Nemathelminthes (today obsolete) by Gegenbaur (1859). Then the taxon Nematoidea has been promoted to the rank of phylum by Ray Lankester (1877) including the family Gordiidae (horsehair worms). In 1919, Nathan Cobb proposed that roundworms should be recognized alone as a phylum. He argued that they should be called **nema**(s) in English rather than "nematodes" and defined the taxon **Nemates** (Latin plural of *nema*). For ITIS, the taxon Nematoda is invalid. Since Cobb was the first to exclude all but nematodes from the group, the valid taxon should be ***Nemates*** Cobb 1919 or ***Nemata*** Cobb 1919.

Phylogeny

The mysterious Gastrotricha seem to hold the key to the "Ecdysozoa debate", but they have been little studied. Whether they are relatives of the nematodes is still unknown.

The relationships of the nematodes and their close relatives among the protostomian Metazoa are unresolved. Traditionally, they were held to be a lineage of their own, but in the 1990s it was proposed that they form a clade together with moulting animals such as arthropods. This group has been named Ecdysozoa. However, the monophyly of the Ecdysozoa was never unequivocally accepted: while most researchers consider at least the placement of arthropods as more distant relatives of annelids — with which they were formerly united — to be warranted, the presumed close relationships of the nematodes and relatives with the arthropods has been a major point of contention.

Even though the amount of data since accumulated in regard to this problem is staggering, the situation seems if anything less clear these days. DNA sequence data, initially strongly supporting the Ecdysozoa hypothesis, has become rather equivocal on ecdysozoan monophyly, and is simply unable to refute either a close or a more distant relationship between the arthropod and nematode lineages. That the roundworms have a large number of peculiar apomorphies and in many cases a parasitic lifestyle confounds morphological analyses. Genetic analyses of roundworms suggest that — as is also indicated by their unique morphological features — the group has been under intense selective pressure during its early radiation, resulting apparently in accelerated rates of both morphological and molecular evolution. Furthermore, no distinctive apomorphies of Ecdysozoa are known; even moulting has recently been confirmed to occur outside the presumed clade.

Conversely, the identity of the closest living relatives of the Nematoda has always been considered to be well resolved. Morphological characters and molecular phylogenies agree with placement of the roundworms as sister taxon to the parasitic horsehair worms (Nematomorpha); together they make up the Nematoida. Together with the Scalidophora (formerly Cephalorhyncha), the Nematoida form the Introverta. It is entirely unclear whether the Introverta are, in turn, the closest living relatives of the enigmatic Gastrotricha; if so, they are considered a clade Cycloneuralia, but there is much disagreement both between and among the available morphological and molecular data. The Cycloneuralia or the Introverta — depending on the validity of the former — are often ranked as a superphylum.

Nematode systematics

Due to the lack of knowledge regarding many nematodes, their systematics is contentious. Traditionally, they are divided into two classes, the Adenophorea and the Secernentea, and initial DNA sequence studies suggested the existence of five clades:

- Dorylaimia
- Enoplia
- Spirurina
- Tylenchina
- Rhabditina

As it seems, the Secernentea are indeed a natural group of closest relatives. But the "Adenophorea" appear to be a paraphyletic assemblage of roundworms simply retaining a good number of ancestral traits. The old Enoplia do not seem to be monophyletic either but to contain two distinct lineages. The old group "Chromadoria" seem to be another paraphyletic assemblage, with the Monhysterida representing a very ancient minor group of nematodes. Among the Secernentea, the Diplogasteria may need to be united with the Rhabditia. while the Tylenchia might be paraphyletic with the Rhabditia.

The understanding of roundworm systematics and phylogeny as of 2002 is summarised below:

Phylum Nematoda

- Basal order Monhysterida
- Class Dorylaimea
- Class Enoplea
- Class Secernentea
    - Subclass Diplogasteria (disputed)
    - Subclass Rhabditia (paraphyletic?)
    - Subclass Spiruria
    - Subclass Tylenchia (disputed)
- "Chromadorea" assemblage

Anatomy

Nematodes are slender, worm-like animals, typically less than 2.5 millimetres (0.10 in) long. The smallest nematodes are microscopic, while free-living species can reach as much as 5 centimetres (2.0 in) and some parasitic species are larger still. The body is often ornamented with ridges, rings, warts, bristles or other distinctive structures.

The head of a nematode is relatively distinctive. Whereas the rest of the body is bilaterally symmetrical, the head is radially symmetrical, with sensory bristles and, in many cases, solid *head-shields* radiating outwards around the mouth. The mouth has either three or six lips, which often bear a series of teeth on their inner edge. An adhesive *caudal gland* is often found at the tip of the tail.

The epidermis is either a syncytium or a single layer of cells, and is covered by a thick collagenous cuticle. The cuticle is often of complex structure, and may have two or three distinct layers. Underneath the epidermis lies a layer of muscle cells. Projections run from the inner surface of these cells towards the nerve cords; this is a unique arrangement in the animal kingdom, in which nerve cells normally extend fibres into the muscles rather than *vice versa*.

The muscle layer surrounds the body cavity, which is filled with a fluid that lacks any form of blood cells. The gut runs down the centre of the cavity.

Digestive system

The oral cavity is lined with cuticle, which is often strengthened with ridges or other structures, and, especially in carnivorous species, may bear a number of teeth. The mouth often includes a sharp stylet which the animal can thrust into its prey. In some species, the stylet is hollow, and can be used to suck liquids from plants or animals.

The oral cavity opens into a muscular sucking pharynx, also lined with cuticle. Digestive glands are found in this region of the gut, producing enzymes that start to break down the food. In stylet-bearing species, these may even be injected into the prey.

There is no stomach, with the pharynx connecting directly to the intestine that forms the main length of the gut. This produces further enzymes, and also absorbs nutrients through its lining. The last portion of the intestine is lined by cuticle, forming a rectum which expels waste through the anus just below and in front of the tip of the tail. The intestine also has valves or sphincters at either end to help control the movement of food through the body.

Excretory system

Nitrogenous waste is excreted in the form of ammonia through the body wall, and is not associated with any specific organs. However, the structures for excreting salt to maintain osmoregulation are typically more complex.

In many marine nematodes, there are one or two unicellular *renette glands* that excrete salt through a pore on the underside of the animal, close to the pharynx. In most other nematodes, these specialised cells have been replaced by an organ consisting of two parallel ducts connected by a single transverse duct. This transverse duct opens into a common canal that runs to the excretory pore.

Nervous system

Four nerves run the length of the body on the dorsal, ventral, and lateral surfaces. Each nerve lies within a cord of connective tissue lying beneath the cuticle and between the muscle cells. The ventral nerve is the largest, and has a double structure forward of the excretory pore. The dorsal nerve is responsible for motor control, while the lateral nerves are sensory, and the ventral combines both functions.

At the anterior end of the animal, the nerves branch from a dense circular nerve ring surrounding the pharynx, and serving as the brain. Smaller nerves run forward from the ring to supply the sensory organs of the head.

The body of nematodes is covered in numerous sensory bristles and papillae that together provide a sense of touch. Behind the sensory bristles on the head lie two small pits, or *amphids*. These are well supplied with nerve cells, and are probably chemoreception

organs. A few aquatic nematodes possess what appear to be pigmented eye-spots, but is unclear whether or not these are actually sensory in nature.

Reproduction

Most nematode species are dioecious, with separate male and female individuals. Both sexes possess one or two tubular gonads. In males, the sperm are produced at the end of the gonad, and migrate along its length as they mature. The testes each open into a relatively wide sperm duct and then into a glandular and muscular ejaculatory duct associated with the cloaca. In females, the ovaries each open into an oviduct and then a glandular uterus. The uteri both open into a common vagina, usually located in the middle of the ventral surface.

Reproduction is usually sexual. Males are usually smaller than females (often much smaller) and often have a characteristically bent tail for holding the female for copulation. During copulation, one or more chitinized spicules move out of the cloaca and are inserted into genital pore of the female. Amoeboid sperm crawl along the spicule into the female worm. Nematode sperm is thought to be the only eukaryotic cell without the globular protein G-actin.

Eggs may be embryonated or unembryonated when passed by the female, meaning that their fertilized eggs may not yet be developed. A few species are known to be ovoviviparous. The eggs are protected by an outer shell, secreted by the uterus. In free-living roundworms, the eggs hatch into larvae, which appear essentially identical to the adults, except for an under-developed reproductive system; in parasitic roundworms, the life cycle is often much more complicated.

Nematodes as a whole possess a wide range of modes of reproduction. Some nematodes, such as *Heterorhabditis* spp., undergo a process called *endotokia matricida*: intrauterine birth causing maternal death. Some nematodes are hermaphroditic, and keep their self-fertilized eggs inside the uterus until they hatch. The juvenile nematodes will then ingest the parent nematode. This process is significantly promoted in environments with a low or reducing food supply.

The nematode model species *Caenorhabditis elegans* and *C. briggsae* exhibit androdioecy, which is very rare among animals. The single genus *Meloidogyne* (root-knot nematodes) exhibit a range of reproductive modes including sexual reproduction, facultative sexuality (in which most, but not all, generations reproduce asexually), and both meiotic and mitotic parthenogenesis.

The genus *Mesorhabditis* exhibits an unusual form of parthenogenesis, in which sperm-producing males copulate with females, but the sperm do not fuse with the ovum. Contact with the sperm is essential for the ovum to begin dividing, but because there is no fusion of the cells, the male contributes no genetic material to the offspring, which are essentially clones of the female.

Free-living species

In free-living species, development usually consists of four molts of the cuticle during growth. Different species feed on materials as varied as algae, fungi, small animals, fecal matter, dead organisms and living tissues. Free-living marine nematodes are important and abundant members of the meiobenthos. They play an important role in the decomposition process, aid in recycling of nutrients in marine environments and are sensitive to changes in the environment caused by pollution. One roundworm of note is *Caenorhabditis elegans*, which lives in the soil and has found much use as a model organism. *C. elegans* has had its entire genome sequenced, as well as the developmental fate of every cell determined, and every neuron mapped.

Parasitic species

Nematodes commonly parasitic on humans include ascarids (*Ascaris*), filarias, hookworms, pinworms (*Enterobius*) and whipworms (*Trichuris trichiura*). The species *Trichinella spiralis*, commonly known as the *trichina worm*, occurs in rats, pigs, and humans, and is responsible for the disease trichinosis. *Baylisascaris* usually infests wild animals but can be deadly to humans as well. *Dirofilaria immitis* are Heartworms known for causing Heartworm disease by inhabiting the hearts, arteries, and lungs of dogs and some cats. *Haemonchus contortus* is one of the most abundant infectious agents in sheep around the world, causing great economic damage to sheep farms. In contrast, entomopathogenic nematodes parasitize insects and are considered by humans to be beneficial.

One form of nematode is entirely dependent upon fig wasps, which are the sole source of fig fertilization. They prey upon the wasps, riding them from the ripe fig of the wasp's birth to the fig flower of its death, where they kill the wasp, and their offspring await the birth of the next generation of wasps as the fig ripens.

A newly discovered parasitic tetradonematid nematode, *Myrmeconema neotropicum*, apparently induces fruit mimicry in the tropical ant*Cephalotes atratus*. Infected ants develop bright red gasters, tend to be more sluggish, and walk with their gasters in a conspicuous elevated position. These changes likely cause frugivorous birds to confuse the infected ants for berries and eat them. Parasite eggs passed in the bird's feces are subsequently collected by foraging *Cephalotes atratus* and are fed to their larvae, thus completing the life cycle of *Myrmeconema neotropicum*.

Colorized electron micrograph of soybean cyst nematode (*Heterodera* sp.) and egg

Plant parasitic nematodes include several groups causing severe crop losses. The most common genera are *Aphelenchoides* (foliar nematodes), *Ditylenchus*, *Globodera* (potato cyst nematodes), *Heterodera* (soybean cyst nematodes), *Longidorus*, *Meloidogyne* (root-knot nematodes), *Nacobbus*, *Pratylenchus* (lesion nematodes), *Trichodorus* and *Xiphinema* (dagger nematodes). Several phytoparasitic nematode species cause histological damages to roots, including the formation of visible galls (e.g. by root-knot nematodes), which are useful characters for their diagnostic in the field. Some nematode species transmit plant viruses through their feeding activity on roots. One of them is *Xiphinema index*, vector of GFLV (Grapevine Fanleaf Virus), an important disease of grapes.

Other nematodes attack bark and forest trees. The most important representative of this group is *Bursaphelenchus xylophilus*, the pine wood nematode, present in Asia and America and recently discovered in Europe.

Agriculture and horticulture

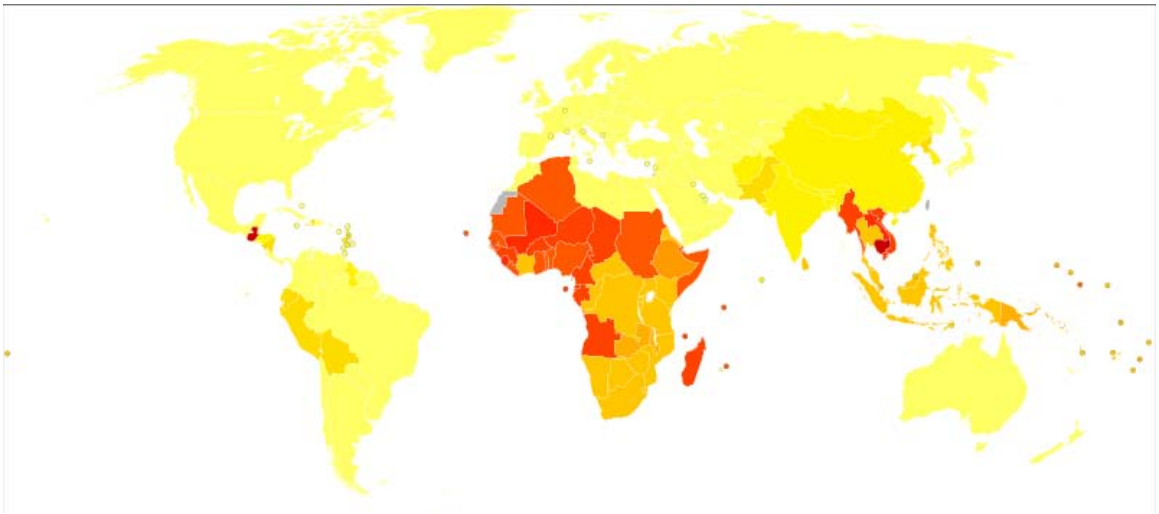
Depending on the species, a nematode may be beneficial or detrimental to plant health. From agricultural and horticulture perspectives, there are two categories of nematode: predatory ones, which will kill garden pests like cutworms, and pest nematodes, like the root-knot nematode, which attack plants and those that act as vectors spreading plant viruses between crop plants. Predatory nematodes can be bred by soaking a specific recipe of leaves and other detritus in water, in a dark, cool place, and can even be purchased as an organic form of pest control.

Rotations of plants with nematode resistant species or varieties is one means of managing parasitic nematode infestations. For example, marigolds, grown over one or more seasons (the effect is cumulative), can be used to control nematodes. Another is treatment with natural antagonists such as the fungus *Gliocladium roseum*. Chitosan is a natural biocontrol that elicits plant defense responses to destroy parasitic cyst nematodes on roots of soybean, corn, sugar beets, potatoes and tomatoes without harming beneficial nematodes in the soil. Furthermore soil steaming is an efficient method to kill nematodes before planting crop.

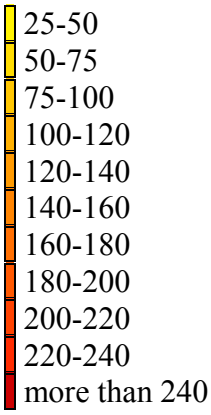
CSIRO has found that there was 13- to 14-fold reduction of nematode population densities in plots having Indian mustard (*Brassica juncea*) green manure or seed meal in the soil.

Hundreds of *Caenorhabditis elegans* were featured in a research project on NASA's STS-107 space mission (which ended in the Space Shuttle Columbia Disaster).

Epidemiology



Disability-adjusted life year for intestinal nematode infections per 100,000 inhabitants in 2002.
no data
less than 25



A number of intestinal nematodes affect human beings. These include ascariasis, trichuriasis and hookworm disease.

Chapter 2

Caenorhabditis Elegans

Caenorhabditis elegans

An adult hermaphrodite *C. elegans* worm

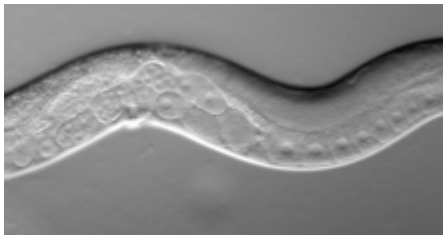
Scientific classification	
Kingdom:	Animalia
Phylum:	Nematoda
Class:	Secernentea
Order:	Rhabditida
Family:	Rhabditidae
Genus:	*Caenorhabditis*
Species:	***C. elegans***

Binomial name

Caenorhabditis elegans
Maupas, 1900

Caenorhabditis elegans is a free-living, transparent nematode (roundworm), about 1 mm in length, which lives in temperate soil environments. Research into the molecular and developmental biology of *C. elegans* was begun in 1974 by Sydney Brenner and it has since been used extensively as a model organism.

Biology



Movement of Wild-type *C. elegans*

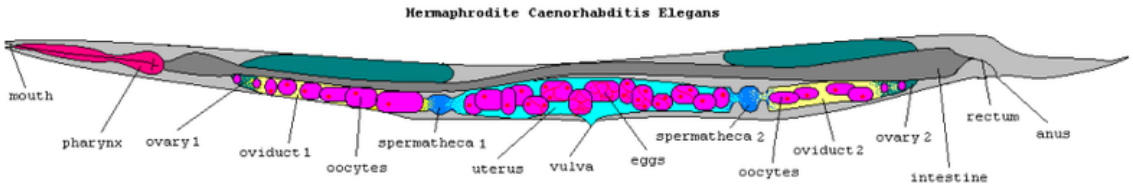
Wild type *C. elegans* hermaphrodite

C. elegans is unsegmented, vermiform, and bilaterally symmetrical, with a cuticle integument, four main epidermal cords and a fluid-filled pseudocoelomate cavity. Members of the species have many of the same organ systems as other animals. In the wild, they feed on bacteria that develop on decaying vegetable matter. *C. elegans* has two sexes: hermaphrodites and males. Individuals are almost all hermaphrodite, with males comprising just 0.05% of the total population on average. The basic anatomy of *C. elegans* includes a mouth, pharynx, intestine, gonad, and collagenous cuticle. Males have

a single-lobed gonad, vas deferens, and a tail specialized for mating. Hermaphrodites have two ovaries, oviducts, spermatheca, and a single uterus.

C. elegans eggs are laid by the hermaphrodite. After hatching, they pass through four juvenile stages (L1–L4). When crowded or in the absence of food, *C. elegans* can enter an alternative third larval stage called the dauer state. Dauer larvae are stress-resistant and do not age. Hermaphrodites produce all their sperm in the L4 stage (150 sperm per gonadal arm) and then switch over to producing oocytes. The sperm are stored in the same area of the gonad as the oocytes until the first oocyte pushes the sperm into the spermatheca (a kind of chamber where the oocytes become fertilized by the sperm). The male can inseminate the hermaphrodite, which will use male sperm preferentially (both types of sperm are stored in the spermatheca). When self-inseminated the wild-type worm will lay approximately 300 eggs. When inseminated by a male, the number of progeny can exceed 1,000. At 20 °C, the laboratory strain of *C. elegans* has an average life span of approximately 2–3 weeks and a generation time of approximately 4 days.

C. elegans has five pairs of autosomes and one pair of sex chromosomes. Sex in *C. elegans* is based on an X0 sex-determination system. Hermaphrodite *C. elegans* have a matched pair of sex chromosomes (XX); the rare males have only one sex chromosome (X0). The sperm of *C. elegans* is ameboid, lacking flagella and acrosomes.



Longitudinal section through the hermaphrodite *C. elegans*

Ecology

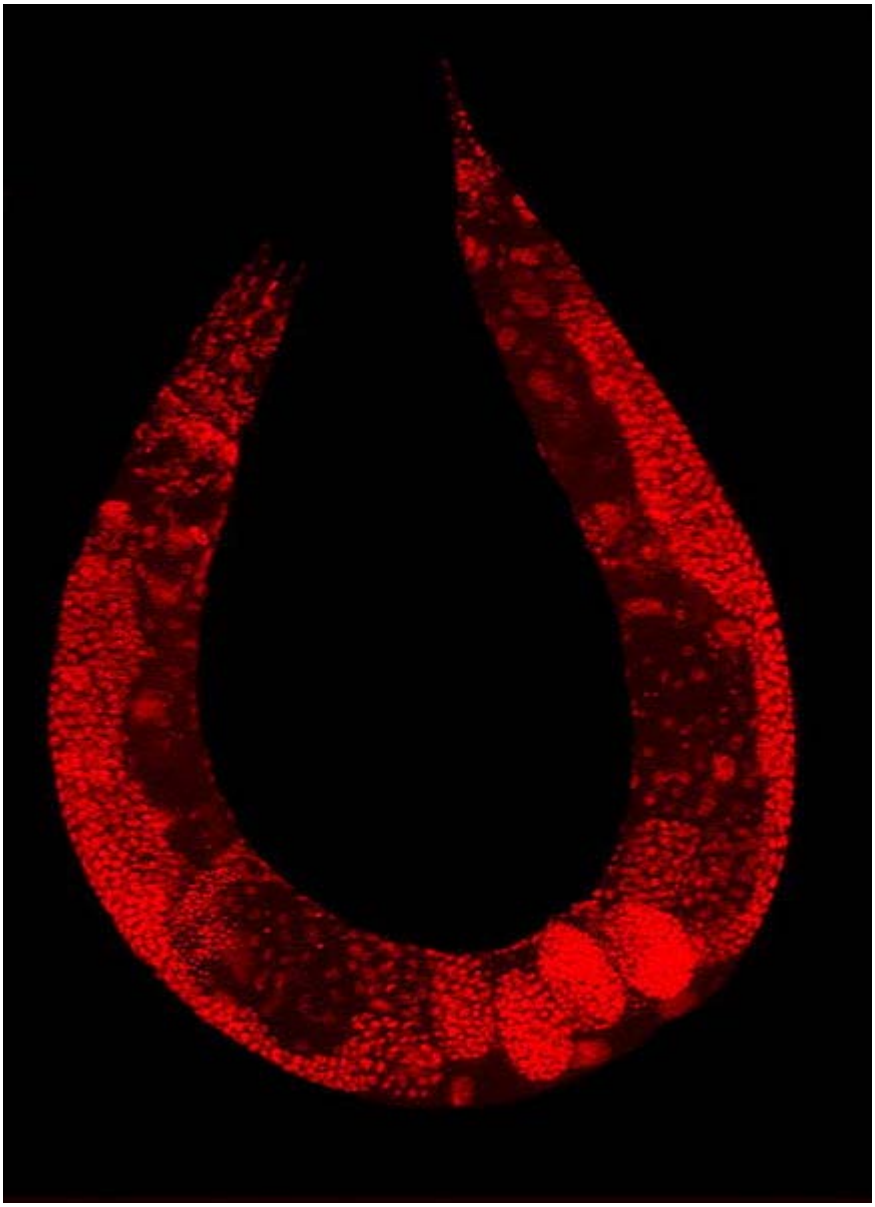
The different *Caenorhabditis* species occupy various nutrient and bacteria rich environments. They do not form self-sustaining populations in soil, as it lacks enough organic matter. *C. elegans* can survive on a diet of a variety of kinds of bacteria (not all bacteria, though), but its wild ecology is largely unknown. Most laboratory strains were found in human-altered situations like gardens and compost piles. Recently, however, growing *C. elegans* has been found to be abundant in rotting organic matter, particularly rotting fruits . Dauer larvae can be transported by invertebrates including millipedes, insects, isopods, and gastropods. When they reach a desirable location they then get off, and at least in the lab they will also feed on the host if it dies.

Nematodes are capable of surviving desiccation, and in *C. elegans* the mechanism for this capability has been demonstrated to be Late Embryogenesis Abundant (LEA) proteins.

Laboratory uses

C. elegans is studied as a model organism for a variety of reasons. It is a multicellular eukaryotic organism that is simple enough to be studied in great detail. Strains are cheap to breed and can be frozen. When subsequently thawed they remain viable, allowing long-term storage.

In addition, *C. elegans* is transparent, facilitating the study of cellular differentiation and other developmental processes in the intact organism. The developmental fate of every single somatic cell (959 in the adult hermaphrodite; 1031 in the adult male) has been mapped out. These patterns of cell lineage are largely invariant between individuals, in contrast to mammals where cell development from the embryo is more largely dependent on cellular cues. In both sexes, a large number of additional cells (131 in the hermaphrodite, most of which would otherwise become neurons), are eliminated by programmed cell death (apoptosis). This aspect has been thoroughly studied in this organism, specifically because of this "apoptotic predictability", which has contributed to the elucidation of some apoptotic genes, mainly through observation of abnormal, apoptosis-surviving nematodes.



Wild-type *C. elegans* hermaphrodite stained with the fluorescent dye Texas Red to highlight the nuclei of all cells

In addition, *C. elegans* is one of the simplest organisms with a nervous system. In the hermaphrodite, this comprises 302 neurons whose pattern of connectivity, or "connectome", has been completely mapped and shown to be a small-world network. Research has explored the neural mechanisms responsible for several of the more interesting behaviors shown by *C. elegans*, including chemotaxis, thermotaxis, mechanotransduction, and male mating behavior.

A useful feature of *C. elegans* is that it is relatively straightforward to disrupt the function of specific genes by RNA interference (RNAi). Silencing the function of a gene in this way can sometimes allow a researcher to infer what the function of that gene may be. The

nematode can either be soaked in or injected with a solution of double stranded RNA, the sequence of which is complementary to the sequence of the gene that the researcher wishes to disable. Alternatively, worms can be fed on genetically transformed bacteria which express the double stranded RNA of interest.

C. elegans has also been useful in the study of meiosis. As sperm and egg nuclei move down the length of the gonad, they undergo a temporal progression through meiotic events. This progression means that every nucleus at a given position in the gonad will be at roughly the same step in meiosis, eliminating the difficulties of heterogeneous populations of cells.

The organism has also been identified as a model for nicotine dependence as it has been found to exhibit behavioral responses to nicotine that parallel those observed in mammals, including acute response, tolerance, withdrawal, and sensitization.

As for most model organisms, there is a dedicated online database for the species that is actively curated by scientists working in this field. The WormBase database attempts to collate all published information on *C. elegans* and other related nematodes. A reward of $4000 has been advertised on their website, for the finder of a new species of closely related nematode. Such a discovery would broaden research opportunities with the worm.

