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Nemata

Strongyloidea and Trichostrongyloidea (Superfamilies): Bursate Nematodes

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Phylum Nemata

Superfamily Strongyloidea

Superfamily Trichostrongyloidea

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Chapter 56

Strongyloidea and Trichostrongyloidea (Superfamilies): Bursate Nematodes

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Introduction

The bursate nematodes include those within the superfamilies Strongyloidea Baird, 1853 and Trichostrongyloidea Cram, 1927. Even though there are new data showing various phylogenetic relationships among and between species in these groups, they will be discussed here under the umbrella of bursate nematodes for the sake of simplicity. Bursate nematodes are distinguished by the presence of a copulatory bursa on the posterior end of the male (**bursa** = purse or pouch; Latin).

In general—although with exceptions—bursate nematodes have relatively stout bodies with a muscular **esophagus** that is not divided into various parts but is narrower at the anterior end and more bulbous and expanded toward the posterior end where the esophagus attaches to the **intestine**. There is always an encircling **nerve ring** around the esophagus and—as in almost all nematodes—the nerve ring slants more posteriad on the ventral aspect of the nematode and slants more anteriad on the dorsal aspect. The **excretory pore** exits the **cuticle** in the general vicinity of the nerve ring and is always situated ventrally (Figure 1). A morphological feature—which is in fact a synapmorphy for the bursate nematodes—is the possession of a **copulatory bursa** in males that is composed of muscular **rays** with **cuticular membranes** connecting them (Figure 2). The copulatory bursa consists of laterally-projecting **cuticular extensions** that surround the **tail** of males in species assigned to both superfamilies, and this structure serves as a grasping/sensory organ equipped with **sensory muscular papillae** (Figure 3). The bursa grasps the female during copulation and enables the male to extend the **spicule** or spicules (Figure 4) into the **reproductive tract** of the female thus facilitating the transfer of ameboid sperms to the female reproductive system. The bursa surrounds the spicules and the **cloaca** (which is the joint opening that drains the intestinal and reproductive systems; Gardner et al., 1994b).

Most species of bursate nematodes that are parasites in the intestines of vertebrates have direct life history patterns and only the definitive host is needed for the parasite to reach sexual maturity; however, some species that occur in organs or tissues such as lungs, muscles, or the central nervous system of their vertebrate hosts, have indirect life history patterns. Examples are species of *Angiostrongylus*, which are normally parasites of rodents, but can infect people living in tropical and subtropical regions with devastating neurological consequences. These species use land-dwelling molluscs (snails and slugs) as intermediate hosts (Alicata, 1991).

Following are discussions of the 2 superfamilies and a few of the highly numerous other families. For additional information on other groups of these animals, see Travassos (1937), Anderson (2000), and the CIH keys by Anderson and colleagues (2009).

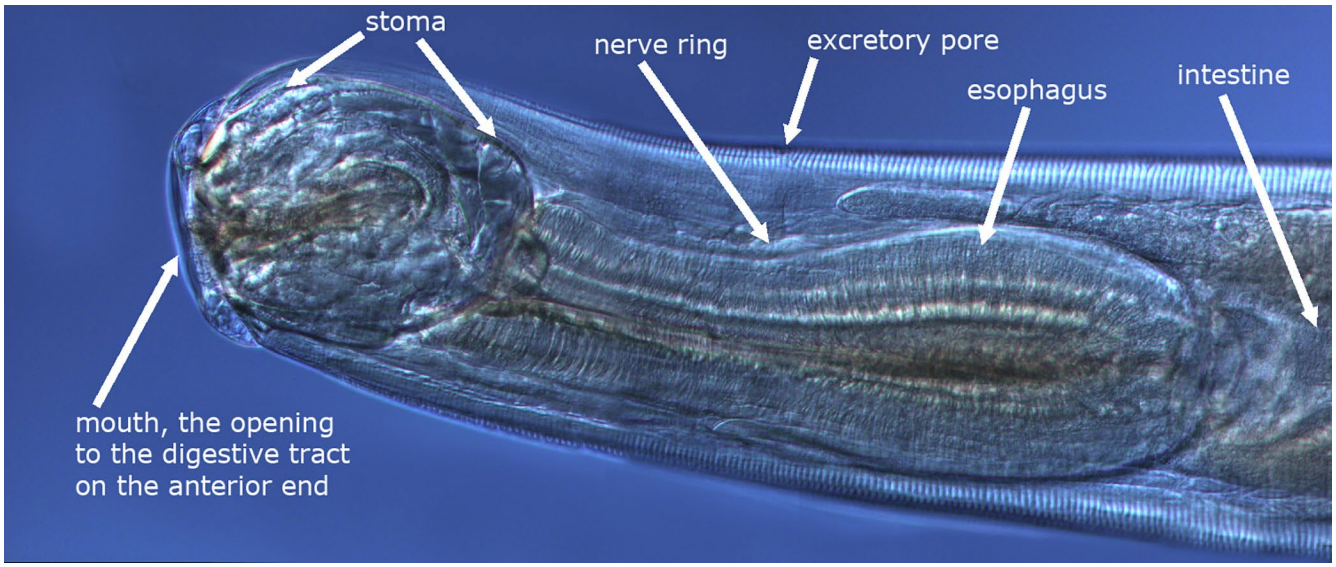

Figure 1. Anterior end of a specimen of *Ransomus rodentorum* with structures labeled. Normarsky micrograph. Source: S. L. Gardner, HWML. License: CC BY 4.0.

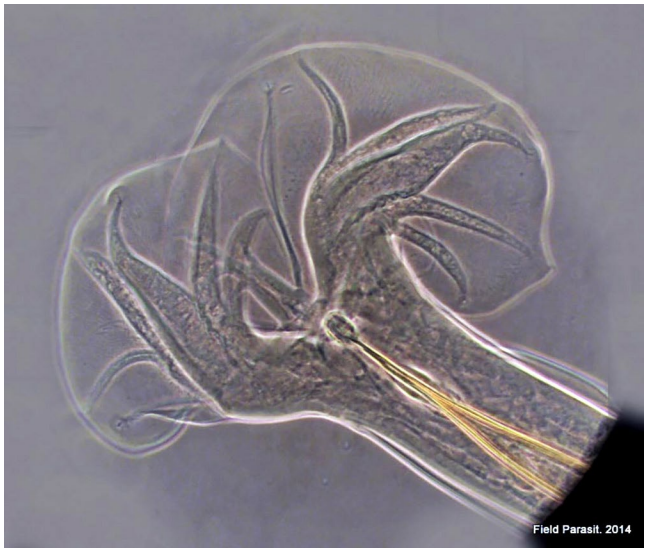

Figure 2. Copulatory bursa of *Vexillata armandae*, a parasite of the small intestine of the coarse-haired pocket mouse *Chaetodipus hispidus*. Collected and imaged at Cedar Point Biological Station, near Ogallala, Nebraska, United States. Source: S. L. Gardner, HWML, 2014. License: CC BY 4.0.

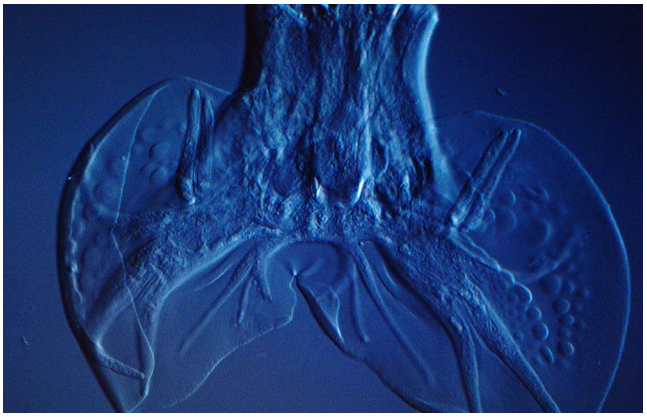

Figure 3. Copulatory bursa of a male of a species of trichostrongyloid nematode from *Ochotona princeps* (order Strongylida, superfamily Trichostrongyloidea) collected in Colorado, United States. Source: D. Tufts, HWML, 2013. License: CC BY 4.0.

Superfamily Strongyloidea Baird, 1853

The strongyloids (also known as strongyles; superfamily Strongyloidea Baird, 1853) comprise a diverse group of parasitic nematodes with a cosmopolitan distribution in vertebrates. Nematodes classified in the superfamily Strongyloidea are defined by several characteristics that are well-established **synapomorphies** (meaning, shared derived characters) for the group, as listed above.

Following are discussions of 2 families within the Strongyloidea, families **Ancylostomatidae** and **Strongylidae**, and several species within those. From the standpoint of human health, the more important of these 2 families is the Ancylostomatidae, commonly known as hookworms.

Hookworms: Family Ancylostomatidae

The ancylostomatid nematodes are commonly known as **hookworms** because of the initial name given them by Goeze (1782), who noted membranous expansions with 2 rib-like structures on the tail of the males, which were collected from the intestine of a European badger (*Meles meles*). Frölich (1789) found similar worms in foxes, also with membranous

Figure 4. Posterior end of male *Ransomus rodentorum* showing bursa and twin/paired spicules. Source: S. L. Gardner, HWML. License: CC BY 4.0.

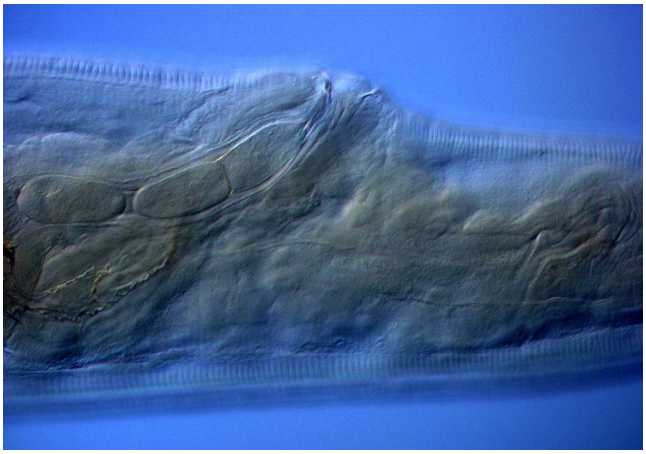

Figure 5. Posterior end showing ovijector of the same individual of *Ransomus* as shown in Figure 1 above. Eggs can be seen being expelled from the body via the muscles of the ovijector. Normarsky micrograph. Source: S. L. Gardner, HWML. License: CC BY 4.0.

expansions in the tails. Frölich called these nematodes Hakenwurm (= hookworm; German).

In their mammalian hosts (including humans), adult hookworms usually live in the duodenum (the anterior part of the small intestine), where they attach to the mucosa, pulling mucosa into their **stomata** and abrading the villi with **cuticularized cutting teeth** or **plates** (Figure 6, left). The cuticularized plates of the stoma rupture the capillaries of the mucosa and the nematode pumps blood rapidly into its intestine with the muscular esophagus. Little of the blood that is extracted from the host is digested or used by the nematode and most of the blood that is pumped out of the capillary beds of the host pass through the intestine of the nematode, out the anus, and directly back into the lumen of the host's gut. As can be seen clearly in the video (available at hookworm.vob (Japan National Institute of Health, n. d.), worms feed heavily on blood, most of which is wasted as it passes directly out of the worms out into the lumen of the intestine, where most of the blood is digested and reabsorbed.

As mentioned above, species in the family Ancylostomatidae are important human pathogens. They generally afflict people in warm-temperate, subtropical, and tropical areas of the world living without access to adequate sanitation facilities and without ready access to shoes (Loukas et al., 2016). Because the main 3 species of hookworms that reside in humans have similar life-histories, pathogenicity, control schemes, symptomatology, and epidemiology, they are treated below as a single topic, with notations where important variations occur. See Table 1 for a list of valid species of *Ancylostoma* (see Drabik and Gardner, 2019). *Necator americanus* is also of concern with respect to human health and will be discussed below, as well.

General Morphology

Ancylostomatid nematodes are robust with the anterior extremity reflexed dorsad. The esophagus is muscular and club shaped with a narrow anterior end and a more swollen posterior part. Esophageal glands are located in the pseudocoel extending posteriad. Cervical papillae or deirids are present near the nerve ring. These nematodes are dioecious with males having a conspicuous and well-developed copulatory bursa, consisting of 2 broad lateral lobes with lateral rays and a smaller dorsal lobe with a multi- or bifurcating dorsal ray (depending on the species) (Figure 6). The bursal rays found laterally are termed the externolateral rays. The dorsal ray is situated in the dorsal part of the bursal lobe. The rays

Figure 6. Left image: Posterior end of a male specimen of *Ancylostoma* from a tuco-tuco (a rodent of the genus *Ctenomys*) in Bolivia. The rays and vellum make up the copulatory bursa. Right image: Anterior end showing the cuticularized hooks of the stoma of the same species of nematode. Source: S. L. Gardner, HWML. License: CC BY 4.0.

Table 1. Species of *Ancylostoma* from mammals including known original hosts, zoogeographic region of occurrence, and nearest approximate geographic collection locality of type specimens. Zoogeographic regions follow Wallace, 1876. Source: Adapted from Drabik and Gardner, 2019. License: CC BY 4.0.

Species of *Ancylostoma*	**Host**	**Biogeographic region**	**Type locality**
A. aliuropodae Xie et al., 2017	*Ailuropoda melanoleuca* (David, 1869)	Palearctic	Fengtongzai Nature Reserve, China
			Brazil
A. bidens Molin, 1861	*Nasua nasu* (Linnaeus, 1766), *Procyon cancrivorous* (Linnaeus, 1766)	Neotropical	Brazil
A. braziliense de Faria, 1910	Canidae, Felidae	Cosmopolitan	Brazil
A. buckleyi Le Roux and Biocca, 1957	*Felis concolor* (Linnaeus, 1771), *Cerdocyon thous* (Linnaeus, 1766)	Neotropical	Leticia Amazonas, Colombia
A. caninum (Ercolani, 1859)	Canidae	Cosmopolitan	Turin, Italy
A. ceylanicum Looss, 1911	Canidae, Felidae, *Homo sapiens*	Ethiopian, Oriental, Palearctic	Sri Lanka
A. conepati (Solanet, 1911)	*Conepatus chinga* (Molina, 1782)	Neotropical	Buenos Aires, Argentina
A. ctenomyos	*Ctenomys steinbachi* Thomas, 1907, *C. boliviensis* Waterhouse, 1848	Neotropical	Bolivia
A. duodenale (Dubini, 1843)	*Homo sapiens* Linnaeus, 1758	Cosmopolitan	Milan, Italy
A. galogoi van der Berghe, 1936	*Otolemur crassicaudatus* (Geoffroy Saint-Hilaire, 1812)	Ethiopian	East Central Africa
A. genettae Macchioni, 1995	*Genetta genetta* (Linnaeus, 1758)	Ethiopian, southeast Palearctic	Scebeli River, Somalia
A. gilsoni Gedoelst, 1917	*Sciurus prevosti* (Desmarest, 1822)	Oriental	Malaysia
A. hescheleri Mönnig, 1938	*Orycteropus afer* (Pallas, 1766)	Ethiopian	South Africa
A. iperodontatum Le Roux and Biocca, 1957	*Acinonyx jubatus* (Schreber, 1775)	Ethiopian	Zambia
A. japonica Fukuda and Katsurada, 1925	*Homo sapiens* Linnaeus, 1758	Palearctic	Japan
A. longespiculatum Mönnig, 1938	*Felis silvestris* Schreber, 1777	Palearctic	South Africa
A. malayanum Alessandrini, 1905	*Ursus* sp.	Palearctic, Oriental	Southeast Asia
A. martinaglai Mönnig, 1931	*Canis mesomelas* Schreber, 1775	Ethiopian	South Africa
A. mephitis Micheletti, 1929	*Ictonyx striatus* (Perry, 1810)	Ethiopian	?
A. minimum (von Linstow, 1906)	*Prionailurus rubiginosus* (Geoffroy Saint-Hilaire, 1831)	Oriental	Sri Lanka
A. mucronatum (Molin, 1861)	*Dasypus novemcinctus* Linnaeus, 1758	Neotropical	Brazil
A. mycetis (Molin, 1861)	*Alouatta* sp.	Neotropical	Brazil
A. paraduodenale Biocca, 1951a	*Leptailurus serval* (Schreber, 1776)	Ethiopian	Zambia—Rome Zoo
A. pluridentatum (Alessandrini, 1905)	*Felis* spp.	Neotropical	Brazil
A. protelesis Macchioni, 1995	*Proteles cristatus* (Sparrman, 1783)	Ethiopian	Scebeli River, Somalia
A. somaliense Macchioni, 1995	*Canis mesomelas* Schreber, 1775	Ethiopian	Scebeli River, Somalia
A. taxidae Kalkan and Hansen 1966	*Taxidea taxus* (Schreber, 1777)	Nearctic	Manhattan, Kansas, United States
A. tubaeforme (Zeder, 1800)	*Felis silvestris* Schreber, 1777	Palearctic	—

are species specific (Drabik and Gardner, 2019). The number and general patterns of rays in the copulatory bursa are also a characteristic found in other male rhabditid nematodes, although they are much reduced in free living and insect parasitic forms (Gardner et al., 1994b). As mentioned earlier, all species of nematodes in the superfamilies Strongyloidea and Trichostrongyloidea have very well-developed copulatory bursae (Gardner et al., 1994a). The paired spicules in these nematodes are setaceous in form with a well-developed vellum (Maggenti, 1981).

Females have a simple, conical tail. The vulva is ventrally located and is usually in the posterior one-third of the body and the uterus is didelphic. About 5% of the daily output of eggs is found in the uteri at any one time; the total production is several thousand per day for as long as 14 or more years for a single female.

Life History

As far as is known, species of *Ancylostoma* and *Necator* mature and mate in the small intestine of their host. Eggs are produced by the thousands and embryos within the thin-shelled eggs develop into 2-, 4-, or several-cell stages by the time they are passed with feces. Species infecting humans cannot be identified by egg structure or size. Eggs that pass out into the environment require warmth, shade, and moisture for continued development. Coprophagous insects may mix the feces with soil and air, perhaps hastening embryogenesis, which is completed within 24 to 48 hours in ideal moist conditions. Newly hatched J_1s have a rhabditiform esophagus with a characteristic constriction at the level of the nerve ring and a basal bulb with a valve. Differentiation of hookworm juveniles from those of *Strongyloides* spp. is difficult for a beginning parasitologist.

First stage juveniles living in the feces deposited by their host feed on bacteria therein and molt their cuticle in 2 to 3 days. Second-stage juveniles (J_2), which also have a rhabditiform esophagus, continue to feed and grow and, after about 5 days, molt to the third stage (J_3) filariform-type of juvenile, which is then infective to a mammal. At this point, the second-stage cuticle may be retained as a loose-fitting sheath until penetration of a new host, or the cuticle may be lost just before the juvenile penetrates. Filariform J_3s have a strongyliform esophagus; that is, with a reduced basal bulb that is not separated from the corpus by an isthmus. It has been shown that the J_3s do not feed and they evidently survive on the stored bacterial soup stored in the intestine. Hookworm J_3s are similar to filariform J_3s of *Strongyloides* spp. but can be distinguished by the tail tip, which is pointed in hookworms and notched in *Strongyloides* spp.

Living in the upper few millimeters of soil, J_3s remain in the water film surrounding soil particles and they never survive freezing or drying out. There is a short, vertical migration in the soil, depending on the weather or time of day. When the ground surface begins to desiccate, they migrate a short distance into the soil, staying ahead of the drying soil. Under ideal conditions, they can live for several weeks using this up and down method to stay alive. When the ground surface is wet, after rain or morning moisture condensation in the form of dew, the juveniles wriggle to the surface, remaining in a resting posture until activated (Haas et al., 2005). They are stimulated into sinusoidal motion called "the dance macabre" by a variety of environmental cues, such as touch, vibration, water currents, heat, light, or carbon dioxide. Warmth and moisture stimulate them to stand upright on their tail, waving to-and-fro in a searching behavior termed **questing** or **nictation**. Warmth and fatty acids in skin induce penetration behavior (Haas et al., 2005).

Infection occurs when J_3s contact a host's skin and burrow into it, and they resume feeding at about this time (Hawdon et al., 1993). They usually shed the second-stage cuticle as they penetrate, but the presence of a cuticle does not preclude resumption of feeding (Kumar and Pritchard, 1994). Juveniles can penetrate any epidermis, although parts most often in contact with the soil, such as hands, feet, and buttocks, are most often attacked. *Necator americanus* (and probably other skin-penetrating nematodes) secrete a variety of enzymes that hydrolyze skin macromolecules (Brown et al., 1999; Crompton, 1989; Yu et al., 1995).