Genome

C. elegans was the first multicellular organism to have its genome completely sequenced. The sequence was published in 1998, although a number of small gaps were present; the last gap was finished by October 2002. The adult hermaphrodite has 959 somatic nuclei. Its gene density is about 1 gene/5kb. Introns are 26% of the genome. There are some large intergenic regions containing repetitive DNA sequences. Many genes are arranged in operons: polycistronic series that are transcribed together. *C. elegans* and other nematodes are the only eukaryotes currently known to have operons. The *C. elegans* genome sequence is approximately 100 million base pairs long and contains approximately 20,100 protein-coding genes. The number of known RNA genes in the genome has increased greatly due to the 2006 discovery of a new class of 21U-RNA gene, and the genome is now believed to contain more than 16,000 RNA genes, up from as little as 1,300 in 2005. Scientific curators continue to appraise the set of known genes, such that new gene predictions continue to be added and incorrect ones modified or removed.

In 2003, the genome sequence of the related nematode *C. briggsae* was also determined, allowing researchers to study the comparative genomics of these two organisms. Work is now ongoing to determine the genome sequences of more nematodes from the same genus such as *C. remanei*, *C. japonica* and *C. brenneri*. These newer genome sequences are being determined using the whole genome shotgun technique which means they are likely to be less complete and less accurate than that of *C. elegans*, which was sequenced using the "hierarchical" or clone-by-clone approach.

The official version of the *C. elegans* genome sequence continues to change as and when new evidence reveals errors in the original sequencing (DNA sequencing is not an error-free process). Most changes are minor, adding or removing only a few base pairs (bp) of DNA. For example, the WS169 release of WormBase (December 2006) lists a net gain of 6 base pairs to the genome sequence. Occasionally more extensive changes are made, as in the WS159 release of May 2006, which added over 300 bp to the sequence.

Evolution

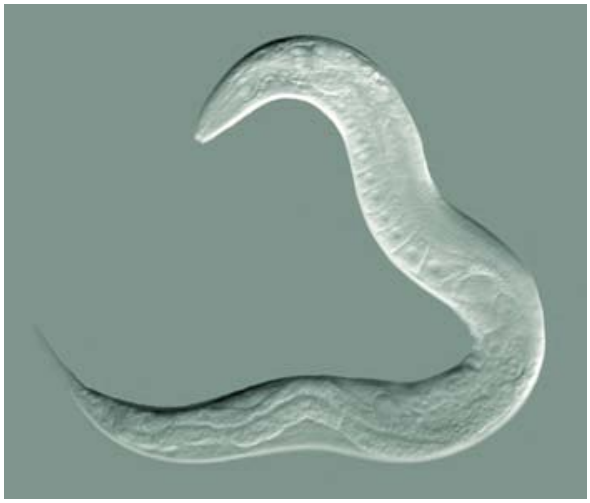
It has been shown that a small number of conserved protein sequences from sponges are more similar to humans than to *C. elegans*. This suggests that there has been an accelerated rate of evolution in the *C. elegans* lineage. The same study found that several phylogenetically ancient genes are not present in *C. elegans*.

RNA interference

RNA interference (RNAi) has been used extensively in *C. elegans* because it can be done by simply feeding the worms transgenic bacteria expressing RNA complementary to the gene of interest. This strategy for gene loss of function experiments is the easiest of all animal models, and thus, scientists were able to knock down 86% of the ~20,000 genes in the worm, establishing a functional role for 9% of the genome.

Incidentally, RNAi does not work nearly as well in other species of worm in the *Caenorhabditis* genus. Although injecting RNA into the body cavity of the animal induces silencing in most species, only *C. elegans* and a few other distantly related nematodes can uptake RNA from the bacteria they eat for RNAi. This ability has been mapped down to a single gene, *sid-2*, which when inserted as a transgene in other species allows them to uptake RNA for RNAi the way *C. elegans* does.

Scientific community



C. elegans hermaphrodite

In 2002, the Nobel Prize in Physiology or Medicine was awarded to Sydney Brenner, H. Robert Horvitz and John Sulston for their work on the genetics of organ development and programmed cell death in *C. elegans*. The 2006 Nobel Prize in Physiology or Medicine was awarded to Andrew Fire and Craig C. Mello for their discovery of RNA interference in *C. elegans*. In 2008 Martin Chalfie shared a Nobel Prize in Chemistry for his work on green fluorescent protein in *C. elegans*.

Because all research into *C. elegans* essentially started with Sydney Brenner in the 1970s, many scientists working in this field share a close connection to Brenner, having either worked as a post-doctoral or post-graduate researcher in Brenner's lab or in the lab of someone who previously worked with Brenner. Because most people who worked in his lab went on to establish their own worm research labs, there is now a fairly well documented "lineage" of *C. elegans* scientists. This lineage was recorded in some detail at the 2003 International Worm Meeting and the results were stored in the WormBase database.

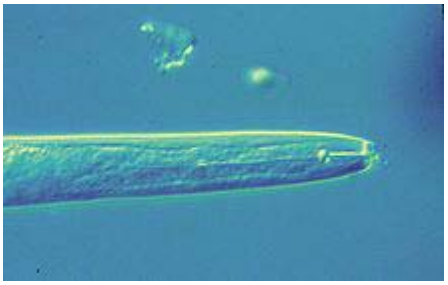
Long-term storage

A 15% glycerol solution is used for the freezing of *C. elegans*. Samples are cooled at 1°C per minute. Freshly starved young larvae survive freezing best. About 35 to 45% of the worms stored in liquid nitrogen survive. The worms can also be stored at −80°C for over ten years, but survival is not as great as for worms stored at −196°C, the temperature of liquid nitrogen.

Chapter 3

Pratylenchus

Pratylenchus penetrans



Pratylenchus, or lesion nematode

Scientific classification	
Kingdom:	Animalia
Phylum:	Nematoda
Class:	Adenophorea
Subclass:	Diplogasteria
Order:	Tylenchida
Superfamily:	Tylenchoidea
Family:	Pratylenchidae
Subfamily:	Pratylenchinae
Genus:	***Pratylenchus***

Species

Species include:

- *Pratylenchus brachyurus*
- *Pratylenchus coffeae*
- *Pratylenchus crenatus*
- *Pratylenchus dulscus*
- *Pratylenchus fallax*
- *Pratylenchus flakkensis*

- *Pratylenchus goodeyi*
- *Pratylenchus hexincisus*
- *Pratylenchus loosi*
- *Pratylenchus minutus*
- *Pratylenchus mulchandi*
- *Pratylenchus musicola*
- *Pratylenchus neglectus*
- *Pratylenchus penetrans*
- *Pratylenchus pratensis*
- *Pratylenchus reniformia*
- *Pratylenchus scribneri*
- *Pratylenchus thornei*
- *Pratylenchus vulnus*
- *Pratylenchus zeae*

Pratylenchus is a genus of plant-parasitic nematodes responsible for root lesions on many plant hosts in temperate regions around the world. Lesion nematodes are migratory endoparasites that enter the host root for feeding and reproduction and move freely through or out of the root tissue. They do not become sedentary in the roots, as do the cyst or root-knot nematodes. Feeding is restricted almost entirely to the cortex of the root. Species differentiation is usually based on morphology; especially by variation in mouthparts known as stylets.

Hosts and symptoms

Brown rotting of roots from lesion nematodes

Boxwood showing symptoms from root lesion nematodes

Root Lesion Nematodes infect a larger variety of hosts than any other type of nematode. *Pratylenchus penetrans* in itself has over 350 hosts. They can range from potatoes and corn, to bananas and wheat. While the range of hosts might be huge, there are specific hosts that Root Lesion Nematodes cause the most significant damage to. *Pratylenchus* is found to be associated with corn more than any other host. The symptoms are fairly similar throughout each of the different hosts. As the nematode enters the host's root, it creates a hole…or lesion… in the root. This initial puncture of the root turns into brown or black necrotic lesions. As the root begins to die, you begin to see secondary symptoms above ground. These symptoms may include stunting, chlorosis in the leaves, wilt like symptoms, and may eventually lead to death. These symptoms can usually be seen in small patches throughout the field as nematodes tend to have a patchy distribution throughout the field. In serious cases, root lesion nematodes can cause significant yield loss.

Environment

Pratylenchus can endure a wide range of environmental conditions. Moist temperate soils are ideal conditions for breeding and migrating underground, but they can persist even in warm and dryer environments. When the nematodes are susceptible to drying they lie quiescent until moisture increases and plants resume growth. Lesion nematodes remain inactive when soil temperatures are below 59°F (15°C); except for P. penetrans, there is little activity until temperatures rise above 68°F (20°C). Sandy soils and weed infestation are other factors that increase the likelihood of Pratylenchus prevalence. Several parameters influence the soil environment, and the optimal conditions may vary between Pratylenchus species. Some important factors that can alter Pratylenchus abundance include: soil moisture, mineral components, temperature, aeration, organic matter, and pH.

Management

While a normal distribution of *Pratylenchus* is patchy, serious cases can destroy an entire field of a crop. Seeing as complete destruction is devastating, management and control are extremely important. There are several different methods that can be used in order to help control or eliminate problems with root lesion nematodes. The genus *Pratylenchus* has several different species and each is controlled in a different manor.

In general there are a couple major ways to manage root lesion nematodes. The first includes soil fumigation and the use of nematicides. Either injected into the soil, or sprayed onto the plants, both of these options introduce a nematode killing substance into the environment that the nematode lives in to hopefully decrease the nematode problem. While this may work with some species, it does not work with all of them and can get expensive. This is normally seen only in more expensive crops where the cost of the nematicides can be offset and well worth it. This can be used for *P. brachyurus* for example.

The next type of control involves water immersion. If infected hosts are transplanted, some may have successful management through a hot water bath. Transplants are immersed in 54˚C water bath for 30 minutes. This practice should be enough to kill the nematodes that are infecting the plant without damaging the plant itself. *P. coffeae* is one of the species that can be controlled by this treatment.

Another possibility includes crop rotation. Different hosts are susceptible to different species of *Pratylenchus*. If you have one species of *Pratylenchus*, planting a crop not susceptible to that specific species will eliminate the problem. Or for *P. neglectus* specifically, a 4-5 year crop rotation will allow the problem to subside.

Finally, the best, and sometimes only form of control with root lesion nematodes is resistance. By selecting plant genotypes that are resistant to affects of the nematodes, you don't have to worry about any other form of control. In the case of *P. vulnus* there is no good way to control it other than to use host resistant varieties. Resistance has been found for several different hosts and species of *Pratylenchus* and more research is constantly being done to find even more.

Life/disease cycle

Root lesion nematodes are migratory endoparasites. This means that they usually feed on the roots internally versus ectoparasites that feed on the roots externally. The migratory endoparasite enters the root by puncturing a hole in an outside cell with its stylet. Once the nematode has access to the inside of the cell, it continues to migrate from one cell to the next feeding along its way. As it moves through the cells, and takes nutrients from them, the cells are left with tiny lesions that eventually become necrotic as the root begins to decay. As the nematode feeds on the cells in order get nutrients, the metacarpal pump (a part of a nematode) begins to "pump" nutrients are ingested into the nematode. Over the course of feeding on a specific root, female nematodes lay single eggs that can hatch

in the root (or if the egg was laid in the soil, it hatches in the soil) and the process begins again with the juvenile nematode. *Pratylenchus* does not need both male and female to reproduce; in fact the females can and do lay eggs without the presence of males. For some species, the number of females drastically outnumbers the number of males.

Importance

The genus *Pratylenchus* is considered one of the most important among plant-parasitic nematodes because of its wide range of hosts and worldwide distribution. They may reduce root growth or inhibit root development by forming local lesions on young roots. The lesions may also lead to secondary infections by fungi or bacteria, increasing the plant's susceptibility. The overall root damage associated with Pratylenchus can cause poor growth, reduce crop yields, or kill off the plant completely. These nematodes hold economic importance if crop loss is drastic and profits decrease.

Chapter 4

Angiostrongylus Cantonensis

Angiostrongylus cantonensis
Scientific classification
Kingdom: Animalia
Phylum: Nematoda
Class: Secernentea
Order: Strongylida
Family: Metastrongylidae
Genus: *Angiostrongylus*
Species: ***A. cantonensis***
Binomial name
Angiostrongylus cantonensis

Angiostrongylus cantonensis is a parasitic nematode (roundworm) which causes Angiostrongyliasis, the most common cause of eosinophilic meningitis in Southeast Asia and the Pacific Basin. The nematode commonly resides in the pulmonary arteries of rats, giving it the nickname the **rat lungworm**. Snails are the primary intermediate hosts, where larvae develop until they are infective.

Humans are incidental hosts of this roundworm, and may become infected through ingestion of larvae in raw or undercooked snails or other vectors, or from contaminated water and vegetables. The larvae are then transported via the blood to the central nervous system (CNS), where they are the most common cause of eosinophilic meningitis, a serious condition that can lead to death or permanent brain and nerve damage. Identified in 1964, Angiostrongyliasis is an infection of increasing public health importance as globalization aids in the geographic spread of the disease.

Infectious agent

Angiostrongylus cantonensis is a helminth of the phylum *Nematoda*, order *strongylida*, and superfamily *metastrongyloidea*. Nematodes are roundworms characterized by a tough outer cuticle, unsegmented bodies, and a fully developed GI tract. The order *Strongylida* includes hookworms and lungworms. *Metastrongyloidea* are characterized as long, slender, threadlike worms that reside in the lungs of the definitive host. *Angiostrongylus costaricensis* is a closely related worm that causes intestinal Angiostrongyliasis in Central and South America.

Epidemiology

Nematodes suspected to be *A. cantonensis* were first identified in the cerebrospinal fluid of a patient with eosinophilic meningitis by Nomura and Lim in Taiwan in 1944. They called the parasite *Haemostrongylus ratti*, and noted that raw food eaten by the patient may have been contaminated by rats. Their paper, however, was not translated from the original Japanese into English until just after the parasite had been recognized in 1964, so their discovery was not widely recognized.

In 1955, Mackerass and Sanders identified the life cycle of the worm in rats, defining snails and slugs as the intermediate host and noting the path of transmission through the blood, brain, and lungs in rats.

Following World War II, *A. cantonensis* spread throughout Southeast Asia and Western Pacific Islands including, Micronesia, Melanesia, Polynesia, and Australia. Cases were soon reported in the following nations: Sumatra, the Philippines, Taiwan, Saipan, New Caledonia, Rarotonga, and Tahiti. In the 1960s even more cases were reported from the region from locations such as: Vietnam, Thailand, Cambodia, Java, Sarawak, the New Hebrides (Vanuatu), Guam and Hawaii.

In 1961, an epidemiological study of eosinophilic meningitis in humans was conducted by Rosen, Laigret, and Bories, who hypothesized that the parasite causing these infections was carried by fish. However Alicata noted that raw fish was consumed by large numbers of people in Hawaii without apparent consequences, and patients presenting with meningitis symptoms had a history of eating raw snails or prawns in the weeks before presenting with symptoms. This observation along with epidemiology and autopsy of infected brains confirmed *A. cantonensis* infection in humans as the cause of the majority of eosinophilic meningitis cases in Southeast Asia and the Pacific Islands.

Since then, cases of *A. cantonensis* infestations have appeared in Okinawa, Ryukyu islands, Honshu, Kyushu, New Britain, American Samoa, Western Samoa, Australia, Hong Kong, Bombay, India, Fiji and most recently mainland China. Other sporadic occurrences of the parasite in its rat hosts have been reported in Madagascar, Cuba, Egypt, Puerto Rico, New Orleans, Louisiana and Nigeria.

In 2004, a captive Yellow-tailed Black Cockatoo (*Calyptorhynchus funereus*) and 2 free-living Tawny Frogmouths (*Podargus strigoides*) suffering neurological symptoms were shown to have the parasite. They were the first non-mammalian hosts discovered for the organism

In recent years, the parasite has been shown to be proliferating at an alarming rate due to modern food consumption trends and global transportation of food products. Scientists are calling for a more thorough study of the epidemiology of *A. cantonensis*, stricter food safety policies, and the increase of knowledge on how to properly consume products commonly infested by the parasite.

Hosts

Intermediate hosts of larvae of for *Angiostrongylus cantonensis* include:

- land snails: *Thelidomus aspera* from Jamaica, *Achatina fulica*, *Satsuma mercatoria*, *Acusta despecta*, *Bradybaena circulus* and *Parmarion martensi* from Hawaii
- freshwater snails: *Pila* sp., *Pomacea canaliculata*
- slugs: *Limax maximus*, *Deroceras laeve*, *Deroceras reticulatum*, *Laevicaulis alte*, *Sarasinula plebeia*, *Lehmannia valentiana* and other species of slugs.

Paratenic hosts of *Angiostrongylus cantonensis* include: predatory land flatworm *Platydemus manokwari* and amphibians *Bufo asiaticus*, *Rana catesbeiana*, *Rhacophorus leucomystax* and *Rana limnocharis*.

Pathogenesis of Human Angiostrongylosis

The presence of parasitic worms burrowed in the neural tissue of the human CNS will cause obvious complications. All of the following will result in damage to the CNS:

1. Direct mechanical damage to neural tissue from the worms' motion
2. Toxic by-products such as nitrogenous waste
3. Antigens released by dead and living parasites

Eosinophilic meningitis

Eosinophilic meningitis is primarily caused by parasitic infestations, specifically infestations of *A. cantonensis*. Like other forms of meningitis, eosinophilic meningitis is marked by the inflammation of the meninges. The meninges become inflamed as the result of dying *A. cantonensis* larvae and the general presence of the young adult worm in the central nervous system. This inflammation can lead to mental retardation, nerve damage, permanent brain damage or death. Eosinophilic meningitis is also defined by the increased volume of eosinophils in the cerebrospinal fluid. In most cases, Eosinophil levels rise to 10 or more eosinophils per μL in the cerebrospinal fluid, accounting for at least 10% of the total CSF leukocyte count.

Role of eosinophils

Eosinophils, which are white blood cells, contain granules in their cytoplasm. These granules contain proteins that are toxic to parasites. When these granules degranulate, or break down, chemicals are released that combat parasites such as *A. cantonensis*. Eosinophils, which are located throughout the body, are guided to sites of inflammation by chemokines when the body is infested with parasites such as *A. cantonensis*. Once at the site of inflammation, Type 2 cytokines are released from helper T cells, which communicate with the Eosinophils, signaling them to activate. Once activated, eosinophils can begin the process of degranulation, releasing their toxic proteins in the fight against the foreign parasite.

Clinical symptoms

The clinical symptoms of Eosinophilic Meningitis are as follows:

- Headaches- a bitemporal character in the frontal or occipital lobe
- Nausea
- Vomiting
- Paresthesias - tingling, prickling, or numbing of skin
- Vertigo
- Low-grade fever or no fever
- Meningismus - neck stiffness and photophobia
- Hyperesthesia
- Paralysis
- Blindness

Treatment

Minimal relief can be provided for the intense headaches brought on by eosinophilic meningitis. Typical analgesics and sedatives provide negligible relief so a lumbar puncture is usually resorted to. Removing CSF at regular 3 to 7-day intervals is the only proven method of significantly reducing cranial pressure. This process is performed until improvement is shown.

Diagnosis

The diagnosis of Eosinophilic Meningitis can be arrived at through detection of elevated cranial pressure and increased volumes of eosinophils. The diagnosis of the cause of eosinophilic meningitis and the presence of *A. cantonensis* is remarkably more difficult. A spinal tap, or a sample of CSF, must be taken to search for *A. cantonensis* worms or larvae. *A. cantonensis* is virtually undetectable in the CSF of half of the infected individuals. Current methods of detecting specific antigens associated with *A. cantonensis* are also unreliable. Consequently, alternative approaches to detect antigen-antibody reactions are being explored, such as Immuno-PCR.

Chapter 5

Aonchotheca Forresteri and Ascaridia

Aonchotheca forresteri

Aonchotheca forresteri	
Scientific classification	
Kingdom:	Animalia
Phylum:	Nematoda
Class:	Adenophorea
Order:	Trichurida
Family:	Trichuridae
Genus:	*Aonchotheca*
Species:	***A. forresteri***
Binomial name	
Aonchotheca forresteri (Kinsella and Pence, 1987)	
Synonyms	

- *Capillaria forresteri* Kinsella and Pence, 1987
- *Aonchotheca forresteri*: Pisanu and Bain, 2004

Aonchotheca forresteri is a parasitic nematode that infects the marsh rice rat (*Oryzomys palustris*) in Florida. Occurring mainly in adults, it inhabits the stomach. It is much more common during the wet season, perhaps because its unknown intermediate host is an earthworm that only emerges when it rains. The worm was discovered in 1970 and formally described in 1987. Originally classified in the genus *Capillaria*, it was reclassified in *Aonchotheca* in 1999. *A. forresteri* is small and narrow-bodied, with a length of 13.8 to 19.4 mm in females and 6.8 to 9.2 mm in males. Similar species such as *A. putorii* differ in features of the alae and spicule (organs in the male), the size of the female, and the texture of the eggs.

Taxonomy

Aonchotheca forresteri was discovered during a survey of the endoparasites of Florida marsh rice rats (*Oryzomys palustris*) by John Kinsella from 1970 to 1972, and is one of several new parasite species in this study, which was done because there were no previous comprehensive studies of the endoparasites of the species. Together with Danny Pence, Kinsella described the worm in a 1987 paper as *Capillaria forresteri*; the specific name honors Donald J. Forrester of the College of Veterinary Medicine, University of Florida. Kinsella and Pence described it as one of many species of *Capillaria*, a large and taxonomically difficult genus. They suggested that it may be closest to some other small species that live in the digestive systems of mammals, such as the very similar *C. putorii*, which is found in a variety of carnivorans in North America and Europe. In 1982, Moravec had placed *Capillaria putorii* and a number of related species in a separate genus, *Aonchotheca*, and in 1999 Pisanu and Bain transferred *Capillaria forresteri* and various other species to that genus from *Capillaria*. Thus, the species is now known as *Aonchotheca forresteri*.

Description

The marsh rice rat (here in Paynes Prairie, Florida, the type locality of *A. forresteri*), is the only known host of *Aonchotheca forresteri*.

Aonchotheca forresteri is a small, narrow-bodied worm. It is narrowest at the front and increases in width to about three fourths of its length. The cuticle, the surface layer, is smooth. Females are 13.8 to 19.4 mm long, averaging 16.9 mm, which makes them substantially longer than female *A. putorii*, and 55 to 70 (average 62) μm wide. The eggs

are smooth, lacking the elaborate pattern on the surface seen in *A. putorii*, and are 53 to 58 (54) µm long and 21 to 24 (21) µm broad. The esophagus, the frontmost part of the digestive system, is 2.9 to 3.9 (3.6) mm long and is lined by 36 to 45 (40) cells known as stichocytes. The vulva is located 66 to 105 (83) µm behind the end of the esophagus and the anus is near the end of the worm, which is rounded.

At 6.8 to 9.2 (7.7) mm, males are only about half as long as females. Their maximum width is 34 to 42 (37) µm. The length of the esophagus is 2.3 to 3.0 (2.6) mm, of which the muscular pharynx makes up 260 to 315 (273) µm, and is lined by 35 to 42 (37) stichocytes. The back region of the worm is 4.5 to 6.2 (5.1) mm long. The back, or rectal, opening of the digestive tube is located near the end of the worm, and the length of the cloaca is 530 to 576 (550) µm. Near the back end, there are two alae (ridges) at the sides (laterally), which are 40 to 55 (46) µm long; these are located at 10 to 15 µm from another, small ala at the tip. In *A. putorii*, the lateral alae are much longer and reach the ala at the tip. The spicule, a spikelike structure that functions in reproduction, is curved at the tip and hardened and has a length of 380 to 426 (406) µm. It is smaller than that of the similar *A. tamiasstriati* from North American chipmunks and larger than that of *A. murissylvatici* from various North American and European small rodents, but about as long as that of *A. putorii*, which however lacks the curved tip.

Distribution and ecology

Marsh rice rats from Paynes Prairie, Alachua County; Cedar Key, Levy County; and Lake Istokpoga, Highlands County, all in Florida, have yielded *A. forresteri*. In Paynes Prairie, the type locality, 82 of 178 animals examined were infected with 1 to 50 (average 10) worms, but in Cedar Key only a single rat contained one worm. The worms were found in the front part, or fundus, of the stomach, with their front ends in the fundal tissue and their back ends projecting into the inside.

In Paynes Prairie, there was no significant difference in rate of infection between males and females, but only 4% of juveniles were infected, compared to 52% of adults. Most species of *Capillaria* occur in multiple hosts, but *A. forresteri* has been found only in the marsh rice rat, even though several other small mammals (the round-tailed muskrat, *Neofiber alleni*; cotton mouse, *Peromyscus gossypinus*; hispid cotton rat, *Sigmodon hispidus*; and marsh rabbit, *Sylvilagus palustris*) occur in Paynes Prairie. The rice rat eats more animal food than any of those, and perhaps *A. forresteri* has an intermediate host that is not eaten by the other species. *A. forresteri* is markedly more prevalent in the wet season (spring) than the dry season (autumn), perhaps because rainfall patterns influence the habits of the rice rat in some way. One possibility is that the intermediate host is an earthworm or other oligochaete worm that moves to the surface when it rains.

Ascaridia

Ascaridia	
Scientific classification	
Kingdom:	Animalia
Phylum:	Nematoda
Class:	Secernentea
Order:	Ascaridida
Suborder:	Ascaridina
Superfamily:	Heterakoidea
Family:	Ascaridiidae
Genus:	***Ascaridia*** Dujardin, 1845
Diversity	
15 species	

Ascaridia is the name of a genus of parasitic nematodes. Members of the genus are primarily intestinal parasites of birds. There are three well known species, namely, *A. galli* found mostly in chicken, *A. dissimilis* in turkeys, and *A. columbae* in pigeons. Lesser known species such as *A. hermaphrodita*, *A. sergiomeirai*, *A. ornata*, *A. nicobarensis* and *A. platyceri* are found in parrots. Among these *A. galli* is the most important and most pathogenic species, responsible for 'ascaridiasis' of poultry. Symptoms of heavy infection include diarrhea, stunted growth, listlessness and enteritis. The eggs of these nematodes are characterized by a thick shell, smooth and ellipsoidal, and composed of three distinct layers. They exhibit direct life cycle involving release of eggs into the soil and then ingesting them subsequently along the food. Eggs are resistant to desiccation, persist for a long time in the environment, and remain directly infective. Therefore control of infection involves the prevention of contamination of feeders and drinkers with faeces; pasture rotation and regular treatment, especially for young birds.

Chapter 6

Ascaris and Ascaris Lumbricoides

Ascaris

Ascaris

Adult female

Scientific classification	
Kingdom:	Animalia
Phylum:	Nematoda
Class:	Secernentea
Order:	Ascaridida
Family:	Ascarididae
Genus:	***Ascaris*** Linnaeus, 1758

Species

Ascaris lumbricoides
Ascaris suum

Ascaris is a genus of parasitic nematode worms known as the "giant intestinal roundworms". One species, *A. suum*, typically infects pigs, while another, *A.*

lumbricoides, affects human populations, typically in sub-tropical and tropical areas with poor sanitation. *A. lumbricoides* is the largest intestinal roundworm and is the most common helminth infection of humans worldwide, an infection known as *ascariasis*. Infestation can cause morbidity, and sometimes death, by compromising nutritional status, affecting cognitive processes, inducing tissue reactions, such as granuloma, and provoking intestinal obstruction or rectal prolapse.