After gaining entry to a blood or lymph vessel, juveniles are carried to the liver via the hepatic portal vein, and then to the heart and the lungs via the pulmonary artery. In the lungs, the juveniles break from the venous capillary beds into the air spaces of the alveoli where they molt to the fourth stage (J_4), leaving behind the cuticle like an abandoned collapsed space suit. At this point, the fourth stage juvenile (J_4) now has an enlarged stoma. The fourth stage juveniles (J_4) are carried by ciliary action of the ciliated columnar epithelial cellular lining of the bronchi and bronchioles up the respiratory tree to the glottis where they coughed up by the host, and—if they are lucky—they may be swallowed and finally arrive in the small intestine. There they attach to the mucosa with their enlarged stoma, begin to grow, and then molt to the adult stage. After further growth, they become sexually mature and the male grasps the female with his copulatory bursa transferring ameboid sperm into the genital tract of the female (Williamson et al., 2003).

At least 5 weeks are required from the time of infection via penetration through the host's epidermis to the beginning of

egg production in the intestine. However, it has been shown that juveniles of *Ancylostoma duodenale* can undergo developmental arrest for up to 38 weeks, their maturation perhaps coinciding with the seasonal return of environmental conditions favorable to transmission (Behnke, 1987). *Ancylostoma caninum*, a widespread hookworm of dogs and other carnivores, manifests developmental arrest or stasis during its tissue migration and then is reactivated in female dogs when they begin lactation, resulting in transmammary transmission to pups (Arasu, 2001). Reactivation of the juveniles is modulated by estrogen and prolactin (Hotez et al., 2004).

Many species of hookworms across several genera occur in humans and domestic and wild mammals globally (Drabik and Gardner, 2019). Hookworms represented by several species infect approximately 500 million people worldwide and are responsible for much morbidity and mortality globally (Loukas et al., 2016).

Following are more details about of some the species of Ancylostomatidae, including *Necator americanus* (Stiles, 1902), *Ancylostoma duodenale* (Dubini, 1843), *A. ceylanicum* Looss, 1911, and *A. caninum* (Ercolani, 1859), especially the implications of their effect on human health.

***Necator americanus* (Stiles, 1902)**

The Latin name for this species translated literally means American killer. This species is also called the New World hookworm and was first discovered in Brazil and then Texas, United States, but it was later found indigenous in Africa, India, Southeast Asia, China, and the southwest Pacific islands. It probably came to the New World with the trade in enslaved people in the 16th through 19th centuries, with both enslaved individuals and their captors contributing to the importation of the pathogen.

This nematode has caused much human suffering and has had a significant negative impact on the economic development of the southern United States as well as other regions of the world in which it occurs (Loukas et al., 2016). Primarily a parasite of tropical and subtropical regions, *Necator americanus* is the most common species of hookworm in humans in most of the world, accounting for about 85% of recorded infections (Hotez et al., 2010). Prior to effective hookworm control in the United States, about 95% of hookworms in the southern states were this species (Behnke, 1987; Loukas et al., 2016).

Necator americanus has a pair each of dorsal and ventral cutting plates surrounding the anterior margin of the stoma (Looss, 1911). In addition, a pair each of subdorsal and subventral teeth are near the rear of the stoma. The duct of the dorsal esophageal gland opens on a conspicuous cone that projects into the stoma. Males are 5 mm- to 9 mm-long with filariform/needlelike spicules that have minute barbs at their tips and are fused distally. Females are 9 mm- to 11 mm-long and their vulva is located in about the middle of their body with a single individual producing about 5,000 to 10,000 eggs per day (Behnke, 1987; Loukas et al., 2016).

***Ancylostoma duodenale* (Dubini, 1843)**

As noted above, *Ancylostoma duodenale* (Looss, 1911; see Figure 7) has a tropical and subtropical worldwide distribution (Loukas et al., 2016). It is known in mines as far north as England and Belgium. Since Lucretius, in the 1st century CE, it was known to cause serious anemia in miners. Mines offer an ideal habitat for egg and juvenile development because of their constancy in temperature and humidity. The problem is apt to occur whenever miners defecate on the open ground, outside of established latrines (Cumming and White, 1917).

The anterior margin of the stoma of *Ancylostoma duodenale* has 2 ventral plates, each with 2 large teeth that are fused at their bases (Looss, 1911). A pair of small teeth is found in the depths of the capsule. The duct of the dorsal esophageal gland runs in a ridge in the dorsal wall of the buccal capsule and opens at the vertex of a deep notch on the dorsal margin of the capsule.

Adult males are 8 mm- to 11-mm-long and have a bursa characteristic for the species. The needlelike spicules have simple tips and are never fused distally. Females are 10 mm- to 13 mm-long, with the vulva located about a third of the body length from the posterior end. A single female can lay from 10,000 to 30,000 eggs per day (Hotez and Pritchard, 1995).

This is the first hookworm for which the life history was fully studied and understood. To demonstrate this early work on this organism, following is a lengthy excerpt from Arthur Looss (1911) from his monograph on the morphology and life cycle of *Ancylostoma duodenale*, based on his work in Egypt in 1896 he wrote:

> In order to study in greater detail the very earliest changes in the larvae after their arrival in a host, without sacrificing large experimental animals, I had attempted to introduce the larvae into rats and guinea pigs, partly along with food or drink …. These attempts never gave rise to a settlement of the larvae in the intestine of the experimental animals … [but the larvae] evidently remained alive for a long time. The subsequent history of these larvae was not investigated further, since in the meantime my attention was drawn in another direction. While engaged on one of the experiments, … a drop of the fluid fell on my left hand between the roots of two fingers. I paid no attention to this moisture which dried up of itself within a few minutes. At the