Morphology

- Adult: cylindrical shape, creamy white or pinkish in color.
- Male: average 15–31 cm and is more slender than female.
- Female: average 20–35 cm in length.

Symptoms

- Bloody sputum
- Cough
- Low-grade fever
- Vomiting worms
- Passing of worm in stool
- Gallstone formation
- Liver abscesses
- Pancreatis
- Pulmonary eosinophilia

Examination

- Abdominal X-ray
- Complete blood count
- Stool ova and parasite exam

Pathology

Lung phase

A.lumbricoides is known as Ascaris pneumonitis. In the lung it causes hemorrhage, inflammation, bacterial infection. It also causes allergy in areas with seasonal transmission. Typically occurs at 6–15 days after initial exposure.

Intestinal phase

The intestinal phase causes malnourishment, intestinal blockage, verminous intoxication. *A.lumbricoides* will move around in the body in response to chemotherapy or fever. Typically occurs at 6 to 8 weeks after initial exposure.

Management

Early diagnosis can be performed by examination of stool for the worm eggs. The spread or infection of *A.lumbricoides* can be controlled by proper disposal of faeces and proper washing of food. Control of helminthiasis is based on drug treatment, improved sanitation and health education.

Defense Mechanism

As part of the parasite defense strategy, Ascaris roundworms secrete a series of inhibitors to target digestive and immune-related host proteases, which include pepsin, trypsin, chymotrypsin/elastase, cathepsins, and metallocarboxypeptidases (MCPs). Ascaris inhibits MCPs by releasing a enzyme known as Ascaris carboxypeptidase inhibitor (ACI). This enzyme binds to the active site of MCP and blocks the cleavage of its own proteins by the host MCP (Sanglas et al., 2008)

Treatment

Infections with *A.lumbricoides* are easily treated with a number of anthelmintic drugs:

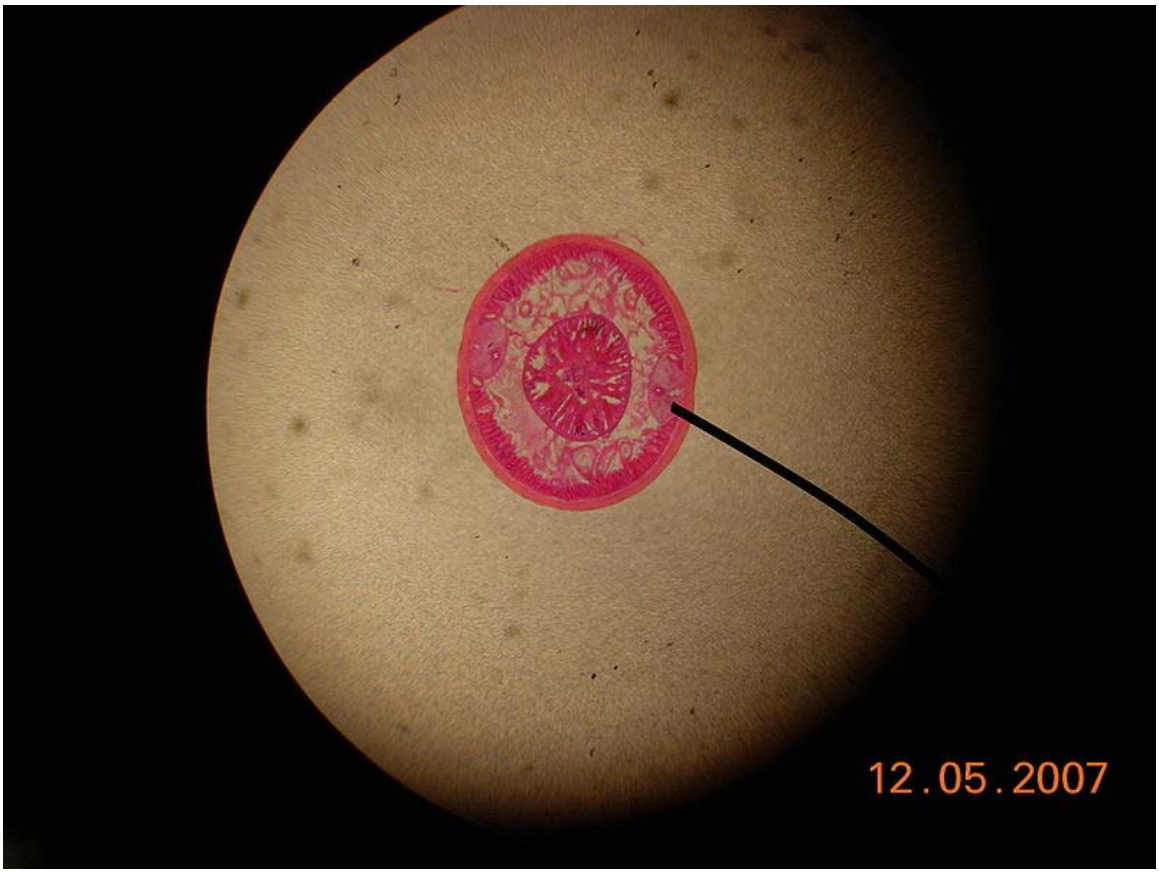
- pyrantel pamoate given as a single dose of 10 mg/kg
- levamisole given as a single dose of 2.5 mg/kg
- mebendazole given as a single dose of 500 mg
- albendazole given as a single dose of 400 mg.sup

The drugs main target is the absorbing cells of the worm. The drugs prevent the worm from absorbing sugar in the intestine which is essential for its survival. This process leads to depletion of energy in worm and its eventual death within few days. The dead worm is then excreted from the gut in the stool. Albendazole is not well absorbed by the intestines and a high fat food or meal should be consumed with each dose.

Many parasitic disease specialists are seeing increased initial incidence and recurrence of roundworm in the U.S. and are thereby increasingly recommending follow up courses of medication to treat internal eggs which have not yet hatched, in addition to the initial treatment period as above. This consists of sporadic treatment with albendazole or similar for a period of three days each month for up to five months after the initial treatment period.

It has been theorized in work by Dr. Hulda Clark that a chemical found in jalapeno peppers may prevent Ascaris infection given the low infection rate in Mexicanos .

More severe cases, blockage of intestine or pancreatic ducts require surgical removal of worms.



Esophagus of an *Ascaris* worm.

Ascaris lumbricoides

Ascaris lumbricoides

An adult female Ascaris worm.

Scientific classification	
Kingdom:	Animalia
Phylum:	Nematoda
Class:	Secernentea
Order:	Ascaridida
Family:	Ascarididae
Genus:	*Ascaris*
Species:	***A. lumbricoides***

Binomial name
Ascaris lumbricoides Linnaeus, 1758

Ascaris lumbricoides is the **giant roundworm** of humans, belonging to the phylum Nematoda. An ascarid nematode, it is responsible for the disease ascariasis in humans, and it is the largest and most common parasitic worm in humans. A quarter of the human population is estimated to be infected by this parasite. Ascariasis is prevalent worldwide and more so in tropical and subtropical countries.

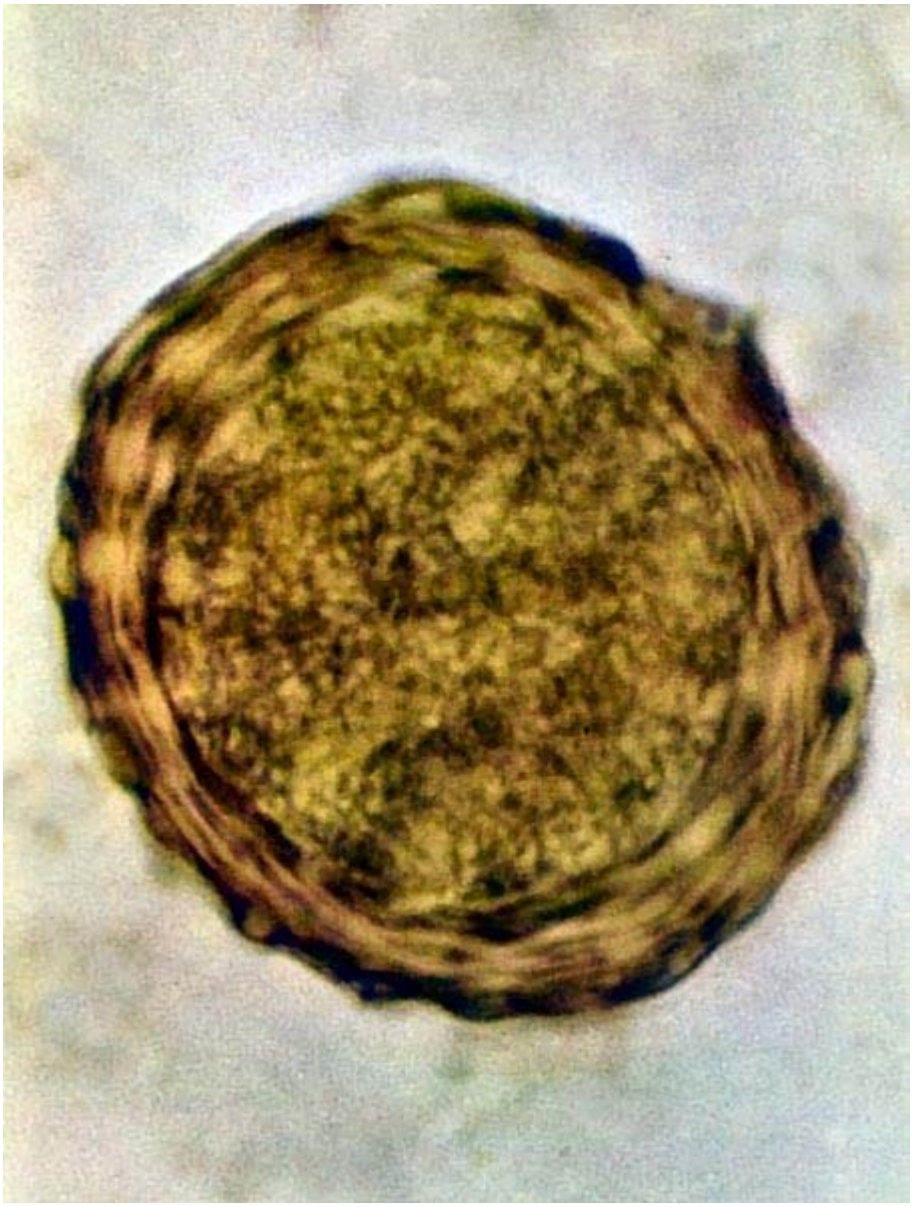
It can reach a length of up to 35 cm.

Life cycle

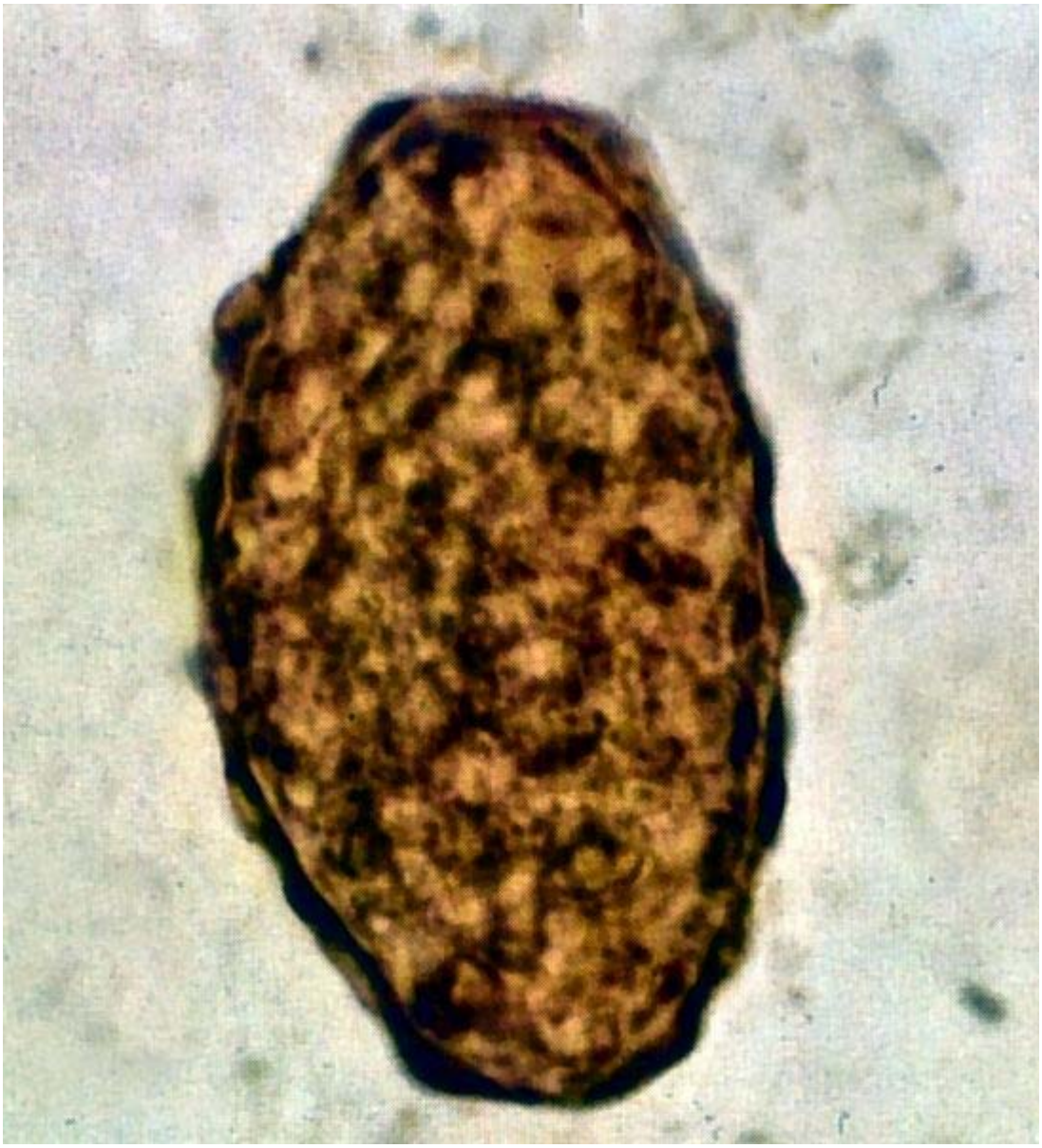
Ascaris lumbricoides, or "roundworm", infections in humans occur when an ingested infective egg releases a larval worm that penetrates the wall of the duodenum and enters the blood stream. From here, it is carried to the liver and heart, and enters pulmonary circulation to break free in the alveoli, where it grows and molts. In 3 weeks, the larvae pass from the respiratory system to be coughed up, swallowed, and thus returned to the small intestine, where they mature to adult male and female worms. Fertilization can now occur and the female produces as many as 200,000 eggs per day for a year. These fertilized eggs become infectious after 2 weeks in soil; they can persist in soil for 10 years or more.

The eggs have a lipid layer, that makes them resistant to the effects of acids and alkalis as well as other chemicals. This resilience helps to explain why this nematode is such a ubiquitous parasite.

Morphology



Fertile egg in human faeces



Infertile egg

Ascaris lumbricoides is characterized by its great size. Males are 2–4 mm in diameter and 15–31 cm long. The males' posterior end is curved ventrally and has a bluntly pointed tail. Females are 3–6 mm wide and 20–49 cm long. The vulva is located in the anterior end and accounts for about a third of its body length. Uteri may contain up to 27 million eggs at a time with 200,000 being laid per day. Fertilized eggs are oval to round in shape and are 45-75 micrometers long and 35-50 micrometers wide with a thick outer shell. Unfertilized eggs measure 88-94 micrometers long and 44 micrometers wide.

Epidemiology

More than 1 billion people are affected by this infection. In the United States there is a reported prevalence of 0.8% of the total population as of 1987. *Ascaris lumbricoides* eggs are extremely resistant to strong chemicals, desiccation, and low temperatures. The eggs can remain viable in the soil for several months or even years.

Eggs of *A. lumbricoides* have been identified in archeological coprolites in the Americas, Europe, Africa, the Middle East, and New Zealand, the oldest ones being more than 24,000 years old.

Infections

Infections with these parasites are more common where sanitation is poor and raw human feces are used as fertilizer.

Prevention

Preventing any fecal-borne disease requires educated hygienic habits/culture and fecal treatment systems once a year. This is particularly important with ascaris because its eggs are usually the most difficult pathogen to kill (besides prions), and the eggs commonly survive 1-3 years. Ascaris lives in the intestine where it lays eggs. Infection occurs when the invisible eggs are eaten. The eggs may get onto vegetables when improperly processed human feces of infected people, are used as fertilizer for food crops. Infection may occur when food is handled without removing or killing the eggs on the hands, clothes, hair, raw vegetables/fruit, or cooked food that is (re)infected by handlers, containers, etc. Bleach will not (necessarily) kill Ascaris eggs but it will remove their sticky film, to allow the eggs to be rinsed away. Ascaris eggs can be reduced by most composting, but to completely kill them may require rubbing alcohol, iodine, specialized chemicals, cooking heat, or "unusually" hot composting (for example, over 120 degrees Fahrenheit for 24 hours). Pests such as house flies, blow flies, roaches, rodents, skunks, etc, can transmit pathogens to anything they touch. For humanure composting to be sanitary it must also prevent the unheated feces from coming into contact with these pests, as well as flood water.

Details of infection process

Infections happen when a human swallows water or food contaminated with unhatched juveniles. The juveniles hatch in the duodenum (1st section of small intestine). They then penetrate the mucosa and submucosa and enter venules or lymphatics. Next they pass through the right heart and into pulmonary circulation. They then break out of the capillaries and enter the air spaces. Acute tissue reaction occurs when several worms get lost during this migration and accumulate in other organs of the body. The juveniles migrate from the lung up the respiratory tract to the pharynx where they are swallowed. They begin producing eggs within 60–65 days of being swallowed. These are produced within the small intestine where the juveniles mature. It might seem odd that the worms

end up in the same place where they began. One hypothesis to account for this behavior is that the migration mimics an intermediate host, which would be required for juveniles of an ancestral form to develop to the third stage. Another possibility is that tissue migration enables faster growth and larger size, which increases reproductive capacity.

Diagnosis and treatment

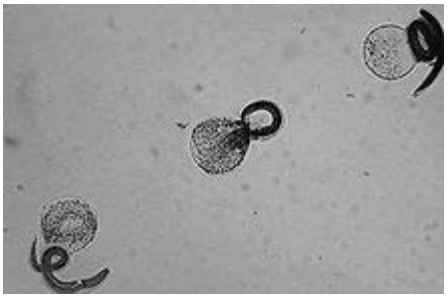
Most diagnoses are made by identifying the appearance of the worm or eggs in feces. Due to the large quantity of eggs laid, physicians can diagnose using only one or two fecal smears.

Infections can be treated with drugs called ascaricides. The treatment of choice is Mebendazole. The drug functions by binding to tubulin in the worms' intestinal cells and body-wall muscles. Nitazoxanide and ivermectin can also be used.

Chapter 7

Baylisascaris

Baylisascaris



Baylisascaris procyonis larvae

Scientific classification	
Kingdom:	Animalia
Phylum:	Nematoda
Class:	Nematoda
Order:	Ascaridida
Family:	Ascarididae
Genus:	*Baylisascaris*

Baylisascaris is a genus of roundworms that infest more than fifty animal species.

Life cycle

Baylisascaris eggs are passed in feces and become active within a month. They can remain viable in the environment for years, withstanding heat and cold. According to University of California, Davis, and the Santa Barbara County Public Health Department, animals become infested either by:

- Swallowing the eggs, or
- Eating another animal infested with *Baylisascaris*.

Disease progression

After an animal swallows the eggs, the microscopic larvae hatch in the intestine and invade the intestinal wall. If they are in their definitive host they develop for several weeks, then enter the intestinal lumen, mature, mate, and produce eggs, which are carried out in the fecal stream. If the larvae are in a paratenic host, they break into the bloodstream and enter various organs, particularly the central nervous system. A great deal of damage occurs wherever the larva tries to make a home. In response to the attack, the body attempts to destroy it by walling it off or killing it. The larva moves rapidly to escape, seeking out the liver, eyes, spinal cord or brain. Occasionally they can be found in the heart, lungs, and other organs. Eventually the larva dies and is reabsorbed by the body. In very small species such as mice, it might take only one or two larvae in the brain to be fatal. If the larva does not cause significant damage in vital organs then the victim will show no signs of disease. On the other hand, if it causes behavioral changes by destroying parts of the brain, the host becomes easier prey, bringing the larva into the intestine of a new host.

Clinical signs in humans

- Skin irritations from larvae migrating within the skin.
- Respiratory discomfort, liver enlargement, and fever due to reaction to larvae migration.
- Eye and brain tissue damage due to the random migration of the larvae.
- Nausea, a lethargic feeling, incoordination and loss of eyesight.
- Severe neurological signs including imbalance, circling and abnormal behavior, caused by extensive tissue damage due to larval migration through the brain, eventually seizures and coma.

Treatment

While worming can rid the intestine of adult *Baylisascaris*, no treatment has been shown to alleviate illness caused by migrating larvae. Despite lack of larvicidal effects, albendazole (20–40 mg/kg/d for 1–4 weeks) has been used to treat many cases.

Baylisascaris *species*

Each *Baylisascaris* species has a host species that it uses to reproduce. The eggs appear in the host species' feces. They can then be ingested by, and infest, a variety of other animals (including humans) that serve as paratenic hosts.

Baylisascaris species include:

- *Baylisascaris procyonis* (of raccoons)
- *Baylisascaris melis* (of European badgers)
- *Baylisascaris transfuga* (of bears)
- *Baylisascaris columnaris* (of skunks) and American badgers)

- *Baylisascaris devosi* (of fishers) and martens)
- *Baylisascaris laevis* (of marmots)

Baylisascaris procyonis

Baylisascaris procyonis, the raccoon parasite, is related to the canine roundworm *Toxocara canis*. It is found in the intestines of raccoons in North America, Japan and Germany. It infests 68 to 82% of some raccoon populations, according to the House Rabbit Society. This parasite can be extremely harmful or deadly to humans.

Baylisascaris columnaris

Skunks carry *Baylisascaris columnaris*, a similar species to *B. procyonis.* Many pet skunks have died from this parasite. According to several skunk experts and Information on Parasites in Skunks by Matt Bolek, Diagnostic Parasitologist, many baby skunks from skunk farms have *B. columnaris* present in their bodies. The exact proportion of new skunks that are infested is unknown. Since the worms are often at too early a stage in development to begin shedding eggs into the feces, a fecal test may not detect the parasite, and the pet should be pre-emptively treated with wormers.

Baylisascaris columnaris is not as prevalent as *B. procyonis.*

Disease prevention

Careful decontamination procedures need to be performed after contact with animal feces. *Baylisascaris* eggs can enter the digestive tract of a person who, for instance, removes dung from his property and then eats without thoroughly washing his hands.

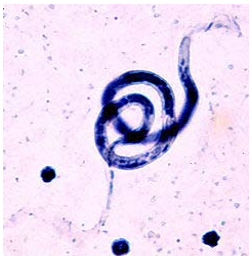
Baylisascaris are highly resistant to decontamination procedures because of their dense cell walls and sticky surface. They can survive hot or freezing weather and certain chemicals, remaining viable for several years. Rats are a known vector, and rat droppings may deposit the eggs into the carpets and interiors of homes.

Bleach can prevent the eggs from sticking, but will not ensure destruction. According to Parasitism in Companion Animals by Olympic Veterinary Hospital, hand washing is an important countermeasure against ingestion, and decontamination of other surfaces is accomplished by thoroughly flaming with a propane torch or treating with lye. According to Bolek, other forms of high heat such as boiling water or steam will accomplish the same result. Children are more likely to be infected than adults because of their tendency to pica, particularly geophagy (eating dirt).

Chapter 8

Brugia Malayi

Brugia malayi



B. malayi, blood smear, Giemsa stain.

Scientific classification	
Kingdom:	Animalia
Phylum:	Nematoda
Class:	Secernentea
Order:	Spirurida
Family:	Onchocercidae
Genus:	*Brugia*
Species:	*B. malayi*

Binomial name
Brugia malayi Brug 1927

Brugia malayi

ICD-10	B74.1
ICD-9	125.

Brugia malayi is a nematode (roundworm), one of the three causative agents of lymphatic filariasis in humans. Lymphatic filariasis, also known as elephantiasis, is a condition characterized by swelling of the lower limbs. The two other filarial causes of lymphatic filariasis are *Wuchereria bancrofti* and *Brugia timori*, which differ from *B. malayi* morphologically, symptomatically, and in geographical extent.

B. malayi is transmitted by mosquitoes and is restricted to South and South East Asia. It is one of the tropical diseases targeted for elimination by the year 2020 by the World Health Organization, which has spurred vaccine and drug development, as well as new methods of vector control.

History of discovery

Identification of a distinct parasite

Lichentenstein and Brug first recognized *B. malayi* as a distinct pathogen in 1927. They reported the occurrence of a species of human filariae in North Sumatra that was both physiologically and morphologically distinct from the *W. bancrofti* microfilariae commonly found in Jakarta and named the pathogen *Filaria malayi*. However, despite epidemiological studies identifying *Filaria malayi* in India, Sri Lanka, China, North Vietnam, and Malaysia in the 1930s, Lichentenstein and Brug's hypothesis was not accepted until the 1940s, when Rao and Mapelstone identified two adult worms in India.

Based on the similarities with *W. bancrofti*, Rao and Mapelstone proposed to call the parasite *Wuchereria malayi* In 1960, however, Buckley proposed to divide the old genus *Wuchereria*, into two genera, *Wuchereria* and *Brugia* and renamed *Filaria malayi* as *Brugia malayi*. *Wuchereria* contains *W. bancrofti*, which so far has only been found to infect humans, and the *Brugia* genus contains *B. malayi*, which infects humans and animals, as well as other zoonotic species.

Identification of different B. malayi strains

In 1957, two subspecies of human infecting *B. malayi* were discovered by Turner and Edeson in Malaysia based on the observation of different patterns of microfilaria periodicity. Periodicity refers to a pronounced peak in microfilariae count during a 24 hour interval when microfilariae are present and detectable in the circulating blood. The basis for this phenomenon remains largely unknown.

- **Nocturnal periodicity:** microfilariae are not detectable in the blood for the majority of the day, but the microfilarial density peaks between midnight and 2 AM nightly.
- **Nocturnal subperiodicity:** microfilariae are present in the blood at all times, but appear at greatest density between noon and 8 PM.

Transmission: Vectors and Reservoirs

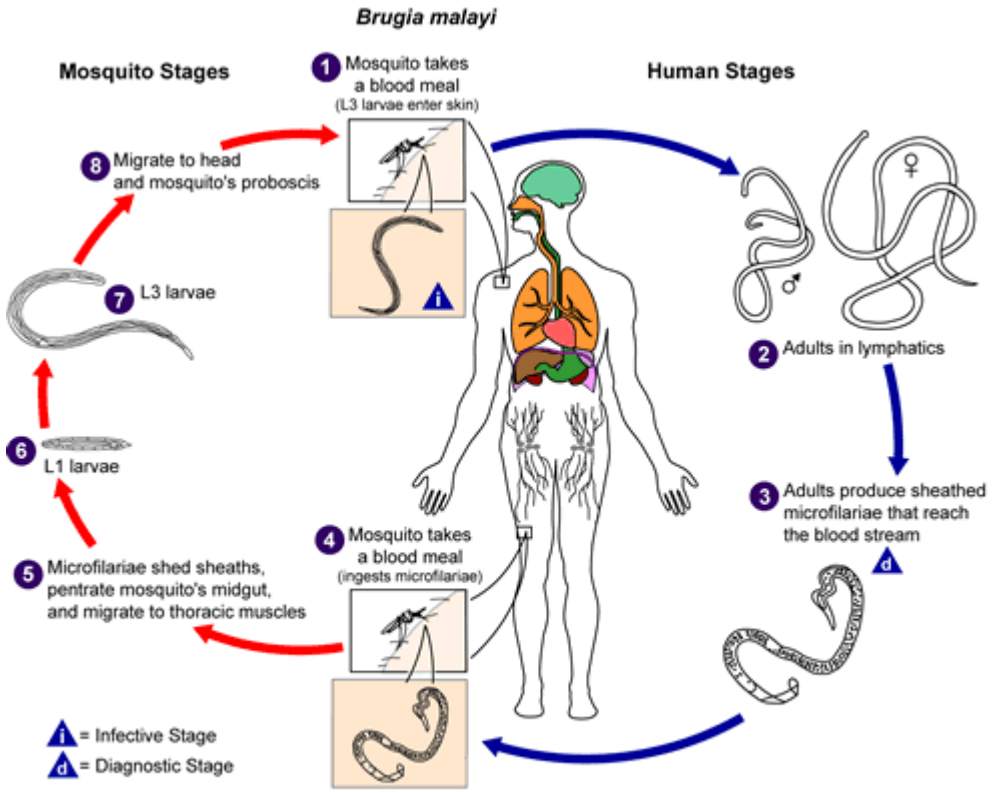
B. malayi is transmitted by a mosquito vector. The principle mosquito vectors include *Mansonia*, *Anopheles*, and *Aedes* mosquitoes. The mosquito serves as a biological vector – it is required for the developmental cycle of the parasite. The geographical distribution of the disease is thus dependent on suitable mosquito breeding habitat.

- The **nocturnal periodic form** is transmitted by *Mansonia* and some Anopheline mosquitoes in open swamps and rice growing areas. These mosquitoes tend to bite at night and appear to only infect humans. Natural animal infections are rare and experimental animals do not retain infection.

- The **nocturnal subperiodic form** is transmitted by *Mansonia* in forest swamps, where mosquitoes bite in the shade at any time. Natural zoonotic infections are common. Cats, dogs, monkeys, slow lorises, civet cats, and hamsters have all been successfully experimentally infected with *B. malayi* from man and may serve as important reservoirs.

The accumulation of many infective mosquito bites—several hundreds to thousands—is required to establish infection. This is because a competent mosquito usually transmits only a few infective L3 larvae, and less than 10% of those larvae progress through all the necessary molting steps and develop into adult worms. Thus those at greatest risk for infection are individuals living in endemic areas—short term tourists are unlikely to develop lymphatic filariasis.

Life cycle



The life cycle of *Brugia malayi*.

Development and replication of *B. malayi* occurs in two discrete phases: in the mosquito vector and in the human. Both stages are essential to the life cycle of the parasite.

Mosquito: The mosquito serves as a biological vector and intermediate host – it is required for the developmental cycle and transmission of B. malayi.
4. The mosquito takes a human blood meal and ingests microfilariae (worm-like sheathed eggs) that circulate in the human blood stream.
5-7 In the mosquito, the microfilariae shed sheaths, penetrate the midgut, and migrate to the thoracic muscles were the microfilariae increase in size, molt, and develop into infective larvae (L1 and L3) over a span of 7–21 days. No multiplication or sexual reproduction of microfilariae occurs in the mosquito.
8-1 The infective larvae (L3) migrate to the salivary glands, enter the proboscis and escape onto human skin when the mosquito takes another blood meal.

Human: B. malayi undergoes further development in the human as well as sexual reproduction and egg production.
1-2 The infective larvae (L3) actively penetrate the skin through the bite hole and develop into adults in the lymphatic system over a span of 6 months. Adult worms can survive in

the lymphatic system for 5–15 years
3. The male and female adult worms mate and the females produce an average of 10,000 sheathed eggs (microfilaria) daily The microfilariae enter the blood stream and exhibit the classic nocturnal periodicity and subperiodicity.
4. Another mosquito takes a blood meal and ingests the microfilariae. Infection depends on the mosquito taking a blood meal during a periodic episode – when microfilariae are present in the bloodstream.

Morphology

Adult

Adult worms resemble the classic nematode roundworm. Long and threadlike, *B. malayi* and other nematode possess only longitudinal muscles and move in an S-shape motion. Adults are typically smaller than adult *W. bancrofti*, though few adults have been isolated. Female adult worms (50 mm) are larger than male worms (25 mm).

Microfilariae

B. malayi microfilariae are 200-275 um in length and have a round anterior end and a pointed posterior end. The microfilariae are sheathed, which stains heavily with Giemsa. The sheath is actually the egg shell, a thin layer that surrounds the egg shell as the microfilariae circulates in the bloodstream. The microfilariae retain the sheath until it is digested in the mosquito midgut.

B. malayi microfilariae resemble *W. bancrofti* and *Loa loa* microfilariae with minor differences that can aid in laboratory diagnosis. *B. malayi* microfilariae can be distinguished by the noncontinuous row of nuclei found in the tip of the tail. There are two terminal nuclei that are distinctly separated from the other nuclei in the tail, whereas the tail of *W. bancrofti* contains no nuclei and *Loa loa* microfilariae nuclei form a continuous row in the tail. *B. malayi* microfilariae also have a characteristic cephalic space ratio of 2:1.

Symptoms

B. malayi is one of the causative agents of lymphatic filariasis, a condition marked by infection and swelling of the lymphatic system. The disease is primarily caused by the presence of worms in the lymphatic vessels and the resulting host response. Signs of infection are typically consistent with those seen in bancroftian filariasis—fever, lymphadenitis, lymphangitis, lymphedema, and secondary bacterial infection—with a few exceptions.

Lymphadenitis

Lymphadenitis, the swelling of the lymph nodes, is a commonly recognized symptom of many diseases. An early manifestation of filariasis, lymphadenitis more frequently occurs in the inguinal area during *B. malayi* infection and can occur before the worms mature.

Lymphangitis

Lymphangitis is the inflammation of the lymphatic vessels in response to infection. It occurs early in the course of infection in response to worm development, molting, death, or bacterial and fungal infection. The affected lymphatic vessel becomes distended and tender, and the overlying skin becomes erythemous and hot. Abscess formation and ulceration of the affected lymph node occasionally occurs during *B. malayi infection*, more readily than in Bancroftian filariasis. Remnants of adult worms can sometimes be found in the ulcer drainage.

Lymphedema (elephantiasis)

The most obvious sign of infection, elephantiasis, is the enlargement of the limbs. A late complication of infection, elephantiasis is a form of lymphedema and is caused by repeated inflammation of the lymphatic vessels. Repeated inflammatory reactions causes vessel dilation and thickening of the affected lymphatic vessels, which can compromise function. The lymphatic system normally functions to maintain fluid balance between tissues and the blood and serves as an integral part of the immune system. Blockage of these vessels due to inflammatory induced fibrosis, dead worms, or granulomatous reactions can interfere with normal fluid balance, thus leading to swelling in the extremities. Elephantiasis resulting from *B. malayi* infection typically affects the distal portions of the extremities. Unlike bancroftian filariasis, *B. malayi* rarely affects genitalia and does not cause funiculitis, orchitis, epididymitis, hydrocele, or chyuria, conditions more readily observed with bancroftian infection.

Secondary bacterial infection

Secondary bacterial infection is common among patients with filariasis. Compromised immune function due to lymphatic damage in addition to lymph node ulcerations and abscesses exposure and impaired circulation due to elephantiasis can cause secondary bacterial or fungal infection. Elephantiasis, in addition to the physical burden of a swollen limb, can be a severely dehabilitating condition given bacterial infection. Part of the WHO’s "Strategy to Eliminate Lymphatic Filariasis" targets hygiene promotion programs in order to alleviate the suffering of affected individuals.

However, clinical manifestations of infection are variable and depend on several factors, including host immune system, infectious dose, and parasite strain differences. Most infections appear asymptomatic, yet vary from individual to individual. Individuals living in endemic areas with microfilaremia may never present with overt symptoms, whereas in other cases, only a few worms can exacerbate a severe inflammatory response.

The development of the disease in humans, however, is not well understood. Adults typically develop worse symptoms, given the long exposure time required for infection. Infection may occur during childhood, but the disease appears to take many years to manifest. The incubation period for infection ranges from 1 month to 2 years and typically microfilariae appear before overt symptoms. Lymphedema can develop within six months and development of elephantiasis has been reported within a year of infection among refugees, who are more immunologically naive. Men tend to develop worse symptoms than women.

Laboratory diagnosis

Tender or enlarged inguinal lymph nodes or swelling in the extremities can alert physicians or public health officials to infection.

With appropriate laboratory equipment, **microscopic examination** of differential morphological features of microfilariae in stained blood films can aid diagnosis—in particular the examination of the tail portion, the presence of a sheath, and the size of the cephalic space. Giemsa staining will uniquely stain *B. malayi* sheath pink. However, blood films can prove difficult given the nocturnal periodicity of some forms of *B. malayi*.

PCR based assays are highly sensitive and can be used to monitor infections both in the human and the mosquito vector. However, PCR assays are time-consuming, labor intensive and require laboratory equipment. Lymphatic filariasis mainly affects the poor, who live in areas without such resources.

The ICT **antigen card test** is widely used in the diagnosis of *W. bancrofti*, but commercial antigens of *B. malayi* have not been historically widely available. However, new research developments have identified a recombinant antigen (BmR1) that is both specific and sensitive in the detection of IgG4 antibodies against *B. malayi* and *B. timori* in ELISA and immunochromatographic rapid dipstick (Brugia Rapid) test. However, it appears that immunoreactivity to this antigen is variable in individuals infected with other filarial nematodes. This research has led to the development of two new rapid immunochromatographic IgG4 cassette tests—**WB rapid** and **panLF rapid**—which detect bancroftian filariasis and all three species of lymphatic filariasis, respectively, with high sensitivity and selectivity.

Management and Therapy

The ["Global Alliance to Eliminate Lymphatic Filariasis"] was launched by the World Health Organization in 2000 with two primary goals: 1) to interrupt transmission and 2) to alleviate the suffering of affected individuals. Mass drug treatment programs are the main strategy for interrupting parasite transmission, and morbidity management, focusing on hygiene, improves the quality of life of infected individuals.

Drugs

A goal of community base efforts is to eliminate microfilariae from the blood of infected individuals in order to prevent transmission to the mosquito. This is primarily accomplished through the use of drugs. The treatment for *B. malayi* infection is the same as for bancroftian filariasis. Diethylcarbamazine (DEC) has been used in mass treatment programs in the form of DEC-medicated salt, as an effective microfilaricidal drug in several locations, including India. While DEC tends to cause adverse reactions like immediate fever and weakness, it is not known to cause any long-term adverse drug effects. DEC has been shown to kill both adult worms and microfilariae. In Malaysia, DEC dosages (6 mg/kg weekly for 6 weeks; 6 mg/kg daily for 9 days) reduced microfilariae by 80% for 18–24 months after treatment in the absence of mosquito control. Microfilariae numbers slowly return many months after treatment, thus requiring multiple drug doses over time in order to achieve long-term control. However, it is not known how many years of mass drug administration is required to eliminate transmission. But currently, there have been no confirmed cases of DEC resistance.

Single doses of two drugs (albendazole-DEC and albendazole-ivermectin) have been shown to remove 99% of microfilariae for a year after treatment and help to improve elephantiasis during early stages of the disease. Ivermectin does not appear to kill adult worms but serves as a less toxic microfilaricide.

Since the discovery of the importance of Wolbachia in the lifecycle of *B. malayi* and other nematodes, novel drug efforts have targeted the endobacterium. Tetracyclines, rifampicin, and chloramphenicol have been effective in vitro by interfering with larvae molting and microfilariae development. Tetracyclines have been shown to cause reproductive and embryogenesis abnormalities in the adult worms, resulting in worm sterility. Clinical trials have demonstrated the successful reduction of *Wolbachia* and microfilariae in onchocerciasis and *W. bancrofti* infected patients. These antibiotics, while acting through a slightly more indirect route, are promising antifilarial drugs.

Hygiene

Secondary bacterial infection is often observed with lymphatic filariasis. Rigorous hygiene practices, including washing with soap and water daily and disinfecting wounds can help heal infected surfaces, and slow and potentially reverse existing tissue damage. Promoting hygiene is essential for lymphatic filariasis patients given the compromised immune and damaged lymphatic systems and can help prevent suffering and disability.

Prevention Strategies

Vaccines

There is currently no licensed vaccine to prevent lymphatic filariasis. However, recent research has produced vaccine candidates with good results in experimental animals. A glutathione-S-transferase, a detoxification enzyme in parasites isolated from Setaria

cervi, a bovine filarial parasite, reduced B. malayi adult parasite burden by more than 82% 90 days post parasite.

Vector control

Vector control has been effective in virtually eliminating lymphatic filariasis in some regions, but vector control combined with chemotherapy produces the best results. It is suggested that 11–12 years of effective vector control may eliminate lymphatic filariasis. Successful methods of *B. malayi* vector control include residual house spraying using DDT and insecticide treated bednets. Mansonia larvae attach their breathing tubes to underwater roots and plants in order to survive. While chemical larvicides have only provided partial control, plant removal would prevent vector development, but would have potential adverse effects on the aquatic environment. Lymphatic filariasis vector control is neglected in comparison to the far more established efforts to control malaria and Dengue vectors. Integrated vector control methods should be applied in areas where the same mosquito species is responsible for transmitting multiple pathogens.

Epidemiology

B. malayi infects 13 million people in south and southeast Asia and is responsible for nearly 10% of the world's total cases of lymphatic filariasis. *B. malayi* infection is endemic or potentially endemic in 16 countries, where it is most common in southern China and India, but also occurs in Indonesia, Thailand, Vietnam, Malaysia, the Philippines, and South Korea. The distribution of *B. malayi* overlaps with *W. bancrofti* in these regions, but does not coexist with *B. timori*. Regional foci of endemicity are determined in part by the mosquito vectors.

Genome deciphered

On September 20, 2007, scientists sequenced the genome of *Brugia malayi* in the paper "Draft Genome for the Filarial Nematode Parasite Brugia malayi" by Elodie Ghedin, et al. Science 317, 1756 (2007); DOI:10.1126/science.1145406. Identifying the genes of this organism might lead to development of new drugs and vaccines.

To decipher the genome, "Whole Genome Shotgun Sequencing" was performed. The genome was found to be approximately 90-95 mega bases in size. The results of the sequencing was then compared to that of the *C. elegans*, along with its prototype *C. briggsae*. These other organisms were incorporated in the study and proved to be important for several reasons:

- comparing genomes using *C. elegans* was extremely beneficial in identifying similar linkages in genes.
    - the researchers found a genomic conservation
    - also found data that supported an absence of conservation at a more local gene level

        - This demonstrated that rearrangements had occurred over time between the *C. elegans* and *B. malayi* and allowed researchers to identify genes or proteins that were more specific to *B. malayi*
        - These unique genes were significant because they could have led to the parasitism seen in *B. malayi*, and would therefore be seen as appropriate targets for future studies.
- gene linkages offer new insight into the evolutionary trend of parasitic genes that could possess clues to further explain their unique ability to successfully survive for many years in human hosts.

Potential for New Drugs to Treat B. Malayi

Sequence comparisons between the two genomes allow us to map *C. elegans* orthologs to *B. malayi* genes. Using these orthology mappings (between *C. elegans* and *B.malayi*) and by incorporating the extensive genomic and functional genomic data, including genome-wide RNAi screens, that already exist for *C. elegans*, we identify potentially essential genes in *B. malayi*. Scientists are hoping to be able to use these genes as potential new drug targets for new drug treatments. The longevity of this parasite complicates treatment because existing drugs target the larvae and, thus, do not completely kill the worms. The drugs often must be taken periodically for years, and the worm can cause a massive immune reaction when it dies and releases foreign molecules in the body. Drug treatments for filariasis have not changed significantly in over 20 years, and with the risk of resistance rising, there is an urgent need for the development of new anti-filarial drug therapies. From the genome sequence, Dr. Ghedin and her co-investigators identified several metabolic pathways containing dozens of gene products that they believe are likely to be helpful for the discovery of more targeted and effective drug therapies.

- Possible new drug targets include:
    - molting
    - nuclear receptors
    - collagens and collagen processing
    - neuronal signaling
    - the *B. malayi* kinome
    - reliance on host (*B. malayi*) and endosymbiont (*Wolbachia*) metabolism.

These potential new targets for drugs or vaccines should provide new opportunities for understanding, treating and preventing elephantiasis.

Endosymbiotic Relationship of *Brugia malayi* with *Wolbachia*

The relationship between the bacteria *Wolbachia* and *B. malayi* is not fully understood. Some theories based on research done with *Wuchereria bancrofti*, another worm that causes filariasis, believe that *Wolbachia* may: aid in embryogenesis of the worm, be responsible for potent inflammatory responses from macrophages and filarial disease, and may be linked to the onset of lyphodema and blindness sometimes associated with *B. malayi* infections. According to a study done by University of Bonn in Ghana,

doxycycline was effective in depleting *Wolbachia* from *W. bancrofti*. It is likely that the mechanism of doxycycline is similar to that in other filarial species, i.e., a predominant blockade of embryogenesis, leading to a decline of microfilariae according to their half-life. This could render doxycycline treatment an additional tool for the treatment of microfilaria-associated diseases in bancroftian filariasis, along with *B. malayi* fiariasis. The doxycycline course of treatment would be much shorter as it would be able to target the adult worm rather than the larvae current treatments kill, and there would be fewer side effects for the infected individual.

Genome use in Transplant Research

Another hopeful use for the research is in the area of transplant research. Because the *B. malayi* genome is the first parasitic genome to have been sequenced, the implications on the mechanism of parasitism in humans are crucial to understand. According to Alan L. Scott, Ph.D., a collaborator at Johns Hopkins University, it is this understanding of how a particular parasite, such as *B. malayi*, which can adapt to humans, that may yield medical benefits far beyond treating elephantiasis. According to the author, "This worm can reside in the host for years and not necessarily cause disease, in fact the less disease the individual has, the more worms there are in circulation. Now that we know those genes don't exist in humans we can target them to control disease." Some of the predicted proteins for these new genes appear to be similar to known immuno-modulator proteins, regulators of the immune system, suggesting that they are involved in deactivating the host's immune system to ensure the parasite remains undetected. Knowledge of these previously unknown immune suppressors could also be of use in organ transplants and to help treat autoimmune disease.

A specific gene of interest is the ***Brugia malayi* MIF** (macrophage migration inhibition factor) gene. Results suggest that ***B. malayi* MIF** may interact with the human immune system during the course of infection by altering the function of macrophages in the infected individual, and studies are currently testing the hypothesis that MIF may be involved in reducing the host’s immune response to the filarial parasite.

According to the Filarial Genome Project being done by The Special Programme for Research and Training in Tropical Diseases (TDR), the *Brugia malayi* MIF gene is expressed in all life-cycle stages of the parasite, and results suggest that *B. malayi* MIF may interact with the human immune system during the course of infection by altering the function of macrophages in the infected individual. TDR also states that studies are currently testing the hypothesis that MIF may be involved in reducing the host’s immune response to the filarial parasite. Understanding how this particular parasite has adapted to humans may help organ transplant researchers by figuring out how to prevent the immune system from attacking the transplanted tissue.

Overall hope for the use of the Genome Sequencing

The genomic information gives us a better understanding of what genes are important for different processes in the parasite’s life cycle. So, it will now be possible to target these

genes more specifically and interrupt its life cycle. And, understanding how this particular parasite has adapted to humans may yield medical benefits far beyond treating elephantiasis, says collaborator Alan L. Scott, Ph.D., of the Bloomberg School of Public Health at Johns Hopkins University. “Parasitic worms are a lot like foreign tissue that has been transplanted into the human body. But unlike baboon hearts or pig kidneys, which the immune system quickly recognizes as foreign and rejects, worms can survive for years in the body. Discovering how they do so may someday benefit transplant surgery,” explained Dr. Scott.

Chapter 9

Caenorhabditis Briggsae and Capillaria Hepatica

Caenorhabditis briggsae

Caenorhabditis briggsae	
Scientific classification	
Kingdom:	Animalia
Phylum:	Nematoda
Class:	Secernentea
Order:	Rhabditida
Family:	Rhabditidae
Genus:	*Caenorhabditis*
Species:	***C. briggsae***
Binomial name	
Caenorhabditis briggsae	

Caenorhabditis briggsae is a small nematode, closely related to *Caenorhabditis elegans*. The differences between the two species are subtle. The male tail in *C. briggsae* has a slightly different morphology than *C. elegans*. Other differences include changes in vulval precursor competence and the placement of excretory duct opening . *C. briggsae* is frequently used to study the differences between it and the more intimately understood *C. elegans*, especially at the DNA and protein sequence level. Several mutant strains of *C. briggsae* have also been isolated that facilitate genetic analysis of this organism . *C. briggsae*, like *C. elegans*, is a hermaphrodite. The genome sequence for *C. briggsae* was determined in 2003.

History

Ellsworth C. Dougherty first recognized the potential of C. briggsae, which had been found by Margaret Briggs in a pile of leaves on the campus of Stanford University in Palo Alto, California, in 1944 and used in her MS studies under the direction of Dr.

Arthur C. Giese (Briggs, 1946; Gochnauer, 2004). Briggs studied the lifecycle of what she identified as Rhabditis sp. in association with bacteria and in various culture media devoid of other organisms. She showed that the population could not be sustained in the absence of bacteria or even on dead bacterial cells; living bacteria were a necessary food source. However, survival of individuals was greater on some bacteria-free media than others.

Habitat

Caenorhabditis briggsae can often be found in compost, garden beds, moist mushrooms or rotting fruit rich with microorganisms and various nutrients. The organism's main habitat is often considered to be the temperate regions of the globe, often accompanying its relatives *C. elegans* and *C. remanei*.

Overview of Genome

The whole genome sequencing project (Stein et al., 2003) revealed that the genomes of C. briggsae and C. elegans have much in common. For example, both worms have the same number of chromosomes (six chromosomes each), similar genome size, and similar numbers of protein coding and non-protein coding genes. Further analysis demonstrated that about 62% of the protein coding genes in C. briggsae have orthologs in C. elegans. Nevertheless, many interesting species-specific features including species-specific genes exist, which serve as the foundation for comparative analysis. In the following subsections, we will describe the C. briggsae genome and compare it with the C. elegans genome.

Comparative genomics with C.elegans

Caenorhabditis briggsae is a soil nematode estimated to have diverged from C. elegans approximately 80-100 million years ago, and yet is morphologically almost indistinguishable from it. Areas of sequence encoding proteins are mostly conserved between the two species while most intergenic and intronic sequence are divergent. Areas of similarity between the sequence of the two organisms can suggest coding exons or point to regulatory regions and to RNA genes missed in standard analysis.

Capillaria hepatica

Capillaria hepatica	
Scientific classification	
Kingdom:	Animalia
Phylum:	Nematoda
Class:	Adenophorea
Subclass:	Enoplia
Order:	Trichurida
Family:	Trichinellidae
Genus:	*Capillaria*
Species:	***C. hepatica***
Binomial name	
Capillaria hepatica Bancroft, 1893	

Capillaria hepatica is a parasitic nematode which causes **hepatic capillariasis** in rodents and numerous other mammal species, including man. The life cycle of *C. hepatica* may be completed in a single host species. However, the eggs, which are laid in the liver, must mature outside of the host body (in the environment) prior to infecting a new host. So the death of the host in which the adults reach sexual maturity, either by being eaten or dying and decomposing, is necessary for completion of the life cycle.

Discovery and taxonomy

This species was first described in 1893, from specimens found in the liver of *Rattus norvegicus*, and named *Trichocephalus hepaticus*. Various authors have subsequently renamed it *Trichosoma hepaticum*, *Capillaria hepatica*, *Hepaticola hepatica* and *Calodium hepaticum*. Currently it is usually referred to as either *Capillaria hepatica* or, less often, *Calodium hepaticum*.

Hosts and distribution

Adults are often found in dozens of rodent species, but also occur in a wide variety of other wild and domestic mammals, and occasionally humans. *C. hepatica* has been found on every continent, and infestation rates of wild-caught rats of up to 100% have been reported.

Usually, Capillaria hepatica is found in rodents, monkeys and other animals. *Capillaria hepatica* is rarely found in humans and at least 40 cases have been reported.

Of the human infections, most have been found in children.

Life cycle

Hosts ingest *C. hepatica* eggs (from sources outlined below) which hatch into first stage larvae (L1). The L1 larvae bore through the intestinal wall and are carried to the liver by the hepatic portal vein. Development from the L1 stage to sexually mature adults occurs in the liver within 18–21 days. Eggs are laid in the liver parenchyma of the host throughout the adult worm's life span, which lasts for about 30–40 days. Up to 938,000 eggs have been reported from the liver of a single rodent host.

As the adult *C. hepatica* begin to die in the liver tissue, their decomposition accelerates the immune response of the host. This response leads to chronic inflammation and encapsulation of the dead worms in collagen fibers, and eventually to septal fibrosis (abnormal connective tissue growth) and cirrhosis of the liver.

Meanwhile, the eggs in the liver exist in a state of arrested development – they are unable to develop into larvae until they spend some time outside of the host, in the environment. Escaping from the liver tissue may be accomplished either by the death and decomposition of the host's body, or by the consumption and digestion of the host by a predator or scavenger. If the host is eaten, the eggs will pass into the environment in the feces of the predator or scavenger. In the environment, eggs require 4–5 weeks to develop, and may remain viable in a dormant state for several more months. Once these "environmentally-conditioned" eggs are eaten by a suitable host, the first stage larvae (L1) hatch in the intestine and continue the life cycle.