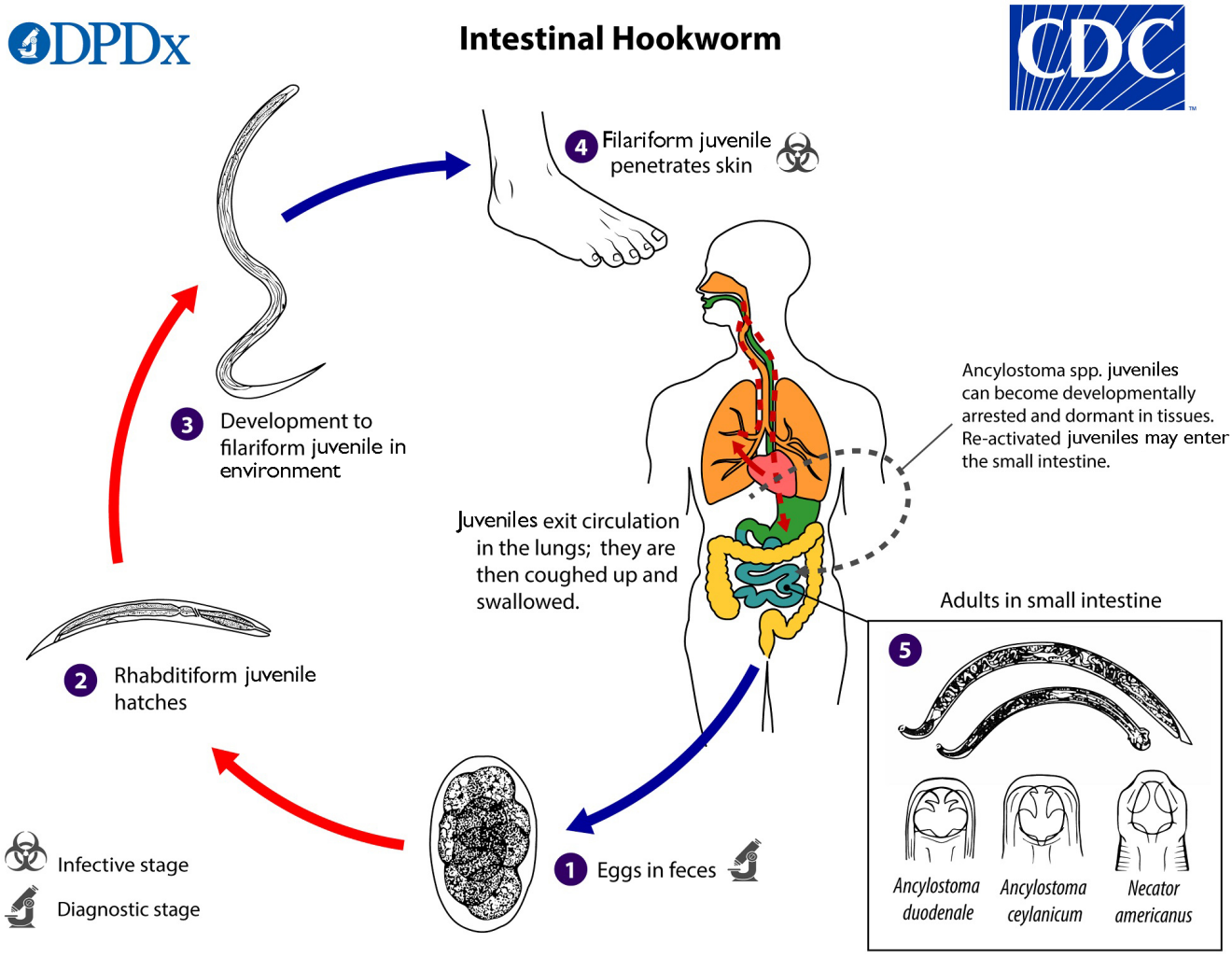

Figure 7. Hookworm (intestinal) causal agents and life cycle. Intestinal hookworm disease in humans is caused by *Ancylostoma duodenale*, *A. ceylanicum*, and *Necator americanus*. Classically, *A. duodenale* and *N. americanus* were considered the 2 primary intestinal hookworm species worldwide, but newer studies show that a parasite infecting animals, *A. ceylanicum*, is also an important emerging parasite infecting humans in some regions. Occasionally juveniles of *A. caninum*, normally a parasite of canids, may partially develop in the human intestine and cause eosinophilic enteritis, but this species does not appear to reach reproductive maturity in humans. Another group of hookworms infecting animals can penetrate the human skin causing cutaneous larva migrans (*A. braziliense*, *A. caninum*, *Uncinaria stenocephala*). Other than *A. caninum*, these parasites do not develop further after their juveniles penetrate human skin. Eggs are passed in the stool (1), and under favorable conditions (moisture, warmth, shade), juveniles hatch in 1 to 2 days and become free-living in contaminated soil. These released rhabditiform juveniles grow in the feces and/or the soil (2), and after 5 to 10 days (and 2 molts) they become filariform (third-stage) juveniles that are infective (3). These infective larvae can survive 3 to 4 weeks in favorable environmental conditions. On contact with the human host, typically bare feet, the juveniles penetrate the skin and are carried through the blood vessels to the heart and then to the lungs. They penetrate into the pulmonary alveoli, ascend the bronchial tree to the pharynx, and are swallowed (4). The juveniles reach the jejunum of the small intestine, where they reside and mature into adults. Adult worms live in the lumen of the small intestine, typically the distal jejunum, where they attach to the intestinal wall with resultant blood loss by the host (5). Most adult worms are eliminated in 1 to 2 years, but the longevity may reach several years. Some *A. duodenale* juveniles, following penetration of the host skin, can become dormant (hypobiosis in the intestine or muscle). These juveniles are capable of re-activating and establishing patent, intestinal infections. In addition, infection by *A. duodenale* may probably also occur by the oral and the transmammary route. *Ancylostoma ceylanicum* and *A. caninum* infections may also be acquired by oral ingestion. *Ancylostoma caninum*-associated eosinophilic enteritis is believed to result following oral ingestion of juveniles, not percutaneous infection. *Necator americanus* does not appear to be infective via the oral or transmammary route. Source: Adapted from United States Centers for Disease Control and Prevention, Division of Parasitic Disease and Malaria, 2019. Public domain.

same time, however, a burning sensation made itself felt on the spot, which grew more intense, while the skin became distinctly reddened. I am still of opinion that the most natural thing was to refer these symptoms to the *Ankylostoma* larvae, which … were present in the drop in great numbers. But the alternative existed that the active agent was either the water, in which the larvae had been kept (in this special case for a long time), and into which they might have discharged irritating products of excretion; or the irritating agency was the *Ankylostoma* larvae themselves. To test this, I let a drop of fluid without larvae fall on to another part of the hand and allowed it to dry. No reaction followed. Then a drop of fluid containing numerous larvae—as at first—was dropped in a third place on the back of the left hand, and was spread out with the handle of the scalpel so gently that the skin was only touched occasionally. Even before the fluid had quite dried up, the burning and reddening of the skin began exactly as before. It was thus clear that the irritating action proceeded from the larvae themselves. In order to see what had become of them I scraped the last remains of the fluid from the skin with the blade of the scalpel, using some pressure, and examined it under the microscope. The *Ankylostoma* larvae, previously so numerous, had disappeared, except for a few specimens; between the epithelial cells which had been scraped off innumerable empty skins were found, burst at the head end, and among them some half desiccated, still feebly motile larvae. The great majority had disappeared, and I saw no better explanation of this disappearance than the assumption that the larvae, casting their envelopes, had penetrated the skin and had thus produced the symptoms described. These symptoms, which were at first local, extended in the course of the next 24 hours over the whole hand, which also swelled considerably. The application of poultices of Goulard's water reduced the swelling in about 3 days, but it completely disappeared only after 6 days. … The fact that the mature *Ankylostoma* larvae not only possessed the power of actively penetrating into the uninjured skin of their host, but that they made energetic use of this power the moment they had an opportunity of doing so was so unusual—from the helminthological standpoint—that to regard it as a mere chance behaviour on the part of the larvae would have seemed to me simply absurd. Its true significance would not perhaps have suggested itself to me so rapidly, had I not become so strongly infected with the parasite in a manner up to that time wholly inexplicable. This infection was a fact; that it had not occurred through the mouth I also regarded as a fact; that the larvae could disappear in the uninjured skin I had just convinced myself on my own person with my own eyes; that this power of penetration into the uninjured skin was accidental and without further significance I considered as out of the question. But if the penetration of the larvae was the starting point of a second path by which they—no matter for the present in what manner—could reach the intestine of their definitive host, then indeed this phenomenon had not only a significance, as was to be expected from the outset, but my own enigmatical infection could be explained. For during my previous investigations I had certainly been careful to keep my hands away from the mouth, or to disinfect them according to the prescribed methods whenever there was the possibility of their coming into contact with the mouth (in eating, etc.); but I had thought nothing of allowing the water permeated with larvae to remain on my hands while manipulating the cultures and the material used for infection. Thus, the larvae had had an ample opportunity to affect an entry from the hands. That their penetration had never produced subjective symptoms is easy to understand; for in the first place the number of larvae entering simultaneously can, under the circumstances, never have been very large, and in the second place, even if a slight itching had been perceptible, there would have been no conceivable reason for seeking its cause in the penetration of *Ankylostoma* larvae into the skin. I may say without exaggeration that I have given earnest and prolonged consideration to all the points here enumerated before coming to a final decision; but in whatever way the facts in question were regarded they all conformed to one theory only, namely that the skin must be another starting point for the larvae from which they could reach the intestine and grow there to sexual maturity.

***Ancylostoma ceylanicum* Looss, 1911**

Ancylostoma ceylanicum was first recorded as a parasite of carnivores in Sri Lanka but is now known from people in Southeast Asia, the East Indies, and the Philippines. A morphologically similar species, *A. braziliense*, is considered to be cosmopolitan in the tropics and is found in domestic and wild carnivores. Although this species has been reported from humans in Brazil, Africa, India, Sri Lanka, Indonesia, and

the Philippines, the infections reported probably were from *A. ceylanicum. Ancylostoma braziliense* is the most common cause of cutaneous larva migrans (creeping eruption) in the southeastern United States and the tropics in the Western Hemisphere.

***Ancylostoma caninum* (Ercolani, 1859)**

Ancylostoma caninum is the most common hookworm of domestic dogs, especially in the Northern Hemisphere. It has been found in humans on at least 5 occasions, and the worm also is a common cause of cutaneous larva migrans (Figure 8). This hookworm is an important cause of eosinophilic enteritis (EE) in northeastern Australia and is now reported in the United States (Croese, 1998). EE causes abdominal pain with peripheral blood eosinophilia but with no eggs evident in the fecal examinations. Evidently the development to maturity of these nematodes in humans is inhibited, but the presence of even 1 immature worm can cause EE. *Ancylostoma caninum* juveniles have been isolated from human muscle and associated with muscle inflammation (Little et al., 1983). This species is also implicated in other pathology involving invasion of human tissues (Loukas et al., 2016).

Human Hookworm Disease

The distinction between hookworm infection and hookworm disease is important. Far more people are infected with hookworms than exhibit overt disease symptoms. The presence and severity of disease depend strongly on 3 factors: 1) Number of worms present, 2) species of hookworm, and 3) nutritional condition and immune status of the infected person. In general, fewer than 25 *Necator americanus* individuals in a person will cause no symptoms, 25 to 100 worms lead to light symptoms, 100 to 500 produce moderate symptoms and considerable damage, 500 to 1,000 result in severe symptoms and grave damage, and more than 1,000 worms cause very grave damage that may be fatal. Because *Ancylostoma duodenale* individuals suck more blood than *N. americanus* ones, fewer *A. duodenale* worms can cause greater disease; for example, 100 *A. duodenale* worms may cause severe symptoms. However, the clinical disease is intensified by nutritional condition, impairment of host's immune response, and other factors.

The human immune response to hookworm infection is complex, but it is clear that hookworms have evolved to modulate the host's defense system. Survival of hookworms appears to depend upon a balance between host immune responses that ultimately protect the parasite. When attached to the host's mucosa, mature hookworms seem to be protected from the host's immune response. In contrast to established adults, newly recruited juvenile worms appear to cause a strong eosinophilic response that expels them from the small intestine (Croese and Speare, 2006).

In addition, several potential mechanisms for evading the host's defense systems have been discovered. For example, *Ancylostoma* spp. secrete a neutrophil inhibition factor that interferes with activation of neutrophils (Pritchard, 1995). *Necator americanus* directly secretes acetyl cholinesterase, which can inhibit or decrease peristaltic movement of the intestine and possibly acts also as an anti-inflammatory factor. It also secretes glutathione-S-transferase and superoxide dismutase, substances that interfere with antibody-dependent, cell-mediated cytotoxicity (ADCC). Nine genes in *N. americanus* code for proteins similar to neutrophil inhibitory factor (Daub et al., 2000). The details involved in the possible immunomodulation by hookworms is not established definitively and is a hotly debated topic (Mortimer et al., 2006).

Epidemiology

A combination of poor sanitation and conducive environmental conditions is necessary for high endemicity of hookworm in people. The disease is restricted to warmer parts of the world (and to specialized habitats, such as mines in more severe climates) because juveniles will not develop to maturity at less than 17 °C, with 23–30 °C being optimal. Freezing temperatures kill eggs and juveniles. Oxygen is necessary for hatching of eggs and juvenile development because their metabolism is aerobic. Thus, juveniles will not develop in undiluted feces or in waterlogged soil. Therefore, soil that is loose with lots of humus and has reasonable drainage and aeration is favorable to the development and survival of juveniles. Both heavy clay and coarse sandy soils are unfavorable for the parasite, the latter because juveniles are also sensitive to desiccation. Alternate drying and moistening are particularly damaging to juveniles; hence, very sandy soils become noninfective after brief periods of frequent rainfall. However, juveniles live in the film of water surrounding soil particles, and even apparently dry soil may have enough moisture to enable survival, particularly below the surface.