Diagnosis and treatment

In human cases, symptoms of hepatic capillariasis include abdominal pain with fever and chills, hepatitis (liver inflammation), ascites (excess fluid in the peritoneal cavity), hepatolithiasis (gallstones in the bile ducts), and hepatomegaly (enlarged liver). Diagnosis is made by finding eggs or adults of *C. hepatica* in liver biopsy samples. The encapsulated eggs and adults may appear as white nodules which measure 2–3 mm in diameter on the surface and interior of the liver at autopsy.

Successful treatment of human cases with thiabendazole or albendazole (with or without corticosteroids) have been reported.

Research uses

The selective liver damage by *C. hepatica* in rodents has been used in model systems to study the extensive regeneration capabilities of the mammalian liver, and for testing antifibrotic drugs.

In Australia, several releases of *Capillaria hepatica* eggs in the field have been unable to control rapidly expanding populations of mice.

Chapter 10

Capillaria Plica and Dracunculus

Capillaria plica

Capillaria plica	
Scientific classification	
Kingdom:	Animalia
Phylum:	Nematoda
Class:	Adenophorea
Subclass:	Enoplia
Order:	Trichurida
Family:	Trichinellidae
Genus:	*Capillaria*
Species:	***C. plica***
Binomial name	
Capillaria plica Rudolph, 1819	
Synonyms	
Pearsonema plica	

Capillaria plica (dog bladder worm) is a parasitic nematode which is most often found in the urinary bladder, and occasionally in the kidneys, of dogs and foxes. It has also been found in the domestic cat, and various wild mammals. Its presence usually produces no clinical symptoms, but in some cases, it leads to hematuria (blood in the urine), cystitis (inflammation of the urinary bladder), or difficulty in urination.

Taxonomy and Description

This species was originally described in 1819, and named *Capillaria plica*. In 1982, the suggestion was made that *C. plica* be transferred to the genus *Pearsonema* Freitas & Mendonça 1960, as *Pearsonema plica*. Currently, both names are used in the literature with roughly equal frequency. For example, searches of the PubMed database performed

on 22 Nov 2008 yielded the same number of hits dated 2000 or later using either *Capillaria plica* or *Pearsonema plica*.

Males are 13–30 mm long and females are 30–60 mm long. Eggs are colorless, oval and pitted, and measure 50-68 µm by 22-32 µm.

Hosts and distribution

Capillaria plica is often found in the urine, urinary bladder or kidneys of dogs and cats in North America, Europe, Asia and Africa. It has also been identified in the urinary bladder and kidneys of several wild mammals in North America and Europe:

- American Badger (*Taxidea taxus*; North America)
- American Mink (*Mustela vison*; in introduced European populations)
- Brown Bear (*Ursus arctos*; Russia)
- Coyote (*Canis latrans*; North America)
- European Badger (*Meles meles*; Europe)
- European mink (*Mustela lutreola*; Europe)
- Fisher (*Martes pennanti*; North America)
- Lynx (*Felis lynx*; Lithuania)
- Marten (*Martes americanus*; North America)
- Masked Shrew (*Sorex cinereus*; North America)
- Northern Short-tailed Shrew (*Blarina brevicauda*; North America)
- Raccoon (*Procyon lotor*; North America)
- Raccoon Dog (*Nyctereutes procyonoides*; Europe)
- Red Fox (*Vulpes vulpes*; North America and Europe)
- Skunk (*Mephitis mephitis*; North America)
- Wolf (*Canis lupus*; Europe)

Life cycle

In dogs and cats, eggs of *Capillaria plica* are released in the urine of the mammalian definitive host. First stage larvae (L1) develop within the eggshell in 30–36 days. When eaten by the intermediate host -- earthworms of the genera *Lumbricus* or *Dendrobaena* -- the L1 larvae hatch in the earthworm's intestine. The larvae burrow through the intestinal wall and become embedded in connective tissue throughout the worm's body. If the earthworm is eaten by a suitable mammalian host, the larvae molt into second stage larvae (L2), burrow through the intestinal wall, and molt again into third stage larvae (L3). The L3 are carried through the circulatory system to the glomeruli of the kidneys. From there, they travel down the ureter to the urinary bladder. By 33 days post-infection, third (L3) and fourth-stage larvae (L4) are found in the urinary bladder. Here they mature into adults and reproduce sexually, shedding fertilized eggs into the urine of the host within about 60 days of infection. Detailed life cycle studies have not been carried out with wild animal definitive hosts.

Prevalence

Prevalence rates of up to 50% in wild hosts and 76% in kennel dogs have been reported.

Clinical symptoms

Most infected animals exhibit no clinical symptoms. In cases of heavy infestation, symptoms may include cystitis (inflammation of the urinary bladder), mild proteinuria (protein in the urine), and hematuria (blood in the urine). Mild inflammation of the ureter has also been reported.

Diagnosis and treatment

Diagnosis in cases of hematuria or cystitis is made by finding eggs in the urine sediment. Successful treatment with levamisole, ivermectin or fenbendazole have been reported.

Dracunculus

Dracunculus	
Scientific classification	
Kingdom:	Animalia
Phylum:	Nematoda
Class:	Secernentea
Order:	Camallanida
Superfamily:	Dracunculoidea
Family:	Dracunculidae
Genus:	***Dracunculus***
Species	

D. alii
D. dahomensis
D. fuelliborni
D. globocephalus
D. insignis
D. lutrae
D. medinensis
D. ophidensis

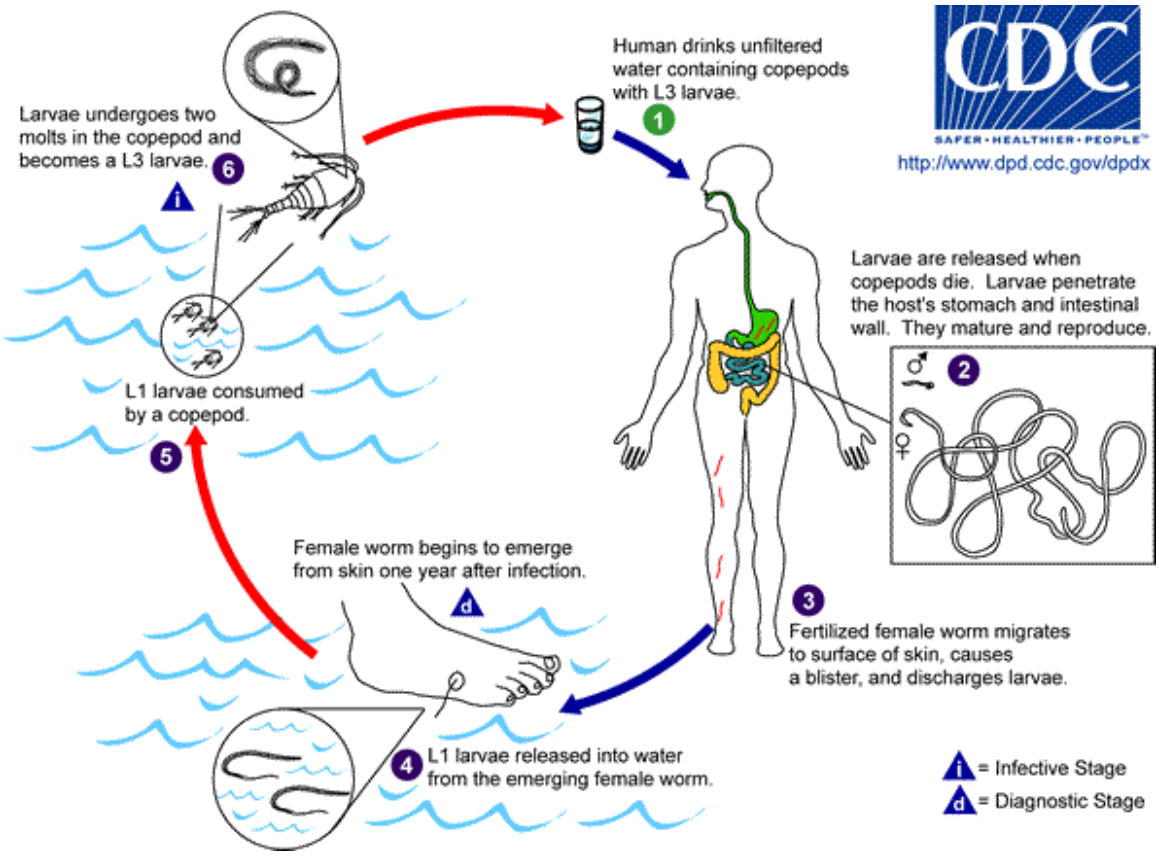
Dracunculus is a genus of spiruroid nematode parasites in the family Dracunculidae. Some species infest humans, and alter their hosts' behaviour in a way that supports the

worm's reproductive cycle. Dracunculus causes a blister to form on its host's foot, causing severe pain and a sensation of heat. The human, soothing this by soaking it in water, causes the blister to burst, allowing reproductive larvae into the water where they can await the next host to infect.

The worms can reach a meter in length. If one simply pulls off the protruding head of the worm, the burst worm will release larvae into the tissue causing further infection. Hence it is important to remove the worm slowly (over a period of weeks). This is typically undertaken by winding the worm onto a stick (say, a matchstick), by a few centimetres each day.

Species

D. medinensis and *D. insignis*



The life cycle of *Dracunculus medinensis*.

The best known species is *D. medinensis*, known commonly as the Guinea worm. This parasite is frequently found in the subcutaneous tissues and muscles of humans, dogs, and sometimes cattle and horses. The medical name for this condition is dracunculiasis. The disease causes cutaneous nodules and subsequent ulcers. The anterior end of the adult female worm protrudes from the host animal's body, most commonly on a lower limb, through an ulcer. When the worm feels the presence of cold water, muscle contractions in

its body cause its uterus (which fills the whole body cavity) to burst, releasing hundreds of thousands of first-stage larvae into the water, where they can find new hosts.

D. insignis infects dogs and wild carnivores, causing cutaneous lesions, ulcers, and sometimes heart and vertebral column lesions. Like *D. medinensis*, it is also known as Guinea worm, as well as *Dragon* or *Fiery Dragon*.

DNA fingerprinting can differentiate between *D. medinensis* and *D. insignis*, which is important to efforts to eradicate dracunculiasis.

Other species

D. fuelliborni parasitizes opossum, *D. lutrae* parasitizes otters, and *D. ophidensis* parasitizes reptiles.

Distribution

In 2011 only four countries still had the human-infecting *Dracunculus medinensis* – and of these, Ghana, Ethiopia and Mali have nearly eliminated it. Of the 1785 cases found in 2010, 1690 were in south Sudan, which is 38 per cent fewer than the number of cases in 2009.

Life cycle

The life cycle was elucidated in 1870 when Alexei Pavlovich Fedchenko of Russia discovered the copepod crustacean intermediate host stages.

Rod of Asclepius

It has been suggested that the symbol once represented a worm wrapped around a rod; parasitic worms such as the guinea worm (*Dracunculus medinensis*) were common in ancient times, and were extracted from beneath the skin by winding them slowly around a stick. According to this theory, physicians might have advertised this common service by posting a sign depicting a worm on a rod. However plausible, no concrete evidence in support of this theory has been adduced.

Chapter 11

Elaeophora Poeli and Elaeophora Sagitta

Elaeophora poeli

Elaeophora	
Scientific classification	
Kingdom:	Animalia
Subkingdom:	Eumetazoa
(unranked):	Bilateria
Superphylum:	Platyzoa
Phylum:	Nematoda
Class:	Secernentea
Subclass:	Spiruria
Order:	Spirurida
Superfamily:	Filarioidea
Family:	Onchocercidae
Genus:	*Elaeophora*
Species:	***E. poeli***
Binomial name	
Elaeophora poeli (Vryburg, 1879)	

Elaeophora poeli is a parasitic nematode found in the aorta, and sometimes the heart, of various cattle throughout Asia, and in parts of Africa. It is a large nematode, with males measuring 45-70 mm long and 200-260 µm wide, and females 40-300 mm long and 350 µm wide. Microfilariae are 340-346 µm long and 7.0-7.5 µm wide. Despite the fact that it lives in nodules (aneurysms) in the walls of the aorta and heart, apparent clinical symptoms of *E. poeli* infestation are seldom reported.

Discovery and nomenclature

This species was first described from Water buffalo in 1879, and named *Filaria poeli*. In 1912, it was transferred to the newly erected genus *Elaeophora* Railliet and Henry 1912. In 1938, a detailed redescription of *E. poeli* was published. In that study, *E. poeli* was determined to be the same animal that previous authors had referred to as *Filaria blini* and *Filaria haemophila*, both isolated from Water buffalo aortas.

Hosts and geographic distribution

E. poeli has been found in several species of cattle: African buffalo (*Syncerus caffer*), Carabao or Water buffalo (*Bubalus bubalus*), and Zebu (*Bos primigenius indicus*). The geographic distribution of this species includes several Asian and African nations: Democratic Republic of Congo, India, Indonesia, Malaysia, Mozambique, the Philippines, Tanzania, Thailand, Uganda, and Vietnam.

Life cycle

The life cycle of *E. poeli* is not known. The adults usually live attached to the inner walls of the aorta. They make aneurysmal (ie. bulging) nodules in the wall of the aorta, which can be up to 2 cm in diameter. The male lives curled up inside the nodule, while the female lives with its head in the nodule and its body free in the lumen (interior space) of the aorta. Presumably the female sheds the offspring (microfilariae) directly into the host's bloodstream. Adults have also been found in nodules on the epicardium of the heart.

Prevalence

The percentage of animals found to be infested in large-scale slaughterhouse studies range from 1.7% in Tanzanian Zebu (*Bos primigenius indicus*) to over 60% in Philippine *Bubalus bubalis*. A study of free-ranging buffalo in Queen Elizabeth National Park, Uganda, yielded a 55% infestation rate.

Clinical significance

The nodules where the filariae reside (as described above) are aneurysms - bulges in the aorta wall - which could conceivably rupture. Corrugated and migratory tract lesions on the inner wall of the aorta and fibrin strands attached to the nodules have also been described. The latter study found narrowing of the aorta down to 1/3 of its usual diameter in some cases.

Despite the presence of nodules 2-cm in diameter on aorta walls and heart tissue, and narrowing of the aorta, almost all studies of *E. poeli* infestation mention a lack of obvious clinical symptoms in infested individuals. One study found a strong correlation between

infestation and visceral pleuresy (inflammation of the membrane that surrounds the lungs).

Elaeophora sagitta

Elaeophora	
Scientific classification	
Kingdom:	Animalia
Subkingdom:	Eumetazoa
(unranked):	Bilateria
Superphylum:	Platyzoa
Phylum:	Nematoda
Class:	Secernentea
Subclass:	Spiruria
Order:	Spirurida
Superfamily:	Filarioidea
Family:	Onchocercidae
Genus:	*Elaeophora*
Species:	*E. sagitta*
Binomial name	
Elaeophora sagitta (Linstow, 1907) Anderson & Bain, 1976	

Elaeophora sagitta is a parasitic nematode found in the heart, coronary arteries and pulmonary arteries of several ruminant species and Water buffalo in Africa. Infestation usually occurs without significant health effects in the Greater kudu (*Tragelaphus strepsiceros*), but may affect cardiac function in some other host species.

Discovery and nomenclature

This species was first described in 1907 from the heart of a Bushbuck (*Tragelaphus scriptus*) from Cameroon, and named *Filaria sagitta*. In 1926, it was transferred to the genus *Cordophilus*, as *Cordophilus sagittus*. In 1976, the genus *Cordophilus* was made a synonym of the genus *Elaeophora*, so this species became *Elaeophora sagitta*.

Hosts and geographic distribution

Adults of *E. sagitta* have been found attached to the inner walls of the chambers and vessels of the heart, as well as the arterioles of the lungs of various hosts: Bushbuck (*Tragelaphus scriptus*), Greater kudu (*Tragelaphus strepsiceros*), bongos (*Tragelaphus eurycerus*), nyala (*Tragelaphus angasii*), common eland (*Taurotragus oryx*), and African Forest Buffalo (*Syncerus caffer nanus*). This species has also been found in unspecified "cattle". Lesions similar to those described in *E. sagitta* infestations were also found in sheep (*Ovis aries*), but the actual parasites were not recovered. *E. sagitta* has been found in several African nations: Cameroon, Kenya, Malawi, Mocambique, the Republic of the Congo, South Africa and Swaziland.

Life cycle

The life cycle of *E. sagitta* has not been studied in detail. It is viviparous, since the female sheds microfilariae, rather than eggs, directly into the blood stream.

Prevalence

Infestation rates as high as 74% and over 90% have been reported in free-ranging kudu. Infestation in a herd of eland (*Taurotragus oryx*) in Kruger National Park was reported as "nearly half of 33" individuals. A slaughterhouse survey in Swaziland yielded a very low prevalence–77 of 18,458 (0.416%) -- of bovine hearts showing lesions typical of *E. sagitta* infestation. In other host species, only isolated cases have been reported.

Clinical significance

E. sagitta adults are typically found in the heart ventricles, as well as coronary and pulmonary arteries, and occasionally coronary veins. They produce aneurysmal (bulging) lesions in the vessel walls which are 1–2 cm in diameter, and have been associated with hypertrophy and dilatation of heart ventricles, thrombosis (blood clots) and myocarditis (inflammation of the heart muscle). The degree of interference with general circulatory function has not been studied in detail. As one author points out, however, if the infested host is fleeing from a lion, only a minor difference in cardiopulmonary efficiency could certainly affect survival.

E. sagitta infestation appears to be clinically benign in greater kudu. Some fatalities in a herd of eland were attributed to *E. sagitta* infestation, though many of the eland were also "heavily" infested with various gastrointestinal parasites. In the Congo, *E. sagitta* infestation was suggested to be one of the factors which led to mortality in several bongos (*Tragelaphus eurycerus*) and one African Forest Buffalo (*Syncerus caffer nanus*)

Chapter 12

Elaeophora Schneideri and Enterobius

Elaeophora schneideri

Elaeophora schneideri	
Scientific classification	
Kingdom:	Animalia
Phylum:	Nematoda
Class:	Secernentea
Order:	Spirurida
Family:	Onchocercidae
Genus:	*Elaeophora*
Species:	***E. schneideri***
Binomial name	
Elaeophora schneideri Wehr & Dikmans, 1935	

Elaeophora schneideri (arterial worm; cause of elaeophorosis, aka "filarial dermatitis" or "sorehead" in sheep; or "clear-eyed" blindness in elk) is a nematode which infests several mammalian hosts in North America. It is transmitted by horse-flies. Infection in the normal definitive hosts, Mule deer or Black-tailed deer, seldom produces clinical symptoms. In other hosts, such as sheep, elk, moose, and goats, infection with *E. schneideri* leads to elaeophorosis. Symptoms of elaeophorosis include necrosis of the muzzle, ears, and optic nerves; lack of coordination (ataxia); facial or lower limb dermatitis; horn deformities; blindness; and death.

Discovery

Symptoms of elaeophorosis were first observed in 1933, in sheep (New Mexico) and mule deer (Utah) infested by an unknown nematode worm. Specimens were first

described as *Macdonaldius* sp. in 1934, and later revised to *Elaeophora schneideri* Wehr and Dikmans, 1935. A more complete description of adults from elk, sheep and deer was published in 1968.

Description

The female adults are 60–120 mm long and 56-89 µm wide, while males are 55–85 mm long and 40-68 µm wide. The microfilariae are 239-279 µm long and 11-15 µm wide.

Hosts and Life cycle

The normal definitive hosts for *E. schneideri* are the Mule deer and Black-tailed deer. It has also been found in several other wild mammalian hosts: White tailed deer, elk, moose, Bighorn sheep, Barbary sheep, Domestic sheep. Infestation was also found in sika deer on Texas ranches. Infestations of cattle, horses or humans have not been reported. The vectors of *E. schneideri* are blood-feeding Horse-flies of the family Tabanidae, genera *Hybomitra*, *Tabanus*, or Silvius.

Life cycle. In the normal definitive host, *E. schneideri* microfilariae are found in the host's skin, particularly around the forehead and poll areas. When a horse-fly feeds on an infected host, it ingests some of these microfilariae. Within a few weeks, the microfilariae develop into infective third-stage larvae (called L3) in the fly's fat body tissue and haemocoel. The mature L3 larvae migrate to the head and mouth-parts of the fly. When the fly feeds on another host, the L3 larvae enter the host's blood stream through the bite wound. They are carried throughout the host's circulatory system, and embed themselves in the walls of the leptomeningeal arteries. After a 2-week maturation period, they migrate to the carotid artery. About 4–6 months later they become sexually mature and begin to produce microfilariae. The adults live for 3–4 years. The microfilariae are released into the host's bloodstream, which carries them to the small capillaries of the skin in the head region. They become lodged in these narrow spaces, and await the next feeding horsefly.

In abnormal definitive hosts, such as sheep and elk, the adults may remain in the smaller arteries of the head and face region, instead of migrating to the carotid artery. In these smaller arteries, they obstruct blood flow to various parts of the head, face, and brain; which leads to the clinical symptoms of elaeophorosis (see below).

Distribution

In the United States, *E. schneideri* has been reported from various wild hosts in 18 states: Arizona, Arkansas, California, Colorado, Florida, Georgia, Louisiana, Montana, Nebraska, New Mexico, North Dakota (in imported animals), Oklahoma, South Carolina, South Dakota, Texas, Utah, Washington and Wyoming. It has also been reported from elk and Black-tailed Deer in Canada.

Species of *Elaeophora* other than *E. schneideri* infest various mammals in Europe, Asia and Africa. One survey of Red Deer (*Cervus e. elaphus*), Fallow deer (*Dama dama*), as well as domestic sheep, cattle and goats in Czechoslovakia yielded no specimens of *E. schneideri*.

Prevalence

Field surveys of wild mammal populations have shown wide variation in the percentage of animals infested with *E. schneideri*. Surveys of all hosts in the southeastern United States generally indicate infection rates in the range of 2-15%. Prevalence rates as high as 50% in mule deer near Durango, Colorado; 78% in black tailed deer herd in Mendocino County, California, 90% in mule deer herds at high elevation sites in Arizona and New Mexico, and 100% (14 of 14 animals) in Texas mule deer have been reported. In areas of high abundance in definitive hosts, the fear of spread to commercial livestock is greatest.

Clinical symptoms

Symptoms of *Elaeophora schneideri* infestation vary among the different mammalian hosts.

In the normal definitive hosts, mule deer and black-tailed deer, infestations are asymptomatic.

In the white-tailed deer, infestation is also often asymptomatic. However, blockage and thickening of coronary, cephalic, brachial and femoral arteries and sublingual food impaction have been reported in this host.

In both moose and elk, infestation can lead to fatality. Blockage of the carotid and other arteries of the head and face region by *E. schneideri* adults restricts local bloodflow, leading to ischemic damage to the brain, optic nerve, ears, muzzle and other facial areas. The results are often blindness; walking in circles or poor coordination (ataxia); dermatitis or gangrene of the ears, muzzle or nostrils; abnormal antler growth; or death.

In the domestic sheep, Barbary sheep, Bighorn sheep, goats, and Sika deer, symptoms are typically dermatological, resulting from inflammatory responses to the microfilariae which accumulate under the skin of the face and ears. The resulting lesions have been described by various authors as "dermal encrustations", "tumorous masses", "raw, bloody dermatitis", or "crusty, scabby lesions" of the head and face. Alopecia, blepharitis, and secondary conjunctivitis have also been observed in sheep. Arterial occulsion may also occur in sheep, but to a lesser degree than in moose and elk.

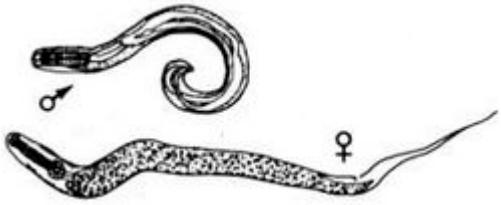
Diagnosis and treatment

Diagnosis involves recovering either adult worms from the arteries after the death of the infested host, or microcercariae from the skin of the face or head. Treatments have been reported for sheep. A combination of tartar emetic (antimony potassium tartrate) and

emetine hydrochloride healed the skin lesions. For elimination of the nematodes, fuadin (stibophen), diethylcarbamazine and piperazine hexahydrate have been suggested. However, repeated administration of diethylcarbamazine runs the risk of fatality due to accumulation of dead worms in the arteries.

Enterobius

Pinworm **ICD 127.4**



Pinworms(U.S.)/Threadworms(U.K.)
(*Enterobius vermicularis*).

Scientific classification	
Kingdom:	Animalia
Phylum:	Nematoda
Class:	Secernentea
Subclass:	Spiruria
Order:	Oxyurida
Family:	Oxyuridae
Genus:	***Enterobius***

Species

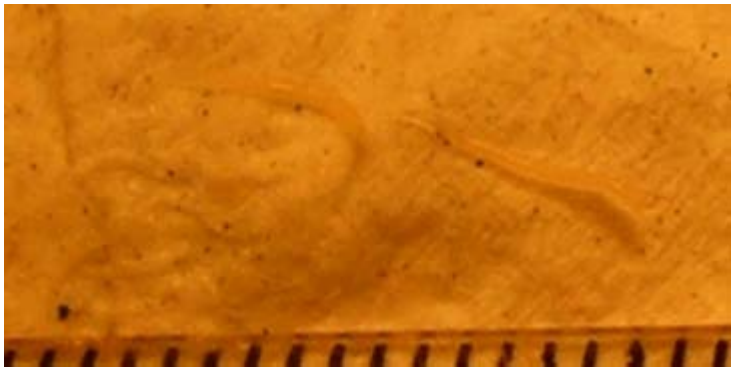
- *Enterobius vermicularis* (Linnaeus, 1758)
- *Enterobius anthropopitheci* (Gedoelst, 1916)
- *Enterobius gregorii* (Hugot, 1983) (disputed)

The **pinworm** (in the United States of America) (genus ***Enterobius***), also known as **threadworm** (in the United Kingdom) or **seatworm**, is a nematode (roundworm) and a common human intestinal parasite, especially in children. The medical condition associated with pinworm infestation is known as enterobiasis, or less precisely as *oxyuriasis* in reference to the family *oxyuridae*.

Classification

The pinworm (genus *Enterobius*) is a type of roundworm, and three species of pinworm have been identified with certainty. Humans are hosts only to *Enterobius vermicularis* (formerly *Oxyuris vermicularis*). Chimpanzees are host to *Enterobius anthropopitheci*, which is morphologically distinguishable from the human pinworm. Hugot (1983) claims there is another species affecting humans, *Enterobius gregorii*, which is supposedly a sister species of *E. vermicularis*, and has a slightly smaller spicule (i.e., sexual organ). Its existence is controversial however; Totkova et al. (2003) consider there to be insufficient evidence, and Hasagawa et al. (2006) contend that *E. gregorii* is a younger stage of *E. vermicularis*. Regardless of its status as a distinct species, *E. gregorii* is considered clinically identical to *E. vermicularis*.

Morphology



Two female pinworms next to a ruler. The markings are one millimeter apart.

The pinworm appears as a white, small and delicate nematode (i.e., roundworm). The adult female has a sharply pointed posterior end, is 8 to 13 millimeters long, and 0.5 millimeter thick. The adult male is considerably smaller, measuring 2 to 5 millimeters long and 0.2 millimeter thick, and has a curved posterior end. The eggs are translucent and have a surface that adheres to environmental objects. The eggs measure 50 to 60 micrometers by 20 to 30 micrometers, and have a thick shell that is flattened on one side. The small size and colorlessness of the eggs make them invisible to the naked eye, except in barely visible clumps of thousands of eggs. Eggs may contain a developing embryo or a fully developed pinworm larva. Inside the host, the larvae grow to 140–150 micrometers in length.

Distribution

The pinworm has a worldwide distribution, and is the most common helminth (i.e., parasitic worm) infection in the United States and Western Europe. In the United States, a study by the Center of Disease Control reported an overall incidence rate of 11.4% among people of all ages. Pinworms are particularly common in children, with prevalence rates in this age group having been reported as high as 61% in India, 50% in England, 39% in Thailand, 37% in Sweden, and 29% in Denmark. Finger sucking has

been shown to increase both incidence and relapse rates, and nail biting has been similarly associated. Because it spreads from host to host through contamination, pinworms are common among people living in close contact, and tends to occur in all people within a household. The prevalence of pinworms is not associated with gender, nor with any particular social class, race, or culture. Pinworms are an exception to the tenet that intestinal parasites are uncommon in affluent communities. The earliest known instance of pinworms is evidenced by pinworm eggs found in coprolite, carbon dated to 7837 BC at western Utah.

Life cycle

The entire life cycle—from egg to adult—takes place in the human gastrointestinal tract of a single human host. Cook et al. (2009) and Burkhart & Burkhart (2005) disagree over the length of this process, with Cook et al. stating two to four weeks, while Burkhart & Burkhart states that it takes from four to eight weeks.

The life cycle begins with eggs being ingested.. The eggs hatch in the duodenum (i.e., first part of the small intestine). The emerging pinworm larvae grow rapidly to a size of 140 to 150 micrometers in size, and migrate through the small intestine towards the colon. During this migration they moult twice and become adults. Females survive for 5 to 13 weeks, and males about 7 weeks. The male and female pinworms mate in the ileum (i.e., last part of the small intestine), whereafter the male pinworms usually die, and are passed out with stool. The gravid female pinworms settle in the ileum, caecum (i.e., beginning of the large intestine), appendix and ascending colon, where they attach themselves to the mucosa and ingest colonic contents. Almost the entire body of a gravid female becomes filled with eggs. The estimations of the number of eggs in a gravid female pinworm ranges from about 11,000 to 16,000. The egg-laying process begins approximately five weeks after initial ingestion of pinworm eggs by the human host. The gravid female pinworms migrate through the colon towards the rectum at a rate of 12 to 14 centimeters per hour. They emerge from the anus, and while moving on the skin near the anus, the female pinworms deposit eggs either through (1) contracting and expelling the eggs, (2) dying and then disintegrating, or (3) bodily rupture due to the host scratching the worm. After depositing the eggs, the female becomes opaque and dies. The reason the female emerges from the anus is to obtain the oxygen necessary for the maturation of the eggs.

Transmission

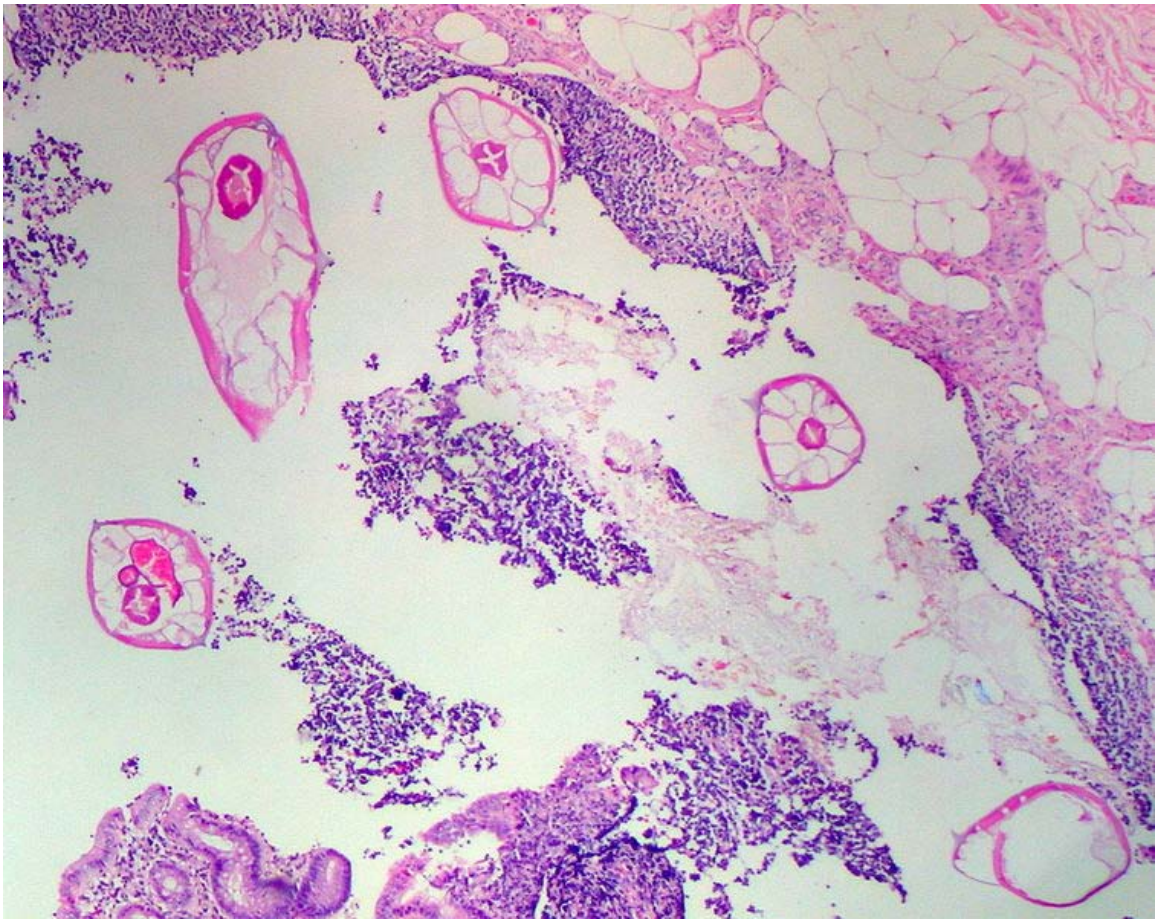
Pinworms spread through human-to-human transmission, by ingesting (i.e., swallowing) infectious pinworm eggs. The eggs are hardy and can remain viable (i.e., infectious) in a moist environment for up to three weeks. They do not tolerate heat well, but can survive in low temperatures: two-thirds of the eggs are still viable after 18 hours at −8 degrees Celsius (18 °F).

After the eggs have been initially deposited near the anus, they are readily transmitted to other surfaces through contamination. The surface of the eggs is sticky when laid, and the

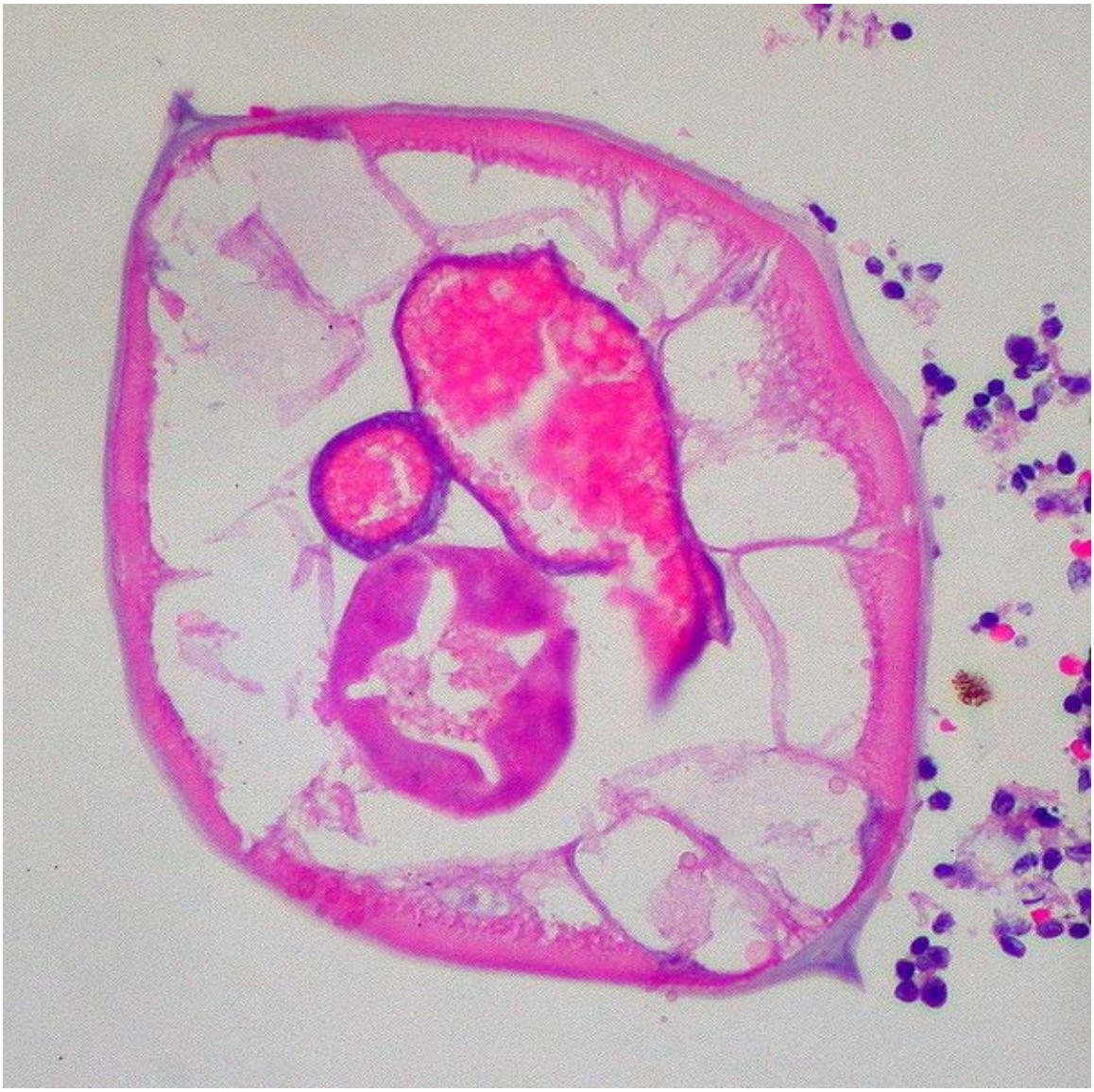
eggs are readily transmitted from their initial deposit near the anus to fingernails, hands, night-clothing and bed linen. From here, eggs are further transmitted to food, water, furniture, toys, bathroom fixtures and other objects. Household pets often carry the eggs in their fur, while not actually being infected. Dust containing eggs can become airborne and widely dispersed when dislodged from surfaces, for instance when shaking out bed clothes and linen. Consequently the eggs can enter the mouth and nose through inhalation, and be swallowed later. Although pinworms do not strictly multiply inside the body of their human host, some of the pinworm larvae may hatch on the anal mucosa, and migrate up the bowel and back into the gastrointestinal tract of the original host. This process is called *retroinfection*. According to Burkhart (2005), when this retroinfection occurs, it leads to a heavy parasitic load and ensures that the pinworm infestation continues. This statement is contradictory to a statement by Caldwelli (1982), who contends that retroinfection is rare and not clinically significant. Despite the limited, 13 week lifespan of individual pinworms, autoinfection (i.e., infection from the original host to itself), either through the anus-to-mouth route or through retroinfection, causes the pinworms to inhabit the same host indefinitely.

Treatment

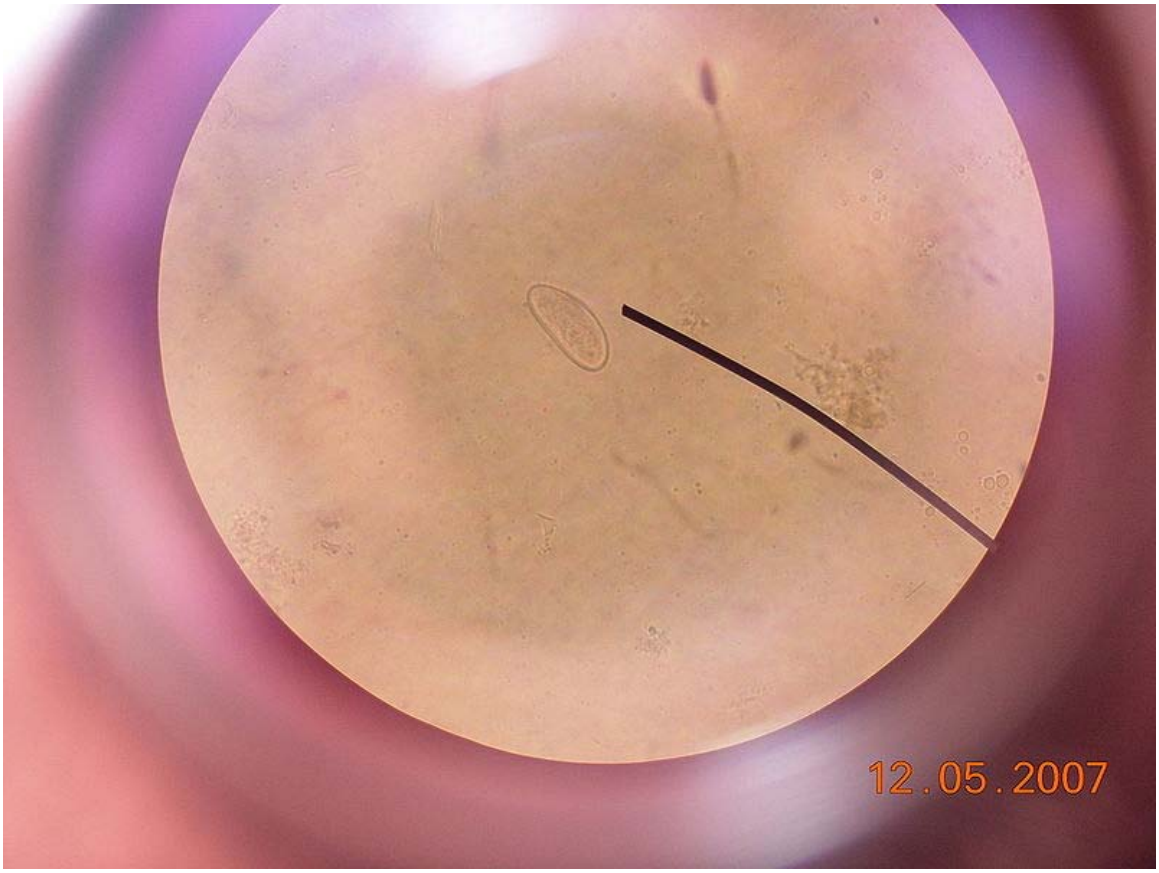
Although hygiene plays a role, medication is the chief treatment. Because the pharmaceutical drugs kill the adult pinworms but not the eggs, retreatment is recommended in two weeks. If one household member spreads the eggs to another, it will be a matter of two or three weeks before those eggs become adult worms and thus amenable to treatment.The benzimidazole compounds albendazole (brand names e.g., Albenza, Eskazole, Zentel and Andazol) and mebendazole (brand names e.g., Ovex, Vermox, Antiox and Pripsen) are the most effective. They work by inhibiting the microtubule function in the pinworm adults, causing glycogen depletion, thereby effectively starving the parasite. A single 100 milligram dose of mebendazole with one repetition after a week, is considered the safest, and is usually effective with cure rate of 96%. Mebendazole has no serious side effects, although abdominal pain and diarrhea have been reported. Pyrantel pamoate (also called pyrantel embonate, brand names e.g., Reese's Pinworm Medicine, Pin-X, Combantrin, Anthel, Helmintox, and Helmex) kills adult pinworms through neuromuscular blockade, and is considered as effective as the benzimidazole compounds. Other medications are piperazine, which causes flaccid paralysis in the adult pinworms, and pyrvinium pamoate (also called pyrvinium embonate), which works by inhibiting oxygen uptake of the adult pinworms. Pinworms located in the genitourinary system (in this case, female genital area) may require other drug treatments. Regardless of the medication used, reinfection is frequent. Asymptomatic infections, often in small children, can serve as reservoirs of infection, and therefore the entire household should be treated regardless of whether or not symptoms are present. Total elimination of the parasite in a household may require repeated doses of medication for up to a year or more.



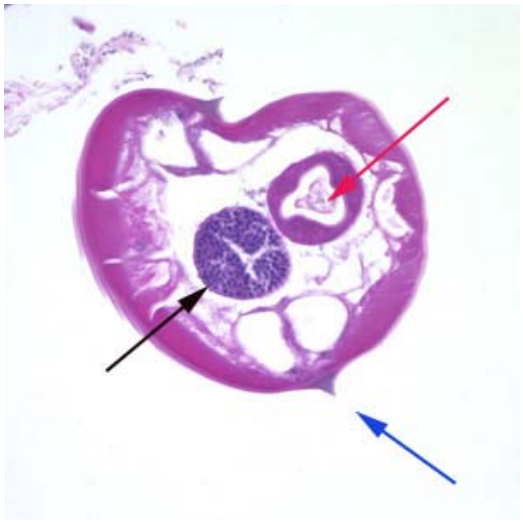
Pinworms are sometimes diagnosed incidentally by pathology. Micrograph of pinworms in the appendix. H&E stain.



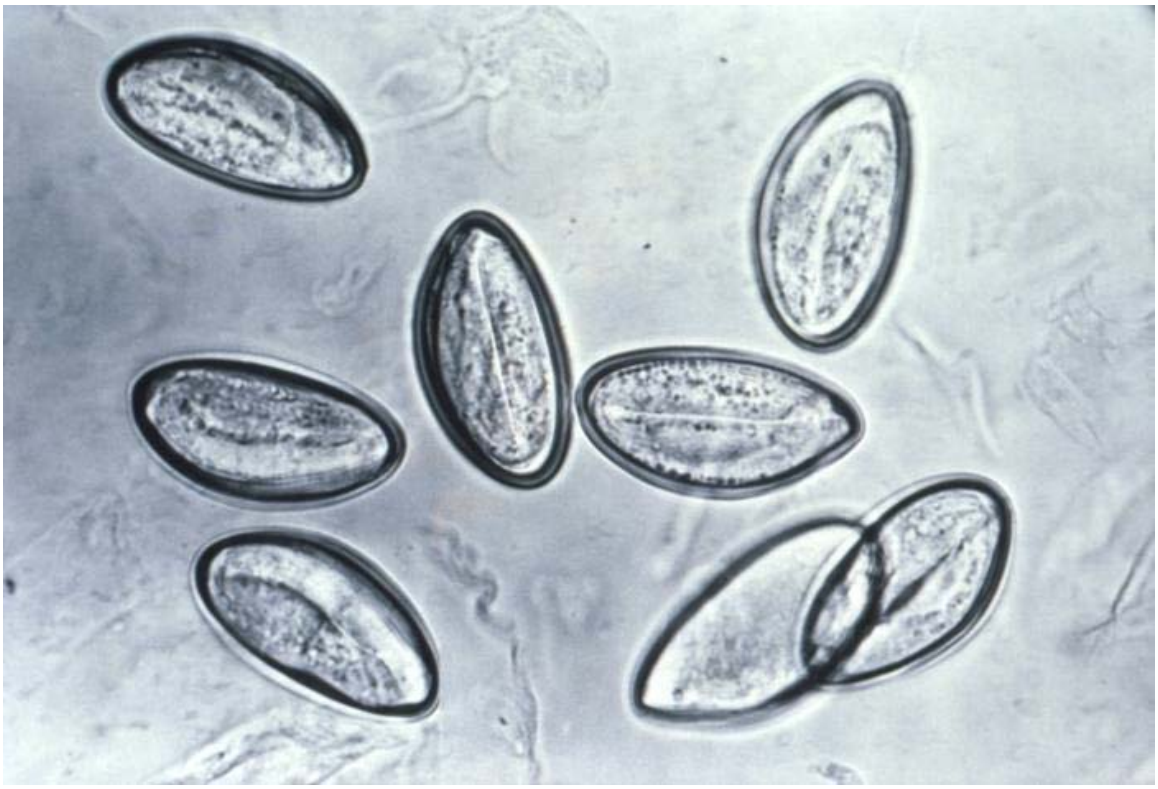
High magnification micrograph of a pinworm in cross section in the appendix. H&E stain.



Enterobius vermicularis egg under a light microscope.



Pinworms are sometimes diagnosed incidentally by pathology. Micrograph of male pinworm in cross section. Alae (blue arrow), intestine (red arrow) and testis (black arrow). H&E stain.



Pinworm eggs are easily seen under a microscope.

This micrograph reveals the cephalic alae in the head region of Enterobius vermicularis.

Chapter 13

Entomopathogenic Nematode

Nematodes emerging from a wax moth cadaver

Entomopathogenic nematodes are soil-inhabiting, lethal insect parasitoids that belong to the phylum Nematoda, commonly called roundworms. The term *entomopathogenic* comes from the Greek word *entomon,* meaning insect, and pathogenic, which means causing disease. Although many other parasitic nematodes cause diseases in plants, livestock, and humans, entomopathogenic nematodes, as their name implies, only infect insects. Entomopathogenic nematodes (EPNs) live inside the body of their host, and so they are designated *endoparasitic*. They infect many different types of soil insects, including the larval forms of butterflies, moths, beetles, and flies, as well as adult crickets and grasshoppers. EPNs have been found in all inhabited continents and a range of ecologically diverse habitats, from cultivated fields to deserts. The most commonly studied genera are those that are useful in the biological control of insect pests, the Steinernematidae and Heterorhabditidae (Gaugler 2006).

Life cycle

Since they are economically important, the life cycles of the genera Heterorhabditidae and Steinernematidae are particularly well known. Although not closely related, phylogenetically, both share similar life histories (Poinar 1993). The cycle begins with an infective juvenile, whose only function is to seek out and infect new hosts. After entering an insect, infective juveniles release an associated mutualistic bacterium. These bacteria

of the genus *Xenorhabdus* or *Photorhabdus*, for steinerernematides and heterorhabditids, respectively—cause host mortality within 48 hours. The nematodes provide shelter to the bacteria, which, in return, kill the insect host and provide nutrients to the nematode. Together, the nematodes and bacteria feed on the liquefying host, and reproduce for several generations inside the cadaver. Steinernematid infective juveniles may become males or females, whereas heterorhabditids develop into self-fertilizing hermaphrodites with later generations producing two sexes. When food resources in the host become scarce, the adults produce new infective juveniles adapted to withstand the outside environment. After about a week, hundreds of thousands of infective juveniles emerge and leave in search of new hosts, carrying with them an inoculation of mutualistic bacteria, received from the internal host environment (Boemare 2002, Gaugler 2006).

Foraging strategies

The foraging strategies of entomopathogenic nematodes vary between species, influencing their soil depth distributions and host preferences. Infective juveniles use strategies to find hosts that vary from ambush and cruise foraging (Campbell 1997). In order to ambush prey, some *Steinernema* species nictate, or raise their bodies off the soil surface so they are better poised to attach to passing insects, which are much larger in size (Campbell and Gaugler 1993). Many *Steinernema* are able to jump by forming a loop with their bodies that creates stored energy which, when released, propels them through the air (Campbell and Kaya 2000). Other species adopt a cruising strategy and rarely nictate. Instead, they roam through the soil searching for potential hosts. These foraging strategies influence which hosts the nematodes infect. For example, ambush predators such as *Steinernema carpocapsae* infect more insects on the surface, while cruising predators like *Heterorhabditis bacteriophora* infect insects that live deep in the soil (Campbell and Gaugler 1993).

Population ecology

Competition and coexistence

Inside their insect hosts, EPNs experience both intra and interspecific competition. Intraspecific competition takes place among nematodes of the same species when the number of infective juveniles penetrating a host exceeds the amount of resources available. Interspecific competition occurs when different species compete for resources. In both cases, the individual nematodes compete with each other indirectly by consuming the same resource, which reduces their fitness and may result in the local extinction of one species inside the host (Koppenhofer and Kaya 1996). Interference competition, in which species compete directly, can also occur. For example, a steinernematid species that infects a host first usually excludes a heterorhabditid species. The mechanism for this superiority may be antibiotics produced by *Xenorhabdus*, the symbiotic bacterium of the steinernematid. These antibiotics prevent the symbiotic bacterium of the heterorhabditid from multiplying (Kaya and Koppenhofer1996). In order to avoid competition, some species of infective juveniles are able to judge the quality of a host before penetration.

The infective juveniles of *S. carpocapsae* are repelled by 24-hour-old infections, likely by the smell of their own species' mutualistic bacteria (Grewal *et al.* 1997).

Interspecific competition between nematode species can also occur in the soil environment outside of hosts. Millar and Barbercheck (2001) showed that the introduced nematode *Steinernema riobrave* survived and persisted in the environment for up to a year after its release. *S. riobrave* significantly depressed detection of the endemic nematode *H. bacteriophora*, but never completely displaced it, even after two years of continued introductions. *S. riobrave* had no effect on populations of the native nematode, *S. carpocapsae*, though, which suggests that coexistence is possible. Niche differentiation appears to limit competition between nematodes. Different foraging strategies allow two species to co-exist in the same habitat. Different foraging strategies separate the nematodes in space and enable them to infect different hosts. EPNs also occur in patchy distributions, which may limit their interactions and further support coexistence (Kaya and Koppenhofer 1996).

Population distribution

Entomopathogenic nematodes are typically found in patchy distributions, which vary in space and time, although the degree of patchiness varies between species (reviewed in Lewis 2002). Factors responsible for this aggregated distribution may include behavior, as well as the spatial and temporal variability of the nematodes natural enemies, like nematode trapping fungus. Nematodes also have limited dispersal ability. Many infective juveniles are produced from a single host could also produce aggregates. Patchy EPN distributions may also reflect the uneven distribution of host and nutrients in the soil (Lewis *et al.* 1998; Stuart and Gaugler 1994; Campbell *et al.* 1997, 1998). EPNs may persist as metapopulations, in which local population fragments are highly vulnerable to extinction, and fluctuate asynchronously (Lewis *et al.* 1998). The metapopulation as a whole can persist as long as the rate of colonization is greater or equal to the rate of population extinction (Lewis *et al.* 1998). The founding of new populations and movement between patches may depend on the movement of infective juveniles or the movement of infected hosts (Lewis *et al.* 1998). Recent studies suggest that EPNs may also use non-host animals, such as isopods and earthworms for transport (Eng *et al.*2005, Shapiro *et al.* 1993) or can be scavengers (San-Blas and Gowen, 2008).