Juveniles are quite sensitive to direct sunlight and survive best in shady locations, such as coffee, banana, or sugarcane plantations. Humans working on such plantations often have preferred defecation sites, not out in the open where juveniles would be killed by sun, of course, but in shady, cool, secluded spots beneficial for juvenile development. Repeated return of people to a defecation site exposes them to continual reinfection. Furthermore, use of preferred defecation sites makes it possible for hookworms to become endemic in otherwise quite arid areas. A higher average number of worms per individual will seed the soil with more eggs, so human defeca-

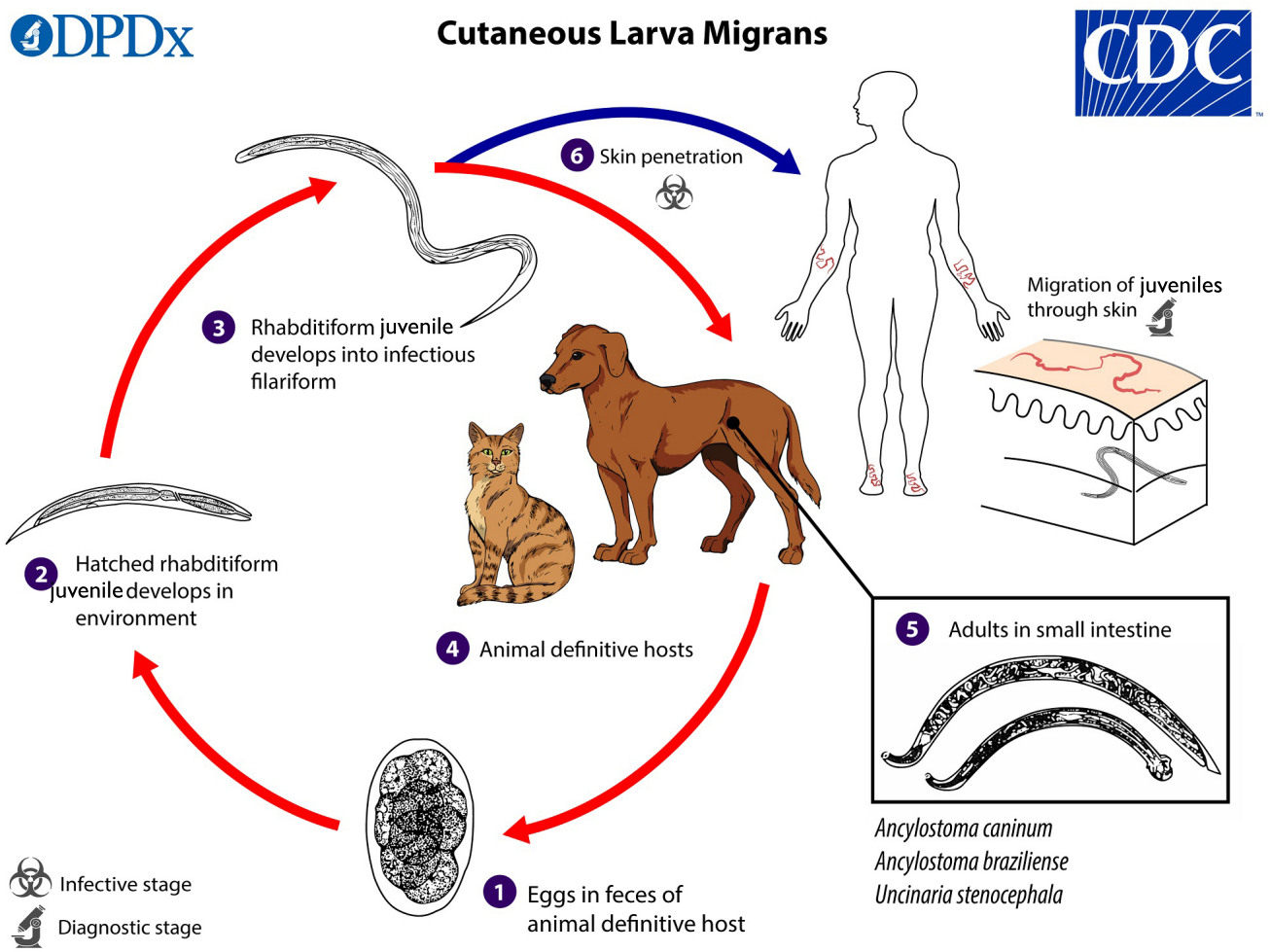

Figure 8. Zoonotic hookworm (extraintestinal) causal agents and life cycle. Some zoonotic hookworm species are capable of infecting humans, but they typically do not develop in the intestine and instead infect extraintestinal sites like the skin. Cutaneous larva migrans (CLM) has been associated with *Ancylostoma caninum*, *A. braziliense*, and *Uncinaria stenocephala*, which are all hookworms of dogs and cats. *Bunostomum phlebotomum*, a cattle hookworm, is also capable of causing short-lived CLM in humans. Cutaneous larva migrans (also known as creeping eruption) is a zoonotic infection with hookworm species that do not use humans as a definitive host, the most common being *A. braziliense* and *A. caninum*. The cycle in the definitive host is very similar to the cycle for the human species, which involves tracheal migration to the small intestine. Some juveniles become arrested in the tissues and serve as the source of infection for pups via transmammary (and possibly transplacental) routes. Mature hookworms reproduce in the small intestine, and eggs are passed in the animal definitive host's stool (1), and under favorable conditions (moisture, warmth, shade), juveniles hatch in 1 to 2 days. The released rhabditiform juveniles grow in the feces and/or the soil (2), and after 5 to 10 days (and 2 molts) they become filariform (third-stage) juveniles that are infective (3). These infective juveniles can survive 3 to 4 weeks in favorable environmental conditions. On contact with the animal host (4), the juveniles penetrate the skin and are carried through the blood vessels to the heart and then to the lungs. They penetrate into the pulmonary alveoli, ascend the bronchial tree to the pharynx, and are swallowed. The juveniles reach the small intestine, where they reside and mature into adults. Adult worms live in the lumen of the small intestine, where they attach to the intestinal wall. Some juveniles become arrested in the tissues and serve as source of infection for pups via transmammary (and possibly transplacental) routes (6). Humans become infected when filariform juveniles penetrate the skin (7). With most species, the juveniles cannot mature further in the human host and migrate aimlessly within the epidermis, sometimes as much as several centimeters a day. Some juveniles may become arrested in deeper tissue after skin migration. Source: Adapted from United States Centers for Disease Control and Prevention, Division of Parasitic Disease and Malaria, 2019. Public domain.

tion on open ground keeps soil contamination high. Use of nightsoil as fertilizer for crops is an especially important factor in parts of East Asia.

Juveniles develop best in near-neutral pH, and acid or alkaline soils inhibit development, as does the acid pH of undiluted feces (pH 4.8 to 5.0). Chemical factors also have an influence. Urine mixed with feces is fatal to eggs, and several strong chemicals that may be added to feces as disinfectants or fertilizers are lethal to free living stages. Salt in the water or soil inhibits hatching and is fatal to juveniles.

Because worms penetrate the epithelial tissues of the host, a habit of going barefoot in tropical countries is an elemental contribution to transmission. The role of skin penetration presumably accounts for a general lack of high correlation of hookworm with *Ascaris lumbricoides* and *Trichuris trichiura* infections, which must be acquired by ingestion (Booth and Bundy, 1992). However, higher egg counts have been reported in instances of hookworm coinfection with *Ascaris lumbricoides*, suggesting a possible synergistic effect (Fleming et al., 2006). This finding may have important implications for control strategies.

Longevity of the worms is important in transmission to new hosts, continuity of infection in a locality, and introduction to new areas. Juveniles can survive in reasonably good environmental conditions for about 3 weeks; in protected sites like mines, they can last for a year. There is some dispute about the life span of adults, but a good estimate is 5 to 15 years. A person who moves from an endemic area loses the infection in about that time. Specifically, *Necator americanus* has been recorded to live up to 15 years and species of *Ancylostoma* have been reported to live from 12 months to 5 years (Nawalinski et al., 1978a; 1978b; Behnke, 1987).

Schad and colleagues (1984) discovered that juveniles will survive in muscles of paratenic hosts. Thus, *Ancylostoma duodenale* can be transmitted through ingestion of undercooked meat, including rabbit, lamb, beef, and pork. Pigs can also serve as transport hosts for *Necator americanus* (Steenhard et al., 2000). Similarly, dogs can be infected with the canid hookworm, *A. caninum*, by ingestion of juveniles in mice, cockroaches, and possibly other paratenic hosts that might be consumed through predation.

Pathogenicity

In addition to causing iron deficiency anemia involving the direct loss of blood from the mucosa of the intestine by the feeding action of adult nematodes, human hookworm disease manifests 3 main phases of pathogenicity: 1) The cutaneous phase or invasion period, 2) pulmonary phase, and 3) intestinal phase. When a juvenile enters an unsuitable host, after the cutaneous phase or invasion period, it may result in cutaneous larva migrans, after which the pathogenic action of the worm is halted. Cutaneous larva migrans is included in the discussion in the section on migration phase or invasion period, below.

Cutaneous phase or invasion period

The cutaneous phase or invasion period begins when juveniles penetrate the host's epithelial tissue. They do little damage to superficial layers, since they seem to slip through tiny cracks between skin scales, or penetrate sweat pores or hair follicles. Juveniles are stimulated to penetrate by host fatty acids and they must remain in a water film for successful penetration (Haas et al., 2005).

Cutaneous larva migrans may occur at this point. See the discussion of this phase, below. At this stage, infection with pyogenic bacteria may result from the nematodes penetrating into the skin and bringing bacteria with them from their previous fecally-laced habitat, causing an urticarial reaction and dermatitis, a condition known as ground itch.

Proteases released by J_3s cause an increase in cellular permeability and disruption of vascular endothelial cell junctions (Williamson et al., 2003). Once in the dermis, however, their attack on blood vessels initiates a tissue reaction that may isolate and kill the worms.

Pulmonary phase

After the cutaneous phase, or invasion period, the worms enter the hepatic portal system, migrate to the liver, then go into the heart, and then to the lungs. More specifically, the pulmonary phase occurs when juveniles break out of the lung capillary bed into alveoli and progress up the bronchi to the throat. Juveniles migrating through the liver, heart, and lungs may cause inflammation in the lungs termed pulmonary pneumonitis (Hotez et al., 2004). Small hemorrhages occur in the alveoli, and in some cases juveniles induce eosinophilic pneumonia, or Loeffler's syndrome. The pulmonary phase is usually asymptomatic, although there may be some dry coughing and sore throat.