Community ecology

Parasites can significantly affect their hosts, as well as the structure of the communities to which they and their hosts belong (Minchella and Scott 1991). Entomopathogenic nematodes have the potential to shape the populations of plants and host insects, as well as the species composition of the surrounding animal soil community.

Entomopathogenic nematodes affect populations of their insect hosts by killing and consuming individuals. When more EPNs are added to a field environment, typically at concentrations of 250,000 individuals per square metre, the population of host insects measurably decreases (Campbell et al. 1998, Strong et al. 1996). Agriculture exploits this

finding, and the inundative release of EPNs can effectively control populations of soil insect pests in citrus, cranberries, turfgrass, and tree fruit (Lewis *et al.* 1998). If entomopathogenic nematodes suppress the population of insect root herbivores, they indirectly benefit plants by freeing them from grazing pressure. This is an example of a trophic cascade in which consumers at the top of the food web (nematodes) exert an influence on the abundance of resources (plants) at the bottom. The idea that plants can benefit from the application of their herbivore's enemies is the principle behind biological control. Consequently, much of EPN biological research is driven by agricultural applications.

Examples of the top-down effects of entomopathogenic nematodes are not restricted to agricultural systems. Researchers at the Bodega Marine Laboratory examined the strong top-down effects that naturally occurring EPNs can have on their ecosystem (Strong *et al.* 1996). In a coastal shrubland food chain the native EPN, *Heterorhabditis heplialus*, parasitized ghost moth caterpillars, and ghost moth caterpillars consumed the roots of bush lupine. The presence *H. heplialus* correlated with lower caterpillar numbers and healthier plants. In addition, the researchers observed high mortality of bush lupine in the absence of EPNs. Old aerial photographs over the past 40 years indicated that the stands where nematodes were prevalent had little or no mass die-off of lupine. In stands with low nematode prevalence, however, the photos showed repeated lupine die-offs. These results implied that the nematode, as a natural enemy of the ghost moth caterpillar, protected the plant from damage. The authors even suggested that the interaction was strong enough to affect the population dynamics of bush lupine (Strong *et al.* 1996).

Not only do entomopathogenic nematodes affect their host insects, they can also change the species composition of the soil community. Many familiar animals like earthworms and insect grubs live in the soil, but smaller invertebrates such as mites, collembolans, and nematodes are also common. Aside from EPNs, the soil ecosystem includes predatory, bacteriovorous, fungivorous and plant parasitic nematode species. Since EPNs are applied in agricultural systems at a rate of 1,000,000 individuals per acre, the potential for unintended consequences on the soil ecosystem appears large. EPNs have not had an adverse effect on mite and collembolan populations (Georgis et al. 1991), yet there is strong evidence that they affect the species diversity of other nematodes. In a golf course ecosystem, the application of *H. bacteriophora*, an introduced nematode, significantly reduced the abundance, species richness, maturity, and diversity of the nematode community (Somaseker *et al.* 2002). EPNs had no effect on free-living nematodes. However, there was a reduction in the number of genera and abundance of plant-parasitic nematodes, which often remain enclosed within growths on the plant root. The mechanism by which insect parasitic nematodes have an effect on plant parasitic nematodes remains unknown. Although this effect is considered beneficial for agricultural systems where plant parasitic nematodes cause crop damage, it raises the question of what other effects are possible. Future research on the impacts EPNs have on soil communities will lead to greater understanding of these interactions.

In aboveground communities, EPNs have few side effects on other animals. One study reported that *Steinernema felidae* and *Heterorhabditis megidis*, when applied in a range

of agricultural and natural habitats, had little impact on non-pest arthropods. Some minimal impacts did occur, however, on non-pest species of beetles and flies (Bathon 1996). Unlike chemical pesticides, EPNs are considered safe for humans and other vertebrates.

Disturbance

Frequent disturbance often perturbs agricultural habitats and the response to disturbance varies among EPN species. In traditional agricultural systems, tilling disturbs the soil ecosystem, affecting biotic and abiotic factors. For example, tilled soils have lower microbial, arthropod, and nematode species diversity (Lupwayi *et al.* 1998). Tilled soil also has less moisture and higher temperatures. In a study examining the tolerances of different EPN species to tillage, the density of a native nematode, *H. bacteriophora*, was unaffected by tillage, while the density of an introduced nematode, *S. carpocapsae*, decreased. The density of a third nematode introduced to the system, *Steinernema riobrave*, increased with tillage (Millar and Barbercheck 2002). Habitat preferences in temperature and soil depth can partially explain the nematodes' different responses to disturbance. *S. carpocapsae* prefers to remain near the soil surface and so is more vulnerable to soil disturbance than *H. bacteriophora*, which forages deeper and can escape the effects of tillage. *S. riobrave* may have responded well to tillage because it is better at surviving and persisting in hotter and drier conditions created by tillage (Millar and Barbercheck 2002). Our datas showed that *Steinernema* sp. found on some Indonesia region showed high adaptation capability when applicated on another region or condition (Anton Muhibuddin, 2008) The response of EPNs to other forms of disturbance is less well defined. Nematodes are not affected by certain pesticides and are able to survive flooding. The effects of natural disturbances such as fire have not been examined.

Applications

Although the biological control industry has acknowledged EPNs since the 1980s, relatively little is understood about their biology in natural and managed ecosystems (Georgis 2002). Nematode-host interactions are poorly understood, and more than half of the natural hosts for recognized Steinernema and Heterorhabditis species remain unknown (Akhurst and Smith 2002). Information is lacking because isolates of naturally infected hosts are rare, so native nematodes are often baited using *Galleria mellonella*, a lepidopteran that is highly susceptible to parasitic infection. Laboratory studies showing wide host ranges for EPNs were often overestimates, because in a laboratory, contact with a host is assured and environmental conditions are ideal; there are no "ecological barriers" to infection (Kaya and Gaugler 1993, Gaugler *et al.* 1997). Therefore, the broad host range initially predicted by assay results has not always translated into insecticidal success.

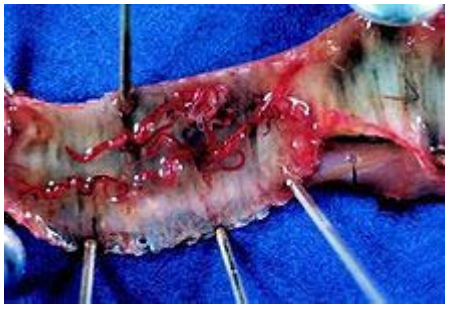
The lack of knowledge about nematode ecology has resulted in unanticipated failures to control pests in the field. For example, parasitic nematodes were found to be completely ineffective against blackflies and mosquitoes due to their inability to swim (Lewis *et al.*1998). Efforts to control foliage-feeding pests with EPNs were equally unsuccessful,

because nematodes are highly sensitive to UV light and desiccation (Lewis *et al.*1998). Comparing the life histories of nematodes and target pests can often explain such failures (Gaugler *et al.* 1997). Each nematode species has a unique array of characteristics, including different environmental tolerances, dispersal tendencies, and foraging behaviors (Lewis *et al.* 1998). Increased knowledge about the factors that influence EPN populations and the impacts they have in their communities will likely increase their efficacy as biological control agents.

Chapter 14

Gapeworm and Hookworm

Gapeworm

Gapeworm

Gapeworms in the trachea of a Ringnecked Pheasant

Scientific classification	
Kingdom:	Animalia
Phylum:	Nematoda
Class:	Secernentea
Order:	Strongylida
Family:	Syngamidae
Genus:	*Syngamus*
Species:	***S. trachea***

Binomial name
Syngamus trachea Montagu, 1811

A **gapeworm** (*Syngamus trachea*) is a parasitic nematode worm infecting the tracheas of certain birds. The resulting disease, known as **gape** or **the gapes**, occurs when the worms clog and obstruct the airway. The worms are also known as red worms or forked worms due to their red color and the permanent procreative conjunction of males and females. Gapeworm is common in young, domesticated chickens and turkeys.

When the female gapeworm lays her eggs in the trachea of an infected bird, the eggs are coughed up, swallowed, then defecated. When birds consume the eggs found in the feces or an intermediate host such as earthworms, snails (*Planorbarius corneus*, *Bithynia tentaculata*, …), or slugs, they become infected with the parasite.

Ivermectin is a drug often used to control gapeworm infection in birds.

Morphology

Males and females are joined together in a state of permanent copulation forming a Y shape "forked worm". They are also known as the "red worm" because of their color. Females are much larger (up to 20mm long) than males (up to 6mm long). The life history of the gapeworm is peculiar in that transmission from bird to bird may be successfully accomplished either directly (by the feeding of embryonated eggs or infective larvae) or indirectly (by ingestion of earthworms containing free or encysted gapeworm larvae they had obtained by feeding on contaminated soil).

Life cycle and pathogenesis

The life cycle is complicated in both its preparasitic and parasitic phases. In the preparasitic phase, L3s develop inside the eggs at which time they may hatch. Earthworms play an important role in the life cycle, serving as transport (paratenic) hosts. Larvae have been shown to remain viable for more than three years encapsulated in earthworm muscles. Other invertebrates may also serve as paratenic hosts and these include terrestrial snails and slugs as well as the larvae of Musca domestica (the common house fly) and Lucilia sericata (the green bottle fly responsible for cutaneous myiasis). The parasitic phase involves substantial migration in the definitive host to reach the predilection site. Young birds are most severely affected with migration of larvae and adults through the lungs causing a severe pneumonia. Lymphoid nodules form at the point of attachment of the worms in the bronchi and trachea. Adult worms also appear to feed on blood. Worms in the bronchi and trachea provoke a hemorrhagic tracheitis and bronchitis with formation of large quantities of mucus, plugging the air passages and, in severe cases, causing asphyxiation. Pheasants appear to be particularly susceptible to infections resulting in mortality rates as high as 25% during outbreaks. The rapidly growing worms soon obstruct the lumen of the trachea and cause suffocation. Turkey poults, baby chicks and pheasant chicks are most susceptible to infection. Turkey poults usually develop gapeworm signs earlier and begin to die sooner after infection than young chickens. Lesions are usually found in the trachea of turkeys and pheasants but seldom if ever in the tracheas of young chickens and guinea fowl. The male worm, in the form of lesions, remains permanently attached to the tracheal wall throughout the duration of its life. The female worms apparently detach and reattach from time to time in order to obtain a more abundant supply of food.

Epidemiology

Earthworm transport hosts are important factors in the transmission of Syngamus trachea where poultry and game birds are reared on soil. The longevity of L3s in earthworms (up to 3 years) is particularly important in perpetuating the infection from year to year. Wild birds may serve as reservoirs of infection and have been implicated as the sources of infections in outbreaks on game-bird farms as well as poultry farms. Wild reservoir hosts may include pheasants, ruffed grouse, partridges, turkeys, magpies, meadowlarks, robins, grackles, jays, jackdaws, rooks, starlings and crows. There is also evidence to suggest that strains of Syngamus trachea from wild bird reservoir hosts may be more infective for domestic birds if they first pass through an earthworm transport host rather than by direct infections via ingestion of L3s or eggs containing L3s. Clinical signs Blockage of the bronchi and trachea with worms and mucus will cause infected birds to gasp for air. They stretch out their necks, open their mouths and gasp for air producing a hissing noise as they do so. This "gaping" posture has given rise to the common term "gapeworm" to describe Syngamus trachea. These clinical signs first appear approximately 1-2 weeks after infection. Birds infected with gapeworms show signs of weakness and emaciation and usually spend much of their time with eyes closed and head drawn back against the body. An infected bird may give its head a convulsive shake in an attempt to remove the obstruction from the trachea so that normal breathing may be resumed. Severely affected birds, particularly young ones, will deteriorate rapidly; they stop drinking and become anorexic. At this stage, death is the usual outcome. Adult birds are usually less severely affected and may only show an occasional cough or even no obvious clinical signs.

Diagnosis

A diagnosis is usually made on the basis of the classical clinical signs of "gaping". Subclinical infections with few worms may be confirmed at necropsy by finding copulating worms in the trachea and also by finding the characteristic eggs in the feces of infected birds. Examination of the trachea of infection birds shows that the mucous membrane is extensively irritated and inflamed. Coughing is apparently the result of this irritation to the mucous lining.

Control and Treatment of Syngamus trachea

Prevention

In the artificial rearing of pheasants, gapes are a serious menace. Confinement rearing of young birds has reduced the problem in chickens compared to a few years ago. However, this parasite continues to present an occasional problem with turkeys raised on range. Confinement rearing of broilers/pullets and caging of laying hens, have significantly influenced the quantity and variety of nematode infections in poultry. For most nematodes, control measures consist of sanitation and breaking the life cycle rather than chemotherapy. Confinement rearing on litter largely prevents infections with nematodes using intermediate hosts such as earthworms or grasshoppers, which are not normally found in poultry houses. Conversely, nematodes with direct life cycles or those that

utilize intermediate hosts such as beetles, which are common in poultry houses, may prosper. Treatment of the soil or litter to kill intermediate hosts may be beneficial. Insecticides suitable for litter treatment include carbaryl, tetrachlorvinphos (stirofos). However, treatment is usually done only between grow-outs. Extreme care should be taken to ensure that feed and water are not contaminated. Treatment of range soil to kill ova is only partially successful. Changing litter can reduce infections, but treating floors with oil is not very effective. Raising different species or different ages of birds together or in close proximity is a dangerous procedure as regards parasitism. Adult turkeys, which are carriers of gapeworms, can transmit the disease to young chicks or pheasants, although older chickens are almost resistant to infection.

Treatment

Thiabendazole (Tresaderm) is currently approved for use only in pheasants and is effective when administered in the feed. Continuous medication of pen-reared birds has been recommended, but is not economical. Several other compounds have been shown effective against S. trachea under experimental conditions. Methyl 5-benzoyl-2-benzimidazole was 100% efficacious when fed prophylactically to turkey poults. 5-isopropoxycarbonylamino-2-(4-thizolyl)-benzimidazole was found to be more efficacious than thiabendazole or disophenol. The level of control with three treatments of cambendazole on days 3-4, 6-7, and 16-17 post-infection was 94.9% in chickens and 99.1% in turkeys. Levamisole (Ergamisol), fed at a level of 0.04% for 2 days or 2 g/gal drinking water for 1 day each month, has proven effective in game birds. Fenbendazole (Panacur) at 20 mg/kg for 3-4 days is also effective.

Hookworm

Necator americanus* and *Ancylostoma duodenale

Scientific classification

Kingdom: Animalia
Phylum: Nematoda
Class: Secernentea
Order: Strongiloidae

Family: Ancylostomatidae

Genus: Necator/Ancylostoma

Species

Species **N. americanus and A. duodenale**

The **hookworm** is a parasitic nematode that lives in the small intestine of its host, which may be a mammal such as a dog, cat, or human. Two species of hookworms commonly infect humans, *Ancylostoma duodenale* and *Necator americanus*. *A. duodenale* predominates in the Middle East, North Africa, India and (formerly) in southern Europe, while *N. americanus* predominates in the Americas, Sub-Saharan Africa, Southeast Asia, China, and Indonesia. Hookworms are thought to infect more than 600 million people worldwide. The *A. braziliense* and *A. tubaeforme* species infect cats, while *A. caninum* infects dogs. *Uncinaria stenocephala* infects both dogs and cats.

Hookworms are much smaller than the larger roundworm *Ascaris lumbricoides*, and the complications of tissue migration and mechanical obstruction so frequently observed with roundworm infestation are less frequent in hookworm infestation. The most significant risk of hookworm infection is anemia, secondary to loss of iron (and protein) in the gut. The worms suck blood voraciously and damage the mucosa. However, the blood loss in the stools is not visibly apparent.

Ancylostomiasis, also known by several other names, is the disease caused when *A. duodenale* hookworms, present in large numbers, produce an iron deficiency anemia by sucking blood from the host's intestinal walls.

Hookworm is a leading cause of maternal and child morbidity in the developing countries of the tropics and subtropics. In susceptible children hookworms cause intellectual, cognitive and growth retardation, intrauterine growth retardation, prematurity, and low birth weight among newborns born to infected mothers. In developed countries, hookworm infection is rarely fatal, but anemia can be significant in a heavily infected individual.

Signs and symptoms

There are no specific symptoms or signs of hookworm infection. As mentioned above, they arise from a combination of intestinal inflammation and progressive iron/protein-deficiency anaemia. Larval invasion of the skin might give rise to intense, local itching, usually on the foot or lower leg, which can be followed by lesions that look like insect bites, can blister ("ground itch"), and last for a week or more. Animal hookworm larvae on penetrating humans may produce a creeping eruption called cutaneous larva migrans. The larvae migrate in tortuous tunnels in between stratum germinativum and stratum corneum of the skin, causing serpigenous vesicular lesions. With advancing movement of the larvae, the rear portions of the lesions become dry and crusty. The lesions are typically intensely pruritic. Coughing, chest pain, wheezing, and fever will sometimes be

experienced by people who have been exposed to very large numbers of larvae. Epigastric pains, indigestion, nausea vomiting, constipation, and diarrhea can occur early or in later stages as well, although gastrointestinal symptoms tend to improve with time. Signs of advanced severe infection are those of anemia and protein deficiency, including emaciation, cardiac failure and abdominal distension with ascites.

Pathophysiology

Morphology

A. duodenale worms are grayish white or pinkish with the head slightly bent in relation to the rest of the body. This bend forms a definitive hook shape at the anterior end for which hookworms are named. They possess well developed mouths with two pairs of teeth. While males measure approximately one centimeter by 0.5 millimeter, the females are often longer and stouter. Additionally, males can be distinguished from females based on the presence of a prominent posterior copulatory bursa.

N. americanus is very similar in morphology to *A. duodenale*. *N. americanus* is generally smaller than *A. duodenale* with males usually 5 to 9 mm long and females about 1 cm long. Whereas *A. duodenale* possess two pairs of teeth, *N. americanus* possesses a pair of cutting plates in the buccal capsule. Additionally, the hook shape is much more defined in *Necator* than in *Ancylostoma*.

Pathology

Hookworm infection is generally considered to be asymptomatic, but as Norman Stoll described in 1962, hookworm is an extremely dangerous infection because its damage is "silent and insidious." There are general symptoms that an individual may experience soon after infection. Ground-itch, which is an allergic reaction at the site of parasitic penetration and entry, is common in patients infected with *N. americanus*. Additionally, cough and pneumonitis may result as the larvae begin to break into the alveoli and travel up the trachea. Then once the larvae reach the small intestine of the host and begin to mature, the infected individual will suffer from diarrhea and other gastrointestinal discomfort. However, the "silent and insidious" symptoms referred to by Stoll are related to chronic, heavy-intensity hookworm infections. Major morbidity associated with hookworm is caused by intestinal blood loss, iron deficiency anemia, and protein malnutrition. They result mainly from adult hookworms in the small intestine ingesting blood, rupturing erythrocytes, and degrading hemoglobin in the host. This long-term blood loss can manifest itself physically through facial and peripheral edema; eosinophilia and pica caused by iron deficiency anemia are also experienced by some hookworm-infected patients. Recently, more attention has been given to other important outcomes of hookworm infection that play a large role in public health. It is now widely accepted that children who suffer from chronic hookworm infection can suffer from growth retardation as well as intellectual and cognitive impairments. Additionally, recent research has focused on the potential of adverse maternal-fetal outcomes when the mother is infected with hookworm during pregnancy.

The disease was linked to nematode worms (*Ankylostoma duodenalis*) from one-third to half an inch long in the intestine chiefly through the labours of Theodor Bilharz and Griesinger in Egypt (1854).

The symptoms can be linked to inflammation in the gut stimulated by feeding hookworms, such as nausea, abdominal pain and intermittent diarrhea, and to progressive anemia in prolonged disease: capricious appetite, pica (or dirt-eating), obstinate constipation followed by diarrhea, palpitations, thready pulse, coldness of the skin, pallor of the mucous membranes, fatigue and weakness, shortness of breath and in cases running a fatal course, dysentery, hemorrhages and edema.

Blood tests in early infection often show a rise in numbers of eosinophils, a type of white blood cell that is preferentially stimulated by worm infections in tissues (large numbers of eosinophils are also present in the local inflammatory response). Falling blood hemoglobin levels will be seen in cases of prolonged infection with anemia.

In contrast to most intestinal helminthiases, where the heaviest parasitic loads tend to occur in children, hookworm prevalence and intensity can be higher among adult males. The explanation for this is that hookworm infection tends to be occupational, so that plantation workers, coalminers and other groups maintain a high prevalence of infection among themselves by contaminating their work environment. However, in most endemic areas, adult women are the most severely affected by anemia, mainly because they have much higher physiological needs for iron (menstruation, repeated pregnancy), but also because customarily they have access to much poorer food than the men.

An interesting consequence of this in the case of *Ancylostoma duodenale* infection is translactational transmission of infection: the skin-invasive larvae of this species do not all immediately pass through the lungs and on into the gut, but spread around the body via the circulation, to become dormant inside muscle fibers. In a pregnant woman, after childbirth some or all of these larvae are stimulated to re-enter the circulation (presumably by sudden hormonal changes), then to pass into the mammary glands, so that the newborn baby can receive a large dose of infective larvae through its mother's milk. This accounts for otherwise inexplicable cases of very heavy, even fatal, hookworm infections in children a month or so of age, in places such as China, India and northern Australia.

An identical phenomenon is much more commonly seen with *Ancylostoma caninum* infections in dogs, where the newborn pups can even die of hemorrhaging from their intestines caused by massive numbers of feeding hookworms. This also reflects the close evolutionary link between the human and canine parasites, which probably have a common ancestor dating back to when humans and dogs first started living closely together.

Life cycle

See the image for the biological life cycle of the hookworms where it thrives in warm earth where temperatures are over 18°C. They exist primarily in sandy or loamy soil and cannot live in clay or muck. Rainfall averages must be more than 1000 mm (40 inches) a year. Only if these conditions exist can the eggs hatch. Infective larvae of *Necator americanus* can survive at higher temperatures, whereas those of *Ancylostoma duodenale* are better adapted to cooler climates. Generally, they live for only a few weeks at most under natural conditions, and die almost immediately on exposure to direct sunlight or desiccation.

Infection of the host is by the larvae, not the eggs. While *A. duodenale* can be ingested, the usual method of infection is through the skin; this is commonly caused by walking barefoot through areas contaminated with fecal matter. The larvae are able to penetrate the skin of the foot, and once inside the body, they migrate through the vascular system to the lungs, and from there up the trachea, and are swallowed. They then pass down the esophagus and enter the digestive system, finishing their journey in the intestine, where the larvae mature into adult worms.

Once in the host gut, *Necator* tends to cause a prolonged infection, generally 1–5 years (many die within a year or two of infecting), though some adult worms have been recorded to live for 15 years or more. On the other hand, *Ancylostoma* adults are short lived, surviving on average for only about 6 months. However, infection can be prolonged because dormant larvae can be "recruited" sequentially from tissue "stores" over many years, to replace expired adult worms. This can give rise to seasonal fluctuations in infection prevalence and intensity (apart from normal seasonal variations in transmission).

Civilian Public Service workers built and installed 2065 outhouses for hookworm eradication in Mississippi and Florida from 1943 to 1947.

They mate inside the host, females laying up to 30,000 eggs per day and some 18 to 54 million eggs during their lifetime, which pass out in feces. Because it takes 5–7 weeks for adult worms to mature, mate and produce eggs, in the early stages of very heavy infection, acute symptoms might occur without any eggs being detected in the patient's feces. This can make diagnosis very difficult.

Summary of Biological Life Cycle

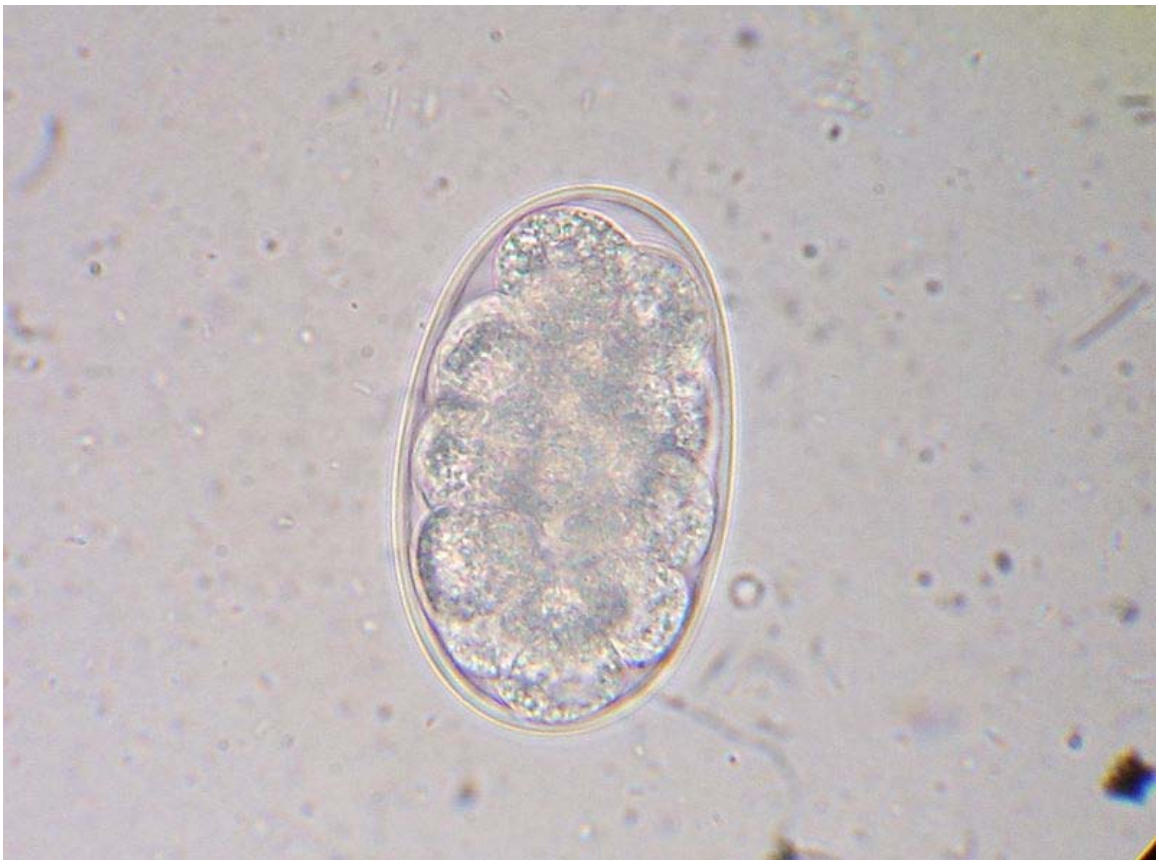
N. americanus and *A. duodenale* eggs can be found in warm, moist soil where they will eventually hatch into first stage larvae, or L1. L1, the feeding non-infective rhabditoform stage, will feed on soil microbes and eventually molt into second stage larvae, L2. L2, which is also in the rhabditoform stage, will feed for approximately 7 days and then molt into the third stage larvae, or L3. L3 is the filariform stage of the parasite, that is, the non-feeding infective form of the larvae. The L3 larvae are extremely motile and will seek higher ground to increase their chances of penetrating the skin of a human host. The L3

larvae can survive up to 2 weeks without finding a host. While *N. americanus* larvae only infect through penetration of skin, *A. duodenale* can infect both through penetration as well as orally. After the L3 larvae have successfully entered the host, the larvae then travel through the subcutaneous venules and lymphatic vessels of the human host. Eventually, the L3 larvae enter the lungs through the pulmonary capillaries and break out into the alveoli. They will then travel up the trachea to be coughed and swallowed by the host. After being swallowed, the L3 larvae are then found in the small intestine where they molt into the L4, or adult worm stage. The entire process from skin penetration to adult development takes about 5–9 weeks. The female adult worms will release eggs (*N. Americanus* about 9,000-10,000 eggs/day and *A. duodenale* 25,000-30,000 eggs/day) which are passed in the feces of the human host. These eggs will hatch in the environment within several days and the cycle with start anew.