Intestinal phase

The intestinal phase is the most important period of pathogenicity. On reaching the small intestine, young worms attach to the mucosa with their strong buccal capsule and teeth, and they begin to feed on blood. Initiation of this phase is accompanied by painful eosinophilic enteritis (see also the discussion about this phase of infection by Looss; 1911). In heavy infections, worms are found from the pyloric stomach to the ascending colon, but usually they are restricted to the anterior third of the small intestine. Worms move from

place to place, and blood loss is exacerbated by bleeding at sites of former attachment (Gilman, 2000). Hookworms produce proteins that inhibit host blood clotting factors (Gan et al., 2009), and these molecules may contribute to bleeding at former feeding sites. Ironically, such anticoagulants may have beneficial medical applications (Friedly, 1996). Worms pass substantially more blood through their digestive tracts than would appear necessary for their nutrition alone, but the reason for this is unknown. Blood loss per worm is about 0.03 ml per day for *Necator americanus* and around 0.26 ml per day for *Ancylostoma duodenale.*

Migration phase

After the cutaneous phase, or invasion period, cutaneous larva migrans (also termed creeping eruption) may occur. It is usually caused by invasive juvenile hookworms of species normally maturing in animals other than humans (however, ground itch and larva migrans may also occur with the normal human hookworms). In cases of cutaneous larva migrans, juveniles manage to penetrate the skin of humans, and although they may migrate into and through the stratum germanitivum, they are incapable of successfully completing migration to the intestine. Before they are overcome by immune effectors, they produce distressing, but rarely serious, complications of the skin.

After entering the top layers of epithelium, juveniles are usually incapable of penetrating the basal layer (stratum germinativum), so they begin an aimless wandering. As they tunnel through skin, they leave a red, itchy wound that usually becomes infected by pyogenic bacteria. Juveniles may live for weeks or months. It is known that some can enter muscle fibers and become dormant (Little et al., 1983). Juveniles can attack skin anywhere on the body, but people's feet and hands are more in contact with the ground and so are most often affected. Thiabendazole is used as a treatment for cutaneous larva migrans.

Species of hookworms from cats, dogs, and other domestic animals are likely to come into contact with people. *Ancylostoma braziliense*, a common hookworm of dogs and cats, appears to be the most common agent throughout its geographic range (Schad, 1994). Travelers from temperate regions who acquire this infection by visiting tropical beaches may encounter difficulty obtaining a correct diagnosis and medication upon returning home (Tremblay et al., 2000).

Severe Infections

Patients with severe infections may lose up to 200 ml of blood per day, but around 40% of the afflicted person's iron may be reabsorbed before it leaves the intestine (Layrisse et al., 1961). Nevertheless, a moderate hookworm infection will gradually produce iron-deficiency anemia as body reserves of iron are used up. Severity of anemia depends on worm load and dietary iron intake of a patient. Anemia during pregnancy can cause serious complications, putting both mother and child at risk. In hookworm endemic regions, iron deficiency resulting from hookworm infection during pregnancy is common (Baidoo et al., 2010). Slight, intermittent abdominal pain, loss of normal appetite, and desire to eat soil (geophagy) are common symptoms of moderate hookworm disease. Certain areas in the southern United States became locally famous for the quality of their clay soil, and people traveled for miles to eat it. In the early 1920s, an enterprising person began a mail-order business, shipping clay to hookworm sufferers throughout the country!

In very severe infections, patients suffer severe protein deficiency, with dry skin and hair, edema, and potbelly in children and with delayed puberty, mental disability, heart failure, and even death. Intestinal malabsorption is not a marked feature of infection with hookworms, but hookworm disease is usually manifested in the presence of malnutrition and is often complicated by infection with other worms and/or malaria.

The drain of protein and iron is catastrophic to a person subsisting on a minimal diet. In addition, the staple foods of some countries, such as cassava, rice, and corn, are poor sources of iron. Chronic malnutrition, particularly in the young, often results in stunted growth and intellectual disability, but treatment for the worms can significantly increase fitness, appetite, and growth (Latham et al., 1990; Stephenson et al., 1989). Impairment in ability to produce IgG results in lowered antibody response to hookworms as well as to other infectious agents.

Diagnosis

Demonstration of hookworm eggs or worms themselves in feces is, as usual for gut parasites, is the only definitive diagnosis of the disease. Demonstration of eggs in direct smears may be difficult, however, even in clinical cases, and one of the several concentration techniques should be used. If estimation of worm burden is necessary, techniques are available that give reliable data on egg counts (Cross, 2000). It is not possible to distinguish *Ancylostoma duodenale* eggs from those of *Oesophagostomum bifurcum* or *Ternidens deminutus*, and this is important in the areas of Africa where *O. bifurcum* and *T. deminutus* are widely prevalent in humans. PCR methods have been described for these identifications, and a multiplex real-time PCR test based on DNA from a 200-µl fecal sample can diagnose species in mixed infections and provide quantitative results that correlate with egg counts (Verweij et al., 2001). However, implementing ad-

vanced molecular diagnostics for routine testing is sometimes not practical (Schindler et al., 2005; Verweij et al., 2001; De Gruijter et al., 2005).

To rid a person of hookworm infection it is neither necessary nor possible to distinguish *Necator americanus* eggs from those of *Ancylostoma* spp., but care should be taken to differentiate *Strongyloides stercoralis* infections. This is not a problem unless some hours pass between time of defecation and time of examination of feces. Then hookworm eggs may have hatched, and juveniles of *Ancylostoma* spp. must be distinguished from those of *S. stercoralis*.

However, it is necessary to be able to distinguish *Necator americanus* and *Ancylostoma* spp. in studies on the efficacy of various drugs or chemotherapeutic regimens because the 2 species are not equally sensitive to particular drugs: *N. americanus* has low sensitivity to ivermectin, in contrast with *Ancylostoma* spp. (Richards et al., 1995). Differentiation can be accomplished by recovery of adults after anthelmintic treatment, culturing juveniles from feces, or molecular identification based on single eggs.

Treatment

Mebendazole or albendazole are commonly used for treatment, as they kill all nematodes. Single-dose therapy is inexpensive and convenient, but reports of drug failure and decreased efficacy for mebendazole suggest that albendazole later emerged the drug of choice (Albonico et al., 2003; Hotez et al., 2010). There is also evidence that populations of *Necator americanus* are becoming resistant to mebendazole in Africa (De Clercq et al., 1997) and *Ancylostoma caninum* shows evidence of resistance to the anthelminthic pyrantel pamoate (Kopp et al., 2007). It has also been found that routine treatment of pregnant women in areas of high hookworm prevalence significantly decreases incidence of infants with very low birthweight (Larocque et al., 2006).

Treatment for hookworm disease should always include dietary supplementation. In many cases, provision of an adequate diet alleviates symptoms of the disease without worm removal.

Control

Control of hookworm disease depends on lowering worm burdens in a population to an extent that remaining worms, if any, can be sustained within nutritional limitations of people without causing symptoms. Mass treatment campaigns do not eradicate the worms but certainly lower the so-called seeding capacity of their hosts. Education and persuasion of a population in sanitary disposal of feces are also vital. Economic dependence on nightsoil in family gardens remains one of the most persistent of all problems in medical parasitology.

Recognizing these factors, the American zoologist Charles W. Stiles persuaded John D. Rockefeller to donate $1 million in 1909 to establish the Rockefeller Sanitary Commission for the Eradication of Hookworm Disease (Ackert, 1952). (The activities of the commission eventually led to the formation of the Rockefeller Foundation and then Rockefeller University.) Beginning state by state and then extending throughout the southeastern United States, the Commission would first survey an area. Residents of the area were examined for infection and then treated with anthelmintics. Thousands of latrines were provided with instructions on how to use and maintain them. As a result of efforts of this and other similar hygiene commissions, hookworm prevalence is now much lower in some areas of the world. Nevertheless, worldwide prevalence of hookworms is still high; between one-fifteenth and one-tenth of the Earth's human population remains infected (Chan, 1997; Hotez et al., 2010; CDC, 2023).

New molecular methods and technologies hold much promise for advances in understanding hookworm biology and implementing control measures. For example, the transcriptome of *Necator americanus* adults has been analyzed (Cantacessi et al., 2010) revealing 18 potential drug targets that lack homologues in the human genome. By inference, this means that drugs can be applied to a human population to rid the worms from humans while relatively no effect is seen on the host itself. Rapid and specific molecular diagnostic methods that clearly differentiate among different species of hookworms are needed in order to begin to achieve effective control (Clements and Alene, 2022). Deep sequencing of the genome of *N. americanus* has recently been carried out in order to identify potential drug resistant markers. Other newer methods of molecular biology are now being implemented in the ongoing battle against hookworm disease (George et al., 2022).

Family Strongylidae Baird, 1853

Family Strongylidae currently contains 1,126 species in 4 subfamilies (see Hodda, 2022).

Members of Strongylidae Baird, 1853 occur in a variety of mammals, especially herbivores such as horses, in which they are a serious veterinary problem. They are commonly recognized as large strongyles (several species of *Strongylus*, of which *S. vulgaris* is the most important) and small strongyles (mostly the numerous species of *Cyathostomum*) (Herd, 1990). Adults of both are found in the large intestine of equines. Eggs pass out in feces, hatch as J_1s, and develop in soil into infective J_3s; the latter retain the cuticle of the J_2 as a close-fitting sheath. These crawl onto vegetation and are eaten by grazing hosts. All undergo a migration and period of development in various tissues, the details of which vary with species.

Developing juveniles of *Strongylus vulgaris* migrate into the arteries of the host, especially the anterior mesenteric artery, where they cause thrombosis and arteritis. After 3 to 4 months in the arteries, young adults migrate to the intestine where they eventually enter the lumen and reach maturity.

In the past, the arterial stages of *Strongylus vulgaris* were shown to be present in 90% to 100% in horses in the United States, and it was the most feared equine parasite (Herd, 1990). *Strongylus vulgaris* remains sensitive to benzimidazole and ivermectin anthelmintics, but cyathostomes are relatively resistant to these drugs. As a result, *S. vulgaris* has almost been eradicated, and small strongyles such as *Cyathostomum* spp. are instead a much bigger problem with horse owners. To aid in diagnosis, a quantitative real-time PCR test has been developed for *S. vulgaris* (Nielsen et al., 2008).