Incubation Period

The incubation period can vary between a few weeks to many months and is largely dependent on the number of Hookworm parasites an individual is infected with.

Diagnosis



Hookworm egg

Diagnosis depends on finding characteristic worm eggs on microscopic examination of the stools, although this is not possible in early infection. The eggs are oval or elliptical, measuring 60 µm by 40 µm, colourless, not bile stained and with a thin transparent hyaline shell membrane. When released by the worm in the intestine, the egg contains an unsegmented ovum. During its passage down the intestine, the ovum develops and thus the eggs passed in feces have a segmented ovum, usually with 4 to 8 blastomeres. As the eggs of both *Ancylostoma* and *Necator* (and most other hookworm species) are indistinguishable, to identify the genus, they must be cultured in the lab to allow larvae to hatch out. If the fecal sample is left for a day or more under tropical conditions, the larvae will have hatched out, so eggs might no longer be evident. In such a case, it is essential to distinguish hookworms from *Strongyloides* larvae, as infection with the latter has more serious implications and requires different management. The larvae of the two hookworm species can also be distinguished microscopically, although this would not be done routinely, but usually for research purposes. Adult worms are rarely seen (except via endoscopy, surgery or autopsy), but if found, would allow definitive identification of the species. Classification can be performed based on the length of the buccal cavity, the space between the oral opening and the esophagus: hookworm rhabditoform larvae have long buccal cavities whereas *Strongyloides* rhabditoform larvae have short buccal cavities.

Recent research has focused on the development of DNA-based tools for diagnosis of infection, specific identification of hookworm, and analysis of genetic variability within hookworm populations. Because hookworm eggs are often indistinguishable from other parasitic eggs, PCR assays could serve as a molecular approach for accurate diagnosis of hookworm in the feces.

Prevention

The infective larvae develop and survive in an environment of damp dirt, particularly sandy and loamy soil. They cannot survive in clay or muck. The main lines of precaution are those dictated by sanitary science:

- Do not defecate in places other than latrines, toilets etc.
- Do not use human excrement or raw sewage or untreated 'night soil' as manure/fertilizer in agriculture
- Do not walk barefoot in known infected areas
- Deworm pet dogs — canine and feline hookworms rarely develop to adulthood in humans (*Ancylostoma caninum*, the common dog hookworm, occasionally develops into an adult to cause eosinophilic enteritis in people), but their invasive larvae can cause an itchy rash called cutaneous larva migrans.

Moxidectin has been released in the United States as part of Advantage Multi (imidacloprid + moxidectin) Topical Solution for dogs and cats. It utilizes moxidectin for control and prevention of roundworms, hookworms, heartworms, and whipworms.

With an estimated 740 million individuals infected, hookworm is a major public health concern in our world today. While hookworm infection may not directly lead to mortality, its effects on morbidity demand immediate attention. When considering disability-adjusted-life-years (DALYs), neglected tropical diseases, including hookworm, rank among diarrheal diseases, ischemic heart disease, malaria, and tuberculosis as one of the most important health problems of the developing world.

It has been estimated that as many as 22.1 million DALYs have been lost due to hookworm. Recently, there has been increasing interest to address the public health concerns associated with hookworm. For example, the Bill & Melinda Gates Foundation recently donated US$34 million to fight Neglected Tropical Diseases including hookworm infection. Former US President Clinton also announced a mega-commitment at the Clinton Global Initiative (CGI) 2008 Annual Meeting to de-worm 10 million children.

Most of these public health concerns have focused on children who are infected with hookworm. This focus on children is largely due to the large body of evidence that has demonstrated strong associations between hookworm infection and impaired learning, increased absences from school, and decreased future economic productivity. In 2001, the 54th World Health Assembly passed a resolution demanding member states to attain a minimum target of regular deworming of at least 75% of all at-risk school children by the year 2010. A 2008 World Health Organization publication reported on these efforts to treat at-risk school children. Some of the interesting statistics were as follows: 1) only 9 out of 130 endemic countries were able to reach the 75% target goal; and 2) less than 77 million school-aged children (of the total 878 million at risk) were reached which means that only 8.78% of at-risk children are being treated for hookworm infection. While there is progress being made, these numbers also remind us of how much work is still to be done.

School-based mass deworming programs have been the most popular strategy to address the issue of hookworm infection in children. School-based programs are extremely cost effective as schools already have an available, extensive, and sustained infrastructure with a skilled workforce that has a close relationship with the community. With little training from a local healthy system, teachers can easily administer the drugs which often cost less than $0.50 per child per year.

Recently, many people have begun to question if the school-based programs are necessarily the most effective approach. An important concern with school-based programs is that they often do not reach children who do not attend school, thus ignoring a large amount of at-risk children. A 2008 study by Massa et al. continued the debate regarding school-based programs. They examined the effects of community-directed treatments versus school-based treatments in the Tanga Region of Tanzania. A major conclusion was that the mean infection intensity of hookworm was significantly lower in the villages employing the community-directed treatment approach than the school-based approach. The community-directed treatment model used in this specific study allowed villagers to take control of the child's treatment by having villagers select their own

community drug distributors to administer the antihelminthic drugs. Additionally, villagers organized and implemented their own methods for distributing the drugs to all children. The positive results associated with this new model highlight the need for large-scale community involvement in deworming campaigns.

Many mass deworming programs also combine their efforts with a public health education. These health education programs often stress important preventative techniques such as: always wearing shoes, washing your hands before eating, and staying away from water/areas contaminated by human feces. But while these may seem like simple tasks, they raise important public health challenges. The fact is that most infected populations are from poverty-stricken areas with very poor sanitation. Thus, it is most likely that at-risk children cannot afford shoes to wear, do not have access to clean water to wash their hands, and live in environments with no proper sanitation infrastructure. Health education, therefore, must address preventive measures in ways that are both feasible and sustainable in the context of resource-limited settings.

Evaluation of numerous public health interventions have generally shown that improvement in each individual component ordinarily attributed to poverty (for example, sanitation, health education, footwear, and underlying nutrition status) often have minimal impact on transmission. For example, one study found that the introduction of latrines into a resource-limited community only reduced the prevalence of hookworm by four percent. Another study in Salvador, Brazil found that improved drainage and sewerage had minimal impact of the prevalence and no impact at all on the intensity of hookworm. This seems to suggest that environmental control alone has minimal effect on the transmission of hookworm. It is imperative, therefore, that more research be performed to understand the efficacy and sustainability of integrated programs that combine numerous preventive methods including education, sanitation, and treatment.

Management

The most common treatment for hookworm are benzimidazoles, specifically albendazole and mebendazole. BZAs kill adult worms by binding to the nematode's β-tubulin and subsequently inhibiting microtubule polymerization within the parasite. In certain circumstances, levamisole and pyrantel pamoate may be used. The 2008 study by Keiser and Utzinger, Efficacy of Current Drugs Against Soil-Transmitted Helminth Infections: Systematic Review and Meta-analysis, examined the relative efficacies of different drug treatments. They found that the efficacy of single-dose treatments for Hookworm infections were as follows: 72% for albendazole, 15% for mebendazole, and 31% for pyrantel pamoate. This substantiates prior claims that albendazole is much more effective than mebendazole for Hookworm infections. Also of note is that the World Health Organization does recommend anthelmintic treatment in pregnant women after the first trimester. It is also recommended that if the patient also suffers from anemia that ferrous sulfate (200 mg) be administered three times daily at the same time as anthelmintic treatment; this should be continued until hemoglobin values return to normal which could take up to 3 months.

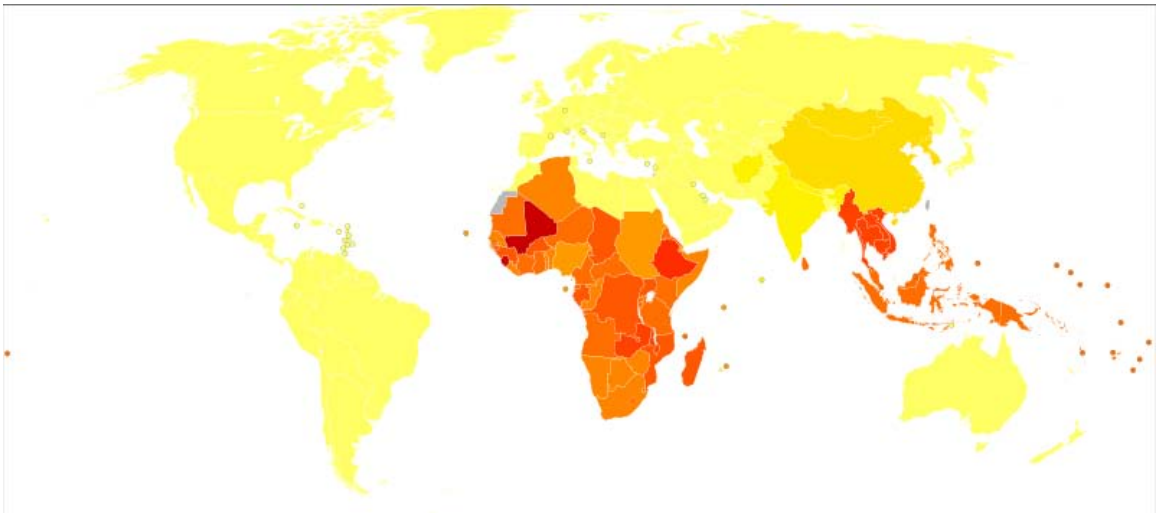
Other important issues related to the treatment of hookworm are reinfection and drug resistance. It has been shown that reinfection after treatment can be extremely high. Some studies even show that 80% of pretreatment hookworm infection rates can be seen in treated communities within 30–36 months. While reinfection may occur, it is still recommended that regular treatments be conducted as it will minimize the occurrence of chronic outcomes. There are also increasing concerns about the issue of drug resistance. Drug resistance has appeared in front-line anthelmintics used for livestock nematodes. Generally human nematodes are less likely to develop resistance due to longer reproducing times, less frequent treatment, and more targeted treatment. Nonetheless, the global community must be careful to maintain the effectiveness of current anthelmintic as no new anthelmintic drugs are in the late-stage development.

The hookworm can be treated with local cryotherapy when it is still in the skin.

Albendazole is effective both in the intestinal stage and during the stage the parasite is still migrating under the skin.

In case of anaemia, iron supplementation can cause relief symptoms of iron deficiency anemia. However, as red blood cell levels are restored, shortage of other essentials such as folic acid or vitamin B12 may develop, so these might also be supplemented.

Epidemiology



Disability-adjusted life year for hookworm disease per 100,000 inhabitants in 2002.
- no data
- less than 10
- 10-15
- 15-20
- 20-25
- 25-30
- 30-35

35-40
40-45
45-50
50-55
55-60
more than 60

It is estimated that between 576-740 million individuals are infected with hookworm today. Of these infected individuals, about 80 million are severely affected. The major etiology of hookworm infection is *N. Americanus* which is found the Americas, sub-Saharan Africa, and Asia. *A. duodenale* is found in more scattered focal environments, namely Europe and the Mediterranean. Most infected individuals are concentrated in sub-Saharan Africa and East Asia/the Pacific Islands with each region having estimates of 198 million and 149 million infected individuals, respectively. Other affected regions include: South Asia (50 million), Latin America and the Caribbean (50 million), South Asia (59 million), Middle East/North Africa (10 million). A majority of these infected individuals live in poverty-stricken areas with poor sanitation. Hookworm infection is most concentrated among the world's poorest who live on less than $2 a day.

Many of the numbers regarding the prevalence of hookworm infection are estimates as there is no international surveillance mechanism currently in place to determine prevalence and global distribution. Some prevalence rates have been measured through survey data in endemic regions around the world. The following are some of the most recent findings on prevalence rates in regions endemic with hookworm.

Darjeeling, Hooghly District, West Bengal, India (Pal et al. 2007)

• 42.8% infection rate of predominantly *N. Americanus* although with some *A. Duodenale* infection

• Both hookworm infection load and degree of anemia in the mild range

Xiulongkan Village, Hainan Province, China (Gandhi et al. 2001)

• 60% infection rate of predominantly *N. Americanus*

• Important trends noted were that prevalence increased with age (plateau of about 41 years) and women had higher prevalence rates than men

Hoa Binh, Northwest Vietnam (Verle et al. 2003)

• 52% of a total of 526 tested households infected

• Could not identify species, but previous studies in North bob reported *N. Americanus* in more than 95% of hookworm larvae

Minas Gerais, Brazil (Fleming et al. 2006)

• 62.8% infection rate of predominantly *N. Americanus*

KwaZulu-Natal, South Africa (Mabaso et al. 2004)

• Inland areas had a prevalence rate of 9.3% of *N. Americanus*

• Coastal plain areas had a prevalence rate of 62.5% of *N. Americanus*

There have also been recent technological developments that will hopefully facilitate more accurate mapping of hookworm prevalence. Some researchers have begun to use geographical information systems (GIS) and remote sensing (RS) to examine helminth ecology and epidemiology. Brooker et al. utilized this technology to create helminth distribution maps of sub-Saharan Africa. By relating satellite derived environmental data with prevalence data from school-based surveys, they were able to create detailed prevalence maps. The study focused on a wide range of helminths, but interesting conclusions about hookworm specifically were found. As compared to other helminths, hookworm is able to survive in much hotter conditions and was highly prevalent throughout the upper end of the thermal range. Hopefully this information along with more detailed prevalence maps can lead to more effective public health measures.

Improved molecular diagnostic tools are another technological advancement that could help improve existing prevalence statistics. Recent research has focused on the development of DNA-based tool that can be used for diagnosis of infection, specific identification of hookworm, and analysis of genetic variability in hookworm populations. Again this can serve as a major tool for different public health measures against hookworm infection. Most research regarding diagnostic tools is now focused on the creation of a rapid and cost-effective assay for the specific diagnosis of hookworm infection. Many are hopeful that its development can be achieved within the next 5 years.

History

The symptoms now attributed to hookworm appear in papyrus papers of ancient Egypt (c. 1500 BCE), described as a derangement characterized by anemia. Avicenna, a Persian physician of the 11th century, discovered the worm in several of his patients and related it to their disease. In later times, the condition was noticeably prevalent in the mining industry in England, France, Germany, Belgium, North Queensland and elsewhere.

Italian physician Angelo Dubini was the modern-day discoverer of the worm in 1838 after an autopsy of a peasant woman. Dubini published details in 1843 and identified the species as *A. duodenale*. Working in the Egyptian medical system in 1852 German physician Theodor Bilharz, drawing upon the work of colleague Wilhelm Griesinger, found these worms during autopsies and went a step further in linking them to local endemic occurrences of chlorosis, which would probably be called iron deficiency anemia today.

A breakthrough came 25 years later following a diarrhea and anemia epidemic that took place among Italian workmen employed on the Gotthard Rail Tunnel. In an 1880 paper, physicians Camillo Bozzolo, Edoardo Perroncito, and Luigi Pagliani correctly hypothesized that hookworm was linked to the fact that workers had to defecate inside the 15 km tunnel, and that many wore worn-out shoes. In 1897, it was established that the skin was the principal avenue of infection and the biological life cycle of the hookworm was clarified. In 1899, American zoologist Charles Wardell Stiles brought this evidence to bear on health issues in the southeast United States, identifying "progressive pernicious anemia" seen in the southern United States was caused by *A. duodenale* and he also identified the other important hookworm species: *U. Necator*. Testing in the 1900s revealed very heavy infestations in schoolage children. In Puerto Rico, Dr. Bailey K. Ashford, an U.S. Army physician, organized and conducted a parasite treatment campaign, which cured approximately 300,000 persons (one-third of the Puerto Rico population) and reduced the death rate from this anemia by 90 percent during the years 1903-1904.

A doctor examines a boy for signs of hookworm in Coffee County, Alabama, 1939.

On October 26, 1909 the Rockefeller Sanitary Commission for the Eradication of Hookworm Disease was organized as a result of a gift of US$1 million from John D. Rockefeller, Sr. The five-year program was a remarkable success and a great contribution to United States public health, instilling public education, medication, field work and modern government health departments in eleven southern states. The hookworm exhibit was a prominent part of the 1910 Mississippi state fair. The program nearly eradicated

hookworm and would flourish afterwards with new funding as the Rockefeller Foundation International Health Division.

In the 1920s, hookworm eradication reached the Caribbean and Latin America, where great mortality was reported among Black people in the West Indies towards the end of the 18th century, as well as through descriptions sent from Brazil and various other tropical and sub-tropical regions.

Early treatment relied on the use of epsom salt to reduce protective mucous, followed by thymol to kill the worms. Later tetrachloroethylene was the leading method. It was not until later in the mid-20th century when new organic drug compounds were developed.

Research

Anemia in pregnancy

It is estimated that a third of all pregnant women in developing countries are infected with hookworm, 56% of all pregnant women in developing countries suffer from anemia, 20% of all maternal deaths are either directly or indirectly related to anemia. Numbers like this have led to an increased interest in the topic of hookworm-related anemia during pregnancy. With the understanding that chronic Hookworm infection can often lead to anemia, many people are now questioning if the treatment of hookworm could effect change in severe anemia rates and thus also on maternal and child health as well. Most evidence suggests that the contribution of hookworm to maternal anemia merits that all women of child-bearing age living in endemic areas be subject to periodic anthelmintic treatment. The World Health Organization even recommends that infected pregnant women be treated after their first trimester. Regardless of these suggestions, only Madagascar, Nepal and Sri Lanka have added deworming to their antenatal care programs.

This lack of deworming of pregnant women is explained by the fact that most individuals still fear that anthelmintic treatment will result in adverse birth outcomes. But a 2006 study by Gyorkos et al. found that when comparing a group of pregnant women treated with mebendazole with a control placebo group, both illustrated rather similar rates in adverse birth outcomes. The treated group demonstrated 5.6% adverse birth outcomes, while the control group had 6.25% adverse birth outcomes. Furthermore, Larocque et al. illustrated that treatment for hookworm infection actually led to positive health results in the infant. This study concluded that treatment with mebendazole plus iron supplements during antenatal care significantly reduced the proportion of very low birth weight infants when compared to a placebo control group. Studies so far have validated recommendations to treat infected pregnant women for hookworm infection during pregnancy.

The intensity of hookworm infection as well as the species of hookworm have yet to be studied as they relate to hookworm-related anemia during pregnancy. Additionally, more

research must be done in different regions of the world to see if trends noted in completed studies persist.

Malaria co-infection

Co-infection with hookworm and *Plasmodium falciparum* is fairly ubiquitous throughout Africa due to spatial congruence. Although exact numbers are unknown, preliminary analyses estimate that as many as a quarter of African schoolchildren (17.8–32.1 million children aged 5–14 years) may be coincidentally at-risk for both *P. falciparum* and hookworm. While original hypotheses stated that co-infection with multiple parasites would impair the host's immune response to a single parasite and increase susceptibility to clinical disease, studies have yielded contrasting results. For example, one study in Senegal showed that the risk of clinical malaria infection was increased in helminth-infected children in comparison to helminth-free children while other studies have failed to reproduce such results. Some hypotheses and studies even suggest that helminth infections may protect against cerebral malaria due to the possible modulation of pro-inflammatory and anti-inflammatory cytokines responses. Furthermore, the mechanisms underlying this supposed increased susceptibility to disease are unknown. For example, it is known that helminth infections cause potent and highly polarized immune response characterized by increased T-helper cell type 2 (T_h2) cytokine and Immunoglobulin E(IgE) production. However, the effect of such responses on the human immune response is unknown. Additionally, both malaria and helminth infection can cause anemia, but the effect of co-infection and possible enhancement of anemia is poorly understood.

Hygiene hypothesis

The hygiene hypothesis states that infants and children who lack exposure to infectious agents are more susceptible to allergic diseases via modulation of immune system development. As Mary Ruebush writes in her book *Why Dirt is Good*, "what a child is doing when he puts things in his mouth is allowing his immune response to explore his environment. Not only does this allow for 'practice' of immune responses, which will be necessary for protection, but it also plays a critical role in teaching the immature immune response what is best ignored." The theory was first proposed by David P. Strachan who noted that hay fever and eczema were less common in children who belonged to large families. Since then, studies have noted the effect of gastrointestinal worms on the development of allergies in the developing world. For example, a study in Gambia found that eradication of worms in some villages led to increased skin reactions to allergies among children.

Although the exact mechanism is unknown, scientists hypothesize that the helper T cells are key players. Allergic diseases, which are immunological responses to normally harmless antigens, are driven by a TH2-mediated immune response. Bacteria, viruses, and parasites, on the other hand, elicit a TH1-mediated immune response which inhibits or down-regulates the TH2 response. TH1 also inhibits the activity of TH17 which is heightened in numerous inflammatory diseases including multiple sclerosis and asthma.

More research is currently being performed to better understand the possible mechanism for the hygiene hypothesis.

Vaccines

While annual or semi-annual mass antihelminthic administration is a critical aspect of any public health intervention, many have begun to realize how unsustainable it is due to aspects such as poverty, high rates of re-infection, and diminished efficacy of drugs with repeated use. Current research, therefore, has focused on the development of a vaccine that could be integrated into existing control programs. The goal of vaccine development is not necessarily to create a vaccine with sterilizing immunity or complete protection against immunity. A vaccine that reduces the likelihood of vaccinated individuals developing severe infections and thus reduced blood and nutrient levels could still have a significant impact on the high burden of disease throughout the world.

Current research focuses on targeting two stages in the development of the worm: the larval stage and the adult stage. Research on larval antigens has focused on proteins that are members of the pathogenesis-related protein superfamily, *Ancylostoma* Secreted Proteins. Although they were first described in *Anyclostoma*, these proteins have also been successfully isolated from the secreted product of *N. americanus*. *N. americanus* ASP-2 (Na-ASP-2) is currently the leading larval-stage hookworm vaccine candidate. A randomized, double-blind, placebo-controlled study has already been performed; 36 healthy adults without a history of hookworm infection were given three intramuscular injections of three different concentrations of Na-ASP-2 and observed for six months after the final vaccination. The vaccine induced significant anti-Na-ASP-2 IgG and cellular immune responses. In addition, it was safe and produced no debilitating side effects. The vaccine is now in a phase one trial; healthy adult volunteers with documented evidence of previous infection in Brazil are being given the same dose concentration on the same schedule used in the initial study. If this study is successful, the next step would be to conduct a phase two trial to assess the rate and intensity of hookworm infection among vaccinated persons. Because the Na-ASP-2 vaccine only targets the larval stage, it is critical that all subjects enrolled in the study be treated with antihelminthic drugs to eliminate adult worms prior to vaccination.

Adult hookworm antigens have also been identified as potential candidates for vaccines. When adult worms attach to the intestinal mucosa of the human host, erythrocytes are ruptured in the worm's digestive tract which causes the release of free hemoglobin which is subsequently degraded by a proteolytic cascade. Several of these proteins that are responsible for this proteolytic cascade are also essential for the worm's nutrition and survival. Therefore, a vaccine that could induce antibodies for these antigens could interfere with the hookworm's digestive pathway and impair the worm's survival. Three proteins have been identified: the aspartic protease-hemoglobinase APR-1, the cysteine protease-hemoglobinase CP-2, and a glutathione S-transferase. Vaccination with APR-1 and CP-2 led to reduced host blood loss and fecal egg counts in dogs. With APR-1, vaccination even led to reduced worm burden. Research is currently stymied at the

development of at least one of these antigens as a recombinant protein for testing in clinical trials.

Hookworm in therapy

Moderate hookworm infections have been demonstrated to have beneficial effects on hosts suffering from diseases linked to overactive immune systems. This is possibly explained by the hygiene hypothesis. Research at the University of Nottingham conducted in Ethiopia observed a small subset of people with hookworm infections were half as likely to experience asthma or hay fever. Potential benefits have also been hypothesized in cases of multiple sclerosis, Crohn's Disease and diabetes.

Research conducted by the Queensland Institute of Medical Research (QIMR) and the Princess Alexandra Hospital (located in Australia) has shown favourable results in clinical trials using hookworms to treat coeliac disease.

Quick Facts

Genus and Species	**Necator americanus**	**Ancylostoma duodenale**
Common Name	New world hookworm	Old world hookworm
Etiologic Agent of:	Necatoriasis, Uncinariasis	Ancylostomiasis, Wakana disease
Infective stage	Filariform larva	
Definitive Host	Human	
Portal of Entry	Usually via skin penetration rather than ingestion	Usually via ingestion rather than skin penetration
Mode of Transmission	Skin > Mouth	Mouth > Skin
Habitat	Small Intestine (jejunum, ileum)	Small Intestine (duodenum, jejunum)
Pathogenic Stage	L3 Larva	
Maturation time in host (days)	49-56	53
Mode of Attachment	Oral attachment to mucosa by sucking	
Mode of Nutrition	Sucking and Ingesting of blood	

Pathogenesis	Larva – ground / dew itch, creeping eruption Adult – IDA Microcytic, Hypochromic Anemia	
Laboratory diagnosis	Concentration methods and Direct Fecal Smear	
Treatment	Albendazole, Mebendazole, or Pyrantel Pamoate	
Length of adult hookworm (mm)	5-9 for males; 9-11 for females	8-11 for males; 10-13 for females
Shape	Head curved opposite to curvature of body, giving a hooked appearance to anterior end	Head continuous in same direction as the body
Egg output per female worm per day	5,000-10,000	10,000-25,000
Blood loss per worm per day (ml)	0.03	0.15-0.23
Temperature at which 90% of eggs hatch (C)	20-35	15-35
Diagnostic Feature - Adult	Semi-lunar cutting plate; Bipartite dorsal ray	Male – Tripartite dorsal ray
Diagnostic Feature - Egg	In Morula	