Oesophagostomum spp. are parasites of primates, rodents, ruminants, and pigs. They are called nodular worms because developing juveniles form nodules in the walls of both the small and large intestines of the host. Adults live in the large intestine. Infections are normally acquired by ingestion of third-stage juveniles (J_3s). Infections in humans are generally considered to be accidentally caused by the zoonotic species of this genus. However, *O. bifurcum* has a high prevalence in humans and nonhuman primates in one small area in Africa (northern Togo and Ghana). Additionally, individuals with infection by species of hookworms have a higher likelihood of also being infected with *O. bifurcum* (Ziem et al., 2006). Infection of humans by these species of nematode typically shows up as a painful abdominal mass that sometimes requires surgical intervention. Eggs of *O. bifurcum* are indistinguishable morphologically from hookworm, but J_3s obtained after fecal culture show clear differences. Although morphologically indistinguishable, *O. bifurcum* from humans and 3 nonhuman primate hosts show relatively high levels of genetic divergence. This observation is consistent with low levels of gene flow between these host-associated populations (Gasser et al., 2006).

Syngamus trachea is the gapeworm of poultry and is called this because adults live in the trachea of their galliform hosts causing the host to gasp and gape with the mouth wide open. The fowl coughs up eggs, swallows them, and then passes them in feces. Juveniles molt twice in the egg to become infective J_3s. Eggs may or may not hatch in soil, and a variety of terrestrial molluscs, earthworms, and arthropods can serve as paratenic hosts.

Syngamus trachea individuals can survive several years in earthworms, and numerous wild bird species serve as reservoirs. Definitive hosts become infected when they swallow embryonated eggs or juveniles. Infective juveniles penetrate the gut wall, are carried by blood to the lungs where they break out into alveoli, and then proceed up to the trachea. At this stage, males remain attached to a female via their copulatory bursa. Young birds are most severely affected and may die with a heavy infection.

Superfamily Trichostrongyloidea Cram, 1927

The superfamily Trichostrongyloidea Cram, 1927 constitutes one of the most diverse and complex taxa within the bursate nematodes (Durette-Desset, 1985; 2009; Hoberg and Lichtenfels, 1994). The group includes more than 1,000 described species approximately 175 genera. These worms have a worldwide distribution and direct life cycle. They occur in the gut and sometimes in the stomach of almost all classes of terrestrial vertebrates (Durette-Desset, 1992).

Some of the species of medical and veterinary importance include *Haemonchus contortus*, *Ostertagia* spp., and *Trichostrongylus* spp., which are discussed briefly below.

Figure 9. Posterior end of a specimen of a trichostrongyloid nematode *Obeliscoides cuniculi*, the stomach nematode of rabbits and hares in North America. This specimen was collected from the stomach of an individual of *Sylvilagus* sp. north of Ogallala, Nebraska, United States. The contracted bursa of this male is visible with small bosses covering the cuticle. Two similar spicules are visible lying parallel which are easily seen in this Normarsky micrograph (NP2380). Source: G. Drabik and S. L. Gardner, HWML, 2018. License: CC BY 4.0.

General Morphology

Trichostrongyloids are usually small, very slender worms, with a small, non-developed **stoma**. Lips around the **mouth** are very reduced or absent, and cuticularized teeth or spines in the stoma are rarely present. The **cuticle** of the **head** may be inflated and some of them are filled with fluid containing hemoglobin that is not host-derived and may be pink when the nematodes are collected alive (Figure 9). Males have a well-developed **copulatory bursa**, and **spicules** vary from simple setaceous to extremely complex falcate or modified hamate in form (see Maggenti, 1981), depending on species and group. Females are usually considerably larger than males. The **vulva** is located anywhere from before the midbody to near the **anus**, depending on the species and group (Figure 10). Worms lay thin-shelled eggs that are in the morula stage (Durrette-Desset et al., 1999).

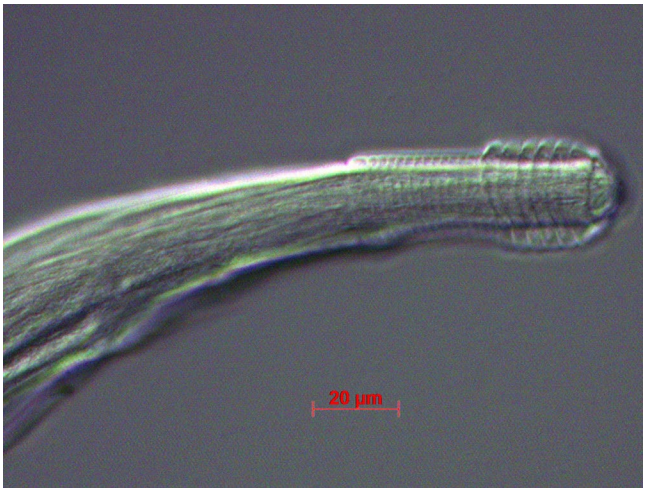

Figure 10. Anterior end of the specimen depicted in Figure 9, a trichostrongyloid nematode *Obeliscoides cuniculi*, the stomach nematode of rabbits and hares in North America. Source: G. Drabik and S. L. Gardner, HWML, 2018. License: CC BY 4.0.

Life Cycles

Life cycles are similar in all species of trichostrongyloid nematodes. For those that have been studied, no intermediate host is required; eggs hatch in soil or water and develop directly into infective J_3s. Some infections may occur through skin, but as a rule juveniles must be swallowed with contaminated food or water. Many trichostrongyloids undergo exsheathment, where J_3s escape the J_2 cuticle during initial infection. The host stimuli that induce production of exsheathing fluid by the J_3 has been extensively investigated. Enormous numbers of juveniles may accumulate on heavily grazed pastures, causing serious or even fatal infections in ruminants and other grazers. A given host usually is infected with several species since their life cycles are similar, and severe pathogenesis results from the cumulative effects of all the worms. Cost to the sheep industry in Australia, for example, is high (McLeod, 1995).

Following is a brief discussion of the families **Trichostrongylidae, Dictyocaulidae**, **Angiostrongylidae**, and **Protostrongylidae**, as well as a few noteworthy species.

Family Trichostrongylidae Leiper, 1912

Many genera and an enormous number of species comprise the family Trichostrongylidae (Durrette-Desset et al., 1999; Hoberg and Lichtenfels, 1994). They are primarily parasites of the stomach or small intestine of all classes of vertebrates, causing great economic losses in domestic animals, especially ruminants, and in a few cases causing disease in humans.

Haemonchus contortus

Haemonchus contortus lives in the so-called fourth stomach (or abomasum) of sheep, cattle, goats, and many wild ruminants. The species has been reported in humans in Brazil and Australia. It is one of the most important nematodes of domestic animals, causing severe anemia in heavy infections (Flach, 2008).

The small stoma contains a single well-developed tooth that pierces a host's mucosa (Emery et al, 2016). The blood this species sucks from this wound gives the transparent worms a reddish color. The large females have white ovaries wrapped around the red intestine, lending it a characteristic red and white appearance and leading to its common names: Twisted stomach worm and barber-pole worm. Prominent cervical papillae are found near the anterior end. The male's bursa is powerfully developed with an asymmetrical dorsal ray. Spicules are 450 µm- to 500 µm-long, each with a terminal barb. The vulva has a conspicuous anterior flap in many individuals but not in all. Frequency of occurrence of the vulvar flap seems to vary according to strain.

Infection occurs when livestock eat forage containing J_3s, which are sheathed in the loosely fitting second-stage cuticle. Exsheathment takes place in the rumen or reticulum of the host animal. Arriving in the abomasum or upper duodenum, worms molt within 48 hours, becoming J_4s with a small buccal capsule having formed. They feed on blood, which forms a clot around the anterior end of the worms. The worms molt for a final time in 3 days and begin egg production about 15 days later. Fourth-stage juveniles can undergo developmental arrest, typically in fall, with maturation to adults occurring in spring. Arrest is considered a mechanism promoting survival and transmission in temperate climates, leading to the spring rise in eggs passed in feces of sheep (Emery et al., 2016).

Anemia, emaciation, edema, and intestinal disturbances caused by these parasites result principally from loss of blood and injection of hemolytic proteins into the host's system. A host often dies with heavy infections, but those that survive usually develop immunity due to specific inflammatory responses in the intestinal mucosa.

Ostertagia Species

Ostertagia spp. are similar to *Haemonchus contortus* in host and location, but they differ in color, being a dirty brown—hence, their common name, brown stomach worm. The buccal capsule is rudimentary and lacks a tooth. Cervical papillae are present. The male bursa is symmetrical. The vulva has a large anterior flap, and the tip of the female's tail bears several cuticular rings.

Their life cycle is similar to that of *Haemonchus contortus* except that J_3s invade gastric glands and elicit nodules. J_3s molt before returning to the lumen, where they feed, molt, and begin producing eggs about 17 days after infection. *Ostertagia* spp. suck blood but not as much as *H. contortus*. Species of *Ostertagia* often undergo developmental arrest as J_4.

Some common species of *Ostertagia* are *O. circumcincta* in sheep, *O. ostertagi* in cattle and sheep, and *O. trifurcata* in sheep and goats. Economic losses in the cattle industry due to *O. ostertagi* and other nematodes probably exceed $600 million per year in the United States alone (Smith and Granfell, 1985).

Trichostrongylus Species

Trichostrongylus spp. are some of the smallest members of the superfamily, seldom exceeding 7 mm in length. Many species parasitize the small intestine of ruminants, rodents, pigs, horses, birds, and humans. They are colorless, lack cervical papillae, and have a rudimentary, unarmed stoma. The male's bursa is symmetrical, with a poorly developed dorsal lobe. Spicules are brown and distinctive in size and shape in each species. The vulva lacks an anterior flap (Anderson, 2000).

Their life cycle is similar to that of *Haemonchus* spp. except that J_3s burrow into mucosa of the anterior small intestine, where they molt. After returning to the lumen, they bury their heads in mucosa and feed, grow, and molt for the last time. Egg production begins about 17 days after infection.

Common species of *Trichostrongylus* are *T. colubriformis* in sheep, goats, cattle, and deer; *T. tenuis* in galliform birds such as grouse, pheasant, chickens, and turkeys; *T. capricola, T. falcatus,* and *T. rugatus* in ruminants; *T. retortaeformis* and *T. calcaratus* in rabbits; and *T. axei* in a wide variety of mammals. Hudson and colleagues (1998) showed that the periodic crashes in populations of British red grouse (*Lagopus lagopus scoticus*) were due to negative impact on fecundity caused by build-up of *T. tenuis* (Cattadori et al., 2005; Hudson et al., 1998).

Approximately 10 species of *Trichostrongylus* have been reported in humans, with records from nearly every country of the world. There are 9 species in Iran alone (Pearson and Schwartzman, 1991). Reported prevalence has varied from very low to as high as 69% in southwest Iran (Sabha et al., 1967) and 70% in a village in Egypt (Lawless et al., 1956).

Pathological conditions are identical in humans and other infected animals. Traumatic damage to intestinal epithelium may be produced by burrowing juveniles and feeding adults. Systemic poisoning by metabolic wastes of the parasites and hemorrhage, emaciation, and mild anemia may develop in severe infections.

Diagnosis can be made by finding characteristic eggs in feces or by culturing juveniles in powdered charcoal. Juveniles are very similar to those of hookworms and *Strongyloides* spp., and careful differential diagnosis is required. Molecular diagnostics are available for the common trichostrongylid species from ruminants (Sweeny et al., 2011).

Treatment and Drug Resistance

Treatment with thiabendazole or with pyrantel pamoate has proven effective. Cooking vegetables adequately will prevent many infections in humans. However, drug resistance in nematodes of livestock has been reported for every class of anthelminthic, and multidrug resistance (MDR) was reported in worms of sheep and goats in the 1980s (Kaplan, 2004; Shoop, 1993). MDR in trichostrongylids infecting small ruminants threatens production throughout the world, but particularly in South America, South Africa, Malaysia, and the United States. Resistance by trichostrongyles to benzimidazole drugs (for example, albendazole, mebendazole, and thiabendazole) is increasing and quite ominous (Conder and Campbell, 1995; Geerts et al., 1997).

Family Dictyocaulidae

Species in this genus are medium-sized nematodes that as adults parasitize the bronchi and trachea and are associated with bronchitis in their hosts. *Dictyocaulus filaria* is an important parasite of sheep and goats, but also infects wild antelope and deer. Adults live in bronchi and bronchioles, where females produce embryonated eggs. Eggs hatch while being carried toward the trachea by ciliary action. First-stage juveniles appear in feces and develop to J_3s in contaminated soil without feeding. Cuticles of both first and second stages are retained by the third stage until the worm is eaten by a definitive host; then cuticles of all these stages are shed together. J_3s penetrate the mucosa of the small intestine and

enter mesenteric lymph nodes. There they undergo 2 molts to become small adults (about 500 µm-long), enter the circulation by way of the thoracic duct, and parasitize the trachea and bronchi. They commonly cause death of their host (Anderson, 2000).

Fully-grown adults are slender and long, with males reaching 80 mm and females 100 mm. The bursa is small and symmetrical; spicules are short and boot-shaped in lateral view. The uterus is near the middle of the body. Other species in horses and cattle are similar to *Dictyocaulus filaria* in morphology and biology.

Family Angiostrongylidae

One of the main pathogenic organisms in the family Angiostrongylidae is *Angiostrongylus cantonensis*, also known as the rat lungworm, detailed below. Other worms in the family are also covered briefly.

Rat Lungworm: *Angiostrongylus cantonensis*

Angiostrongylus cantonensis was first discovered in pulmonary arteries and the heart of domestic rats in China in 1935. Later the worm was found in many species of rats and bandicoots, and it may mature in other mammals throughout Southeast Asia, the East Indies, Madagascar, and Oceania, with infection rates as high as 88%. As a parasite of rats, it attracted little attention, but 10 years after its initial discovery it was found in the spinal fluid of a 15-year-old boy in Taiwan. It has been discovered since in humans in Hawaii, Tahiti, the Marshall Islands, New Caledonia, Thailand, Vanuatu, the Loyalty Islands, and other places in the Eastern Hemisphere. It is now known to exist in Louisiana (United States), the West Indies, and the Bahamas (Raccurt et al., 2003).

This is another illustration of the value of basic research in parasitology to medicine, because when the medical importance of this parasite was realized, the reservoir of infection in rats already was known. Surveys of parasites endemic to wild fauna of the world remain the first step in understanding epidemiology of zoonotic diseases.

Morphology

Angiostrongylus cantonensis is a delicate, slender worm with a simple mouth and no lips or stoma. Males are 15.5 mm- to 25 mm-long, whereas females attain lengths of 19 mm to 34 mm. The bursa is small and lacks a dorsal lobe. Spicules are long, slender, and about equal in length and form. An inconspicuous gubernaculum is present. In females the intertwining of intestine and uterine tubules gives the worm a conspicuous barber-pole appearance. The vulva is about 0.2 mm in front of the anus. Eggs are thin-shelled and unembryonated when laid. Eggs are not produced in human infections.

Life Cycle

Eggs are laid in the pulmonary arteries, carried to capillaries, and break into air spaces, where they hatch. Juveniles migrate up the trachea, are swallowed, and are expelled with feces.

Many types of molluscs serve as intermediate hosts, including slugs and aquatic and terrestrial snails. Terrestrial planarians, freshwater shrimp, land crabs, and coconut crabs serve as paratenic hosts. Frogs have been found naturally infected with infective juveniles (Ash, 1968). Experimentally, Cheng (1965) infected American oysters and clams, and Wallace and Rosen (1966) succeeded in infecting crabs. All juveniles thus produced were infective to rats.

When eaten by a definitive host, J_3s undergo an obligatory migration to the brain, which they leave 4 weeks later as subadults. In rats, the time from infection to egg appearance in feces is about 6 weeks.

Epidemiology

Humans or other mammals become infected when they ingest J_3s. There may be several avenues of human infection, depending on the food habits of particular groups of people (Alicata, 1991; Cross, 1987). In Tahiti it is a common practice to catch and eat freshwater shrimp raw or to make sauce out of their raw juices. It is also possible to eat slugs or snails accidentally with raw vegetables or fruit. In Thailand and Taiwan, raw snails are often considered a delicacy. Infective juveniles escape from slugs and can be left behind in their mucus trail on vegetables over which they crawl (Heyneman and Lim, 1967; Ming et al., 2017). Such juveniles have been found on lettuce sold in a public market in Malaysia. Fish can serve as paratenic hosts in some circumstances. Thus, although the epidemiology of angiostrongyliasis is not completely known, ample opportunities for infection exist.

Pathology

For many years a disease of unknown cause was recognized in tropical Pacific islands and was named eosinophilic meningoencephalitis. Patients with this condition have high eosinophil counts in peripheral blood and spinal fluid in about 75% of cases and increased lymphocytes in cerebrospinal fluid. Neural disorders commonly accompany these symptoms, particularly cranial nerve involvement. It is now known that *Angiostrongylus cantonensis* is at least one cause of this condition.

The presence of worms in blood vessels of the brain and meninges, as well as that of free-wandering worms in brain tissue, or subdural and subarachnoid spaces, results in serious damage. Some effects of such infection are severe headache, fever in some cases, muscle paralysis and speech im-

pairment, stiff neck, coma, and death. The clinical symptoms mimic migraine, brain tumor, and psychoneurosis. In nonsusceptible hosts such as mice and guinea pigs, interleukin-5 activates eosinophils that kill the worms (Sugaya et al., 1997).

Diagnosis and Treatment

When the symptoms described appear in a patient in areas of the world where *Angiostrongylus cantonensis* exists, angiostrongyliasis should be suspected. It should be kept in mind that many of these symptoms can be produced by hydatids, cysticerci, flukes, *Strongyloides* spp., *Trichinella* spp., various juvenile ascarids, and possibly other lungworms. Alicata (1963) and Ash (1968) differentiated the juveniles of several species of metastrongylids that could be confused with *A. cantonensis*.

Albendazole shows promise in treating infection, but no anthelminthic appears reliably therapeutic. Dead worms in blood vessels and the central nervous system may be more dangerous than live ones. A spinal tap to relieve headache may be recommended (Ansdell et al., 2018.

Other Species in the Family Angiostrongylidae

Angiostrongylus costaricensis parasitizes mesenteric arteries of many species of rodents in Central America and South America, southern North America, and Cuba (Morera, 1985). Cases in humans have been diagnosed from countries in North America, Central America, South America, and several Caribbean islands. Worms mature in mesenteric arteries and their branches. In humans, most damage is to the wall of the intestine, especially cecum and appendix, which become thickened and necrotic, with massive eosinophilic infiltration. Abdominal pain and high fever are the most evident symptoms. These intestinal disorders are caused by pathogenic changes that affect blood vessels, or pseudo-neoplastic tissue thickening. No symptoms of meningoencephalitis are noted, unlike the symptoms that are typical in infections due to *A. cantonensis*.

Angiostrongylus vasorum is a serious, emerging disease of dogs (Morgan et al., 2005). It has been reported from many countries in Europe, North America, South America, and Africa. Adults localize in the right ventricle and pulmonary arteries of dogs and other canids and causes labored breathing, exercise intolerance, weight loss, abdominal and lumbar pain, heart failure, and sudden death. Snails and slugs can serve as experimental intermediate hosts, and frogs as transport hosts. However, the role of different infection sources for wild and domestic canids remains undetermined (Morgan et al., 2005). Genetic studies indicate that transmission occurs between wild and domestic canids (Jeffries et al., 2010).

Family Protostrongylidae

Protostrongylus rufescens parasitizes bronchioles of ruminants in many parts of the world. Its intermediate hosts are terrestrial snails, in which it develops to the third stage. The definitive host is infected when it eats the snail along with forage. Mountain sheep in America are seriously threatened by this and related species, which take a heavy toll on lambs every spring. Hibler and colleagues (1972) demonstrated transplacental transmission of *Protostrongylus* spp. in bighorn sheep.

Umingmakstrongylus pallikuukensis is a parasite in lungs of muskoxen in the Canadian Arctic. It has a snail intermediate host and its transmission dynamics are being radically altered by global warming (Kutz et al., 2004; 2005).

Other Trichostrongyloidea Species

In addition to species from ruminants already mentioned, *Cooperia curticei* (family Trichostrongylidae), *Nematodirus spathiger*, and *N. filicollis* (family Molineidae) often occur in the same host as other trichostrongyles and, together, cause much damage. *Hyostrongylus rubidus* (family Trichostrongylidae) is a serious pathogen of swine and can cause death when present in large numbers. *Heligmosomoides polygyrus* (family Heligmosomidae, *H. polygyrus = Nematospiroides dubius*) in mice and *Nippostrongylus brasiliensis* (family Heligmonellidae) in rats are easily kept in the laboratory, and they serve as important tools for research on nematode biochemistry, immunology, life cycles, and other topics (Anderson, 2000).

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